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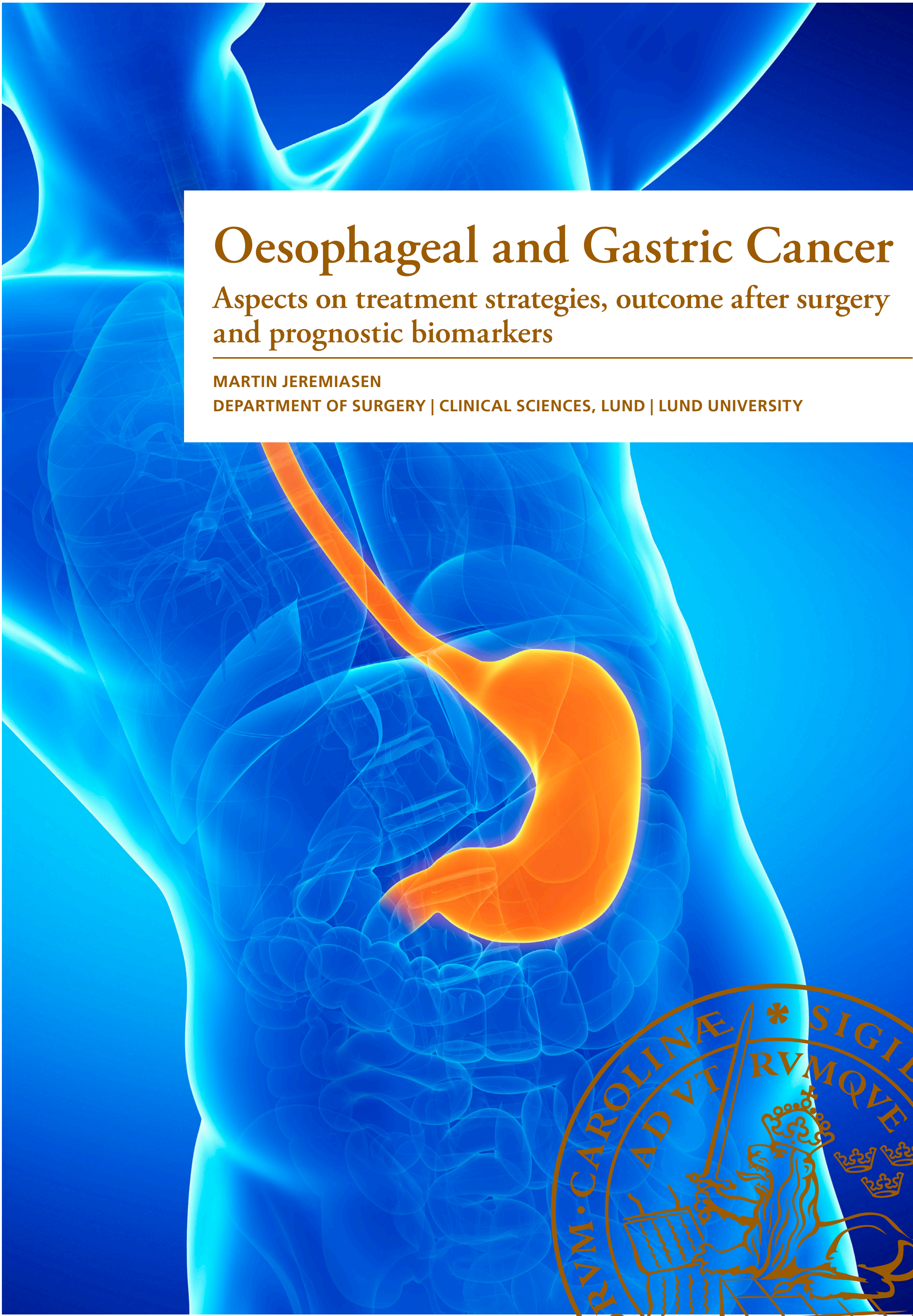

Oesophageal and Gastric Cancer

Aspects on treatment strategies, outcome after surgery and prognostic biomarkers

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Oesophageal and Gastric Cancer

Aspects on treatment strategies, outcome after surgery and prognostic biomarkers

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LUND UNIVERSITY

DOCTORAL DISSERTATION

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Abstract

Background: Approximately 1200 individuals are diagnosed with oesophageal or gastric cancer annually in Sweden. The 5-year survival rate for these patients does not surpass 20%. Surgery and/or oncological therapy is the mainstay of modern cancer treatment regimens for these tumours. The aims of this thesis were: 1. To evaluate current trends in Swedish oesophagogastric (OG) cancer treatment in a national and international context; 2. To investigate the potential benefit of the three-stage thoracoabdominal gastrectomy with resection of the distal two-thirds of the oesophagus and anastomosis with a long Roux-en-Y limb at the level of the vena azygos (THX-ABD) for Siewert type II & III tumours at the gastrooesophageal junction (GOJ); and 3. To evaluate the prognostic value of tumour-associated macrophages (TAMs) in patients with OG cancer.

Methods: Data were derived from the national quality registers for OG cancer in Sweden, the Swedish National Register for Oesophageal and Gastric cancer (NREV) (Papers I and II) and the Netherlands, the Dutch Upper GI Cancer Audit (DUCA) (Paper II). Papers III and IV were based on two separate retrospective cohorts of patients with OG cancer operated on at Skåne University Hospital. In Paper IV, tissue microarrays were created using tissue samples from the primary tumours and then immunohistochemistry (IHC) was applied with validated antibodies against $CD68^+$, $CD163^+$ and $MARCO^+$ macrophages. For detection, light microscopy was used.

Results: Neoadjuvant treatment before surgery became increasingly prevalent, surgical procedures for OG cancer were centralized to fewer hospitals and there was a significant improvement in short-term mortality after gastric cancer surgery in Sweden 2007-2016 (Paper I). Lower annual hospital volumes of OG cancer resections were observed in Sweden compared to the Netherlands. Neoadjuvant treatment rates were significantly lower in Sweden for both oesophageal and gastric cancer and Sweden had lower adjusted 30-day and/or in-hospital mortality after gastrectomies than the Netherlands (Paper II). After THX-ABD the in-hospital mortality was 2.4%, the R0-resection rate was 84% and overall 5-year survival was 27%. Additional thoracic lymph node dissection did not seem to improve long-term survival (Paper III). High infiltration of both $CD68^+$ and $CD163^+$ but not $MARCO^+$ macrophages in tumour nests (TN) was significantly associated with poor prognosis in a stepwise manner in patients with OG cancer not subjected to any neoadjuvant treatment before surgery. High infiltration of $CD68^+$ macrophages remained an independent prognostic factor for worse survival in the adjusted analysis (Paper IV).

Conclusions: The results from NREV 2007-2016 show significant improvements in several important quality indicators of care for patients with OG cancers in Sweden. Well-maintained national quality registers allow for international comparisons where significant differences in patient and treatment characteristics were evident between Sweden and the Netherlands. The THX-ABD can be performed with high rates of R0-resections and low in-hospital mortality. Since long-term prognosis is not superior to other less extensive procedures for tumours in the same locations, the THX-ABD should only be used when less extensive surgical methods are not an option to achieve R0. Infiltrating TAMs in TN can be used to predict outcome after surgery for OG cancer.

