UNIVERSITEIT GENT

FACULTY OF MEDICINE AND HEALTH SCIENCES

Academic Year 2013 – 2014

RECURRENCE PATTERNS FOLLOWING MULTIMODALITY TREATMENT OF ESOPHAGEAL CARCINOMA

Hendrik THOEN

Promotor: Prof. Dr. W. Ceelen
Co-promotors: Prof. Dr. T. Boterberg and Prof. Dr. P. Pattyn

Dissertation presented in the 2nd Master year
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Date: 14/04/2014

Hendrik Thoen Prof. Dr. W. Ceelen

PREFACE

It has been 2 years since I started to work on this master thesis. During this period, I had an interesting and intriguing introduction to the world of medical research. Although it was sometimes time consuming, I certainly believe it was time well spent. I learnt a lot during the course of this master thesis. This was further enhanced by the subject's interdisciplinary character. It gave me the chance to learn and gain experience at both the Gastro-Intestinal Surgery and Radiotherapy Departments of Ghent University Hospital. Although both disciplines use totally different methods to treat patients, it was fascinating to observe how both are combined to provide the optimal standard of care to cure patients with esophageal carcinoma.

This master thesis was not the work of one person. In the past 2 years, I had the privilege to be surrounded with a substantial number of people advising, helping and supporting me.

First of all, I would like to thank prof. Ceelen, my promotor. We had numerous interesting discussions which have made a valuable contribution to the results presented in this master thesis. His constructive criticism helped to put things in perspective and to think twice before accepting new results. Despite his busy schedule, he made time for discussions and to answer my many questions. I am convinced that this work would not have been the same without his input and guidance.

I would also like to thank prof. Boterberg. With his critical attitude and scientific mind, he played an important role in the realization of this master thesis. He was my central point of contact at the Radiotherapy Department and personally gave me my first training in radiotherapy planning software (Eclipse). When I spent yet another weekend at the Radiotherapy Department to work on my thesis, prof. Boterberg was the only one I met. Even then, he made time for discussion. Together, we analyzed the CT images of several patients to decide whether suspicious lesions were to be considered as malignant or not. I am sure that his experience and knowledge have considerably contributed to the accuracy and validity of this master thesis.

During the past 2 years, I also had several meetings with prof. Pattyn. I would like to thank him for his genuine interest in my master thesis. In our conversations, he gave me interesting comments and remarks which have also contributed to the final results of this work.

As the reader will notice, a substantial part of the underlying analysis of this master thesis took place at the Radiotherapy Department. There, I received help and support from several people. First of all, I would like to thank ir. De Gersem for his technical support. His

programming skills were essential for successful conversion of different data formats and extraction of planning data. Without his input, several aspects of this master thesis would simply not have been possible. I would also like to thank Bruno and Cristina for their help with contouring of the thoracic lungs of the included patients. As will be shown, their efforts were essential to achieve some of the most important results presented in this work. Further, I would also like to thank prof. Vanderstraeten and Frank to arrange for and provide me with my first training in Pinnacle, a radiotherapy planning tool which was often used in this master thesis.

In the course of this master thesis, I also met Dr. van Daele, who is doing her PhD at the Department of Gastro-Intestinal Surgery. I would like to thank her for her interest in my work and for the time she made to discuss the results of this master thesis. As her PhD is focused on esophageal cancer, I hope that some of the presented results in this work will be of use for her and perhaps might be a start to elaborate further in the near future.

Two weeks before the deadline, I also got into contact with prof. Mareel. Although we only had one discussion so far, I hope that more will follow. With his scientific expertise and experience, he already provided me multiple suggestions to further investigate several aspects of this work. Therefore, I would like to thank him for sharing his ideas and giving me interesting input to identify possible future steps in this research.

At times, it is also important to relax and to free the mind to be able to continue with new energy. I would like to thank my closest friends Vincent, Lynn, Jeroen, Filip, Eline, Bart and Sanne for the relaxing and entertaining moments we had in this sometimes hectic period and for their continued invitations despite numerous rejections.

I would also like to thanks my parents. Without their unconditional support, it would not have been possible to start my studies in Medicine. This was definitely not evident, as I had already completed another academic study in the past.

Last, but definitely not least, I would like to thank my girlfriend Nele. With her exceptional eye for detail and excellent English language skills, she has made an important contribution to the final form of this master thesis. I am convinced that her support gave me the additional strength to obtain the results presented in this master thesis. Undoubtedly, she is now the most important person in my life. Therefore, I would like to dedicate this master thesis to her.

Yours sincerely,

Hendrik Thoen, Brussels, 07/04/2014.

LIST OF ABBREVIATIONS

5-FU	:	5-Fluorouracil	LN	:	Lymph Node
AC	:	Adenocarcinoma	LNR	:	Lymph Node Ratio
ARDS	:	Acute Respiratory Distress Syndrome	MRD	:	Mean Radiation Dose
CEA	:	Carcinoembryonic Antigen	MRI	:	Magnetic Resonance Imaging
CI	:	Confidence Interval	OR	:	Odds Ratio
CS	:	Cisplatinum	pCR	:	pathological Complete Response
CT	:	Computed Tomography	PET	:	Positron Emission Tomography
DVH	:	Dose-Volume Histogram	PLUNC	:	Planning system of the University of North Carolina
EUS	:	Endoscopic Ultrasound	pNR	:	pathological No Response
FDG	:	Fluorodeoxyglucose (18F)	pPR	:	pathological Partial Response
FNA	:	Fine Needle Aspiration	PTV	:	Planning Target Volume
GERD	:	Gastro-Esophageal Reflux Disease	RI	:	Respiratory Insufficiency
GRATIS	:	George's RAdiotherapy Treatment design System	ROI	:	Region-Of-Interest
GUH	:	Ghent University Hospital	SCC	:	Squamous Cell Carcinoma
HR	:	Hazard Ratio	SD	:	Standard Deviation
ICRU	:	International Commission on Radiation Units	TNM	:	Tumor, Node, Metastasis
IMRT	:	Intensity Modulated Radiotherapy	TR	:	Total Range
IQR	:	Inter-Quartile Range	TRG	:	Tumor Regression Grade (Mandard)

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SAMENVATTING

Inleiding: Wereldwijd is slokdarmkanker de 8ste meest voorkomende maligniteit en de 6de belangrijkste oorzaak van kanker-gerelateerde sterfte. Slokdarmkanker heeft een uitgesproken dodelijk karakter, zelfs voor curatief behandelde patiënten met lokaal gevorderd carcinoom. De meeste van deze patiënten sterven aan de gevolgen van tumor recidieven. In dit eindwerk werd een gedetailleerde studie uitgevoerd van de recidiefpatronen die optreden na een curatieve multimodale behandeling van het lokaal gevorderd slokdarmcarcinoom. Deze behandeling bestond uit neoadjuvante chemoradiotherapie gevolgd door chirurgie. Om de locoregionale controle van de behandeling te onderzoeken, werd via extractie van bestralingsparameters bepaald hoeveel locoregionale recidieven optraden in het bestralingsveld. Tijdens de analyse van de recidiefpatronen werd opgemerkt dat opvallend veel patiënten longmetastasen hadden ontwikkeld. Als mogelijke verklaring voor dit verschijnsel werd radiotherapie-geïnduceerde verspreiding van longmetastasen vooropgesteld. Daarom werd ook de relatie bepaald tussen de bestralingsdosis op de longen en het risico op ontwikkeling van longmetastasen.

Methoden: Een retrospectieve studie van patiënten met lokaal gevorderd slokdarmkanker werd uitgevoerd. Alle geïncludeerde patiënten werden curatief behandeld met neoadjuvante chemotherapie (5-FU en/of cisplatinum) en radiotherapie (totale dosis van 36 of 45 Gy) gevolgd door Ivor-Lewis oesofagectomie. Alle operaties vonden plaats in het Universitair Ziekenhuis Gent. Demografische, klinische en pathologische patiëntkarakteristieken werden verzameld via raadpleging van de elektronische en/of papieren patiënten dossiers. Recidieven werden gedetecteerd via klinisch onderzoek en/of medische beeldvorming. Indien mogelijk, werd de aanwezigheid van een recidief bevestigd via weefselbiopsie. Voor alle patiënten werden alle tumorrecidieven in rekening gebracht tot de dood of het laatste contact. Recidieven werden geclassificeerd als locoregionaal of op afstand. Om te bepalen of locoregionale recidieven gelegen waren in het bestralingsveld, werd eerst een beeldfusie uitgevoerd tussen een radiotherapie planning CT en een follow-up CT met de tumorrecidieven. Vervolgens werd een Region-Of-Interest (ROI) getekend op de planning CT rond elk locoregionaal recidief en werd daarvan de gemiddelde bestralingsdosis (GBD) geëxtraheerd. Een locoregionaal recidief werd als in het bestralingsveld beschouwd indien de GBD van de corresponderende ROI ten minste 95% bedroeg van de totale dosis. Om de

mogelijke radiotherapie-geïnduceerde verspreiding van longmetastasen te onderzoeken, werd het thoracaal gedeelte van de longen ingetekend op de beschikbare planning CT's. De dosis-volume histogrammen (DVHs) werden dan geëxporteerd voor patiënten met en zonder longmetastasen. Vervolgens werd de D_8 gecategoriseerd en gebruikt als verklarende variabele om het risico op longmetastasen te voorspellen. Dit werd verder onderzocht in een multivariabel model door te corrigeren voor leeftijd, klinisch stadium, thoracale tumor locatie en pathologische respons. De invloed van de D_8 werd ook bepaald op de overleving in een multivariabel model na correctie voor dezelfde variabelen, aangevuld met de lymfeklierverhouding (Eng. LNR).

Resultaten: Tachtig patiënten werden geïncludeerd in de analyse van de recidiefpatronen. Hiervan hadden 29% klinisch stadium II en 69% stadium III (TNM 7de editie). Voor 2 patiënten was stadiëring niet mogelijk. De mediane follow-up duur was 19 maanden. Vierenzestig procent van de patiënten had tumorrecidief. Locoregionale recidieven en metastasen op afstand werden gedetecteerd in resp. 39% en 59%. De grote meerderheid van de patiënten met locoregionaal recidief had ook metastasen op afstand (87%). Meest frequent traden metastasen op afstand op in de longen (29%), het bot (19%) en de lever (19%). Vijftien van 95 locoregionale recidieven (16%) werden gedetecteerd in het bestralingsveld in 6 patiënten (8%). Significant meer longmetastasen werden gedetecteerd bij patiënten met een D_8 op de longen tussen 15.7 en 29.7 Gy vergeleken met patiënten met lagere of hogere dosissen (67% versus 33%, P = 0.041). In een univariabel model hadden de patiënten met een D_8 tussen 15.7 en 29.7 Gy een 3.06 keer hoger risico om longmetastasen te ontwikkelen (95% CI: 1.02-9.13, P = 0.045). Dit verhoogd risico bleef significant in een multivariabel model (OR: 3.32, 95% CI: 1.00-10.96, P = 0.050). Bovendien hadden deze patiënten na correctie een 2.44 keer hoger risico op kanker-gerelateerde sterfte (95% CI: 1.03-5.80, P = 0.043).

Conclusie: Recidief op afstand was het dominante recidiefpatroon bij patiënten curatief behandeld met multimodale therapie voor het lokaal gevorderd slokdarmcarcinoom. De longen waren het meest aangetaste orgaan (29%). Slechts 8% van de patiënten hadden locoregionaal recidief in het bestralingsveld, wat een excellente locoregionale controle aantoont van de gebruikte therapie. De meerderheid van patiënten met locoregionaal recidief had echter ook metastasen op afstand (87%). Dit suggereert de nood aan de ontwikkeling van meer effectieve systemische therapie. Patiënten met een D_8 op de longen tussen 15.7 en 29.7 Gy hadden een 3.32 keer hoger risico om longmetastasen te ontwikkelen en stierven 2.44 keer sneller. Dit indiceert dat de verspreiding van longmetastasen gestimuleerd wordt door radiotherapie, maar enkel indien de dosis op de longen zich bevindt in een bepaald bereik.

ABSTRACT

Introduction: Esophageal carcinoma is the 8th most prevalent malignancy and the 6th leading cause of cancer-related death worldwide. It has a pronounced deadly potential, even for patients treated with curative intent for locally advanced cancer. For these patients, the principal cause of death is tumor recurrence. In this master thesis, a detailed analysis was performed of the recurrence patterns developing after curative multimodality treatment of patients with locally advanced esophageal carcinoma. Multimodality treatment consisted of neoadjuvant chemoradiation followed by surgery. The number of locoregional recurrences occurring within the radiation target volume was investigated by extraction of radiation dose parameters. During analysis of the recurrence patterns, it was noticed that lung metastases were detected in a conspicuous amount of patients. It was then proposed that the dissemination of lung metastases might be enhanced by the radiotherapy treatment. Therefore, the relation between the radiation dose on the lungs and the risk of developing lung metastases was also assessed in this master thesis.

Methods: A retrospective study was performed of patients with locally advanced esophageal cancer. All included patients were curatively treated with concurrent neoadjuvant chemotherapy (5-FU and/or cisplatinum) and radiotherapy (total dose of 36 Gy or 45 Gy) followed by Ivor-Lewis esophagectomy. Surgery was performed at Ghent University Hospital for all patients. The patients' demographic, clinical and pathological characteristics were obtained via consultation of the electronic and/or paper medical records. Tumor recurrences were detected by clinical examination and/or medical imaging (CT, MRI, FDG-PET or bone scan). If possible, a biopsy had been performed to confirm the diagnosis of recurrent esophageal cancer. For each patient, all tumor relapses were recorded until death or the last patient contact. Recurrences were classified as locoregional or distant. To determine whether locoregional failures were located with the radiation field, first image fusion of radiotherapy planning CT with follow-up CTs was performed. A Region-Of-Interest (ROI) was then contoured on the planning CT at the location of and surrounding each locoregional relapse. Subsequently, the mean radiation dose (MRD) was extracted for each ROI. A locoregional recurrence was considered to occur within the radiation field if the MRD of its corresponding ROI was at least 95% of the prescribed dose. To examine the possible enhancement of lung metastases by radiotherapy, the thoracic part of the lungs was contoured on each available planning CT. The dose-volume histograms (DVHs) were then extracted for patients with and

without lung metastases. Based on these DVHs the D_8 was categorized and used as explanatory variable in a univariate model to compare the occurrence of lung metastases between both groups. The independence of the D_8 in predicting lung metastases was further investigated in a multivariate model by correcting for age, clinical stage, thoracic tumor location and pathological response. The D_8 was also used to predict survival of the included patients, first in a univariate and subsequently in a multivariate analysis by correcting for the same variables and adding the lymph node ratio.

Results: Eighty patients were included in the recurrence pattern analysis, of which 29% had clinical stage II and 69% stage III (TNM 7^{th} edition). Staging data were incomplete for 2 patients. The median follow-up time was 19 months. Sixty-four percent of patients experienced tumor relapse. Locoregional and distant failure were detected in 39% and 59% of patients respectively. The vast majority of the locoregionally relapsed patients (87%) also had distant tumor recurrence. The most frequent sites of distant failure were the lungs (29%), the bone (19%) and the liver (19%). Fifteen of 95 (16%) locoregional relapses were detected within the radiation field in 6 patients (8%). None of the patients with in-field failure had a pathological complete response, while all had distant metastases. Significantly more lung metastases were detected in patients with a D_8 on the lungs between 15.7 and 29.7 Gy compared to patients with lower or higher doses (67% versus 33%, P = 0.041). In a univariate model, patients with D_8 between 15.7 and 29.7 Gy had a 3.06 times higher risk to develop lung metastasis (95% CI: 1.02-9.13, P = 0.045). This increased risk remained significant in a multivariate model (OR: 3.32, 95% CI: 1.00-10.96, P = 0.050). Furthermore, these patients had a 2.44 times higher risk of cancer-related death after correction for demographic and clinicopathological variables (95% CI: 1.03-5.80, P = 0.043).

Conclusion: Distant relapse was the predominant recurrence pattern experienced by patients curatively treated with multimodality therapy for locally advanced esophageal carcinoma. The lungs were the most frequent site of tumor recurrence. Only 8% of patients had locoregional recurrence within the radiation field. This demonstrates the excellent in-field control of the used multimodality treatment. Nevertheless, the vast majority of patients with locoregional recurrence had simultaneous distant relapse (87%). This clearly suggests the need for more effective systemic therapies. Patients with a D_8 on the lungs between 15.7 and 29.7 Gy, had a 3.32 times higher risk of developing lung metastasis and died 2.44 times faster from esophageal cancer. This is a strong indication that dissemination of lung metastasis might be enhanced by radiotherapy, but only if the lungs receive doses within a certain specified range.

I. INTRODUCTION

I.1. Epidemiology

Esophageal carcinoma is the 8th most prevalent cancer worldwide, having an incidence rate of 16.2 per 100 000 person-years.[1] The incidence is considerably smaller in Belgium, where an age standardized rate (using the World Standard Population) of 9.3 per 100 000 person-years was observed in 2008.[2] Although it is a relatively rare disease, esophageal cancer has a pronounced lethal potential. It is the 6th leading cause of cancer-related death in the world.[1] In 2005, around 498 000 new cases were diagnosed globally. Furthermore, a mortality-to-incidence ratio (MIR) of 83.6% was attained, as an estimated number of 416 500 patients died of esophageal carcinoma in that same year.[3] In Belgium, a remarkably smaller MIR of 71.0% was perceived in 2008 [2], pointing to the good availability and high quality of the health care services in our country.

Adenocarcinoma (AC) and squamous cell carcinoma (SCC) are the two most common histological types. Together they account for more than 90% of all esophageal cancers.[4] SCC still is the most prevalent esophageal cancer worldwide, but during the past decades considerable epidemiological changes took place in the developed countries. While the incidence of SCC remained relatively stable or even decreased, a remarkable increase in the incidence of AC was demonstrated. AC even became the fastest increasing cancer in the US in the 2000s [5], where it increased 6-fold between 1975 and 2000.[6, 7] Furthermore, similar epidemiological changes were observed in several European countries. The highest incidence of AC in the world is found in the UK, with an incidence rate of 7 per 100 000 person-years.[8, 9]. In the Netherlands, the incidence of AC increased by a factor of 3.2 for males and by 2.4 for females in the period 1989-2003.[10] In Belgium, the incidence of AC increased for males by a factor of 2 between 1999 and 2008, while for females no significant change was observed.[2] As a consequence of the evolutions described above, AC has become the predominant esophageal cancer type of the Western world.

Regardless of the histological type, esophageal cancer is a deadly malignancy. Overall 5-year survival ranges between 10-25%.[5, 11, 12]. In Belgium, a 5-year survival of 22.8% was observed in the period 2004-2008.[13] The marked lethality of esophageal carcinoma can be explained by the late onset of symptoms caused by the cancer.[14] At the time of diagnosis, more than 50% of patients have either unresectable tumors or metastasized disease.[11] Furthermore, up to 65% of patients curatively treated have locoregional or distant recurrence

within 5 years.[15] Survival after recurrence is extremely poor, with a median survival of only 10.5 months.[16]

It is clear from the above considerations that esophageal cancer is becoming an increasing health problem in our regions, mainly due to the rising incidence of AC. Moreover, low survival and high recurrence rates persist. It is, therefore, of great importance to conduct further research in the domain of esophageal carcinoma, in order to investigate the reasons for treatment failure and to improve survival of these patients.

I.2. Pathogenesis

I.2.1. Tumor types

As stated in paragraph I.1, SCC and AC are the most common types of esophageal cancer. Other much less prevalent esophageal carcinomas are e.g. mucoepidermoid, adenosquamous or small cell carcinoma. Rarely, carcinoids, lymphomas or non-epithelial tumors such as leiomyosarcomas or melanomas can affect the esophagus.[17] In the following section, we will only discuss the risk factors of the 2 most prevalent types of esophageal cancer: SCC and AC.