Key words: Oesophageal cancer, gastric cancer, national quality registers, gastrooesophageal junction and tumour-assosciated macrophages.

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To Ida, Hugo & Jakob

Content

List of publications

This thesis is based on the following original papers, which are referred to by their Roman numerals in the text:

I. Jeremiasen M, Linder G, Hedberg J, Lundell L, Björ O, Lindblad M, Johansson J. Improvements in esophageal and gastric cancer care in Sweden-population-based results 2007-2016 from a national quality register. Dis Esophagus. 2019 Oct 11. pii: doz070. doi: 10.1093/dote/doz070. [Epub ahead of print]

II. Busweiler LAD*, Jeremiasen M*, Wijnhoven BPL, Lindblad M, Lundell L, van de Velde CJH, Tollenaar RAEM, Wouters MWJM, van Sandick JW, Johansson J, Dikken JL. International benchmarking in oesophageal and gastric cancer surgery. BJS Open. 2018 Oct 19;3(1):62-73.
* These authors contributed equally to this manuscript.

III. Jeremiasen M, Walther B, Djerf P, Staël von Holstein C, Zilling T, Hermansson M, Falkenback D, Johansson J. Thoracoabdominal gastrectomy and distal 2/3 esophageal resection with wide lymph node dissection for type II and III adenocarcinoma at the gastro-esophageal junction. Am J Surg. 2019 Aug;218(2):329-334.

IV. Jeremiasen M, Borg D, Hedner C, Svensson M, Nodin B, Leandersson K, Johansson J and Jirström K. Tumour-associated $CD68^+$, $CD163^+$ and $MARCO^+$ macrophages as prognostic biomarkers in chemoradiotherapy-naïve oesophageal and gastric adenocarcinoma. Submitted to Oncoimmunology, Jan 20 2020.

Thesis at a glance

	Question	Methods	Results	Conclusion
I	How have trends in oesophageal and gastric (OG) cancer treatment changed in Sweden during 2007-2016?	Retrospective study of 12,242 patients with OG cancer registered in NREV 2007-2016.	More neoadjuvant treatment, centralization of surgery and lower 30-day survival after gastric surgery.	Improvement in several important indicators for quality of care in OG cancer surgery.
II	How do potential differences in OG cancer care affect short-term outcome after surgery in Sweden compared to the Netherlands?	Comparison of 1029 Swedish and 3410 Dutch patients with OG cancer resections 2012-2014 registered in NREV and DUCA, respectively.	Older patients, less comorbidity and less neoadjuvant treatment in Sweden. Lower 30-day/in-hospital mortality after gastric resections in Sweden.	Marked differences in patient selection, use of neoadjuvant treatment and short-term outcomes after gastric resections.
III	Is there a benefit of the long Roux-en-Y oesophagogastrectomy (THX-ABD) in patients with Siewert II & III tumours of the GOJ?	Single institution retrospective study of 83 patients with large Siewert II & III tumours of the GOJ operated on during 1986-2011.	THX-ABD can be performed with low in-hospital mortality (2.4%) and high R0-rate (84%). No impact on long-term survival.	THX-ABD should only be used when less extensive surgical methods are not an option to achieve R0.
IV	Is there a prognostic value of infiltration of $CD68^+$, $CD163^+$ and $MARCO^+$ macrophages in patients with OG cancer?	Retrospective study of 174 chemoradio-therapy-naïve patients with OG cancer operated on during 2006-2010.	$CD68^+$ and $CD163^+$ but not $MARCO^+$ macrophages in TN correlate with overall survival (OS) in OG cancer.	High infiltration of $CD68^+$ and $CD163^+$ macrophages in TN is prognostic for decreased OS in OG cancer.

Abbreviations

5-FU	5-fluorouracil
AJCC	American Joint Committee on Cancer
APC	Argon plasma coagulation
CD	Cluster of differentiation
$CD68^+$	CD68-positive macrophage
$CD163^+$	CD163-positive macrophage
CIN	Chromosomal instability
CRT	Chemoradiotherapy
CT	Computed tomography
DICA	Dutch Institute for Clinical Auditing
DUCA	Dutch Upper GI Cancer Audit
EBV	Epstein-Barr virus
EMR	Endoscopic mucosa resection
ESD	Endoscopic submucosal dissection
ESGE	European Society of Gastrointestinal Endoscopy
FLOT	Fluorouracil, calcium folinate, oxaliplatin, docetaxel
GI	Gastrointestinal
GOJ	Gastro-oesophageal junction
GS	Genomically stable
Gy	Gray
HER2	Human epidermal growth factor receptor 2
HR	Hazard ratio
IEBLD	Index of estimated benefit from lymph node dissection
IHC	Immunohistochemistry
MARCO	Macrophage receptor with collagenous structure
$MARCO^+$	MARCO-positive macrophage
MDC	Multi-disciplinary conference

MSI	Microsatellite instability
NOGCA	The British National Oesophago-Gastric Cancer Audit
NREV	Swedish National Register for Oesophageal and Gastric Cancer
OG	Oesophagogastric
OS	Overall survival
PAMP	Pathogen-associated molecular pattern
PET	Positron emission tomography
PIN	Personal identification number
PSA	Prostate specific antigen
QoL	Quality of life
RCT	Randomized controlled trial
SCC	Squamous cell carcinoma
SECC	The Swedish Oesophageal and Cardia Cancer Register
SFÖAK	The Swedish Association for Upper Gastrointestinal Surgery
SKL	Sveriges Kommuner och Landsting
SWEGIR	The Swedish Gastrointestinal Register
TAM	Tumour-associated macrophage
TCGA	The Cancer Genome Atlas
THX-ABD	Total gastrectomy including two-thirds of the distal oesophagus and reconstruction with a long Roux-en-Y limb.
TMA	Tissue microarray
TME	Tumour microenvironment
TN	Tumour nest
TTE	Transthoracic oesophagectomy
UICC	International Union Against Cancer

Introduction

Oesophageal and gastric cancer are global health problems affecting millions of people around the world every year. According to GLOBOCAN 2018 oesophageal cancer is placed seventh in terms of cancer incidence and is the sixth most common cause of cancer-related death worldwide whereas the corresponding ranks for gastric cancer are fifth and third, respectively (1). In Sweden in 2018, around 800 individuals were diagnosed with oesophageal cancer and 400 with gastric cancer (2). Due to the often late diagnosis of tumour disease, the majority of these patients will not be eligible for treatment with curative intention. Typically, 5-year survival rate for the whole cohort of patients does not surpass 20% (3).

The last two decades have seen the advent of many new concepts of treatment and care of oesophagogastric (OG) cancer patients such as neoadjuvant/perioperative oncological therapy in addition to surgery, minimally invasive surgical techniques and centralization of complicated surgical procedures for tumours in these locations to high volume centres. A change of guidelines is often supported by elegantly designed randomized controlled trials (RCT-s) with robust outcomes. Examples of this include the MAGIC- and CROSS-studies' impact on the use of oncological therapy (4, 5) in addition to surgery for OG tumours and the MIRO-study advocating hybrid oesophagectomy ahead of open surgery for mid- to distal oesophageal cancers (6). However, there are limitations in the generalizability of outcomes of RCTs, mandating complementary and confirmatory information preferably from real-world data in well-defined population-based studies, as exemplified by prospective data retrieved from national registers (7). Assuming adequate coverage and high validity of data in registers, as in the Swedish National Register for Oesophageal and Gastric Cancer register (8), these can reflect general clinical practice and generate real-world evidence when certain methods or strategies are implemented widely. National registers also harbour the opportunity for benchmarking within or between countries.