I.2.2. Risk factors

The risk of both SCC and AC increases with age, with an average age at diagnosis of 67 years.[4] For both types, esophageal cancer is clearly more prevalent in males than in females. Depending on the geographical region, male:female ratios of 2:1 to 4:1 are obtained for SCC.[18] This male predominance is even higher for AC, especially in the Western countries. In the US, a male:female ratio of as high as 7:1 was achieved in the period 1993-2002.[19] Besides age and gender, several other risk factors are known to be associated with esophageal carcinoma. An overview of these risk factors is given in Table 1.[11, 20-22]

Tobacco use is associated with an increased risk in both SCC and AC. This increased risk is related to the direct exposure of the esophagus to carcinogenic substances in cigarette smoke, including nitrosamine.[23] Alcohol is associated with an increased risk in SCC, but not in AC.[24, 25] In the pathophysiology of SCC, the alcohol metabolite aldehyde, a known carcinogen, most likely plays a predominant role. This is supported by the fact that mutations in the alcohol metabolizing enzymes are associated with an increased risk of SCC.[26] There is evidence that alcohol abuse and smoking interact in the pathogenesis of SCC. In one study a multiplicative effect was found, with a 13-fold increase of the risk for heavy smokers (30 or

more cigarettes per day) and drinkers (4 or more glasses alcohol per day) compared to the non-smoking/non-drinking category.[27]

Gastro-esophageal reflux disease (GERD) is one of the strongest risk factors for AC. This risk is independent of the presence of a Barrett's esophagus. One Swedish case-control study found that the risk of AC was almost 8 times as high among persons with weekly GERD symptoms compared to persons without symptoms.[28] The exact mechanisms through which GERD plays a role in the pathogenesis of AC remain unclear, but it is assumed that chronic irritation and inflammation of the esophageal mucosa by frequent reflux of gastric acid and bile are possible carcinogenic processes.

SCC	AC
Tobacco use (+)	Tobacco use
Alcohol abuse (+)	Symptomatic gastro-esophageal reflux disease (+)
Achalasia (+)	Barrett's esophagus (+)
Caustic injury of the esophagus (+)	Obesity
History of thoracic irradiation (+)	History of thoracic irradiation (+)
Low socioeconomic status, poverty	Medications: anticholinergics, beta-agonists
Frequent consumption of extremely hot beverages	Hiatal hernia
Low fruit and vegetable intake	Low fruit and vegetable intake
Frequent consumption of red meat	Frequent consumption of red meat
Black race	White race
Genetic factors: TERT and p53 polymorphisms	Genetic factors: cyclin D1 and TERT polymorphisms
Plummer-Vinson syndrome (+)	
History of head and neck cancer (+)	
Non-epidermolytic palmoplantar keratoderma (+)	
Opium consumption	
Lye (NaOH) ingestion	
Poor oral hygiene	

Table 1: Known risk factors associated with SCC and AC. The plus sign (+) indicates the strongest risk factors, giving an increased risk by a factor of four or more.

Another important risk factor of AC is the Barrett's esophagus.[28] In a Barrett's esophagus the normal stratified squamous epithelium of the esophagus is replaced by metaplastic columnar epithelium. Although the Barrett's esophagus is considered to be a protective adaptive mechanism of the distal esophagus against an increased environmental acidity, it is also associated with chronic inflammation and, hence, with increased oxidative stress, DNA damage and carcinogenesis. The risk of AC in patients with Barrett's esophagus has been estimated to be 0.12 - 0.5% per year and is highest in patients with high-grade dysplasia of the

esophagus [29]. In one study, 16% of patients with high-grade dysplasia developed AC after an average follow-up time of 7.2 years.[30] In a meta-analysis, it was concluded that 6% of the patients with high-grade dysplasia developed AC per year.[31]

Obesity is also an important risk factor of AC. Although the increased risk of AC in obese patients remains rather limited compared to GERD and the Barrett's esophagus, it is to be considered as a major risk factor due to the widespread prevalence of obesity in the world. Furthermore, it is thought that the increasing incidence of obesity in the developed countries is adding to the rising incidence of AC. There are two possible mechanisms that may explain the role of obesity in the pathogenesis of AC. First, obesity increases the intra-abdominal pressure and hence stimulates the process of gastro-esophageal reflux. This reflux causes chronic inflammation, which in the long term may promote carcinogenesis. Another possible mechanism is the secretion of adipokines and cytokines by the adipose tissue itself, as these substances may stimulate tumor development.[32]

I.2.3. Mechanisms of tumor spread

Locally advanced esophageal cancer may directly invade the adjacent structures. These include, but are not limited to, the trachea, the bronchi, the pleura, the aorta, the heart and the stomach.[33] However, tumor metastasis, either present at diagnosis or occurring as recurrence after treatment with curative intent, is the main cause of death of patients with esophageal carcinoma. Similar to most other cancers, two main routes of tumor dissemination exist in the body, i.e. lymphogenic and hematogenic metastasis. A third, be it less frequent, pattern of tumoral spread in esophageal cancer is serosal metastasis.

An important factor explaining the deadly potential of esophageal carcinoma is the unique anatomy of the lymphatics.[34, 35] The esophagus has a complex network of lymphatic vessels penetrating into the mucosa, which is not present in the other hollow gastro-intestinal organs such as e.g. the stomach.[36] The lymphatic drainage system of the esophagus consists of longitudinal and transverse components. The longitudinal vessels form an extensive submucosal lymphatic network along the axis of the esophagus. Small transverse vessels originate in the lamina propria and connect this submucosal system to the esophageal mucosa. Other transverse vessels pierce the muscularis propria and connect the longitudinal system intermittently to the regional lymph nodes in the peri-esophageal tissue or drain directly into the ductus thoracicus.[37, 38] Due to this unique anatomy, lymphatic spread of a superficial tumor can occur rapidly if only the lamina propria is breached. The longitudinal interconnections explain the existence of so called skip metastases, by which lymph node

metastases occur in distant stations without invasion of the regional lymph nodes. These distant lymphogenic metastases, e.g. cervical lymph nodes in lower third tumors or abdominal lymph nodes in upper third tumors, were found to develop in 37 to 40% of the patients with esophageal carcinoma.[39, 40] Via the intermittent transverse connections, the tumor can spread to the regional lymph nodes, but also directly to the ductus thoracicus. From there, it can reach the venous circulation and spread hematogenously to the distant organs. In this way, even superficial tumors quickly become systemic diseases, which adds to the poor survival and high recurrence rate regardless of the extensive multimodal therapies currently employed to treat esophageal carcinoma.

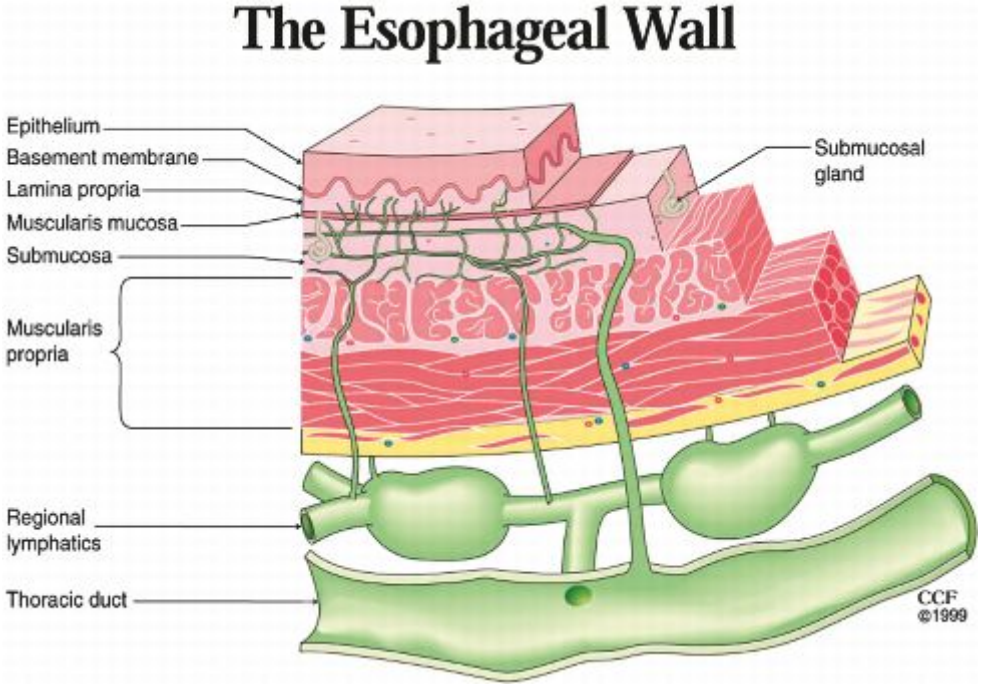


Figure 1: The lymphatic drainage system of the esophageal wall. Retrieved February, 28th, from Cleveland Clinic website: http://www.clevelandclinicmeded.com.

When the invading tumor cells reach the blood vessels, hematogenous metastasis may occur. Esophageal cancer most frequently spreads to the liver and the lungs. Occasionally, the tumor metastasizes to the bone and the adrenals. Less frequent sites are the skin, the muscles, the brain, the thyroid, the heart, the spleen, the stomach, the gallbladder, the intestines, the pancreas and the kidneys.[33, 41, 42]

Hematogenous metastasis might be accelerated by surgery. The rationale behind this phenomenon is that most growth factors, chemokines and cytokines involved in surgical wound healing also promote tumor invasion and angiogenesis.[43] Another proposed mechanism of iatrogenic hematogenous spread is radiation induced metastasis. There is a multitude of experimental evidence supporting the existence of this phenomenon, although

clinical evidence remains inconclusive up to date.[44] Nevertheless, it will be demonstrated in this master thesis that there are indications that this mechanism might also play a non-negligible role in clinical practice.

A third type of tumor metastasis is serosal dissemination in the pleural, peritoneal or pericardial cavities. A possible mechanism explaining the serosal spread of esophageal carcinoma, is the exfoliation of free cancer cells by locally advanced cancer which has invaded the pleural, pericardial or peritoneal cavities. Another possible mechanism is surgery induced serosal dissemination. In an autopsy study of 69 patients who died from esophageal carcinoma, the patterns of metastasis were compared between two groups: one group was treated with esophageal resection, while the other group received non-surgical treatment (mainly radiotherapy). Serosal cancer dissemination (pleural, peritoneal and pericardial) occurred in 36% of the resected patients, while in only 25% of the non-resected patients. This difference was non-significant (P = 0.097), but yet the authors suggested that surgery might increase the incidence of serosal metastasis.[33] Similarly, iatrogenic dissemination caused by the operation itself has already been described as a possible mechanism of peritoneal carcinomatosis in patients treated with curative surgery of gastric carcinoma.[45]

I.3. Diagnosis

I.3.1. Symptoms

At the time of diagnosis, patients with esophageal cancer most frequently present themselves with dysphagia and/or weight loss. An overview of the most common symptoms and their frequency is given in Table 2.

Symptoms	Frequency (%)	Symptoms	Frequency (%)
Dysphagia	74.0	Chronic cough	10.8
Weight loss	57.3	Hoarseness	6.1
Heartburn	20.5	Hematemesis	5.6
Odynophagia	16.6	Cervical adenopathy	5.5
Dyspnea	12.1	Hemoptysis	3.6

Table 2: Most prevalent symptoms of esophageal cancer at the time of diagnosis, adopted from Daly et al.[4]

I.3.2. Diagnostic investigations

When the diagnosis of esophageal carcinoma is suspected based on the clinical symptoms of the patient, an esophagogastroduodenoscopy is performed. If a suspicious lesion is detected, multiple mucosal biopsies are obtained.[46] The diagnosis is then confirmed after pathological examination of these biopsies.

I.3.3. Staging

After confirmation of the diagnosis, cancer staging is performed in accordance with the TNM (Tumor invasion, Nodal status and Metastasis) classification to decide on further tumor management.[47] T and N stadium are determined by endoscopic ultrasound, supplemented with fine-needle aspiration (EUS-FNA) if indicated, which has an accuracy of 72% for overall staging and 90% for nodal staging.[48] The M stadium is evaluated by a CT scan of the thorax and abdomen. Optionally, an FDG-PET-CT scan is performed to detect distant metastasis or to allow metabolic evaluation of the effect of induction therapy.[49]

I.4. Treatment

I.4.1. General

The treatment of esophageal carcinoma is dependent on the tumor stage. Esophagectomy alone is the standard treatment for early esophageal cancer, which includes T_{is} (carcinoma in situ) and $cT_{1-3}N_0M_0$ (non-obstructing and node negative disease).[50] Patients with significant comorbidity or non-resectable locoregional tumors are treated with definitive chemoradiotherapy according to the Herskovic scheme.[51] Metastatic disease (M_1) is approached with palliative treatment, depending on the symptoms and preferences of the patient.

All patients included in this thesis had locally advanced cancer, i.e. $cT_3N_0M_0$ with obstructing tumors or threatened circumferential resection margin, operable $cT_4N_0M_0$ or $cT_{1-4}N_{1-3}M_0$ (operable and node positive disease). These cancers are treated with curative intent with multimodality therapy. In a large part of the developed world, including Belgium, the Netherlands and the US, trimodality therapy is used, consisting of neoadjuvant chemoradiotherapy followed by surgery.[22, 52-54]

I.4.2. Surgery

There are a multitude of procedures to perform a surgical resection of the esophagus. Among these, the Ivor-Lewis esophagectomy is a widely used and well-known technique. In this technique, first a laparotomy is performed to mobilize the stomach. After closing the abdomen, a right thoracotomy is done to resect the major part of the esophagus (starting at the level of the azygos vein) together with the proximal stomach. In a final step, the remaining part of the stomach is pulled up in the thorax and attached to the proximal esophagus using a stapler device to make the gastro-esophageal anastomosis. [55, 56] To further reduce the chance of recurrence and to perform accurate pathological staging, a 2-field

lymphadenectomy is commonly performed during surgery, consisting of a systematic lymph node removal in the chest and the abdomen. Abdominal lymph node removal is usually limited to the paracardial nodes, the nodes along the lesser curvature and the nodes along the celiac, hepatic and left gastric arteries.

I.4.3. Neoadjuvant chemoradiotherapy

Neoadjuvant chemoradiotherapy involves concurrent administration of chemotherapy and radiotherapy in order to achieve tumor down-sizing and down-staging, before surgery is performed. This way the chance of an R0 resection, i.e. the entire removal of macro- and microscopic tumor tissue, is increased and better locoregional control is achieved.[57] This advantage is obtained through the synergistic effect of chemo- and radiotherapy, since chemotherapy offers the ability to induce radiation sensitization. In this process, the effect of radiotherapy is enhanced by synergistic DNA damage, cell cycle synchronization and inhibition of repair and resistance pathways.[58, 59] Theoretically, chemotherapy also has potential to decrease the risk of distant spread due to targeting of micrometastases, which are undetectable by the current staging techniques.[60, 61]

It has long been questioned whether neoadjuvant chemoradiotherapy had the potential to improve survival. Recently, a meta-analysis provided strong evidence that neoadjuvant chemoradiation followed by surgery indeed has a significant survival benefit over treatment with surgery alone.[62] In this analysis, a hazard ratio for all-cause mortality (HR) of 0.78 (95% CI: 0.70-0.88, $P < 0.0001$) was obtained in favor of the neoadjuvant group. Furthermore, comparable hazard ratios were obtained for both SCC and AC. In the most recent trial included in the meta-analysis (the CROSS trial), the median overall survival was 49.4 months in the neoadjuvant group versus 24.0 months in the surgery-only group (HR of 0.66, 95% CI: 0.50-0.87, $P < 0.003$).[63]

Two meta-analyses of 1 116 and 1 308 patients respectively, which compared the recurrence patterns after neoadjuvant chemoradiation and surgery as opposed to surgery alone, concluded unanimously that the locoregional cancer recurrence rate was significantly lower in the neoadjuvant treatment group. Moreover, both concluded that no significant differences were obtained for distant recurrence rates.[64, 65] This result was contradicted recently (2014) by the analysis of recurrence patterns of the patients treated in the CROSS trial.[54] As expected, the neoadjuvant group had significantly lower locoregional (34% vs. 14%, $P < 0.001$) recurrences as the surgery-only group. However, also a significant reduction of both

hematogenous recurrence (35% vs. 29%, P = 0.025) and peritoneal carcinomatosis were obtained (14% vs. 4%, P < 0.001).

In summary, it can be concluded that neoadjuvant chemoradiotherapy gives a modest, but significant benefit in survival compared to surgery alone. This is mainly achieved by better locoregional control, but there is evidence indicating that the reduction of hematogenous and serosal recurrences may also play a limited role. However, despite these recent improvements in survival, the prognosis at diagnosis of locally advanced esophageal cancer generally remains poor. There is, therefore, a clear need for optimization of the existing treatment modalities or development of new and more effective therapies.

I.4.4. Pathological response

The pathological investigation of the resected specimen offers unique possibilities to evaluate the tumor response to neoadjuvant therapy. A pathological complete response (pCR) is obtained when no signs of local tumor are found in the resected sample (ypT_0N_0). This pCR is an important prognostic factor to predict survival in patients treated with multimodality therapy.

Several studies have demonstrated a significant improvement in survival for patients with pCR. In the literature, 3 and 5-year survival rates of patients with pCR are found to be in the range of 49-80% and 34-62% respectively. The reported rates for patients with no pCR are considerably lower with a 3-year survival rate between 27-45% and a 5-year survival between 18-41%.[57]. This improvement in survival is linked to a significant decrease in recurrence rate for patients with pCR. Rohatgi et al. found a recurrence rate of 19% in the pCR group, while recurrence rates of 33 and 39% were obtained for the patients with partial and no response respectively (P = 0.04). Furthermore, the overall and disease-free survival in the pCR and the partial response group were significantly longer than in the no-response group.[66] Meguid et al. drew similar conclusions and found in their multivariate analysis that pathologic response was the only factor associated with tumor recurrence or death.[67]

Mandard et al. developed a classification system of 5 tumor regression grades (TRGs) to describe the different responses of the esophageal tumor to neoadjuvant treatment. In this system, the lowest grade (TRG1) corresponded with a pCR (only fibrosis, no tumor cells), while the highest grade (TRG5) was characterized by a complete absence of regressive changes (negligible fibrosis). TRG2 until TRG4 described intermediate responses, with a higher grade involving more tumor cells and less fibrosis. In their multivariate analysis, only

tumor regression (i.e. TRG1-3 versus. TRG4-5) was significantly associated with disease-free survival ($P < 0.001$).[68]

The above considerations demonstrate that patients with substantial tumor responses have significant advantages regarding survival, especially patients with a pCR. Improvement of the pCR rate is, therefore, to be considered as the most important surrogate endpoint in esophageal cancer treatment.

I.5. Recurrence patterns

From the preceding section, it is concluded that neoadjuvant chemoradiation has substantially enhanced locoregional tumor control. Furthermore, distant recurrence has become the predominant failure pattern. Most probably, this distant failure can be attributed to distant micrometastases which were already present at diagnosis (or which developed in the time period between diagnosis and surgery, during neoadjuvant treatment) and did not respond well to chemotherapy. These recurrences may be only treatable with better systemic therapies, as they were already beyond local control at the time of diagnosis.

On the other hand, as stated in section I.4.3, strong evidence indicates that neoadjuvant therapy significantly increases survival through its improvements in locoregional control. It is, therefore, quite likely that survival can be even further increased if efforts are made to optimize locoregional therapy. In this respect, it is interesting to know if locoregional recurrences are located in- or outside the radiation field. In the first case, dose escalation would seem the appropriate action as a higher dose would destroy more tumor tissue and, hence, reduce the chance of recurrence. On the other hand, when recurrences are located outside the radiation field, it would seem preferable to expand the radiation field to improve locoregional control. In any case, the number of recurrences within the radiation target volume can be considered as a strong indicator of the strength of the locoregional effect of the combined multimodality treatment.

In the abovementioned CROSS trial publication [54], it was also investigated whether recurrences after neoadjuvant chemoradiotherapy and surgery were in- or outside the irradiated volume. It was found that only 5% of patients had recurrences within the planning target volume (PTV), demonstrating excellent in-field control of the multimodality treatment. However, the dose parameters, i.e. the radiation doses at the tumor relapse locations, were not specified, not enabling us to quantify the results. This was the only relevant study we found in the literature, investigating the relation between tumor relapses and radiation target volume.

I.6. Aims

The aim of this master thesis is to perform a detailed analysis of the recurrence patterns occurring after neoadjuvant chemoradiation and Ivor-Lewis esophagectomy of patients curatively treated for locally advanced esophageal carcinoma. Thorough knowledge of these recurrence patterns has to enable us to further optimize the treatment of esophageal cancer. More in particular, we investigated the relation between the occurrence of locoregional relapses and the radiation target volume by extraction of radiation dose parameters. To our knowledge, this has never before been published in the literature.

During the course of this master thesis, it was noticed that a conspicuous amount of patients had lung metastases. It was proposed that tumor dissemination from primary esophageal cancer to the lungs might be enhanced by radiotherapy treatment. Therefore, the relation between the radiation dose on the lungs and the risk of developing lung metastasis was investigated by extraction of dose parameters. Again, no publications were found in the literature indicating the possible existence of radiation induced enhancement of lung metastasis in patients treated for esophageal carcinoma.

II. METHODS

II.1. Study design and inclusion criteria

A retrospective study was performed of patients treated with chemoradiotherapy plus surgery for cancer of the esophagus between 2003 and 2012. The collection of patient data was terminated on 31/10/2013. To be included in the study, patients had to fulfill the following criteria:

1. Diagnosis of locally advanced carcinoma ($cT_3N_0M_0$, $cT_4N_0M_0$ or $cT_{1\text{-}4}N_{1\text{-}3}M_0$, see also section I.4.1) of the thoracic or abdominal part of the esophagus. Esophageal cancer was diagnosed by esophagogastroduodenoscopy and biopsy. Local staging was performed using EUS-FNA, while distant staging was done by CT or FDG-PET-CT of the thorax and abdomen.
2. Neoadjuvant therapy consisted of concurrent radiotherapy and chemotherapy. The planned radiation dose was 36 Gy or 45 Gy. Irradiation was performed in 20 or 25 fractions of 1.8 Gy during 4 or 5 weeks respectively. The planned chemotherapy consisted of 5-fluorouracil (5-FU) and/or cisplatinum. 5-FU (800 mg/m³) was administered during days 1 to 4 of the first and the last week of the radiotherapy treatment, while cisplatinum (80 mg/m³) was only given on day 1 of the first and the last week. Three to 4 weeks after completion of the neoadjuvant therapy, the tumor response was evaluated by a CT of the thorax and abdomen. If no progression of tumor was observed on the CT, a surgical procedure was planned. Neoadjuvant therapy was either administered at Ghent University Hospital (GUH) or at peripheral hospitals.
3. Surgery was performed by Ivor-Lewis esophagectomy 4 to 5 weeks after completion of the neoadjuvant therapy. All surgical procedures were done at GUH between 2003 and 2012.

II.2. Patient characteristics

II.2.1. General

Of all patients included in the study, demographic, clinical and pathological data were collected. To start this data collection, a database was available within the Department of Gastro-Intestinal Surgery. For each patient, these data were completed mainly by analysis of the electronic medical records, but also the paper medical records were consulted, to the extent necessary.

II.2.2. Demographic variables

For all patients, the age at cancer diagnosis and the gender were determined.

II.2.3. Clinical variables

The clinical variables contain information obtained during diagnosis and the staging process. From the pathological examination reports of the biopsies taken during esophagogastroduodenoscopy, we extracted the tumor differentiation grade and tumor histology. From the EUS examinations, we obtained the clinical T and N stage (according to the TNM classification, edition 7), the endoscopic tumor distance (expressed in cm from the incisor teeth) and the tumor length (cm). Lastly, the clinical M stage and the tumor location (upper, middle or lower third of the thoracic esophagus) were determined on CT or FDG-PET-CT.

II.2.4. Pathological variables

The pathological variables comprise information acquired during pathological examination of the resected esophageal specimen and lymph nodes. From the pathological reports, the following variables were obtained: pathological pT and pN stage (TNM 7^{th} edition), positive proximal or distal resection margins, the total number of investigated lymph nodes, the number of lymph nodes invaded by tumor

+cells and the lymph node ratio (the number of invaded lymph nodes divided by the total number of lymph nodes). From the pT and pN stages we could determine the number of patients with a pCR (ypT_0N_0, see section I.4.4).

II.3. Follow-up

II.3.1. Duration

All patients were followed until death or until the last patient contact. As stated in section II.1, the status of all surviving patients was last checked on 31/10/2013. In this study, the follow-up duration is defined as the elapsed period (expressed in months) between surgery and death or last follow-up of the patient. When patients were followed up in peripheral hospitals, their physicians (GP or specialist) were contacted via telephone to obtain their survival status.

II.3.2. Investigations

Three months after surgery, a first re-evaluation was performed consisting of a clinical exam, blood tests (including CEA determination, only for AC) and CT or FDG-PET-CT to detect possible tumor recurrences. This was then followed by an analogous reassessment every 4 to 6 months. If a possible tumor relapse was detected, additional investigations were performed

according to its location. These could include additional CT/FDG-PET-CT scans, MRI scans, bone scintigraphies or biopsies. For each tumor recurrence, the date of first detectable signs was extracted. Based on these dates the disease-free survival, from to the date of surgery, was then determined for each patient.

II.4. Recurrence pattern analysis

II.4.1. Detection

Tumor recurrences were detected by clinical examination or medical imaging (CT, FDG-PET-CT, MR or scintigraphy). If possible, a biopsy was carried out to prove tumor relapse. If follow-up took place at GUH, the medical imaging data (and their reports) were available via PACS and reports about the clinical and pathological examinations were consultable in the electronic medical records. The necessary recurrence data (medical imaging and reports) of the patients followed up in peripheral hospitals, were obtained by contacting the corresponding physicians by mail and/or phone.

For each patient, all recurrences appearing during the entire follow-up duration (until death or last follow-up) were documented and included in the analysis. This contrasts with the conventional approach used by most other studies, which traditionally only take the first relapse into account together with the recurrences occurring within an arbitrary period of 1 month of the first (which are then considered to appear simultaneously). Afterwards, no more relapses are documented and the classification of patients with locoregional and/or distant failure (see II.4.2) is based on the location of the first recurrences. As a result, patients who have distant metastasis 2 months after the occurrence of a first locoregional relapse are incorrectly classified as having only locoregional failure, simply because of incomplete recurrence pattern registration. To avoid this problem, it was chosen in this master thesis to document all tumor recurrences until patient death or the last patient contact.

II.4.2. Classification

We classified the recurrences as local, regional or distant. Local failure arises from residual malignant cells at the primary tumor site, which were not eliminated by the treatment. After surgery, local recurrences typically occur at the level of the gastro-esophageal anastomosis in the remaining proximal part of the esophagus. Less frequently, local recurrences are seen in the gastric wall or at the circumferential resection margin. Regional failure is defined as recurrence within the regional lymph nodes of the esophagus. Local and regional relapses are

grouped under the term locoregional failure. Distant failure includes the distant (non-regional) lymph node, the serosal and the hematogenous recurrences (see also I.2.3).

To classify the lymph nodes recurrences, several lymph node maps were used. The cervical, thoracic and abdominal lymph nodes were classified according to the maps presented by the American Head and Neck Society [69], the International Association for Study of Lung Cancer (IASLC) [70] and the Japanese classification of gastric carcinoma [71] respectively. Other (indicated by the letter O) lymph nodes were grouped as axillary (O1), mesenteric (O2), iliac (common and external, O3) and inguinal (O4). The used lymph node maps are presented in Figure 2 to Figure 4. For a detailed description of the anatomic borders of these lymph node stations, we refer to the corresponding references.

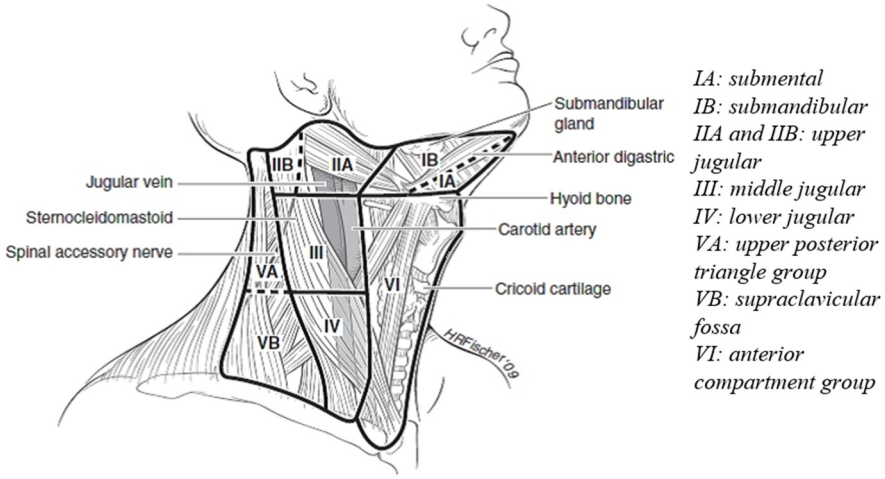
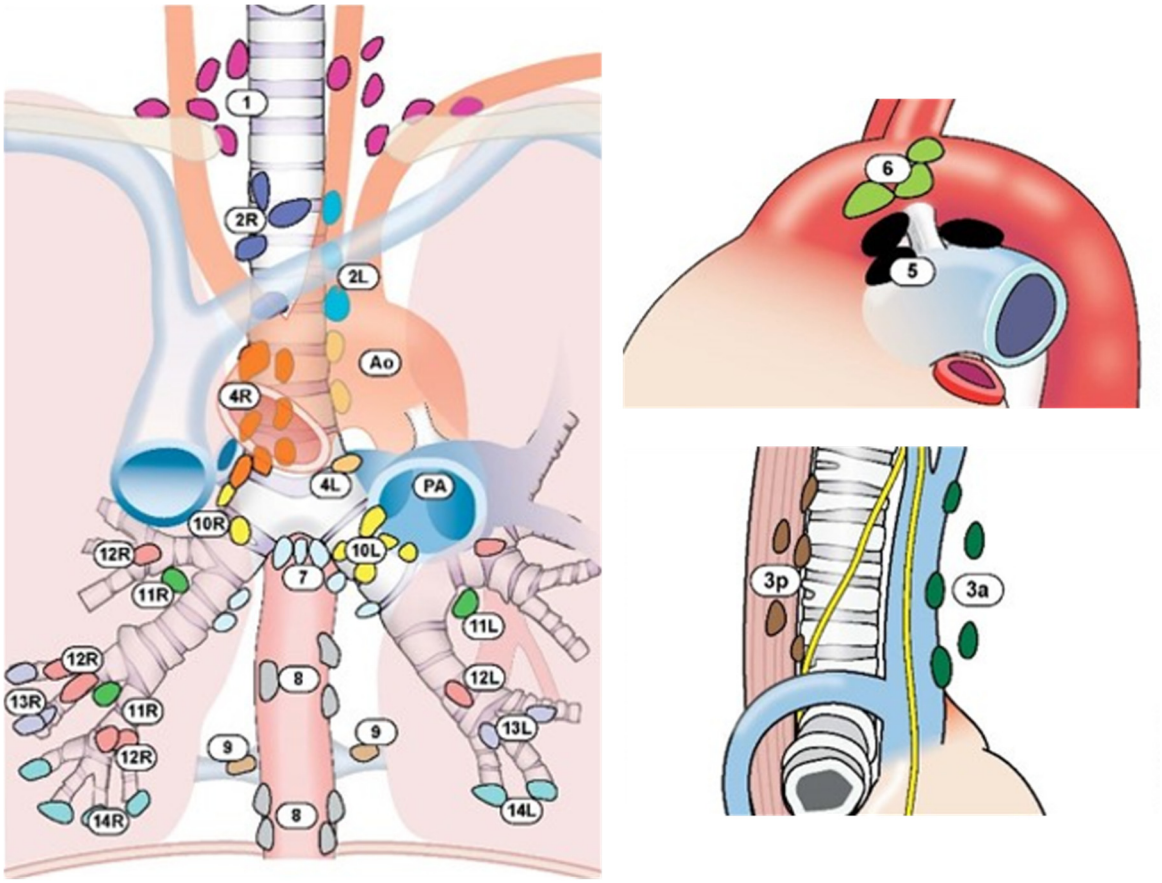


Figure 2: Cervical lymph node map according to the American Head and Neck Society. Figure adopted from Konturek and Barczynski.[72].

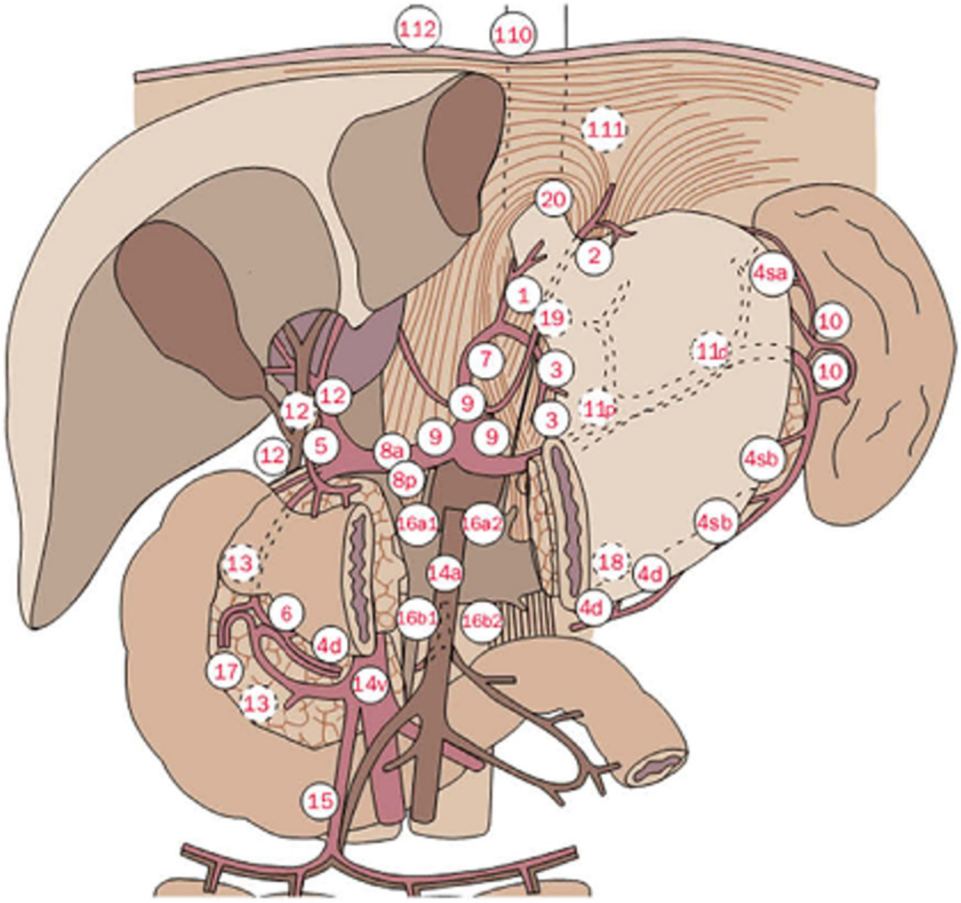
To be able to distinguish regional from distant lymph node recurrences, it is necessary to identify the regional lymph nodes of the esophageal drainage area. In this study, we started from the regional esophageal lymph node classification according to Casson et al.[73] However, in the supraclavicular group, we only included the para-esophageal lymph nodes above the level of the clavicles (i.e. the cervical para-esophageal nodes) as regional nodes. All nodes along the internal jugular vein and the sternal notch nodes, were considered as distant nodes. This is in correspondence with the definition of the regional esophageal lymph nodes according to the 7th edition of the TNM classification: *"the regional lymph nodes, irrespective of the site of the primary tumor, are those in the esophageal drainage area including celiac axis nodes and para-esophageal nodes in the neck, but not supraclavicular nodes."*[47]



1: low cervical, supraclavicular and sternal notch nodes
2R: right upper paratracheal
2L: left upper paratracheal
3a: pre-vascular
3p: retrotracheal
4R: right lower paratracheal
4L: left lower paratracheal
5: subaortic
6: para-aortic
7: subcarinal
8: paraesophageal (below carina)
9: pulmonary ligament
10: hilar
11: interlobar
12: lobar
13: segmental
14: subsegmental

Figure 3: Thoracic lymph node map according to the IASLC. Figure adopted from Rusch et al.[70]

In the Casson classification, the para-esophageal lymph nodes below the carina (group 8 of the thoracic map) are further divided into an 8M (the middle thoracic para-esophageal nodes, from the carina to the lower border of the inferior pulmonary vein) and an 8L group (the lower thoracic para-esophageal nodes, from the lower border of the inferior pulmonary vein to the esophagogastric junction). We also included this division in our analysis. Table 3 gives the definition of the regional esophageal lymph nodes used in this master thesis, based on the above considerations (the letters T and A refer to the thoracic and abdominal lymph node maps of Figure 3 and Figure 4 respectively).



110: lower thoracic paraesophageal
111: supradiaphragmatic
112: posterior mediastinal
1: right paracardial
2: left paracardial
3: along lesser curvature
4d: along right gastroepiploic vessels
4sb: along left gastroepiploic vessels
4sa: along short gastric vessels
5: suprapyloric
6: infrapyloric
7: along left gastric artery
8a: along common hepatic artery (anterosuperior group)
8p: along common hepatic artery (posterior group)
9: along celiac artery
10: around splenic hilum
11p: along proximal splenic artery
11d: along distal splenic artery
12a: in hepatoduodenal ligament (along hepatic artery)
12b: in hepatoduodenal ligament (along bile duct)
12p: in hepatoduodenal ligament (behind the portal vein)
13: on posterior surface of pancreatic head
14v: along superior mesenteric vein
14a: along superior mesenteric artery
15: along middle colic vessels
16a1: in aortic hiatus
16a2: around abdominal aorta (from upper margin of celiac trunk to lower margin of left renal vein)
16b1: around abdominal aorta (from lower margin of left renal vein to upper margin of inferior mesenteric artery)
16b2: around abdominal aorta (from upper margin of inferior mesenteric artery to aortic bifurcation)
17: on anterior surface of pancreatic head
18: along inferior margin of pancreas
19: infradiaphragmatic
20: in esophageal hiatus of diaphragm

Figure 4: Abdominal lymph node map according to the Japanese classification of gastric carcinoma. Figure adopted from Matzinger et al.[74]

Lymph node group	Description	Lymph node group	Description
T1	Cervical para-esophageal	T9	Pulmonary ligament
T2R	Right upper paratracheal	T10	Hilar
T2L	Left upper paratracheal	A111	Supradiaphragmatic
T3p	Prevertebral	A1	Right paracardial
T4R	Right lower paratracheal	A2	Left paracardial
T4L	Left lower paratracheal	A7	Left gastric artery
T5	Subaortic	A8a	Common hepatic artery (anterosup.)
T6	Anterior mediastinal	A8p	Common hepatic artery (posterior)
T7	Subcarinal	A9	Celiac
T8M	Middle thoracic para-esophageal	A11p	Proximal splenic artery
T8L	Lower thoracic para-esophageal	A11d	Distal splenic artery

Table 3: Definition of the regional esophageal lymph nodes used in this master thesis.

II.4.3. Criteria

Both pathological and radiological criteria were used to classify tissue anomalies as malignant or benign. Pathological examination of a lesion biopsy identifying malignant tumor cells of the same type as the primary esophageal cancer was sufficient to prove tumor recurrence. However, not all suspicious lesions were eligible for biopsy. Those had to be assessed exclusively by medical imaging. Hence, radiological criteria had to be used to distinguish benign from malignant lesions.

To diagnose lymph node recurrence with medical imaging techniques, it is essential to identify metastatic lymph nodes on the acquired image data. Often, the primary radiological criterion to identify a malignant lymph node is its size. To be classified as malignant, it typically has to have a short axis diameter larger than 1 cm. However, several studies have shown that size alone is far from accurate in the diagnosis of malignant lymph nodes of the esophagus, as many non-invaded lymph nodes also meet this criterion. On the other hand, malignant nodes often have smaller sizes and therefore do not satisfy this criterion.[75, 76] To overcome this problem, we used several other criteria to identify lymph node recurrences. In this master thesis, the following lymph node characteristics were used as indicators of tumor relapse:

1. The presence of a necrotic center on CT or MRI. This is a known morphological characteristic of malignant processes.
2. Progressive growth on consecutive imaging acquisitions. If, for example, an enlarged lymph node had been detected on one follow-up CT, but had disappeared on the next, it was not classified as malignant.