The scientific community is in agreement that Siewert type I tumours of the gastro-oesophageal junction (GOJ) are best treated with oesophagectomy and Siewert type III tumours by means of extended abdominal gastrectomy. Still, controversy exists regarding the optimal approach for surgery of Siewert type II tumours at the GOJ. By virtue of their location on the border between the thoracic and abdominal cavity, these tumours pose the question as to which surgical approach offers the best opportunity for an R0-resection, radical lymph node clearance and long-term survival. Some argue the superiority of the transthoracic two-field *en* bloc oesophagectomy (TTE) according to Ivor Lewis (9) with better access to the lower and middle part of the mediastinum and hence better lymph node clearance (10). Others propose that the transhiatal extended gastrectomy will suffice as long as a free proximal oesophageal resection margin is achieved with only an abdominal approach (11, 12). The total gastrectomy with resection of the distal two-thirds of the oesophagus, lymph node dissection on both sides of the diaphragm, and a long Roux loop reconstruction (THX-ABD) is a rarely used method for large tumours of the GOJ (13). It incorporates both the principles for the TTE and extended abdominal gastrectomy and has been the surgical procedure of choice in our institution when neither the TTE nor the extended abdominal gastrectomy is an option due to risk of non-radical resection margins for large Siewert type II/III tumours.

The definition of a biomarker is “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” (14). Biomarkers are increasingly used in modern medicine for a wide array of purposes, for example monitoring disease activity in prostate cancer patients with prostate-specific antigen (PSA) and analyzing the susceptibility of breast tumours to treatment with oestrogen inhibitors. Biomarkers analyzed in the specimen removed during surgery can also be used for prognostication of long-term survival. Hitherto, our group has published numerous reports on the prognostic impact of several different biomarkers in relation to oesophagogastric cancer (15-17) but not on the potential prognostic significance of macrophages in these cancers.

Background

Embryology/anatomy and histology

The endoderm covers the surface of the embryonic yolk sac. Folding of the embryo during the first 8 gestational weeks creates a tube within the embryo where the endoderm forms the inner lining of the embryonic gut. Mesenchymal cells derived from the mesoderm differentiate into connective tissues, smooth muscle of the gut and also the serous covering of the organs of the future gastrointestinal (GI) tract. The oesophagus has its origin from the middle part, whereas the stomach stems from the distal segment of the foregut.

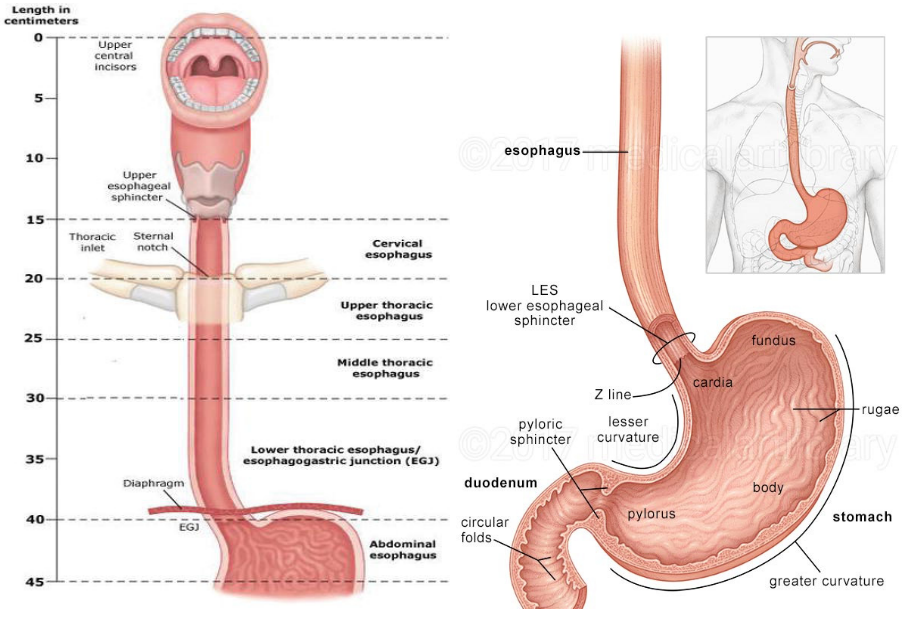

Figure 1a & b: Gross anatomy of the oesophagus and stomach. Figure 1a reprinted by permission from IntechOpen. Figure 1b reprinted by permission from Medical Art Library.

Anatomically the oesophagus is a long hollow tube that spans approximately 25 centimetres from the cricopharyngeal muscle in the neck to the OG junction below the diaphragm. It is divided into three separate parts, which is of great importance for the choice of surgical approach in oesophageal cancer surgery. The cervical (upper) part runs from the cricopharyngeal muscle to the thoracic inlet. Arterial blood supply comes from the inferior and superior thyroid vessels and more caudal from the tracheal arteries. The thoracic (middle) part runs from the thoracic inlet to the hiatus. Branches from the bronchial arteries, originating from the descending aorta, mainly supply this part of the oesophagus. The abdominal (lower) part runs from the hiatus to the oesophagogastric junction. This part receives its blood supply predominantly from branches of the left gastric artery, but also from branches of the splenic artery. Lymphatic drainage from the upper part of the oesophagus down to the level of the tracheal bifurcation is mainly directed cranially whereas the lower half of the oesophagus predominantly drains downwards towards the cisterna chyli.

The stomach is a saccular organ that begins at the OG junction cranially and extends to the pyloric ring. The stomach is further divided into five different parts: the cardia, fundus, corpus, antrum and pylorus. Arterial blood supply is rich and mainly originates from the coeliac trunk and its branches (left/right gastric, gastroduodenal and short gastric arteries).

Histologically the composition of the oesophageal wall is different due to the lack of an outer serosal coating. The oesophageal wall comprises mucosa, submucosa, muscularis propria and adventitia. The mucosa consists of an inner layer of the nonkeratinized squamous epithelium; a delicate layer of connective tissue, the lamina propria and finally a layer of smooth muscle fibres, the muscularis mucosae. An irregular layer of connective tissue forms the submucosa which also harbours adipose cells and fairly large arterial vessels. A rich network of longitudinal lymphatic vessels is present in the superficial layers of the submucosa, which may partly explain the high risk of both skip lesions and early lymphogenic metastatic spread from relatively small malignant lesions in the oesophagus. The muscularis propria in the upper third of the oesophagus consists of striated skeletal muscle, the middle third of both striated skeletal and smooth muscle, whereas the lower third is made up solely of smooth muscle. The adventitia consists of loose connective tissue that envelopes the oesophagus and connects it with adjacent structures.

At the GOJ the nonkeratinized squamous epithelium changes to the simple columnar epithelium of the stomach at the Z-line. The lamina propria, the muscularis mucosae and muscularis propria of the oesophagus are continuous with the same layers in the stomach. Instead of the adventitia the stomach is covered by a layer of simple squamous mesothelium, the serosa (visceral peritoneum).