3. Positive on FDG-PET. Although FDG-PET is known for its limited specificity, it has very high sensitivity. Hence, in combination with the other criteria, it was used as a positive indicator of tumor recurrence.
4. Multiplicity. The presence of large numbers of neighboring, enlarged lymph nodes was considered to be a malignant characteristic.
5. Lesion size. An enlarged lymph node was considered to have a larger potential to be malignant, although we used no strict separation criterion of 1 cm.

In general, the two first criteria were used as the predominant criteria to determine the presence of tumor recurrence, while the other three were mainly used as additional arguments in the case of doubt.

Analogous criteria were used for the detection of hematogenous metastases. Additionally, a positive bone scan was used as a positive indicator of bone metastases.

In all cases, the radiological medical imaging reports were used as a guidance to identify tumor relapses. Furthermore, a radiation oncologist was consulted if there were any doubts about the malignant character of a suspicious lesion.

II.5. Radiation field analysis

II.5.1. General

As stated in the introduction, one of the main goals of this master thesis is to determine whether the locoregional tumor recurrences are located within the radiation field. In summary, the following procedure was followed to determine this. First, image fusion was performed for each patient between planning CT and each follow-up CT containing tumor recurrences. All locoregional recurrences were then delineated on the fused image set, defining new Regions-Of-Interest (ROIs). Finally, the radiation dose parameters of these newly defined volumes were extracted, to determine whether tumor recurrences were located within the radiation field.

II.5.2. Radiotherapy planning systems

In the period 2003-2012, several radiotherapy planning systems were used at the GUH Department of Radiation Oncology: PLUNC (or PLanUNC, PLanning system developed at the University of North Carolina), GRATIS (George's RAdiotherapy Treatment desIgn System) and Eclipse. Up to the beginning of 2009, all radiotherapy plans were made with PLUNC. Afterwards, the use of the PLUNC software was discontinued and it was replaced by the GRATIS and Eclipse systems, which are both still used at present. To extract the radiation

parameters from the PLUNC plans, we had to perform several data conversions. An overview of all involved steps is given in Figure 5. These steps are further explained in the following sections.

II.5.3. Data acquisition

As the use of PLUNC was stopped several years ago, its radiotherapy plan data were not available anymore on the data servers of the local computer network. Instead, the data were stored in a DVD archive. To retrieve the radiotherapy plan data of the PLUNC patients, we had to explore this archive and had to copy the data from DVD to an accessible location on one of the data servers. For each patient, this plan data contained three components: the anastructs (or the delineated anatomical structures, being the PTV and the organs at risk as e.g. the lungs, the liver, etc.), the planning CT and the actual radiotherapy plan(s). As multiple plans were often found per patient, we had to consult the paper-based medical records to be able to transfer the correct radiotherapy plan. Afterwards, we converted the PLUNC data to GRATIS format (step 1 of Figure 5). The plan data of the original GRATIS and of the Eclipse patients were readily accessible via the network and no further actions had to be taken.

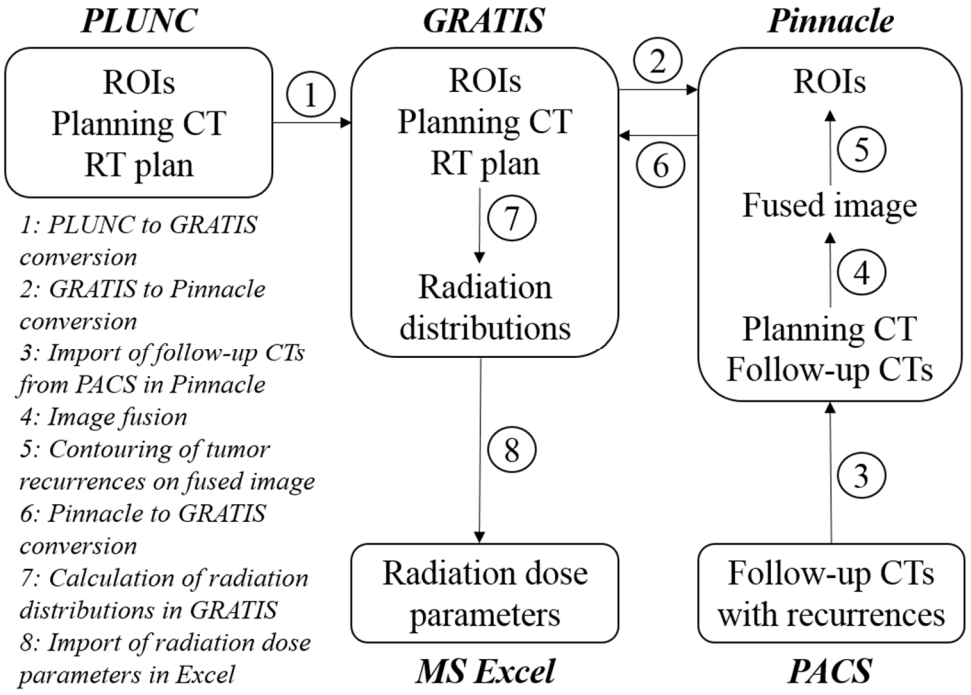


Figure 5: Overview of all steps needed to extract the radiation dose parameters from the patients planned with the PLUNC system.

II.5.4. Contouring

For the PLUNC patients, contouring of new Regions-Of-Interest (ROIs) was done with the Pinnacle radiotherapy planning system, as the PLUNC software was not available anymore.

For simplicity, the contouring of the GRATIS patients was also done with the Pinnacle software. Hence, again a data conversion had to be performed, in which the GRATIS data were now converted to a Pinnacle compatible format (step 2 of Figure 5). After import of the follow-up CTs containing the locoregional tumor recurrences (step 3), image fusion was performed in Pinnacle (step 4). ROIs were then contoured on the fused image sets around each tumor recurrence (step 5). Lastly, all data (including the new ROIs) were converted back to GRATIS format (step 6).

In Eclipse, the delineation of new ROIs could be done directly without the need of additional conversion steps. From the above considerations, it follows that 2 radiotherapy planning systems (Eclipse and Pinnacle) were used for contouring of the tumor recurrences after image fusion. We received training from professional dosimetrists to be able to work with both systems. Furthermore, professional support was available if any technical difficulties were experienced.

Irrespective of the planning system, we used the same methodology to contour the locoregional tumor recurrences. A regional lymph node recurrence occurring on a follow-up CT was delineated on the planning CT after image fusion, by drawing a contour fully encircling the tumoral lesion. A local anastomotic recurrence was assumed to occur in the proximal esophagus at the gastro-esophageal anastomosis. This anastomosis was easily recognizable on a follow-up CT by the presence of the metal staples connecting the proximal esophagus and the stomach. After image fusion, the anastomotic location could thus be determined on the planning CT. The proximal esophagus was then contoured on this CT around the location of the anastomosis to determine its radiation dose. Local recurrences occurring in the gastric wall were not included in our radiation field analysis, since dose parameters could not be determined accurately due to stomach mobilization as part of the surgical procedure.

To study the effect of radiation induced enhancement of lung metastases (see section III.4.3), the thoracic part of the lungs (beneath the suprasternal notch) was contoured for all patients from whom the planning CT was available. Only the thoracic part of the lungs was chosen, because the planning CT of some patients did not reach above the suprasternal notch. Hence, in this way it was possible to define a uniform and well-defined lung volume for all patients. The contouring of the thoracic lungs was done with the help of professional dosimetrists.

II.5.5. Image fusion

For patients with follow-up at GUH, follow-up CTs were obtained via PACS. If follow-up took place at the peripheral hospitals, follow-up CTs were imported from the retrieved DVDs containing the medical imaging exams.

To fuse the planning CT with a follow-up CT containing tumor recurrences, rigid registration was performed. With the planning CT as reference image, rigid registration implies that another image, the follow-up CT, is matched as good as possible with the reference by linear transformations (translations and/or rotations). Both the Eclipse and Pinnacle software contained algorithms to perform this registration automatically. However, these automatic registrations were often not accurate enough to perform a precisely localized delineation of tumor recurrences on the planning CT. This is because the follow-up CT was often non-linearly deformed with respect to the planning CT, due to different positions of the patient (hands above or beside the body), ventilation (in- or expiration) and/or the use of different CT beds (flat or hollow). To minimize these errors, we used a stepwise approach to register the images. In a first step, the automatic registration algorithms were used to obtain globally well-matched images. For each tumor recurrence, we then verified if both images also matched locally by visual comparison of well recognizable anatomical structures as e.g. the carina or a vertebra at a certain level. If this was not the case, the follow-up CT was translated and/or rotated manually until an acceptable visual match was obtained.

II.5.6. Radiation dose extraction

For the locoregional tumor recurrences occurring beyond the reach of the planning-CT, the exact radiation dose could not be determined and hence was assumed to be zero, since these relapses were located far away from the PTV. For the other recurrences, the radiation dose distributions were calculated in each ROI, based on the radiotherapy plans. For the PLUNC and GRATIS patients, this was done in GRATIS (step 7 of Figure 5). The radiation dose parameters of all ROIs of all patients were then automatically imported in MS Excel for further processing (step 8). For the Eclipse patients, the dose parameters had to be extracted per patient and imported manually in MS Excel.

Irrespective of the system, the following radiation dose parameters were extracted for each ROI: the mean dose D_{μ}, the median dose D_{50}, the maximum dose D_{max}, the minimum dose D_{min} and the standard deviation D_{σ}. Furthermore, the (cumulative) dose-volume histograms (DVHs) were also extracted for each ROI. The precision of the DVHs was 0.01 Gy. These DVHs are commonly used to analyze dose distributions during radiotherapy simulation.[77]

In summary, they represent for each dose the percentage of ROI volume receiving a given dose or more.

Only patients treated with neoadjuvant chemoradiotherapy at GUH were included in the radiation field analysis. For all these patients, the prescribed dose on the tumor volume was 36 Gy. Accordingly, a tumor recurrence was considered as occurring within the radiation field if it received a mean radiation dose of 34 Gy or more. The cut-off of 34 Gy was chosen in accordance with the standard of the International Commission on Radiation Units (ICRU), which states that the dose in the PTV has to at least 95% of the prescribed radiation dose.[78] The patients neoadjuvantly treated in peripheral hospitals were not included in this analysis, because we did not retrieve their treatment plans (they are included only in the survival analysis).

II.6. Statistical analysis

II.6.1. General

To perform the statistical analysis we used SPSS (Statistical Product and Service Solutions) Statistics for Windows, version 21.0. For all statistical tests, statistical significance was retained if $P \leq 0.05$.

II.6.2. Statistical methods

To determine whether a continuous variable was normally distributed, histograms were made and the Shapiro-Wilk test was applied. Non-normal distributions of continuous variables were compared with the Mann-Whitney U test for 2 groups or the Kruskal-Wallis test for 3 groups.

The (Pearson's) chi-square test was used to compare frequencies of categorical variables between different groups. If the frequency was 10 or less in a certain category, the Fisher's exact test was used instead. Logistic regression analysis was used to identify independent explanatory variables of a dichotomous outcome.

To estimate disease-specific and disease-free survival, Kaplan-Meier curves were used. Differences in survival between groups were evaluated using the log-rank test. Cox regression analysis was used to identify independent predictors of survival in a multivariate analysis.

II.6.3. Descriptive statistics

To describe normally distributed variables, we used the arithmetic mean, standard deviation (SD) and total range (TR). Non-normally distributed variables were described by the median, interquartile range (IQR) and total range.

II.7. Ethical committee

An approval of the GUH Ethical Committee was obtained before consulting and using the data of the patients included in this master thesis.

III. RESULTS

III.1. Population characteristics

III.1.1. Sample size

In total, 94 patients were identified who met the inclusion criteria (see section II.1). All 94 patients, further mentioned as Group 1, were included in the analysis of disease-specific and disease-free survival, irrespective of the follow-up duration. However, as this master thesis is mainly focused on the study of tumor recurrence patterns, the follow-up duration of the patients had to be long enough for the recurrences to develop. Therefore, in every analysis other than the survival analysis, 14 additional patients were excluded, leaving a total of 80 patients. We further refer to these patients as Group 2. From the excluded patients, 9 died from short or long term postoperative complications and, therefore, were not able to develop recurrences. The other 5 patients had not yet developed recurrences, but were excluded because the follow-up duration was less than 12 months. Hence, a period of 12 months was taken as a minimum follow-up duration for recurrence-free patients. No statistically significant differences were obtained between both groups in the distribution of all analyzed variables.

III.1.2. Demographic variables

For both groups, the descriptive statistics of the demographic variables are presented in Table 4. In Group 1 the median age was 64 (IQR: 54-71), while the patients of Group 2 had a median age of 62 years (IQR: 53-70). More than 80% of the patients in this study were male.

	Group 1 (N = 94)			**Group 2 (N = 80)**		
Variable	**Median**	**IQR**	**TR**	**Median**	**IQR**	**TR**
Age (years)	64	54-71	41-78	62	53-70	41-78
Variable	**Values**	**#**	**%**	**Values**	**#**	**%**
Gender	Male	77	81.9	Male	66	82.5
	Female	17	18.1	Female	14	17.5

Table 4: Descriptive statistics of the demographic variables of Group 1 and 2. As stated in the text, Group 2 contains all patients from Group 1, except the deaths due to postoperative complications and the recurrence-free patients with follow-up duration of less than 12 months. IQR: interquartile range, TR: total range.

III.1.3. Treatment variables and follow-up duration

The follow-up duration and the treatment variables (prescribed dose, type of chemotherapy and chemotherapy stopped) are given in Table 5. In Group 1 the median follow-up duration was 17 months (IQR: 9-28 months). In Group 2, a median follow-up duration of 19 months was obtained (IQR: 12-32 months). The prescribed dose was 36 Gy for the large majority (±95%) of patients. Importantly, all patients included in the study received the full prescribed dose and no radiotherapy treatments were prematurely stopped. The same did not apply to the administration of chemotherapy. Four patients from Group 2 and one additional patient from Group 1 did not complete the full chemotherapy regimen due to the occurrence of side effects. All patients except 2 received concurrent 5-FU and cisplatinum. One patient was treated with 5-FU only, due to the presence of IgA nephropathy as contra-indication for cisplatinum. Another patient received only cisplatinum due to practical considerations (personal preference to limit the number of chemotherapy administrations).

	Group 1 (N = 94)			**Group 2 (N = 80)**		
Variable	**Median**	**IQR**	**TR**	**Median**	**IQR**	**TR**
Follow-up (months)	17	9-28	0-112	19	12-32	3-112
Variable	**Values**	**#**	**%**	**Values**	**#**	**%**
Prescribed dose	36 Gy	89	94.7	36 Gy	76	95.0
	45 Gy	5	5.3	45 Gy	4	5.0
Chemotherapy type	5-FU/CS	92	97.9	5-FU/CS	78	97.5
	5-FU only	1	1.1	5-FU only	1	1.3
	CS only	1	1.1	CS only	1	1.3
Chemotherapy stopped	yes	5	5.3	yes	4	5.0
	no	89	94.7	no	76	95.0

Table 5: Descriptive statistics of the follow-up duration and the treatment variables for Group 1 and 2. IQR: interquartile range, TR: total range, 5-FU: 5-fluorouracil, CS: cisplatinum.

III.1.4. Clinical variables

The descriptive statistics of the clinical variables (see II.2.3 for more information) are presented in Table 6. As can be concluded from the table, more than 70% of the esophageal cancers were ACs, while only a minority had SCC. Accordingly, approximately 70% of the tumors were located in the distal third of the thoracic esophagus. The median endoscopic distance from the upper incisor teeth and tumor length were 33 and 5 cm respectively. Most tumors were moderately (G_2 or intermediate grade) or poorly (G_3 or high grade) differentiated. The less aggressive, well differentiated (G_1 or low grade) tumors were only

present in less than 10% of patients. For 9 patients from Group 2 and one additional patient from Group 1, the tumor grade was not assessed in the medical reports (G_X). All patients in this study had clinical stage II (29.4%) or III (70.6%), as this study included only patients with locally advanced cancer. Hence, more than 80% of patients had clinical T_3 stage, while around 80% of patients had node positive disease.

	Group 1 (N = 94)			Group 2 (N = 80)		
Variable	**Median**	**IQR**	**TR**	**Median**	**IQR**	**TR**
Endoscopic distance (cm)(*)	33	30-35	21-40	33	30-36	21-40
Tumor length (cm)(**)	5	3-7	1-12	5	4-7	1-12
Variable	**Values**	**#**	**%**	**Values**	**#**	**%**
Tumor histology	SCC	26	27.7	SCC	22	27.5
	AC	67	71.3	AC	57	71.3
	Other(***)	1	1.1	Other(***)	1	1.3
Tumor differentiation	G_1	8	9.5	G_1	6	8.5
	G_2	48	57.1	G_2	42	59.2
	G_3	28	33.3	G_3	23	32.4
	G_X	10		G_X	9	
Thoracic tumor location	Upper 1/3	2	2.2	Upper 1/3	2	2.6
	Middle 1/3	24	26.4	Middle 1/3	22	28.2
	Lower 1/3	65	71.4	Lower 1/3	54	69.2
	Unknown	3		Unknown	2	
Clinical stage group	Stage II	27	29.4	Stage II	23	29.4
	Stage III	65	70.6	Stage III	55	70.6
	Unknown	2		Unknown	1	
Clinical T stage	cT_1	1	1.1	cT_1	1	1.3
	cT_2	14	14.9	cT_2	10	12.5
	cT_3	78	83.0	cT_3	68	85.0
	cT_4	1	1.1	cT_4	1	1.3
Clinical N stage	cN_0	16	17.4	cN_0	16	20.5
	cN_+	76	82.6	cN_+	62	79.5
	cN_X	2		cN_X	2	

Table 6: Descriptive statistics of the clinical variables for Group 1 and 2. Unknown data are not included in calculation of the percentages. (*) Patients with unknown endoscopic distance: N = 18 for Group 1 and N = 17 for Group 2. (**) Patients with unknown tumor length: N = 30 for Group 1 and N = 27 for Group 2. (***) One patient was included with a mucoepidermoid carcinoma. IQR: interquartile range, TR: total range, SCC: squamous cell carcinoma, AC: adenocarcinoma.

III.1.5. Pathological variables

Table 7 contains the descriptive statistics of the pathological variables (see II.2.4 for more information). The median number of examined and positive (invaded by tumor) lymph nodes were 14 (IQR: 11-20) and 1 (IQR: 0-2) respectively. Fifteen patients (18.8%) from Group 2 and an additional two patients (18.1%) from Group 1 had a pathological complete response. Nevertheless, most patients still had stage II or stage III tumors after neoadjuvant treatment. One patient was classified as stage IV, because pathological examination demonstrated the presence of an isolated metastatic nodule in the adipose tissue of the omentum minus. Approximately 25% of patients had pathological stage ypT_0, while around half of patients had node negative disease at pathological examination. Only 3 patients had a positive proximal or distal resection margin.

	Group 1 (N = 94)			**Group 2 (N = 80)**		
Variable	**Median**	**IQR**	**TR**	**Median**	**IQR**	**TR**
Examined lymph nodes	14	11-20	3-38	14	11-20	3-38
Positive lymph nodes	1	0-2	0-13	1	0-2	0-13
Lymph node ratio (%)	4.0	0.0-10.5	0.0-71.4	4.0	0.0-9.2	0.0-71.4
Variable	**Values**	**#**	**%**	**Values**	**#**	**%**
Pathological stage group	pCR	17	18.1	pCR	15	18.8
	Stage I	16	17.0	Stage I	14	17.6
	Stage II	28	29.8	Stage II	26	32.5
	Stage III	32	34.0	Stage III	25	31.3
	Stage IV(*)	1	1.1	Stage IV	0	0.0
Pathological T stage	ypT_0	23	24.7	ypT_0	21	26.6
	ypT_1	12	12.9	ypT_1	11	13.9
	ypT_2	18	19.4	ypT_2	15	19.0
	ypT_3	40	43.0	ypT_3	32	40.5
	ypT_X	1		ypT_X	1	
Pathological N stage	ypN_0	45	47.9	ypN_0	39	48.8
	ypN_1	37	39.4	ypN_1	33	41.3
	ypN_2	9	9.6	ypN_2	6	7.5
	ypN_3	3	3.2	ypN_3	2	2.5
Positive resection margin	yes	3	3.2	yes	3	3.8
	no	91	96.8	no	77	96.3

Table 7: Descriptive statistics of the pathological variables of Group 1 and 2. Unknown data are not included in calculation of the percentages. (*) This patient had pathological stage IV because of the

presence of an isolated metastatic nodule in the adipose tissue of the omentum minus. IQR: interquartile range, TR: total range, pCR: pathological complete response.

III.2. Survival

III.2.1. Postoperative complications

Nine of 94 patients (9.6%) died from short or long-term postoperative complications. Two of these patients died within 30 days, giving a 30-day mortality rate of 2.1%. An additional six patients deceased within 90 days, yielding a 90-day mortality rate of 8.4%. An overview of the postoperative complications leading to death is given in Table 8.

Patient	Complications	Time until death (days)
1	Septic shock with multiple organ failure.	4
2	Hypovolemic shock due to massive gastro-intestinal bleeding.	18
3	Candidemia, pneumonia with RI.	39
4	Gastric necrosis with fistulisation leading to pleural collections and RI.	42
5	Anastomotic leakage causing aspiration pneumonia and RI.	47
6	Anastomotic laceration, pneumonia with ARDS, septic shock.	49
7	Tracheal and esophageal rupture after repeated dilatation of strictures.	72
8	Mediastinitis evolving to septic shock and RI.	83
9	Bilateral pneumonia evolving to ARDS and RI.	140

Table 8: Short and long term complications after Ivor-Lewis esophagectomy. RI: respiratory insufficiency, ARDS: acute respiratory distress syndrome.

III.2.2. Disease-specific and disease-free survival

Forty-seven patients died during the follow-up period of this study. Thirty-six (76.6%) of these patients deceased due to local and/or distant disease recurrence. As stated in section III.2.1, nine (19.1%) of these patients died from early or long term postoperative complications. One patient committed suicide, while for another patient the cause of death was unknown.

Kaplan-Meier plots of the disease-specific and disease-free survival are presented in Figure 6. We obtained a median disease-specific survival of 31.0 months (95% CI: 16.8-45.2 months). The corresponding 3-year and 5-year survival rates were 48.2% (95% CI: 35.9-60.5%) and 37.1% (95% CI: 23.6-50.6%) respectively.

On the other hand, the median disease-free survival was 14.0 months (95% CI: 10.5-17.5 months), while the 3-year and 5-year disease-free survival rates were both equal to 36.5% (95% CI: 25.1-47.9%).

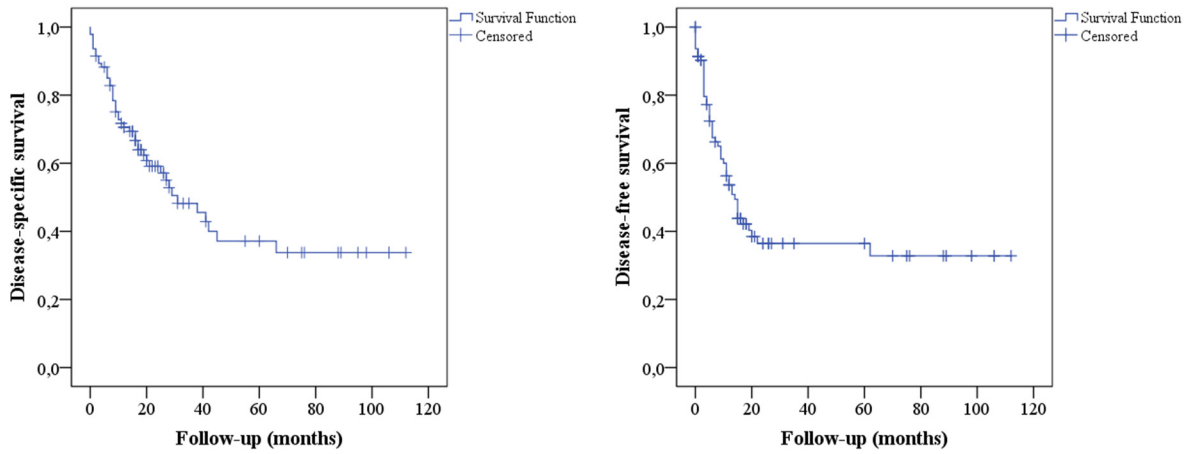


Figure 6: Kaplan-Meier plots of the disease-specific (left) and disease-free survival (right).

III.2.3. Pathological response

For each patient, the tumor response to neoadjuvant chemoradiation can be determined by comparison of the clinical and pathological stages. Table 9 gives the number of patients for each possible combination of clinical and pathological stage.

	pCR	Stage I	Stage II	Stage III	Stage IV
Stage II	8 (8.7%)	2 (2.2%)	9 (9.8%)	8 (8.7%)	X
Stage III	9 (9.8%)	14 (15.2)	18 (19.6%)	23 (25.0%)	1 (1.1%)

Table 9: The number of patients for each combination of clinical (rows) and pathological (columns) stage. pCR: pathological complete response. Ninety-two patients were included in this analysis, as 2 patients had unknown clinical stage.

We combined all these different groups into 3 pathological response categories with complete (pCR), partial (pPR) or no (pNR) response respectively. The partial response category contained the tumors without pCR, but with a pathological stage being smaller than the clinical stage. If the pathological stage was greater than or equal to the clinical stage, the tumor was classified as having no response. Taking the above considerations into account, the pCR, pPR and pNR categories contained 17 (18.5%), 34 (37.0%) and 41 (44.6%) patients respectively. Figure 7 contains the disease-specific and disease-free survival curves per response category, excluding the postoperative deaths (see III.2.1). The corresponding median survival and the 3-year and 5-year survival rates are presented in Table 10 and Table 11.

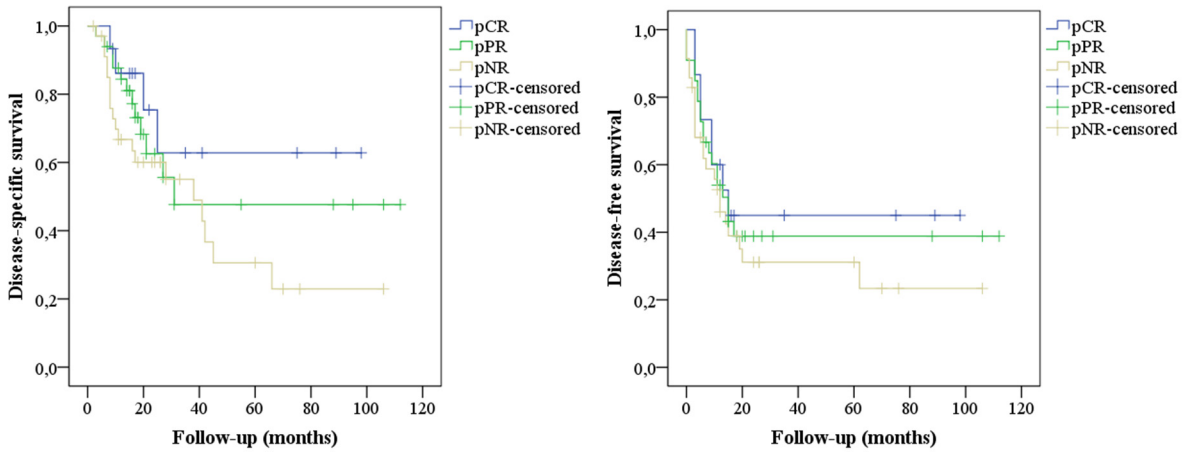


Figure 7: Disease-specific (left) and disease-free (right) survival per response group (pCR, pPR and pNR). Patients who died resulting from postoperative complications were not included in the analysis, hence N = 85.

Response	Median (months)	3-year (%)	5-year (%)
pCR	Not reached	62.8 (32.0-93.6)	62.8 (32.0-93.6)
pPR	31.0(*)	47.7 (24.4-71.0)	47.7 (24.4-71.0)
pNR	38.0 (17.1-58.9)	55.1 (37.1-73.1)	30.6 (10.0-51.2)

Table 10: Descriptive statistics of the disease-specific survival. The 95% confidence intervals are shown in brackets. (*) The 95% confidence interval could not be calculated in SPSS.

Response	Median (months)	3-year (%)	5-year (%)
pCR	15.0 (4.6-25.3)	45.0 (19.1-70.9)	45.0 (19.1-70.9)
pPR	15.0 (8.9-21.1)	38.9 (21.3-56.5)	38.9 (21.3-56.5)
pNR	12.0 (9.7-16.3)	31.2 (14.5-47.9)	31.2 (14.5-47.9)

Table 11: Descriptive statistic of the disease-free survival. The 95% CIs are shown in brackets.

The log-rank test was used to compare survival distributions. Comparison of the disease-specific survival distributions of the pCR and pPR groups, the pCR and pNR groups and the pPR and pNR groups yielded P-values of 0.444, 0.100 and 0.253 respectively. Similar comparisons for the disease-free survival, provided P-values of 0.690, 0.302 and 0.459 respectively. Hence, although the best survival statistics were generally obtained for the pCR group (or the poorest for the pNR group), no significant differences in survival were obtained between any two groups.

Taking the pCR group as reference, Cox regression analysis was used to compute the hazard ratios (HRs) in the pPR and pNR groups respectively. The crude HRs were then corrected for several factors known to independently influence survival. As the total sample size was limited in this study, we chose to correct only for 2 demographic and 2 clinical variables: age, gender, clinical stage and thoracic tumor location. The thoracic tumor location was also included in the multivariate analysis, because of its previously demonstrated influence on

survival.[79]. Moreover, it is also included as independent variable in the prognostic grouping of the TNM 7th edition classification for esophageal cancer.[47] The crude and adjusted HRs are presented in Table 12. Distinct HRs were calculated for 2 different events: cancer-related death and tumor recurrence. No statistically significant crude HRs were obtained. However, after adjustment in a multivariate analysis, the patients with no response had a 1.78 (95% CI: 1.03-3.08, P = 0.039) times higher risk to die from esophageal cancer than the patients with a pathological complete response. The risk for tumor recurrence was also elevated in the pNR group (adjusted HR: 1.33, 95% CI: 0.88-1.99), although it was not statistically significant (P = 0.178).

	Risk of cancer-related death		Risk of tumor recurrence	
Response	**Crude HR**	**Adjusted HR**	**Crude HR**	**Adjusted HR**
pPR	1.55 (0.50-4.82) P = 0.448	0.88 (0.23-3.33) P = 0.854	1.18 (0.52-2.69) P = 0.699	0.87 (0.34-2.25) P = 0.777
pNR	1.55 (0.90-2.65) P = 0.114	1.78 (1.03-3.08) P = 0.039	1.23 (0.82-1.84) P = 0.318	1.33 (0.88-1.99) P = 0.178

Table 12: Crude and adjusted hazard ratios (HRs) in the pPR and pNR groups with respect to the pCR group. The adjusted HR was obtained by correcting for age, gender, clinical stage and thoracic tumor location. HRs were calculated for cancer-related death and tumor recurrence. The 95% CIs are shown in brackets.

III.2.4. Other prognostic factors

Besides the tumor response to neoadjuvant therapy, we investigated the influence of several other possible risk factors on tumor recurrence and cancer-related death: gender, histology, clinical stage, tumor grade, thoracic tumor location, tumor length, lymph node ratio (LNR) and number of positive lymph nodes detected in the resected specimen. As in the preceding section, crude HRs were calculated first for each factor in a univariate analysis. These HRs were then adjusted in a multivariate regression model by correcting for age, gender, clinical stage and thoracic tumor location. Postoperative deaths (see section III.2.1) were again excluded from this analysis. The crude and adjusted HRs of all investigated risk factors are presented in Table 13.

Three risk factors were identified with significant influence on tumor recurrence and cancer-related death: the tumor grade, the LNR and the number of positive lymph nodes. Patients with high grade tumors had a significantly increased risk of tumor recurrence (adjusted HR: 2.21, 95% CI: 1.20-4.09, P = 0.011) and cancer-related death (adjusted HR: 5.23, 95% CI: 2.36-11.60, P < 0.001) compared to patients with low or intermediate grade tumors. Patients with a LNR of 10% or higher had a 2.55 (adjusted HR, 95% CI: 1.26-5.15, P = 0.009) times higher risk of having tumor relapse and a 4.01 (adjusted HR, 95% CI: 1.90-8.47, P < 0.001)

times higher risk of dying from esophageal cancer than patients with a LNR smaller than 10%. Lastly, for each positive lymph node found during pathological examination of the resected specimen, the risks of tumor recurrence and cancer-related death increased by factors of 1.29 (adjusted HR, 95% CI: 1.14-1.46, P < 0.001) and 1.42 (adjusted HR, 95% CI: 1.22-1.67, P < 0.001) respectively.

			Crude			Adjusted		
Variable	**Values**	**Risk**	**HR**	**95% CI**	**P-value**	**HR**	**95% CI**	**P-value**
Gender	Male (Ref.)	Death	1.20	0.52-2.74	0.668	1.12	0.46-2.72	0.800
	Female	Recurrence	1.00	0.49-2.05	0.995	0.94	0.43-2.06	0.877
Histology (*)	SCC (Ref.)	Death	2.00	0.77-5.20	0.153	2.42	0.85-6.91	0.098
	AC	Recurrence	1.74	0.87-3.49	0.119	1.95	0.89-4.27	0.096
Clinical stage	Stage II (Ref.)	Death	1.40	0.67-2.95	0.375	1.45	0.68-3.11	0.342
	Stage III	Recurrence	1.56	0.81-2.98	0.184	1.44	0.74-2.80	0.277
Tumor grade	G_1/G_2 (Ref.)	Death	4.07	2.07-8.02	< 0.001	5.23	2.36-11.60	< 0.001
	G_3	Recurrence	2.21	1.24-3.95	0.007	2.21	1.20-4.09	0.011
Thoracic	Upper 2/3 (Ref.)	Death	0.90	0.63-1.27	0.529	0.91	0.63-1.33	0.631
location	Lower 1/3	Recurrence	0.93	0.70-1.24	0.607	0.94	0.69-1.29	0.717
Tumor length	Continuous	Death	0.90	0.75-1.07	0.226	0.91	0.75-1.11	0.353
		Recurrence	1.01	0.89-1.15	0.863	1.02	0.88-1.19	0.797
LNR	< 10% (Ref.)	Death	3.16	1.58-6.32	0.001	4.01	1.90-8.47	< 0.001
	≥ 10%	Recurrence	2.12	1.14-3.96	0.018	2.55	1.26-5.15	0.009
Positive LNs	Continuous	Death	1.38	1.18-1.61	< 0.001	1.42	1.22-1.67	< 0.001
		Recurrence	1.27	1.12-1.44	< 0.001	1.29	1.14-1.46	< 0.001

Table 13: Crude and adjusted hazard ratios (HRs) of the possible demographic, clinical and pathological risk factors for esophageal cancer-related death and tumor recurrence. The adjusted HRs are corrected for age, gender, clinical stage and thoracic tumor location. Ref.: reference value, LNR: lymph node ratio, LNs: lymph nodes. (*) The patient with mucoepidermoid carcinoma was excluded from the analysis.

III.3. Recurrence patterns

III.3.1. General

As stated in section III.1.1, the 80 patients of Group 2 (excluding 9 deaths due to postoperative complications and excluding 5 recurrence-free patients with follow-up duration of less than 12 months) were included in the analysis of the tumor recurrence patterns.

During follow-up, 63.8% of patients suffered from tumor relapse. The tumor recurrence patterns were classified as local, regional or distant (see also section II.4.2). Figure 8 shows the number of relapsed patients per recurrence pattern. The local and regional recurrence rates were 12.5% and 35.0% respectively, while a locoregional (local, regional or both) recurrence rate of 38.8% was obtained. Locoregional failure was combined with distant failure in as

many as 87.1% of cases. Indeed, the most important pattern of failure was distant tumor relapse, given the attained distant recurrence rate of 58.8%.

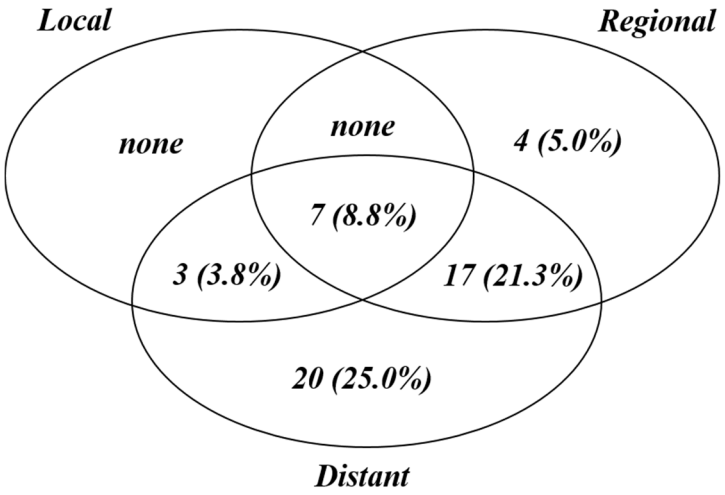


Figure 8: The number of relapsed patients per recurrence pattern (local, regional, distant or a combination). The percentages express the recurrence rates in the total group of 80 patients.

III.3.2. Local recurrences

Of the 10 patients experiencing local failure, 7 had anastomotic tumor recurrence (in the proximal esophagus), while 3 patients had local tumor relapse in the gastric wall. All patients with local recurrence had simultaneous distant relapse.

III.3.3. Regional recurrences

Twenty-eight patients had regional lymph node recurrences (see Table 3 for a definition of the regional lymph nodes of the esophagus). Most patients with regional tumor relapse had recurrences in multiple lymph node groups. Figure 9 contains the number of patients with tumor relapse for each of the lymph node groups of the esophageal drainage area. Most regional lymph nodes were found above or around the level of the carina. The most frequently affected lymph nodes were those in the upper and lower paratracheal groups (9 patients with 2R, 4 with 2L, 10 with 4R and 8 with 4L nodes), the upper thoracic and cervical para-esophageal groups (7 patients with 3p and 8 patients with 1R nodes), the subcarinal group (5 patients) and the para-aortic group (6 patients). Regional recurrences in the lymph node groups of the lower thoracic and the abdominal area were not frequent (occurring in 3 patients or less). No patients were found with hilar (group 9), left gastric (group 17 or A7) or splenic (group 19 or A11p/A11d) lymph node relapses.