History of oesophageal surgery

Like many other fields of surgery, the first mention of oesophageal surgery originates in the context of trauma. The Smith Surgical Papyrus, the oldest known text on traumatic injuries detailing medical practices in ancient Egypt 3000-2500 BC, contains information on a patient with a traumatic “wound to the throat penetrating the gullet” (18). The concept of wound suturing is mentioned for the first time in history. Many centuries later in the 1700s both extraction of foreign bodies, oesophagotomy and surgical repair of a strictured cervical oesophagus were described (19, 20). Significant advances were made during especially the latter half of the 19th century in the field of general surgery. Thoracic surgery was avoided though due to problems in maintaining ventilation during surgery with an open chest. “Surgery should halt at the pleura“, stated Theodor Billroth, the famous 19th century German surgeon. In 1877 Czerny described the first successful resection of a cervical oesophageal tumour. The patient had an oesophagostomy and a gastrostomy but no restoration of gastrointestinal continuity. Later on, the introduction of positive pressure intratracheal anaesthesia paved the way for further advances in thoracic surgery. In 1913 Franz Torek performed the first successful oesophageal resection for cancer with restoration of gastrointestinal continuity via a left thoracotomy (21). An external rubber tube was used as reconstruction and the patient survived for 13 years after surgery. The first attempt of an oesophageal resection in Lund was made in 1914 by Jacques Borelius (22). The patient did not survive his second postoperative day. Open thoracic surgery presented the surgeon with the problem of severe complications such as pneumothorax, risk of anastomotic leakage and mediastinitis. Challenges that the modern oesophageal surgeon of today still has to deal with.

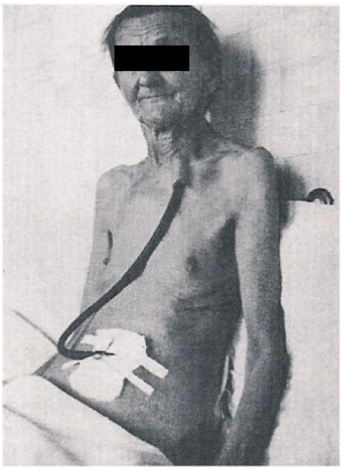

Figure 2: Post-operative result of Torek´s first successful oesphagectomy. Reprinted by permission from Elsevier.

Survival rates after oesophageal surgery were dismal for many years and it was not until after the Second World War that improved peri-operative care led to lower post-operative mortality rates. During this early era of oesophageal surgery both Kelling (23) and Kirschner (24) used the subcutaneous route for an anastomosis in the neck using the transverse colon and a gastric pedicle, respectively, for reconstruction. Zaaijer performed the first successful transthoracic resection of a cancer of the cardia (25) and in 1933 Ohsawa reported the first series of patients with cancer of the distal oesophagus and cardia who successfully underwent oesophageal resection with oesophagogastrostomy through open thoracotomy (26). Ivor Lewis' two-phase procedure (9) including laparotomy and right thoracotomy for oesophageal resections of the middle third portion was described in 1946 and today is still the most widely accepted surgical method for tumours in this location.

History of gastric surgery

The first reference to gastric ulcer disease in the literature was made during the first century AD (27) although it was not until the 16th century when revived interest in post-mortem autopsies triggered a growing interest from the scientific community. The first known operation on a stomach was thought to be the removal of a knife from the stomach of a professional knife thrower in Prague in 1602 (28). In 1881, after many years of surgical training in animal models, Billroth and co-workers in Vienna carried out the first successful partial gastrectomy in a 44-year-old woman with a pyloric carcinoma (29). Billroth claimed that success was not by chance, emphasizing the importance of thorough knowledge of anatomy, physiology and surgical skills among his staff, many of whom later became prominent surgical leaders around Europe. In 1885 von Hacker, another of Billroth's pupils, reported on a patient with a large pyloric carcinoma in whom the intended surgical method of resection and gastroduodenal anastomosis was not possible (30). A jejunal loop anastomosed to the stomach above the tumour followed by resection of the tumour, closure of the cut ends of the stomach and duodenum resulted in what has later been known in a modified version as the Billroth II operation. In 1897 Roux described his "en-y"-loop (31) and in the same year the first successful total gastrectomy was performed by Schlatter in Zurich (32). Reconstruction was carried out by means of an oesophagojejunostomy. Numerous modifications to the above surgical procedures were made during the 20th and 21st centuries, a period of time during which, among other things, have seen the rise and fall of surgery for peptic ulcer disease and the introduction of minimally invasive techniques. Nevertheless, many of the basic concepts developed by these founders of gastric surgery still apply today.

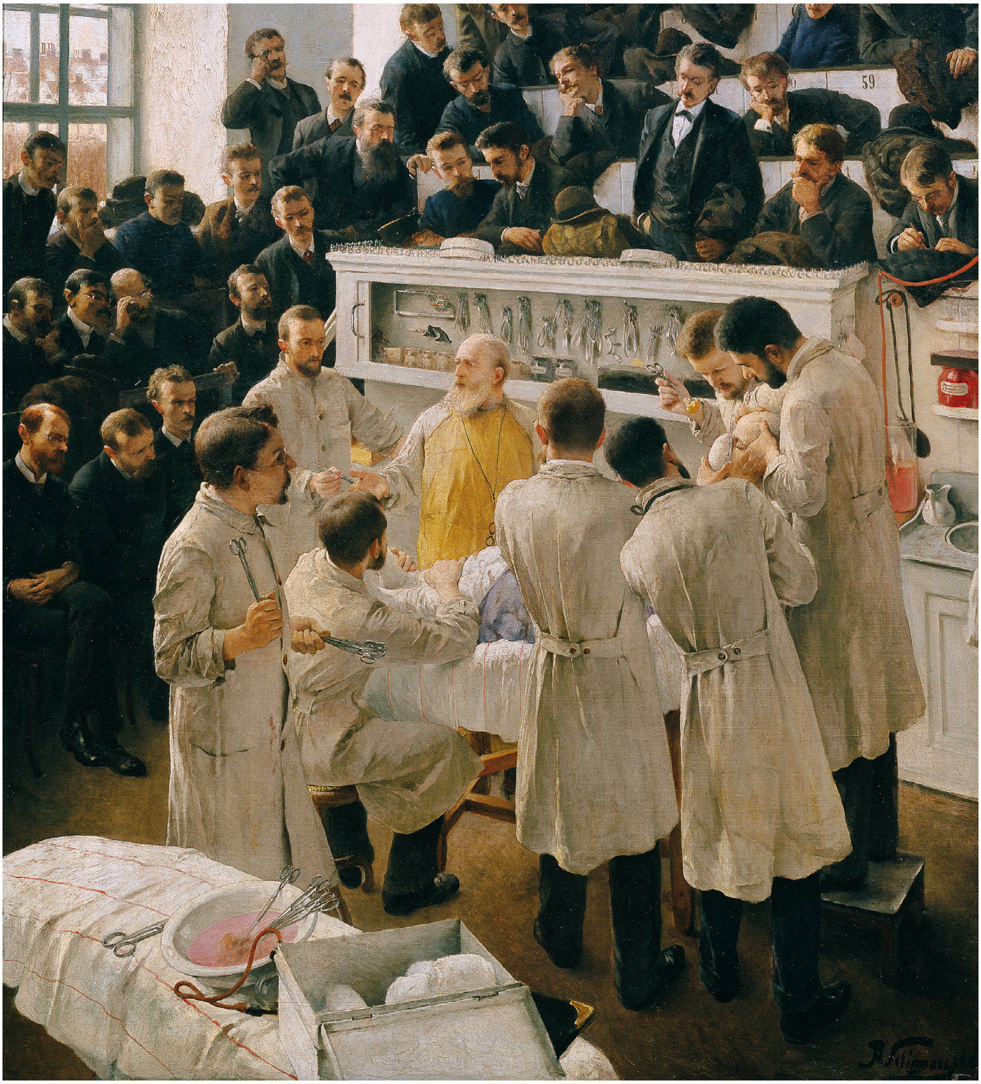

Figure 3: Theodor Billroth performing surgery in front of his pupils in Vienna in the late 1890s. Published under the Creative Commons Attribution-Share Alike 4.0 International License.