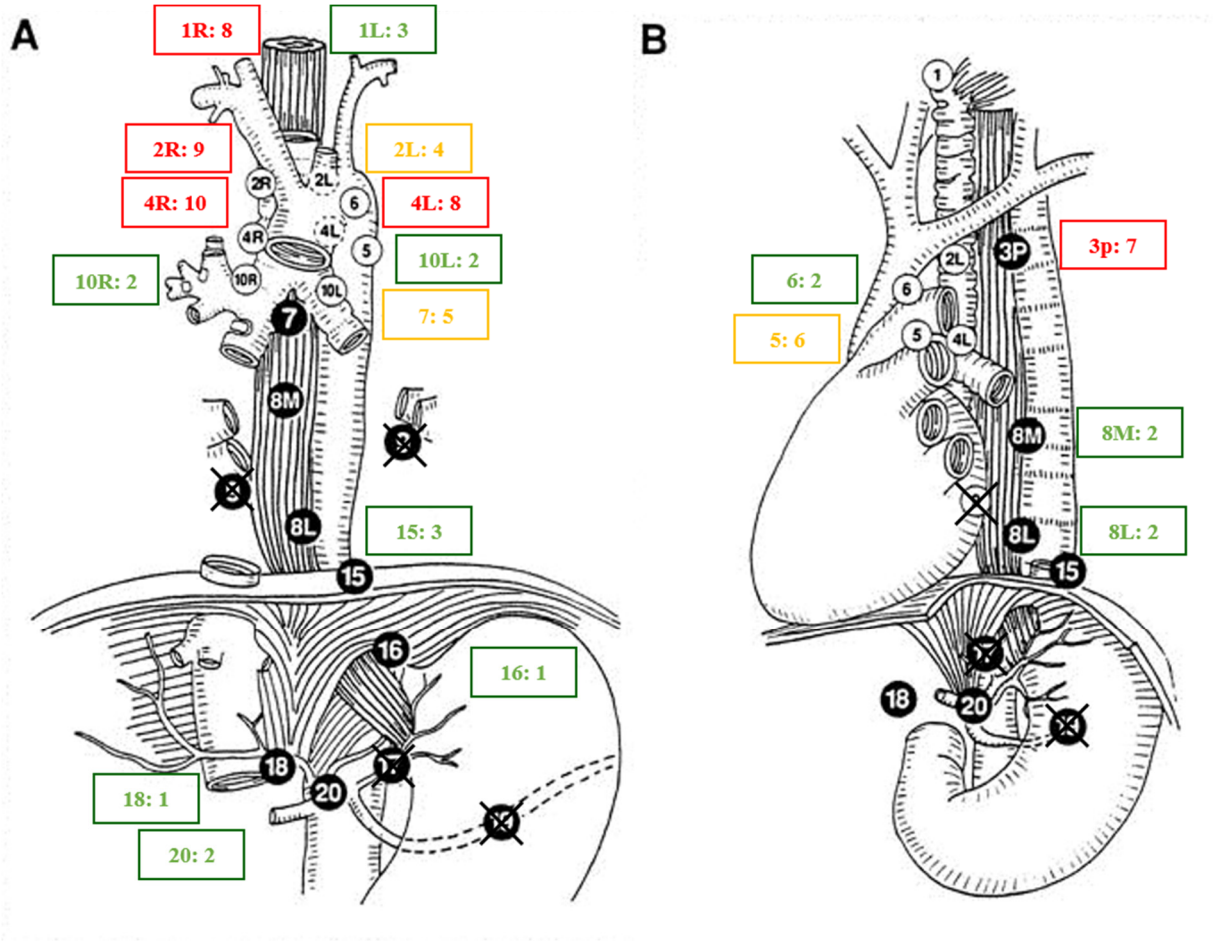


Figure 9: Frontal (A) and left lateral (B) view of the upper gastro-intestinal tract, with indication of the regional lymph node groups of the esophagus. The number of relapsed patients is given per lymph node group. Groups with 3 or less relapsing patients are indicated in green, with 4 to 6 patients in orange and with 7 or more patients in red. Groups 15, 16, 17, 18, 19 and 20 correspond with groups A111 (supradiaphragmatic), A1/A2 (paracardial), A7 (left gastric), A8a/A8p (common hepatic), A11p/A11d (splenic) and A9 (celiac) respectively (see also Table 3). Figure adapted from Casson et al.[73]

III.3.4. Distant recurrences

Distant recurrences were detected in 47 of 80 (58.8%) patients. As explained in section II.4.2, distant failure includes the distant (non-regional) lymphatic, the hematogenous and the serosal recurrences. Figure 10 contains the number of relapsed patients per distant recurrence category. Thirty-seven patients (or 78.7% of patients with distant failure) had hematogenous recurrence. Distant lymph node relapses were detected by 25 patients (53.2%), while 7 patients (14.9%) had serosal recurrence. Many patients with distant lymphatic and/or serosal spread also had hematogenous metastasis.

Table 14 gives the number of detected hematogenous and serosal tumor relapses classified per organ. The lungs were clearly most affected, as 28.8% of patients had lung metastasis. Other frequent sites of failure were the liver and the bone, with each 18.8% of patients having tumor relapse in these organs. The most prevalent serosal site of failure was the pleura, with 6 patients experiencing pleural tumor recurrence. Muscle recurrences were detected in 5

patients, while tumor relapses in each of the other affected organs (adrenal, skin, brain, etc.) occurred in 3 patients or less.

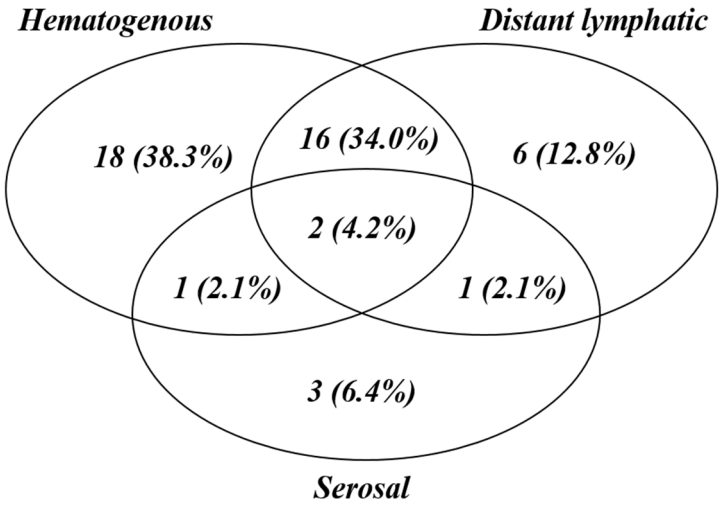


Figure 10: The relapsed number of patients for different categories of distant tumor recurrence. The percentages express the specific distant recurrence rates in 47 patients with distant failure.

Site of failure	#	%	Site of failure	#	%
Lungs	23	28.8	Brain	3	3.8
Liver	15	18.8	Peritoneum	2	2.5
Bone	15	18.8	Kidney	2	2.5
Pleura	6	7.5	Breast	1	1.3
Muscle	5	6.3	Colon	1	1.3
Adrenal	3	3.8	Gallbladder	1	1.3
Skin/subcutis	3	3.8			

Table 14: The number of patients with hematogenous or serosal metastasis according to site of failure. The percentages refer to the total sample size of Group 2 (N = 80).

The distribution of the distant lymph node metastasis is presented in Figure 11. Most patients with distant lymph node recurrence relapsed in multiple groups. Distant lymph node recurrences were frequently detected along the upper part of the abdominal aorta, below the level of the celiac trunk: 13 patients experienced para-aortic lymph node relapse between the level of the left renal vein and the inferior mesenteric artery (group A16b1), while 6 patients relapsed in the para-aortic lymph nodes between the level of the celiac trunk and the left renal vein (group A16a2). Lymph node recurrences were also commonly detected in the cervical zone along the internal jugular veins, as 11 patients had positive nodes in group CIV. Other regularly occurring groups were the axillary (6 patients with O1 nodes) and anterior mediastinal (4 patients with T3a nodes) categories. For the other affected distant lymph node groups, tumor recurrence was detected less frequently (in 3 patients or less).

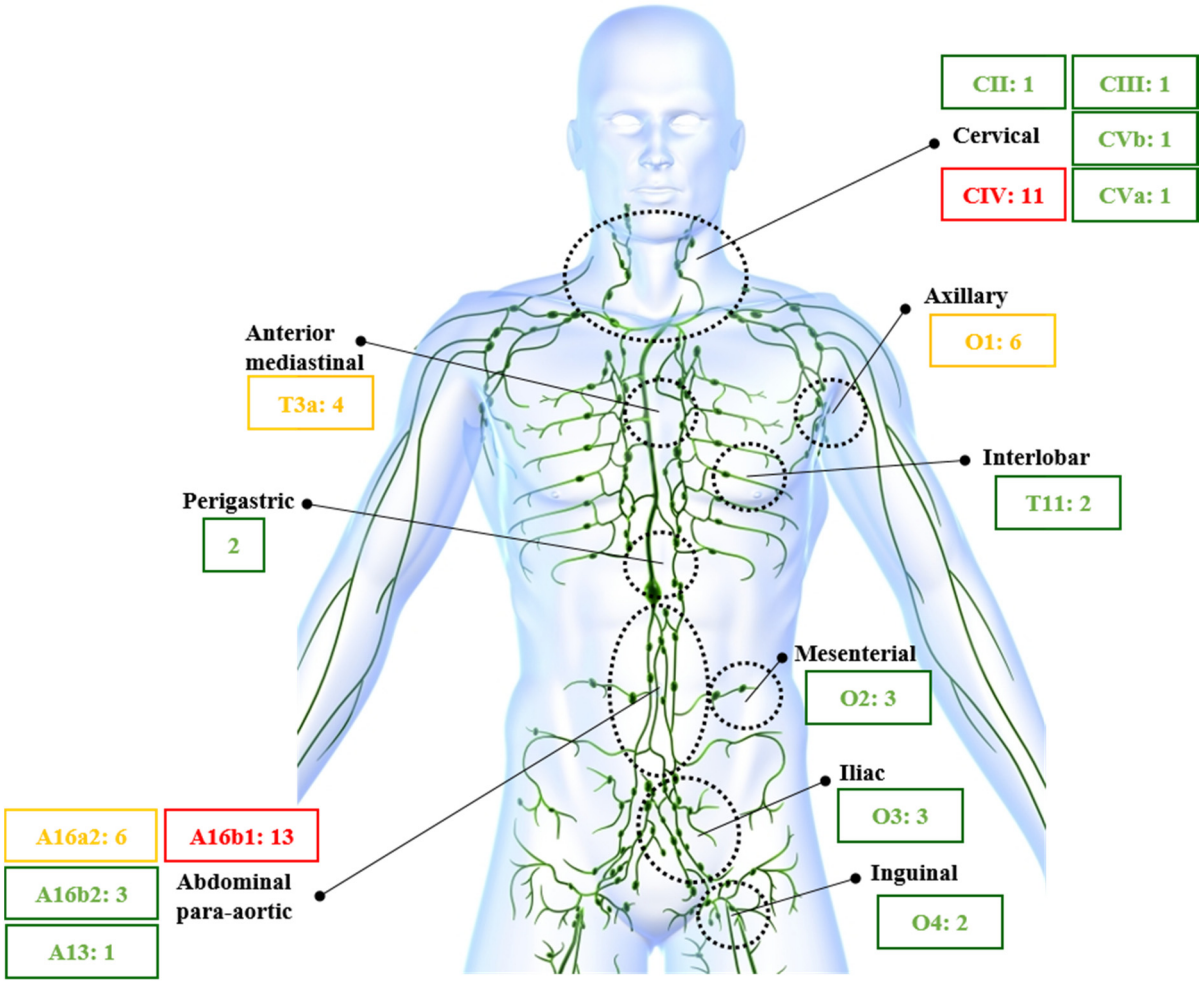


Figure 11: The number of patients with distant lymph node recurrence per lymph node group. The letter 'C' indicates the cervical groups (see Figure 2), 'T' the thoracic groups (see Figure 3), 'A' the abdominal groups (see Figure 4) and 'O' the other groups. The perigastric nodes could not be classified accurately, due to mobilization of the stomach as part of surgery. Groups with 3 or less relapsing patients are indicated in green, groups with 4 to 6 patients in orange and groups with 7 or more patients in red.

III.4. Radiation field analysis

III.4.1. General

In total, 79 of the 94 patients of Group 1 were irradiated at GUH, while the other 15 received radiotherapy at peripheral hospitals. The electronic planning data of 4 patients treated at GUH were not available for analysis. Hence, the radiotherapy plans of 75 patients were available to extract the radiation dose parameters. Patients who died from short-term or long-term postoperative complications (see section III.2.1) and recurrence-free patients with follow-up duration of less than 12 months were again excluded from the analysis, because their follow-up duration was considered to be too short for the development of tumor recurrences.

III.4.2. Locoregional recurrence within the radiation field

Thirty-one patients were identified with locoregional recurrence (see also Figure 8). From 3 of these patients we could not extract the radiation doses on the recurrence locations, because they were irradiated in peripheral hospitals and their radiotherapy plans were not retrieved. However, for one of these 3 patients, the descriptions in the electronic medical reports stated that the locoregional recurrences were entirely located outside the radiation field. For the other two patients, we decided that their tumor recurrences were not within the radiation field based on comparison of the locations of the primary tumor (distal esophagus) and tumor recurrences (cervical para-esophageal or upper paratracheal lymph nodes). The radiation dose could also not be extracted for one locoregionally relapsed patient, irradiated at GUH. This was because the electronic radiotherapy plan of this patient could not be found in the archive. However, since this patient had a recurrent upper paratracheal lymph node for a primary tumor of the distal esophagus which was not present at the time of radiotherapy, this relapse was also considered to occur outside the radiation field.

As mentioned in section III.3.2, 3 patients had local recurrences in the gastric wall. Although these patients were irradiated at GUH, the radiation dose for these recurrences could not be determined, because of stomach mobilization at surgery (as also explained in section II.5.4). Furthermore, since these relapses were local, we could not rule out if they were located outside the radiation field based on the distance between recurrence and primary tumor.

Ninety-eight locoregional recurrences were detected in 31 patients, yielding an average of 3.2 recurrences per patient. For 95 recurrences (excluding the local relapses in the gastric wall) we could determine if they were located within the radiation field. The radiation dose parameters could be extracted from the radiotherapy plans for 92 locoregional recurrences. As explained in section II.5.6, a relapse was considered to occur within the radiation target volume if the mean radiation dose (MRD) in its surrounding ROI was 34 Gy or higher. A histogram containing the number of locoregional recurrences per MRD category is presented in Figure 12. Fifty-one locoregional recurrences (55.4%) were located completely outside the radiation field, as their MRD was 10 Gy or less. We obtained 11 relapses (12.0%) with MRD between 10 and 20 Gy, while 15 recurrences (16.3%) had an MRD between 20 and 34 Gy. Fifteen (16.3%) locoregional relapses were located within the radiation target volume, since they had an MRD of 34 Gy or higher. These in-field recurrences were observed in 6 patients.

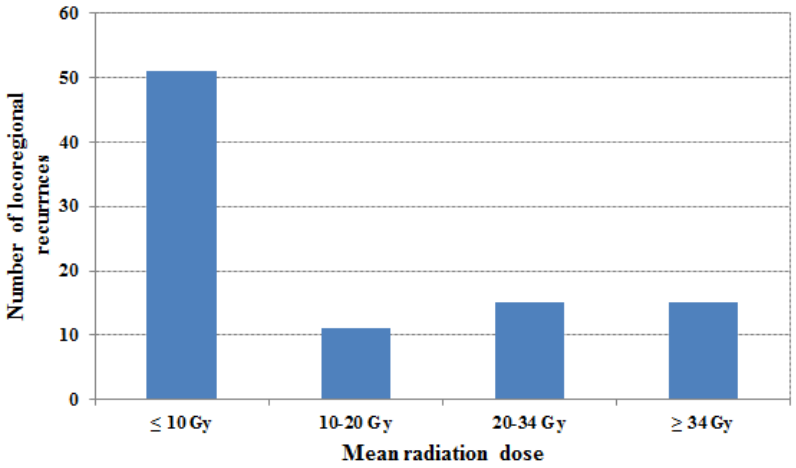


Figure 12: The number of locoregional recurrences per MRD category. The relapses with MRD of 34 Gy or higher were assumed to occur within the radiation target volume. The radiation dose was extracted for a total of 92 locoregional recurrences.

Hence, according to the above analysis, 6 patients were identified with locoregional relapse within the radiation field. With a total of 77 patients (again excluding the 3 patients with local recurrence in the gastric wall), this means that 7.8% of patients, curatively treated with chemoradiotherapy followed by Ivor-Lewis esophagectomy, had tumor relapse within the radiation target volume.

An overview of all detected in-field recurrences is given in Table 15. The 15 in-field relapses, consisting of 14 regional lymph node metastases and one local anastomotic recurrence, occurred on 12 different locations. The prevascular (T3a), retrotracheal (T3p) and lower para-esophageal (T8L) lymph node groups contained 2 in-field recurrences each, while for the other groups only one tumor relapse was observed within the radiation field.

Category	Description	#	Category	Description	#
T3a, LN	Prevascular	2	**T4R, LN**	Right upper paratracheal	1
T3p, LN	Retrotracheal	2	**T5, LN**	Subaortic	1
T8L, LN	Lower para-esophageal	2	**T6, LN**	Para-aortic	1
T1R, LN	Right cervical para-esophageal	1	**T8M, LN**	Middle para-esophageal	1
T2L, LN	Left upper paratracheal	1	**A1, LN**	Right paracardial	1
T4L, LN	Left lower paratracheal	1	**Local**	Anastomotic recurrence	1

Table 15: Overview of the locoregional recurrences which occurred within the radiation field. A total of 15 in-field relapses were detected. Note that the T3a nodes were also included in this analysis, although strictly speaking they are not considered as regional lymph nodes of the esophagus (see Table 3). LN: lymph node.

To detect possible particularities in characteristics of the patients with in-field failure, we compared all variables of Table 4 to Table 7 between the patients with and without (including the patients with no locoregional relapses) recurrences within the radiation field. Distributions

of nominal variables were compared with the chi-square test, while distributions of ordinal and continuous variables were examined with the Mann-Whitney U test. Starting from the patients of Group 2 and excluding the 3 patients with local recurrence in the gastric wall, no significant differences were found in any of the demographic, clinical and pathological variables.

We also investigated the differences in pathological response between both groups. Compared to the patients with no in-field recurrences, the prevalence of a pathological complete response was not significantly different in the group with in-field relapses (P = 0.591, Fisher's exact test). It is remarkable, however, that no patients with in-field recurrence had a pathological complete response (3 had pPR and 3 had pNR).

All patients with in-field relapses developed distant metastases. Five of 6 patients died from the consequences of hematogenous metastases in multiple organs. For all these patients, 2 or more organs were simultaneously affected. One patient had only distant (axillary) lymph node metastases, but died from the complications of an anastomotic local recurrence. The distribution of the distant patterns of recurrence for the patients with in-field relapse is given in Table 16. The lungs and the abdominal lymph nodes were the most frequent sites of failure, containing 4 recurring patients each. The bones and the muscles were affected in 50% of patients with in-field locoregional recurrence. In total, the distant recurrences were detected in 23 different anatomical locations for 6 patients with in-field relapse. This amounts to approximately 4 distant recurrences per patient, illustrating the widespread tumor dissemination in patients with locoregional recurrences within the radiation target volume.

Distant recurrence	**#**	**%**	**Distant recurrence**	**#**	**%**
Lung	4	66.7	Brain	1	16.7
Abdominal LNs	4	66.7	Cervical LNs	1	16.7
Bone	3	50.0	Thoracic LNs	1	16.7
Muscle	3	50.0	Iliac LNs	1	16.7
Liver	2	33.3	Inguinal LNs	1	16.7
Axillary LNs	2	33.3			

Table 16: Distribution of the distant hematogenous and lymphatic recurrences of the in-field relapsed patients. The percentages refer to the number of patients with in-field recurrence (N = 6). Positive abdominal lymph nodes were detected in the A16a2, A16b1, A16b2 and perigastric groups, one positive distant thoracic node in the T11 group and positive cervical nodes in the CII, CIII and CV groups.

The influence of in-field recurrences on survival was investigated with the Kaplan-Meier method. Figure 13 contains the disease-specific and disease-free survival curves of the patients with in-field relapses compared to the patients with recurrences (locoregional and/or distant) occurring outside the radiation field only. Using the log-rank test, clearly non-

significant differences were obtained in disease-specific (P = 0.826) and disease-free (P = 0.912) survival between both groups.

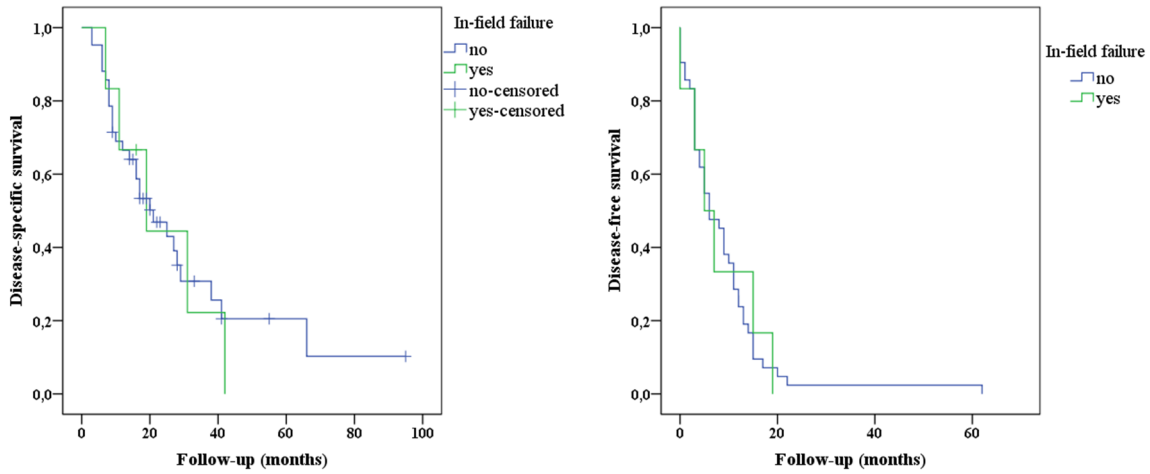


Figure 13: Disease-specific (left) and disease-free (right) survival of the patients with in-field tumor recurrence compared to the patients with distant and/or locoregional recurrence outside the radiation field only.