Epidemiology

Oesophageal cancer was the seventh most common form of cancer worldwide in 2018, according to GLOBOCAN (1) whereas gastric cancer was placed fifth on the same list. In 2018, oesophageal and gastric cancers were the sixth and third most common causes of cancer-related death worldwide. Globally, squamous cell carcinoma (SCC) of the oesophagus accounts for around 85% of the new cases annually with a marked predominance in Eastern Asia and Southern Africa. In regions with a high incidence, SCC has no gender specificity, whereas in low-incidence regions SCC is more common in men. In contrast, the Western hemisphere has seen a pronounced increase in adenocarcinoma of the oesophagus where the annual incidence in many countries today surpasses that of SCC. Adenocarcinoma became the predominant form of oesophageal cancer in Sweden in the mid-2000s. Incidence rates of oesophageal cancer for women have been fairly stable since the 1970s in Sweden, whereas men have experienced a sharp increase in incidence during the same period, mainly because an increase of adenocarcinoma. In 2018 the male:female (M:F) ratio of incidence for adenocarcinoma was 4.7:1 in Sweden (2).

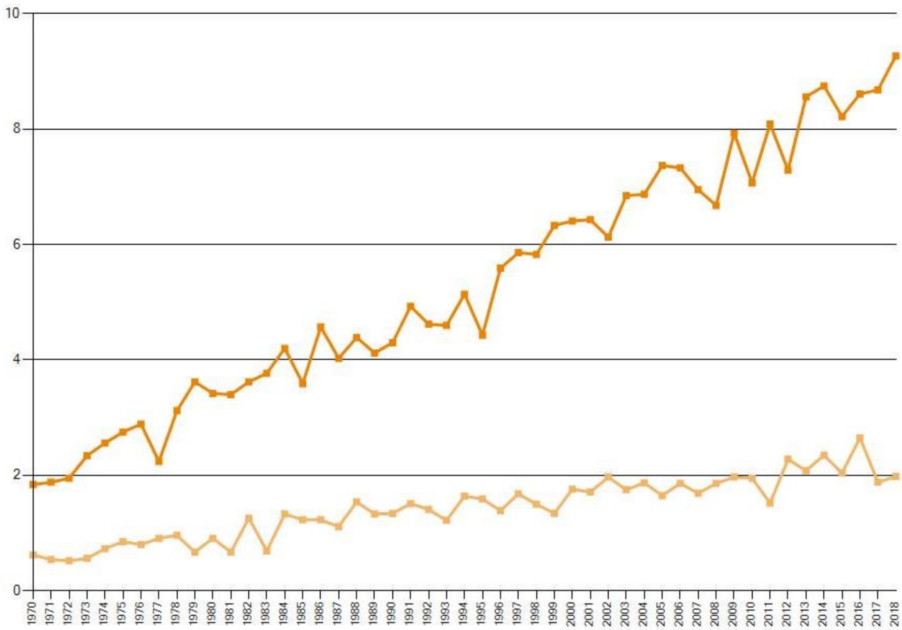

Figure 4: Incidence of oesophageal and gastrooesophageal junction adenocarcinoma in Sweden 1970-2018. New cases/100000 inhabitants (crude rate). Dark yellow curve - males and light yellow curve - females. Reprinted by permission from Socialstyrelsen.

Conversely, the incidence for adenocarcinoma of the stomach both worldwide and in Sweden has been falling consistently during the last decades. Part of the decline is attributed to the recognition of *Helicobacter pylori*, dietary and other environmental risks as causative agents. Geographically, incidence rates are highest in Eastern Asia, Eastern Europe and South America. Over 70% of gastric cancers are found in developing countries. The majority of patients with oesophagogastric cancer are diagnosed between the ages of 65-80 years (33).

Figure 5: Incidence of gastric adenocarcinoma in Sweden 1970-2018. New cases/100000 inhabitants (crude rate). Dark yellow curve - males and light yellow curve - females. Reprinted by permission from Socialstyrelsen.

Aetiology

Oesophageal SCC is linked to direct toxic influence of carcinogens to the epithelial lining. Tobacco smoke and alcohol, especially in combination, are strong risk factors (34, 35). Achalasia (36), consumption of hot beverages (37) and radiation therapy, all of which cause mechanical injury to the oesophageal mucosa, might also influence the risk of developing SCC. High intake of red meat (38) and low intake of fresh fruit and vegetables (39) both increase the cancer risk. The best-known risk factor not only for Barrett's oesophagus, but also for adenocarcinoma of the oesophagus, is gastro-oesophageal reflux (40). Male sex, tobacco smoke and obesity are other risk factors (41, 42), whereas the presence of *Helicobacter pylori* and high intake of fresh fruit and vegetables lowers the risk of oesophageal adenocarcinoma development (43). One of the most important risk factors for gastric cancer is the presence of *Helicobacter pylori* infection (44, 45). Several nutritional factors such as low intake of fresh fruits, high intake of salty foods and high alcohol consumption might also be risk factors for gastric cancer (46). Socioeconomic factors such as marital status, level of education and income also have an impact on the risk of developing gastric cancer, according to a study by Lagergren et al (47).

Recent advances in the genetic characterization of OG cancer have been presented by The Cancer Genome Atlas Network (48, 49). Five different subtypes have been identified based on their specific genetic alterations: oesophageal SCC (ESCC), chromosomally unstable (CIN), Epstein-Barr-virus-positive (EBV), tumours with microsatellite instability (MSI) and genomically stable (GS) as outlined in Figure 6.

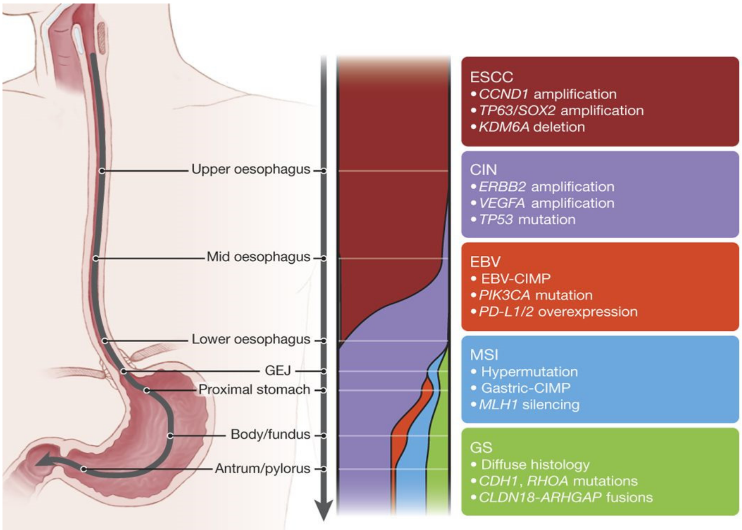

Figure 6: The Cancer Genome Atlas (TCGA) subtypes of OG cancer. Reprinted from Nature by permission from Springer under the terms of the Creative Commons Attribution 4.0 International License.