In summary, only a minority (7.8%) of patients treated with neoadjuvant chemoradiation followed by Ivor-Lewis esophagectomy had tumor relapse within the radiation field. No statistically significant differences in clinicopathological characteristics could be found for this group. However, a pathological complete response was observed in none of these patients. Importantly, all patients showed extensive tumor dissemination in the organs or distant lymph nodes, while no significant differences were obtained in survival compared to the patients with recurrences outside the radiation field only.

III.4.3. Radiation induced enhancement of lung metastasis

Lung metastasis was the most frequent pattern of hematogenous metastasis experienced by the patients in our study. A total of 23 patients (or 28.8% of Group 2) developed lung metastasis after multimodality treatment (see Table 14), which corresponds to 48.9% of patients with distant metastasis and to 56.1% of patients with distant organ metastasis (serosal and/or hematogenous recurrence). These lung recurrence rates are considerably higher than we would expect based on data found in the literature. In one study of 838 patients with esophageal carcinoma, 147 patients had distant metastasis (M1) at the time of diagnosis.[80] Lung metastases were detected in only 19.7% of these patients (as opposed to 48.9% in the current study). As no treatment had yet been given, this percentage can be considered as an estimation of the number of patients developing lung metastasis during the normal course of the disease, without possible alterations of the metastasis pattern by treatment modalities, such as neoadjuvant chemoradiation. Another study investigated the recurrence patterns of

138 relapsed patients treated for esophageal carcinoma with curative intent.[81] All patients underwent esophagectomy and only a clear minority received neoadjuvant therapy (23%, chemoradiation or chemotherapy only). Hence, the recurrence patterns in this study can be considered to occur in patients mainly treated with surgery alone. Of the 92 patients with distant organ metastasis in this study, only 29.3% patients were detected with lung metastasis. This is again considerably smaller than the 56.1% we found in the current study. In the 2 abovementioned studies and the current one, similar methods were used to detect the lung metastases (i.e. mainly on CT). On the other hand, different studies have distinct methodologies and varying population characteristics. Hence, comparison of different studies has to be handled with caution and needs a manifest critical approach. Nevertheless, despite these methodological limitations, it remains a fact that 2 to 2.5 times more lung metastases were detected in the current study compared to both other studies. This is at least to be considered as remarkable. Therefore, since more lung metastases were detected than is expected to occur during the natural course of the disease or after treatment with surgery alone, we hypothesized that neoadjuvant therapy might be responsible for lung metastasis induction. More in particular, we investigated the possible mechanism of radiation induced enhancement of lung metastasis in the treatment of esophageal carcinoma.

If radiotherapy would have an influence on the dissemination of lung metastasis, there should be noticeable differences in radiation dose on the lungs between patients with and without lung metastasis. Furthermore, these differences would be predominantly located in a relatively small lung volume in close proximity of the esophageal tumor, because this volume receives the highest radiation dose. Hence, to determine possible differences in radiation dose on the lungs, the DVHs of the lungs were analyzed for the smallest lung volumes which received the highest minimum doses. In Figure 14, the DVHs of the lungs are presented of the patients with and without lung metastases. By visual analysis of these DVHs, we noticed that the dispersion of the minimum doses received by a certain (small) percentage of the volume (e.g. 8% of total lung volume), seemed larger for the patients without lung metastases. This was confirmed when we determined the IQR of the distributions of the D_8 for both patient groups, as this was 9.1 Gy for the patients with and 15.7 Gy for the patients without lung metastases.

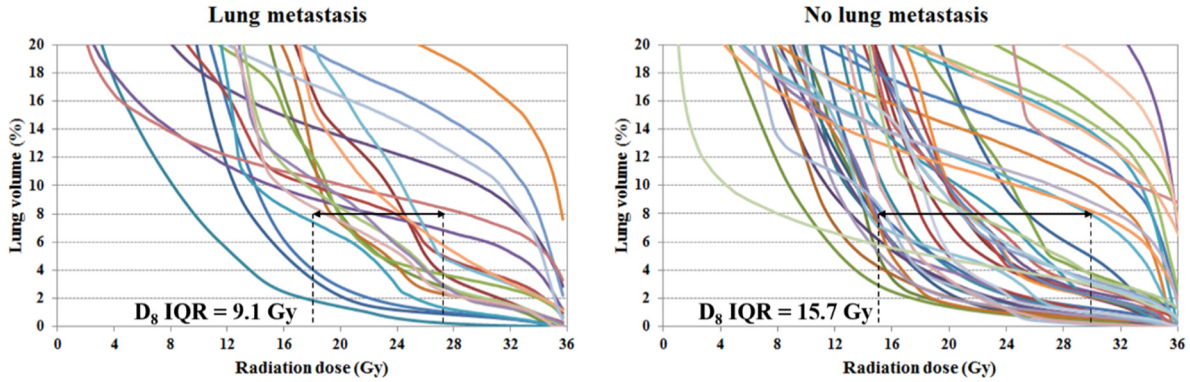


Figure 14: DVHs of the lungs of the patients with (left) and without (right) lung metastases. The IQR of the D_8 (the minimum dose received by 8% of the total lung volume) is indicated by the black arrow between the dotted lines.

This was further investigated through plotting of the histograms representing the distribution of the D_8 in both patient groups, see Figure 15. From this figure we can derive that the D_8 distribution peaks around 20 Gy for the patients with lung metastases. Furthermore, the D_8 is normally distributed for these patients, which is shown by the Shapiro-Wilk test (P = 0.606, in this test the null hypothesis assumes that the variable is normally distributed and, hence, it cannot be rejected). Being normally distributed, the D_8 can be accurately described by the mean and standard deviation, for which we obtained 22.7 Gy and 6.97 Gy respectively. On the other hand, the D_8 of the patients without lung metastases, is clearly not normally distributed (P = 0.003). This histogram shows 3 peaks, one around 13 Gy, one around 20 Gy and one around 33 Gy. Therefore, it seems that the group without lung metastases contained both more patients with lower and higher doses to the lungs, compared to the patients with lung metastasis.

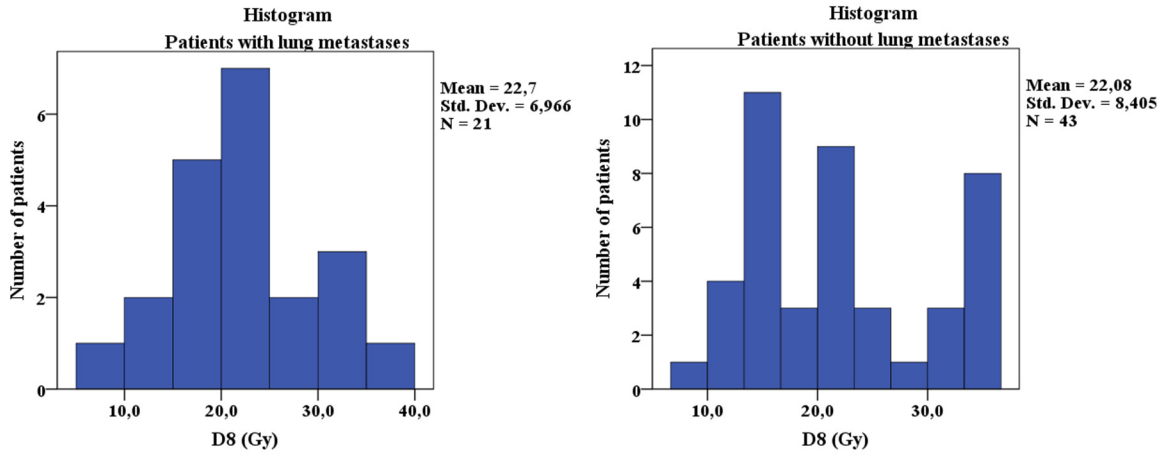


Figure 15: Histograms illustrating the distribution of the D_8 in the patients with (left) and without (right) lung metastases.

To further investigate this interesting observation, we divided the patients of both groups in two categories, one category with a D_8 between 15.7 and 29.7 Gy and another with a D_8 smaller than 15.7 Gy or larger than 29.7 Gy. We calculated these cut-off values by taking the mean D_8 of the patients with lung metastases and adding or subtracting one standard deviation. A frequency table containing the number of patients with or without lung metastasis for each D_8 category is given in Table 17. As can be derived from this table, 39.5% of the patients without lung metastases had a D_8 between 15.7 and 29.7 Gy, while for the patients with lung metastases this was as many as 66.7%. Furthermore, this difference is statistically significant (P = 0.041, chi-square test). Lung metastasis was the only pattern of recurrence with significant higher prevalence in the group with D_8 between 15.7 and 29.7 Gy. No other significant differences were found between both groups in the occurrence of local recurrence, regional recurrence, distant lymph node recurrence, distant serosal recurrence and other hematogenous metastases (bone, liver, etc.).

		D_8 < 15.7 Gy or > 29.7 Gy	**15.7 Gy ≤ D_8 ≤ 29.7 Gy**
Lung meta	no	26 (60.5%)	17 (39.5%)
	yes	7 (33.3%)	14 (66.7%)
Total		33 (51.6%)	31 (48.4%)

Table 17: Frequency table containing the number of patients with or without lung metastasis for both D_8 categories.

Univariate logistic regression analysis, with the presence/absence of lung metastasis as dichotomous outcome, showed a statistically significant higher risk to develop lung metastasis if a D_8 was received between 15.7 and 29.7 Gy, with an odds ratio of 3.06 (95% CI: 1.02-9.13, P = 0.045). To eliminate possible confounders, we also applied a multivariate logistic regression analysis, in which several other variables were included that could have an influence on the occurrence of lung metastasis. We included one demographic variable (age), two clinical variables (thoracic tumor location and clinical stage) and one pathological variable (pathological complete response) in this model. The results of this analysis are presented in Table 18. From the results of this table, it can be deduced that the D_8 was the only independent variable significantly predicting the occurrence of lung metastasis, with an odds ratio of 3.32 (95% CI: 1.00-10.96, P = 0.050).

Differences in distributions of other possible confounders increasing the risk of lung metastasis between 2 (D_8 < 15.7 Gy or > 29.7 Gy and 15.7 Gy ≤ D_8 ≤ 29.7 Gy) or 3 groups (D_8 < 15.7, 15.7 Gy ≤ D8 ≤ 29.7 Gy and D_8 > 29.7 Gy), were investigated with the chi-square test for categorical variables and with the Mann-Whitney U test (2 groups) or Kruskal Wallis

test (3 groups) for continuous variables. In none of these tests, significant differences were found in the distributions of the tumor differentiation grade, the tumor length, the LNR and the number of positive lymph nodes.

Variable	Values	Odds ratio	95% CI	P-value
D_8	15.7 Gy ≤ D_8 ≤ 29.7 Gy	3.32	1.00-10.96	0.050
	< 15.7 Gy or > 29.7 Gy	Reference		
Age	Continuous	1.00	0.94-1.06	0.936
Thoracic location	Distal 1/3	1.06	0.58-1.93	0.863
	Proximal/middle 1/3	Reference		
Clinical stage	Stage III	3.44	0.81-14.52	0.093
	Stage II	Reference		
pCR	no	1.16	0.28-4.77	0.839
	yes	Reference		

Table 18: Results of the multivariate regression analysis to predict the occurrence of lung metastasis.

It follows from the above that the D_8 has a significant influence on the incidence of lung metastasis. Moreover, this might also have an impact on survival. Therefore, we also investigated the influence of the D_8 on disease-specific survival. The Kaplan-Meier survival curves of the patients in the two D_8 categories are presented in Figure 16. Applying the log-rank test, a significant difference in disease-specific survival was found (P = 0.047) between both categories. The median survival, 3-year and 5-year survival rates of the patients with D_8 < 15.7 Gy or > 29.7 Gy were 42 months (95% CI: 0.0-88.0 months), 66.2% (95% CI: 47.2-85.2%) and 49.7% (95% CI: 28.1-71.3%) respectively. For the patients with D_8 between 15.7 and 29.7 Gy, these values were 21 months (95% CI: 10.6-31.4 months), 37.7% (95% CI: 17.2-58.3%) and 28.3% (95% CI: 6.0-50.6%) respectively.

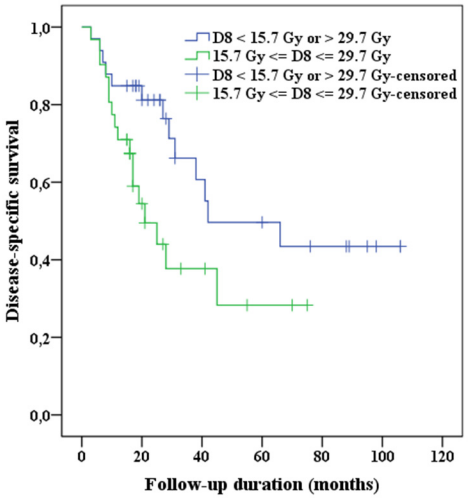


Figure 16: Disease-specific survival for the patients in both D_8 categories.

A univariate Cox regression analysis was then performed to compare the risk of dying from esophageal cancer in the two D_8 categories. A borderline significant hazard ratio of 2.06 (95% CI: 0.99-4.30, P = 0.053) was obtained, indicating a higher risk of cancer-related death for the category with D_8 between 15.7 and 29.7 Gy. This was further elaborated in a multivariate analysis, taking the same variables as in Table 18 and one additional pathological variable (the LNR) into account. We chose this additional variable because of its pronounced influence on survival (see Table 13). The results of this multivariate analysis are presented in Table 19. From these results follows that, after correction for several clinicopathological variables, the D_8 has become a statistically significant independent predictor of cancer-related death, with patients with D_8 between 15.7 and 29.7 Gy having a 2.44 times higher risk (95% CI: 1.03-5.80, P = 0.043) of dying from esophageal cancer than patients with D_8 < 15.7 Gy or D_8 > 29.7 Gy.

Variable	Values	Hazard ratio	95% CI	P-value
D_8	15.7 Gy ≤ D_8 ≤ 29.7 Gy	2.44	1.03-5.80	0.043
	< 15.7 Gy or > 29.7 Gy	Reference		
Age	Continuous	1.03	0.99-1.07	0.220
Thoracic location	Distal 1/3	0.89	0.58-1.36	0.588
	Proximal/middle 1/3	Reference		
Clinical stage	Stage III	1.58	0.63-3.93	0.328
	Stage II	Reference		
pCR	no	1.48	0.46-4.76	0.515
	yes	Reference		
LNR	≥ 10%	3.54	1.48-8.49	0.005
	< 10%	Reference		

Table 19: Results from the multivariate Cox regression analysis to identify the independent predictors of cancer-related death.

In summary, it is concluded from the above that the D_8 was a significant independent predictor of both the occurrence of lung metastasis and cancer-related death. Hence, it seems plausible that the patients with a D_8 between 15.7 and 29.7 Gy die faster from esophageal cancer due to the higher rate of lung metastases in this group, as it was the only recurrence pattern that differed significantly between both groups. Furthermore, this is evidence to support the hypothesis that radiotherapy enhances the dissemination of lung metastases. This enhancement is only observed when subtherapeutical doses are administered to a small percentage of the lung volume surrounding the primary esophageal tumor.

IV. DISCUSSION

IV.1. Limitations

A retrospective study was performed in this master thesis, implying that the general limitations of a retrospective analysis did also apply for this work. Important examples of these limitations are selection and information bias. These result from lack of control in the patient selection process and the acquisition of relevant clinicopathological data respectively. We had to rely mainly on data being available in the electronic and/or paper-based medical records. Although it was possible to extract much relevant data in this way, it was not always possible to obtain a complete data set for all patients. This resulted in missing values for several characteristics as e.g. the tumor length and differentiation grade, decreasing statistical power of the tests in which they were used. Despite this limitation, however, the tumor differentiation grade was identified as a major determinant of survival.

Tumor recurrences were mostly detected on CT images and biopsies were not always taken to prove malignancy of suspicious lesions. Consequently, some lesions might be falsely detected as tumor recurrences. In this respect, a known problem is the difficulty to distinguish reactive (inflammatory) lymph nodes from malignant ones, as both appear enlarged on CT images. To minimize the detection of false positives, however, we used several other criteria than size (e.g. the presence of a necrotic center) to determine whether lesions were malignant or not (see II.4.3 for an overview).

To determine if a recurrence occurred in the radiation field, rigid registration of planning and follow-up CTs was performed. Rigid registration implies image fusion by linear transformations (translations and/or rotations) without the use of non-linear deformations. Most follow-up CTs, however, were non-linearly deformed with respect to their planning CTs due to the use of different beds (flat vs. hollow), different positions of the hands/legs, pronounced weight loss, operative changes, ventilation differences (in- vs. expiration), etc. After rigid registration, therefore, positioning errors exist between planning and follow-up CTs which impede the precise determination of the tumor recurrence locations on the planning CT. To minimize these positioning errors we used a stepwise approach, as described in section II.5.5. Non-rigid registration methods, however, which perform deforming operations with the use of vector mapping might even yield better results.[82] Unfortunately, no such algorithms were available in the used image registration software.

IV.2. Survival

IV.2.1. Postoperative complications

Thirty-day and 90-day postoperative mortality rates of 2.1%, respectively 8.4% were obtained in this master thesis. These rates correspond to the values found in the literature. In one study of 143 patients curatively treated for esophageal carcinoma with transthoracic esophagectomy (of whom 28 received chemoradiation), a 30-day mortality rate of 2.1% was achieved.[83] In the CROSS trial, 30-day and 90-day mortality rates were found of 4.3%, respectively 7.3% in a total of 96 patients undergoing multimodality treatment.[84] In a meta-analysis of 6 RCTs, comparing postoperative mortality and morbidity in patients receiving multimodality treatment with surgery alone, a 90-day mortality of 11.9% was attained in the 328 neoadjuvantly treated patients.[85]

IV.2.2. Disease-specific and disease-free survival

A median disease-specific survival of 31 months was observed. Taking into account the 2 non-disease specific deaths, this corresponds with a median overall survival of 29 months. Three and 5-year survival rates were obtained of 48% and 37% respectively, while the median disease-free survival was 14 months. These survival characteristics are in accordance with the respective ranges found in the literature. In a systematic review of 38 studies (3 RCTs, 12 prospective cohort studies and 23 retrospective studies) of patients neoadjuvantly treated for esophageal carcinoma published between 2000 and 2008, a median overall survival was reported between 16 months until ‘not reached’. Three- and 5-year survival rates ranged between 22-64% and 16-59% respectively.[57] The median disease-free survival was specified in only 2 studies, with values of 16 [86] and 9 [87] months. In most studies included in this review, the chemotherapy regimens consisted of administration of 5-FU and cisplatinum (such as in the current one), but the dose and timing during the radiotherapy course varied widely. Moreover, many different radiotherapy regimens were used, with prescribed doses ranging between 30 and 60 Gy. Differences in therapeutic strategies, therefore, might explain at least in part the relatively broad survival ranges found in the literature. On the other hand, different patient selection criteria and, consequently, different patient characteristics might also explain the observed survival differences.

In the CROSS trial, a median overall survival of 49 months was obtained for the patients treated with neoadjuvant chemoradiation. Three- and 5-year survival rates were reported of 58% and 47% respectively.[63] These values are substantially better than the survival characteristics found in this study. These discrepancies might again be explained by

differences in patient characteristics and treatment protocol. In the CROSS trial, 38% and 61% of patients had clinical N_0 and N_1 stage respectively. However, as many as 83% of patients (of Group 1, see Table 6) had clinical N_1 stage in the current study. As the nodal stage is an important prognostic factor, it is expected that a substantially higher fraction of patients with node positive disease will have a substantial impact on the survival rate of the entire group. Secondly, important differences exist between both studies considering the administered neoadjuvant therapy. In the CROSS trial, chemotherapy consisted of paclitaxel and carboplatin. This was combined with concurrent radiotherapy with a total dose of 41.4 Gy. In the current study, however, chemotherapy consisted of 5-FU and cisplatinum and most patients (95%) received radiotherapy with a total dose of 36 Gy. Hence, it is plausible that better survival was obtained in the CROSS trial due to a higher intrinsic sensitivity of esophageal cancer cells to carboplatin and paclitaxel as opposed to the classical combination of 5-FU and cisplatinum. Additionally, more effective tumor destruction might have been obtained as a result of radiotherapy administration with the higher total radiation dose of 41.4 Gy.