Treatment with curative intent-oesophageal cancer

The anatomical location of the oesophagus, which stretches through three different anatomical compartments (neck, thorax and abdomen), demands a tailored treatment approach based on the anatomical location of a tumour. For early tumours with only superficial growth, endoscopic treatment modalities are preferred. The treatment options with curative intent for patients with more advanced oesophageal cancers include direct surgery, surgery in combination with oncological therapy (radio- and or chemotherapy) or definitive chemoradiotherapy (CRT). Before surgery, the surgeon must consider four important questions that influence the choice of surgical approach:

Which surgical method offers the best chance of an R0-resection?

An R0-resection, defined as free proximal, distal and circumferential resection margins, is strongly correlated to improved survival in multivariable analysis in several studies on oesophageal and GOJ-cancer (11, 50). The extent of free proximal resection margin has also been shown to influence long-term outcome, but the optimal length of free margin is a matter of controversy (51, 52).

How extensive does the lymph node dissection have to be?

The rich lymphatic network in the oesophageal wall with small lymph vessels penetrating as superficial as the lamina propria, carries the risk of early lymphatic spread and dissemination of tumour disease. The pre-operative staging process with computed tomography (CT) of the neck/thorax and abdomen, positron emission tomography (PET) and sometimes endoscopic ultrasound, aims to clarify the potential lymphatic spread of cancer before a treatment recommendation can be made. As long as suspected positive lymph nodes are not classified as distant metastatic lymph node spread, which rules out curative treatment, the surgical method chosen should ideally include all lymph node stations with suspicion of cancerous involvement to optimize long-term survival. The number of harvested lymph nodes during surgery is an indirect measure of the extent of dissection and hence more lymph nodes imply wider dissection. In the setting of non-neoadjuvant treatment there is evidence that the more lymph nodes that are harvested during surgery, the better the prognosis (53, 54). Van der Schaaf et al (55) questioned this view based on results from a nationwide Swedish cohort showing no potential benefit for more lymph nodes harvested during surgery. Another study based on the results from the CROSS-trial (5) showed a potential benefit for patients with more dissected lymph nodes in the surgery alone arm, but no benefit in the group of patients who received neoadjuvant CRT (56). Adequate lymph node dissection also contributes to improved post-operative staging of patients which leads to better prognostication of long-term outcome. Peyre et al showed that patients having undergone oesophagectomy with eight or more metastatic lymph nodes harvested during surgery had an almost 100% risk of systemic disease (57). Similar results have been shown by Johansson (58) and Omloo (59). In addition, the ratio of positive nodes:total number of nodes has been shown to be of prognostic value regardless of whether neoadjuvant CRT was administered before surgery (60).

How to balance the above two aspects with the risk of post-operative morbidity/mortality?

Extensive surgical procedures imply more physiological stress to the patient and consequently a higher risk of complications. A shorter proximal resection margin can be accepted when performing extended abdominal gastrectomy in order to avoid thoracotomy in GOJ cancers, and neck dissection can in a similar fashion be avoided in surgery for tumours of the middle or proximal thoracic oesophagus. The transhiatal oesophagectomy with a cervical anastomosis in the neck, popularized by Orringer (61), omits thoracotomy and proper thoracic lymph node dissection, to avoid morbidity and potential mortality related to the thoracotomy. On the other hand, a cervical anastomosis carries a higher risk of anastomotic leakage compared to a thoracic anastomosis, whereas a leakage in the latter one generally causes more morbidity/mortality. In early superficial Barrett lesions T1b (sm1, low risk) N0M0 the risk of lymph node metastasis was shown to be around 2% and in T1b (sm1, high risk) N0M0 9% (62). The obvious risks and benefits of an organ-sparing endoscopic procedure in patients such as these must be communicated with the patient. Individual patient characteristics (co-morbidity, age, personal preferences, etc) also influence the choice of surgical procedure.

How to balance the first two aspects with preservation of Quality of Life (QoL)?

The optimal goal for patients who receive treatment with curative intent for oesophageal cancer is long-term survival with preserved QoL. Surgery for oesophageal cancer often has great negative impact on QoL in the short term but also in the longer perspective (63). The occurrence of early post-operative complications is also a determinant for worse QoL in the longer term (64). The choice of specific surgical procedures might influence QoL after surgery (65), and when making treatment recommendations for high cervical oesophageal cancers, CRT with curative intent is often preferred due to the significant morbidity associated with surgery in this location.

Surgical methods for oesophageal cancer

Cervical cancer

The predominant form of tumour in this location is SCC. It can sometimes be difficult to distinguish this form of oesophageal cancer from the hypopharyngeal segment. The proximity to the larynx and other vital anatomical landmarks in the neck makes surgery in this location very challenging. To achieve a proximal free margin, laryngectomy with permanent tracheostoma is often needed. Alternatives for reconstruction are a gastric tube (often associated with problems with regurgitation and aspiration) whereas patients with a colonic interposition or a free jejunal graft do not experience these complications to the same extent.

Based on the obvious risks and disadvantages of surgery for patients with tumours in this location, the current treatment recommendation in Sweden is combined definitive CRT including radiation doses of at least 50 Gray (Gy) with 1.8-2.0 Gy/fraction in addition to chemotherapy based on 5-FU and cisplatin/oxaliplatin (66).

Thoracic cancer

Oesophageal tumours located in the thoracic portion can be either SCC or adenocarcinoma. The latter ones are predominantly located in the middle or lower segments. There are numerous alternatives of surgical procedures available, all with their inherent advantages and disadvantages. A gastric conduit is the most preferred method of reconstruction. A three-field McKeown approach with an anastomosis in the neck is favourable when operating on patients with tumours in the proximal part of the thoracic oesophagus (67). It can also be used if the surgeon for any reason wants to avoid an intrathoracic anastomosis in favour of an anastomosis in the neck. A transhiatal oesophagectomy (61) is preferred by many surgeons. The transhiatal approach with a neck anastomosis does not include thoracotomy and hence is not afflicted with the morbidity related to the thoracic incision. In contrast to the transhiatal approach, the *en bloc* two-field approach *ad modum* Ivor Lewis (9) with reconstruction by means of a gastric tube, includes both abdominal and thoracic incisions and the possibility of adequate two-field lymph node dissection in both the abdomen and thorax (Figure 7). The choice of surgical method is often influenced by local preferences among surgeons/institutions and even shows large national differences (68). The benefit of a formal three-field lymph node dissection (neck/thorax/abdomen) is a matter of debate. Some argue for both better staging and a potential survival benefit with acceptable morbidity after this extensive surgery

(69, 70). Others argue against it due to increased morbidity and no obvious improvement in long-term survival (71).