IV.2.3. Pathological response

A total of 18.5% of patients in this study had a pathological complete response, corresponding with the pCR rates found in the literature (typically between 13 and 49%, with an average of 25.8%).[57] The median disease-specific survival was not reached for the pCR group, while 3 and 5-year survival rates of both 62.8% were obtained. Survival was clearly reduced in the no-response group, with median disease-specific survival of 38 months and corresponding 3 and 5-year survival rates of 55.1% and 30.6% respectively. Nevertheless, no significant differences in survival were obtained between both groups, most likely as a result of lack of statistical power. Patients with no response, however, had a 1.78 times higher risk of dying from esophageal cancer than patients with a complete response after correction for age, gender, clinical stage and thoracic tumor location in a multivariate analysis. This increased risk was statistically significant (95% CI: 1.03-3.08, P = 0.039). Accordingly, the risk of tumor recurrence was increased for patients with pNR, although it was not statistically significant. The survival benefit and reduced risk of tumor recurrence for patients with a pathological complete response have already been demonstrated by several authors. Meguid et al. found that pathological response was the only factor associated with tumor recurrence or death in a multivariate analysis. Compared to the patients with pCR, the chance of recurrence was 2.23 (P = 0.012) times higher for the patients with no response. Furthermore, all relapsed

patients died within a year after detection of tumor recurrence.[67] Barbour et al. compared the disease-specific survival of patients with major (0-10% residual tumor) and minor (> 10% residual tumor) pathological responses. In their multivariate analysis, patients with minor response had a 1.85 (95% CI: 1.15-2.86, P = 0.011) times higher risk of cancer-related death than patients with major tumor response.[88]

IV.2.4. Other prognostic factors

The tumor differentiation grade, the number of positive lymph nodes (detected during pathological examination of the resected specimen after neoadjuvant therapy) and the LNR were all identified in this study as significant predictors of cancer-related death and tumor recurrence, even after adjustment for demographic and clinical variables (see Table 13).

The tumor differentiation grade is a well-known prognostic factor for patients curatively treated with surgery for advanced esophageal carcinoma.[89] The substantial reduction in survival of patients with poorly differentiated tumors has led to incorporation of the tumor grade as prognostic grouping variable into the latest (7^{th}) version of the TNM classification system.[90] However, this classification is developed with clinicopathological data of patients treated with surgery alone and did not include data of patients treated with neoadjuvant therapy. Evidence is scarce considering the prognostic value of the tumor differentiation for patients neoadjuvantly treated with chemoradiation, because most studies focus on the pathological response as the most important prognostic factor. In one study, in which 61% of patients received preoperative chemoradiotherapy, it was demonstrated that favorable tumor grade (well or moderately differentiated) was significantly associated with long-term survival of 5 years or more. Furthermore, the tumor grade was the strongest risk factor of actuarial 5-year survival, which was even stronger than a pathological complete response for the neoadjuvantly treated patients.[91] This is a clear indication that the tumor grade has important prognostic implications for patients treated with neoadjuvant chemoradiation and that it might be even more important in predicting long-term survival than the presence of a pathological complete response. This is supported by the results in the current study, as patients with high grade tumors had a 5.23 (95% CI: 2.36-11.60, P < 0.001) times higher chance of dying from esophageal cancer than those with low or intermediate grade tumors. Moreover, this influence on survival was considerably higher than the influence of a pCR (see Table 12).

In the literature, it has repeatedly been shown that a positive pathological lymph node status is to be considered as an independent predictor of poor survival. Moreover, survival further

decreases with increasing number of positive lymph nodes detected during pathological examination. This has not only been demonstrated for patients treated with surgery alone [92, 93], but also for patients after neoadjuvant treatment.[94] Besides the number of positive lymph nodes, the LNR has also been put forward in the literature as an independent predictor of survival for neoadjuvantly treated patients.[95, 96] The current study confirms the independent prognostic values of both the absolute number of positive lymph nodes and the relative LNR for patients treated with preoperative chemoradiation. Patients with a LNR of 10% or more had a 4.01 (95% CI: 1.90-8.47, $P < 0.001$) times higher risk of esophageal cancer-related death than patients with LNR of smaller than 10%. Furthermore, the prognostic value of the LNR was again considerably higher than that of a pathological complete response. This again shows that the prognosis of patients treated with neoadjuvant chemoradiation is significantly determined by more than only the pathological tumor response.

The importance of the tumor differentiation grade and the lymph node status after neoadjuvant chemoradiation in predicting survival, correlates with the fact that distant tumor recurrence is the main concern in patients treated for esophageal carcinoma (see section IV.3). It is common knowledge that poorly differentiated tumors have the highest potential to metastasize distantly. Furthermore, a high LNR detected during pathological examination can be considered as a strong indication of the metastatic potential of the primary tumor pre-existing before multimodality treatment. These observations support the hypothesis that most relapsed patients had already developed micrometastases at the time of diagnosis and were actually beyond locoregional control. Furthermore, this also might explain why a large number of patients with pathological complete response still develop tumor recurrence.

IV.3. Recurrence patterns

In the current study, a total of 64% of the included patients developed tumor recurrence after a minimum follow-up duration of 12 months for non-relapsed patients. The locoregional and distant recurrence rates were 39% and 59% respectively. Furthermore, locoregional failure was accompanied by distant recurrence in as many of 87% of cases and only 5% of patients had isolated locoregional failure. Seventy-nine percent of patients with distant failure had hematogenous relapse. Most affected organs were the lungs, the liver and the bone, corresponding with the distribution of the most prevalent recurrence patterns found in the literature for patients neoadjuvantly treated for esophageal carcinoma.[67, 97, 98]

In a review comparing the recurrence patterns of 23 non-randomized trials of patients treated with preoperative chemoradiation, locoregional, distant and total recurrence rates were found between 0-39%, 19-70% and 19-80% respectively (including only the studies published after 1990 and excluding one study with median follow-up of only 10 months).[99] Hence, relatively broad ranges are found in the literature. Most likely, this is explained by the differences in patient characteristics (e.g. varying number of patients with clinical stage II and stage III), in treatment protocols (different radiotherapy and chemotherapy regimens) and follow-up duration between studies. This makes a direct comparison rather difficult. Nevertheless, the distant and total recurrence rates obtained in the current study are located within the given ranges. On the other hand, the attained locoregional recurrence rate is rather high and corresponds with the highest rates found in the literature. This is probably explained by the fact that in this master thesis all recurrences were documented occurring between the first day after esophagectomy and the patient death or last follow-up (see also section II.4.1). It was noticed that several locoregional recurrences developed several months after detection of a first distant relapse. These locoregional failures would have remained undetected, if only the tumor relapses appearing within one month of the first would have been taken into account (which is the methodology used in most studies).

The recurrence rates found in the CROSS trial of the neoadjuvantly treated patients are considerably smaller than those found in the current study, with locoregional, distant and total recurrence rates of 14%, 32% and 35% respectively.[54] This might again be explained by the lower fraction of cN_1 patients and the possible higher sensitivity of distant micrometastases to carboplatin and paclitaxel, as stated in section IV.2.2. Furthermore, registration of tumor relapses was stopped one month after occurrence of the first, resulting in a possible underestimation of the locoregional recurrences. In the CROSS trial, only 3% of patients developed isolated recurrence, while as many as 77% of patients with locoregional failure had combined distant relapse. These observations are consistent with the results of the current study (5% and 87% respectively).

It is clear from the above considerations that distant (hematogenous) failure is the predominant problem in patients with locally advanced esophageal carcinoma and that only a minor fraction of patients develops isolated locoregional tumor recurrence. This again suggests that most relapsed patients already had distant recurrence in the form of undetectable micrometastases at the time of diagnosis. This has important implications for the used treatment methods, as only a clear minority of patients would benefit from more extensive locoregional control by expansion of the irradiated volume or using 3-field instead of 2-field

lymphadenectomy. Moreover, extended (3-field) lymphadenectomy implies a higher postoperative complication grade and higher proportion of postoperative deaths. This is difficult to justify given the limited expected influence on tumor recurrence and survival.[100-102]. Given the fact that systemic disease is the major problem for patients with locally advanced esophageal carcinoma, it seems appropriate to concentrate on research and development of better systemic therapies.

IV.4. Radiation field analysis

IV.4.1. Locoregional recurrences within the radiation field

Only 7.8% of the included patients had locoregional recurrence within the radiation field. Further, all of these patients had developed distant failure and, consequently, survival did not differ from patients with only recurrences outside the radiation field. In the CROSS trial, a total of 5.2% of patients were identified with recurrences inside the PTV. Furthermore, as many as 82% of these patients experienced synchronous distant metastasis.[54] Although a different methodology (i.e. without dose extraction) was used to determine whether recurrences were inside the radiation field, these figures are fairly comparable to the results obtained in the current study.

Other than the relapses inside the radiation field (with MRD of 34 Gy or higher), several recurrences were detected with MRD between 10 and 34 Gy. These recurrences were not classified as borderline, because it is not possible to accurately determine the position of these relapses with extraction of the radiation dose only. This can be explained as follows. In the modern IMRT (Intensity Modulated Radiation Therapy) and, to a lesser extent, in the non-IMRT 3D conformal radiotherapy techniques, a relatively large region surrounding the PTV still receives a considerable radiation dose. As a result, an ROI with mean dose of e.g. 30 Gy can still be located at a fair distance of the PTV and can not be considered as a recurrence at the border of the target volume (but rather as a true out-field recurrence). Hence, without additional position information it is not possible to determine accurately whether a relapse is located at the border of the PTV. It was chosen not to elaborate this further in this master thesis due to time constraints.

Most likely, the patients with in-field relapse have highly radiotherapy-resistant tumors. This is supported by the fact that none of these patients had a pathological complete response. No significant differences could be identified between patients with and without in-field recurrences in the included clinicopathological characteristics. As only 6 patients had in-field recurrence, this might be explained due to lack of statistical power. On the other hand, it is

likely that biological differences on a cellular and/or molecular level exist which determine the intrinsic radiotherapy resistant properties of esophageal tumor cells. Investigation of these properties, however, was beyond the scope of this master thesis.

The radiation resistant properties of in-field tumor recurrences might be present prior to the utilization of radiotherapy and, hence, might be intrinsic to certain tumor subtypes. On the other hand, they also might be induced during radiotherapy. It is plausible that there is a natural selection of cells of the primary tumor with more radioresistant properties which have the ability to survive in harsh microenvironmental conditions induced by radiotherapy, such as hypoxia. This process is also called the tumor-bed-effect (see section IV.4.2 for a more detailed explanation of this phenomenon). As hypoxia resistance is a well-known property of metastatic cells [103-105], the induced radioresistance might go hand in hand with increased metastatic potential of surviving tumor cells. The hypothesis that radioresistance (be it intrinsic or induced) and metastatic potential might be related, is confirmed by the results of this master thesis. All patients with in-field recurrence had concurrent distant metastases. Furthermore, metastases on approximately 4 different locations were detected per in-field relapsed patient, hence, indicating the pronounced malignant properties of radioresistant tumors.

It is assumed that dose escalation would not be a good approach for the patients with in-field recurrence, due to the presumed low radiation sensitivity of their tumors. Dose escalation would also be ineffective because most patients with in-field recurrence (100% in this study) developed distant metastasis in the same manner as most other patients with locoregional recurrence. The latter observation even questions the relevance to distinguish patients with locoregional recurrences in- or outside the radiation field, because the occurrence of distant metastases is the core problem. Nevertheless, irrespective of the high prevalence of distant metastases in locoregionally relapsed patients, it can be concluded that chemoradiation followed by surgery yields excellent in-field control, since only a clear minority (7.8%) of patients developed in-field relapse. This result has to be put in perspective, however, and is of rather limited importance given the high prevalence of distant metastases in patients neoadjuvantly treated for locally advanced carcinoma. Hence, it seems that most patients fail distantly, despite excellent locoregional control of the combined multimodality therapy. This is again a clear call for the development of more effective systemic therapies.

IV.4.2. Radiation induced enhancement of lung metastasis

Twenty-nine percent of patients developed lung metastasis, corresponding with 56% of patients with distant organ metastasis. Consequently, lung metastasis was the most prevalent distant recurrence found in this master thesis. It has been demonstrated in this study that patients with a D_8 between 15.7 and 29.7 Gy had a statistically significant higher risk to develop lung metastases after correcting for age, thoracic tumor location, clinical stage and pathological complete response (odds ratio: 3.32, 95% CI: 1.00-10.96, P = 0.050). In other words, this means that patients receiving minimum subtherapeutical doses between 15.7 and 29.7 Gy in a small lung volume surrounding the primary tumor, have a higher risk of developing lung metastases. Indeed, the effect seems to exist only for patients with a D_8 within a specific range. This suggests that lower doses might not be sufficient to demonstrate the effect, while higher doses might cause too much lung destruction for the effect to occur.

The patients with D_8 between 15.7 and 29.7 Gy had a 2.44 (95% CI: 1.03-5.80) times higher chance of cancer-related death than other patients. Moreover, this risk was statistically significant (P = 0.043) after correction for several clinicopathological variables. It is reasonable to assume that this higher risk is explained by the significantly higher prevalence of lung metastases in this group. This is strengthened by the fact that no other differences in recurrence pattern were found between both patient groups.

In the literature, radiation induced enhancement is described by several mechanisms. Two of these mechanisms are of particular interest, because its existence is supported by extensive experimental evidence. The first effect is called the tumor-bed-effect and is caused by irradiation of the primary tumor. The second mechanism describes metastasis enhancement as a result of radiation induced damage of the normal tissues surrounding the primary tumor.[44] Evidently, a combination of both effects might be possible.

The tumor-bed-effect is a classical concept in radiation oncology. It describes the mechanism in which the metastatic potential of tumor cells is increased through radiation induced reduction of the growth rate of the primary tumor. It seems that this reduced growth rate provides tumor cells with more time to adapt to radiation induced modifications of the tumor microenvironment, such as hypoxia. As tumor cells are genetically unstable, adaptations occur due to these hypoxic conditions and cells of the primary tumor with more invasive and metastatic capabilities are selected.[106] There is much experimental evidence supporting the existence of the tumor bed effect.[107-111] However, to our knowledge, no convincing clinical evidence has yet been published.

Another effect is the radiation induced damage of normal tissues surrounding the primary tumor. The extracellular matrix of capillaries seems to be a preferential location for tumor cell adherence. When capillaries are damaged by irradiation, their extracellular matrix is directly exposed to the blood. Consequently, when viable tumor cells are present in the circulation, adherence of these cells to extracellular matrix proteins is facilitated. This way, concentration of metastatic tumor cells might take place in the irradiated tissue, hence, leading to development of metastases.[44] There is a multitude of experimental evidence supporting the hypothesis of lung metastasis enhancement by prior irradiation of the lungs.[112-121] Interestingly, subtherapeutical doses were used in most of these studies. On the other hand, clinical evidence remains scarce. Only a limited number of case studies have been found in the literature suggesting the existence of radiation induced enhancement of metastases in clinical practice. In most of these studies, metastases were detected within an irradiated part of the skin after radiotherapy treatment of distantly located tumors.[44, 122, 123]

By combining both mechanisms described above, the radiation induced enhancement of lung metastases from esophageal carcinoma might be explained as follows. Due to the anatomical location of the esophagus, both primary tumor and surrounding lung tissue are irradiated simultaneously. Hence, some of the irradiated tumor cells might survive and be transformed into cells with pronounced metastatic potential due to the tumor-bed-effect. Viable metastatic tumor cells are then released from the surrounding microenvironment into the circulation. These are carried in the circulation to the lung capillaries, which have their extracellular matrix directly exposed to the blood, due to radiation induced damage. Next, adherence of tumor cells to the extracellular matrix proteins occurs with extravasation into the interstitial lung tissue leading, eventually, to development of pulmonary metastases. It has to be stressed, however, that this it is a purely hypothetical explanation and, therefore, more research is needed to support this theory.

As stated above, much experimental data are available to support the hypothesis of radiation induced enhancement of metastasis. Nevertheless, clinical data are scarce and, to date, failed to confirm the extensive amount of experimental evidence. The results of this master thesis are, therefore, the first to provide clear indications that this effect might also take place in clinical practice. Importantly, the effect in this study was not limited to a number of cases, but appeared systematically in a larger group of patients. Moreover, a dose-response relationship was derived, suggesting a causal relationship between the radiation dose on the lungs and the induction of lung metastases. This relationship suggests that the D_8 on the lungs has to be lower than 15.7 Gy to prevent radiation-induced occurrence of lung metastases. Although

more work has to be performed to confirm these results and to refine the dose criteria to include in radiotherapy planning guidelines, we have at least a value to start from in our quest to improve the treatment and survival of patients with esophageal cancer.

V. CONCLUSIONS AND FUTURE WORK

The results of this master thesis have shown that neoadjuvant chemoradiation followed by Ivor-Lewis esophagectomy provides excellent locoregional control, given only 7.8% of the included patients had in-field recurrence. It has been demonstrated, however, that the majority of relapsed patients fails distantly (92.2%). This suggests that the vast majority of these patients already had distant spread of the disease in the form of undetectable micrometastasis at the time of diagnosis and that they were actually beyond locoregional control. Consequently, one might expect that intensification of locoregional therapies such as dose escalation, radiation field extension or three-field lymphadenectomy would not have a major influence on survival. Future work should, therefore, be mainly directed towards improvement of distant control. In this respect, it seems important to support the development of new, more effective systemic therapies. On the other hand, it also seems appropriate to integrate more chemotherapy in the standard treatment of locally advanced esophageal cancer. The addition of adjuvant chemotherapy for patients with increased risk of distant metastasis should be considered to improve these patients' survival. In order to identify increased risk patients, it would be necessary to restage all patients after surgery and to adapt treatment accordingly. The results of this study indicate that the tumor differentiation grade and pathological variables such as the pCR and the LNR could possibly be included as new staging variables in future restaging systems.

In this master thesis, all included patients with in-field recurrences had extensive distant failure. Furthermore, none of these patients had a pathological complete response. This supports the hypothesis that radioresistant and metastatic tumor cell properties frequently coexist and might develop under similar circumstances. It has been proposed that these properties might be induced by radiotherapy as a result of the tumor-bed-effect. This effect would explain, at least in part, the multitude of distant metastases in patients with locally advanced esophageal cancer. Therefore, future work should address if differences exist between the cellular properties of tumor cells before and after neoadjuvant treatment (e.g. the presence of certain adhesion molecules). Comparison of cellular properties could be performed between the tumor samples obtained during initial staging and after surgical resection.

We have demonstrated in this master thesis that subtherapeutical doses on a small lung volume surrounding the primary tumor were significantly associated with the occurrence of

lung metastasis. More in particular, it has been shown that after correction for demographic, clinical and pathological variables, the D_8 had to be lower than 15.7 Gy or higher than 29.7 Gy to significantly reduce the risk of lung metastasis. These results are, to our knowledge, the first to provide clear indications that radiation induced enhancement of metastasis also takes place in clinical practice. Therefore, these results should be confirmed by other studies. In this respect, it would be interesting to investigate the occurrence of lung metastases in completed RCTs comparing neoadjuvant therapy followed by surgery and surgery alone, such as the CROSS trial. The occurrence of significantly more lung metastases in the multimodality group would confirm our results and would provide more arguments to start prospective studies.

One might expect that the induced lung metastases would occur within a lung volume surrounding the primary tumor having received subtherapeutical doses. Therefore, it would be interesting to investigate the precise locations of the lung metastases and to extract the corresponding radiation dose parameters. Due to time constraints, however, we were not able to investigate this further. Finally, it is important to determine whether decrease of the D_8 on the lungs below 15.7 Gy is technically and therapeutically possible. Therefore, it would be of interest to review the radiotherapy plans of the patients included in this study to investigate the possibility of lung dose reduction without substantially reducing the dose received by the primary tumor.

As overall conclusion, it can be stated that still much work has to be done to improve the life expectancy of patients diagnosed with locally advanced esophageal carcinoma. Nevertheless, it is hoped that the results of this master thesis have provided more insight in the recurrence patterns of these patients and that this knowledge can be used to guide future directions in the treatment of this deadly malignancy.

VI. REFERENCES

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