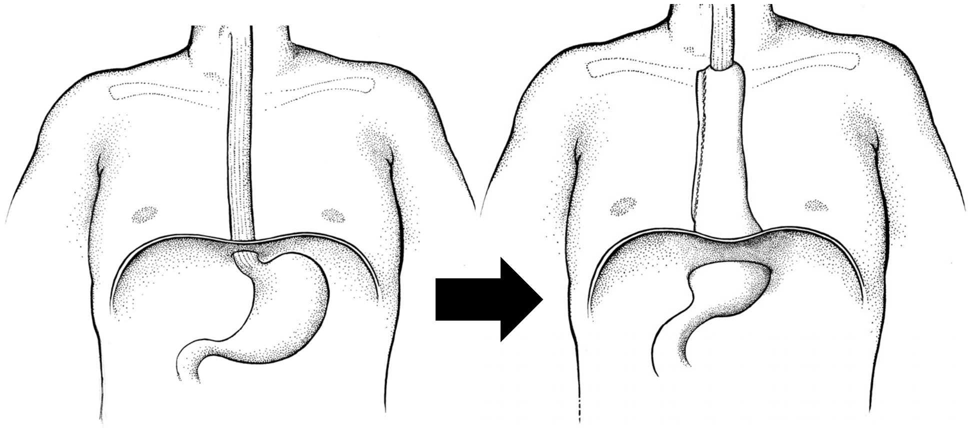

Figure 7: The gastic tube reconstrution *ad modum* Ivor Lewis.

The colonic interposition is an alternative method of reconstruction after resection for oesophageal cancer. Some authors are in favour of this method (72) whilst others argue against it as a primary method of reconstruction (73).

Minimally invasive oesophagectomy, either performed as a total minimally invasive procedure with thoracoscopy and laparoscopy or as a hybrid procedure with both open and thoraco- or laparoscopic approach, has gained widespread acceptance over the last decade as an alternative to the open approach (74). Mariette et al showed a significant reduction of intra- and post-operative complications, especially pulmonary complications, after hybrid oesophagectomy compared to open surgery (6).

GOJ cancer

The anatomical location of the cardia, on the border between the oesophagus and the stomach, still causes debate over the optimal surgical approach for GOJ-cancer. The most widely accepted classification for tumours in the cardia is the Siewert classification (75). It classifies tumours with their epicentre located 1-5 cm above the GOJ as Siewert I tumours. In conformity with tumours of the distal oesophagus, these tumours are best treated with oesophagectomy (76). Tumours located with their epicentre between 2-5 cm below the GOJ are classified as Siewert III tumours.

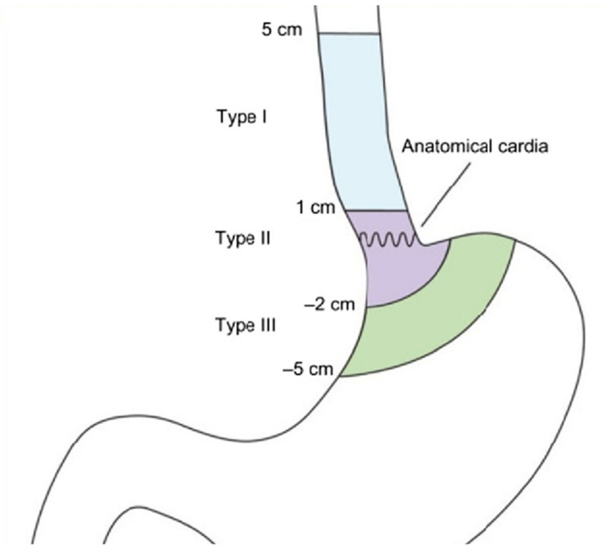

Figure 8: Siewert classification of tumours of the gastrooesophageal junction. Reprinted by permission from Springer Nature.

Most surgeons agree that abdominal gastrectomy is the procedure of choice for tumours in this location (11, 12). The Siewert II or true cardia tumours with their epicentre located between 1 cm above and 2 cm below the GOJ can be removed using the principles of oesophagectomy, gastrectomy or a mix of both procedures. Siewert et al further argued that since an R0-resection can be achieved by means of an extended abdominal gastrectomy, an oesophagectomy offers no advantage because the pattern of lymphatic spread is mainly directed downwards toward the lymph nodes which are dissectible from the abdominal incision (11). Opposed to this view, different groups reported on a higher prevalence of positive lymph nodes in the mediastinum after surgery for Siewert II tumours of the cardia and hence argued for the *en-bloc* two-field oesophagectomy as the best procedure (10, 77).

Combining these two procedures in one, Johansson et al (13) et al showed the three-stage thoracoabdominal gastrectomy with resection of the distal two-thirds of the oesophagus and anastomosis with a long Roux-en-Y limb at the level of the vena azygos (THX-ABD) to be an alternative to the two-field *en bloc* oesophagectomy. This procedure offers both generous proximal and distal resection margins as well as detailed lymph node dissection in both abdomen and chest (Figure 9).

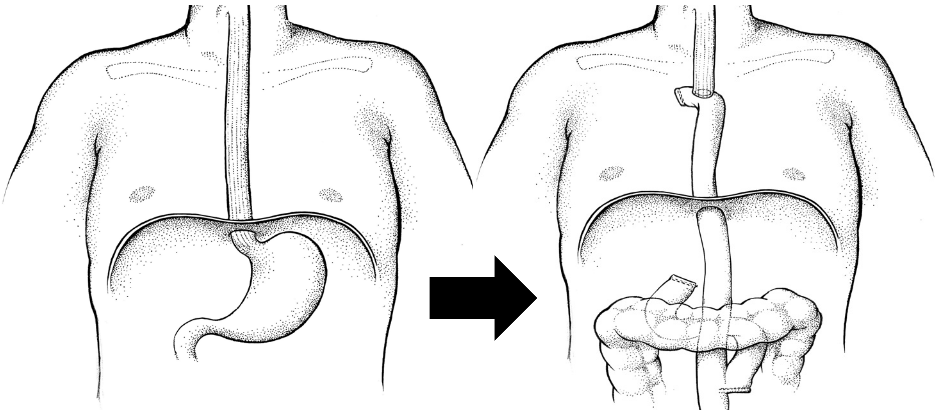

Figure 9: The three stage thoracoabdominal gastrectomy with resection of the distal two-thirds of the oesophagus and anastomosis with a long Roux-en-Y limb at the level of the vena azygos (THX-ABD).

The scientific evidence in favour of the Merendino procedure with a jejunal interposition between the oesophagus and stomach in cancer surgery (78) is confined to surgical resection of early mucosal cancers with low suspicion of lymph node involvement (79).

Treatment with curative intent – Gastric cancer

In line with the surgical treatment of tumours of the oesophagus and GOJ, the goal of the procedure is an R0-resection with adequate clearance of lymph nodes. In clinical practice, a distal gastric resection with a Billroth II reconstruction for distal tumours or a total gastrectomy with a Roux-en-Y-loop for tumours located in the middle or proximal stomach are the standard procedures for gastric cancer. Resection of the spleen is nowadays only indicated in the case of tumours located along the major curvature of the corpus or fundus part of the stomach or in patients with a clear involvement of lymph nodes in the splenic hilum (80).

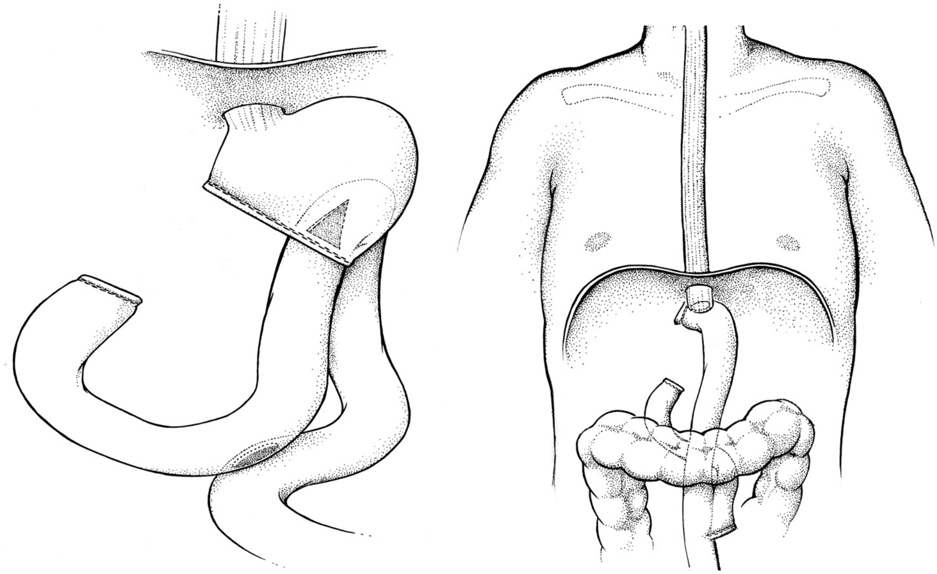

Figure 10: The Billroth II (B2) reconstruction for distal gastric cancer (left) and the Roux-en-y reconstruction after total abdominal gastrectomy for proximal cancer (right).

Laparoscopic gastric cancer surgery was introduced by Kitano (81) and Goh (82) in the early 1990s. Most evidence is once again available from Asian countries such as China, Japan and Korea (83). Conclusions drawn from several studies show a decrease in blood loss during surgery and shorter in-hospital stays after surgery with laparoscopic compared to open gastrectomy. Similar short-term oncological outcome can be achieved but longer operating time for the laparoscopic approach is standard. These results have been reproduced in the Netherlands, from where similar results have been reported of their experience with the introduction of laparoscopic gastrectomy (84, 85), even though a higher rate of anastomotic leakage was noted in the laparoscopic group compared to the group who underwent open total gastrectomy.

The importance of lymph node dissection in gastric cancer surgery has been studied extensively. Much of the evidence comes from Asia, especially Japan where patterns of metastatic lymph node dissemination have been described (86). In brief, lymph node stations closest to the primary tumour, i.e. those lymph node stations most likely to be tumour-invaded early, are labelled N1 (D1-lymphadenectomy), lymph node stations next in line for tumour dissemination are labelled N2 (N1+N2=D2-lymphadenectomy) and peripheral lymph nodes even further away from the primary tumour are labelled N3 (N1+N2+N3=D2+-lymphadenectomy). A D1+ lymphadenectomy refers to a D1 lymphadenectomy plus stations 8a, 9, and

11p. The D1+ lymphadenectomy is the most common type of lymphadenectomy in Sweden.

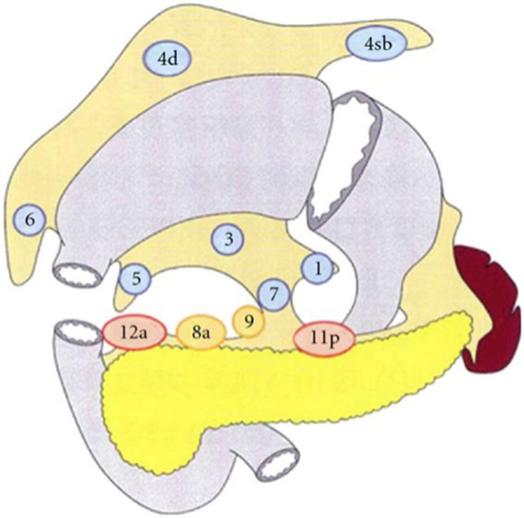

Figure 11: Distal gastric resection**:** D1: 1, 3, 4, 5, 6, 7 (blue), D1+: 1, 3, 4, 5, 6, 7, 8, 9 (blue and yellow) and D2: 1, 3, 4, 5, 6, 7, 8, 9, 11, 12a (blue, yellow and red). Reprinted by permission from the Japanese Gastric Cancer Association under the Creative Commons Attribution 4.0 International License.

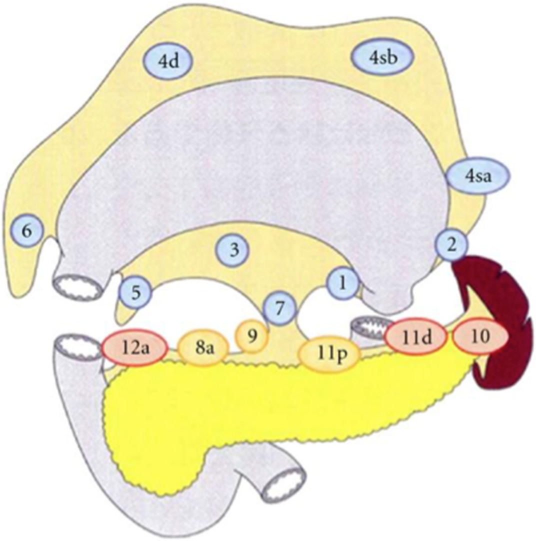

Figure 12: Total gastrectomy**:** D1: 1–7 (blue), D1+: 1–9, 11p (blue and yellow) and D2: 1–12a (blue, yellow and red). Reprinted by permission from the Japanese Gastric Cancer Association under the Creative Commons Attribution 4.0 International License.

Evidence from Taiwan (87) shows improved survival in patients with D2+-resection for gastric cancer, whereas the famous Dutch D1 vs. D2-study failed to show any superiority for more extensive lymph node dissection (88). The long term follow-up of the same study showed a slight benefit with extended lymph node dissection for patients with N2-disease (89). Extensive lymph node dissection implies more complications but can be advocated if major adverse events can be avoided. Bursectomy, with complete removal of the peritoneal sheet covering the mesocolon transversum and pancreas, is not mandatory in gastric surgery, although some support for its benefits in advanced cases in T3-T4 cancers was presented by Fujita et al (90).

Endoscopic resections

Endoscopic resection with curative intent is indicated in OG cancer whenever free profound and lateral resection margins can be achieved. The risk of concurrent lymph node metastasis must also be very low, which is why endoscopic resections are generally only recommended for patients with early tumours with mucosal or low risk submucosal invasion (T1aN0M0 or T1bsm1N0M0 with no risk factors). The two major techniques for endoscopic resections are endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD). Both techniques resect at the same depth with the difference that larger lesions will be dissected with piecemeal technique using EMR, while ESD allows for *en bloc* resections.

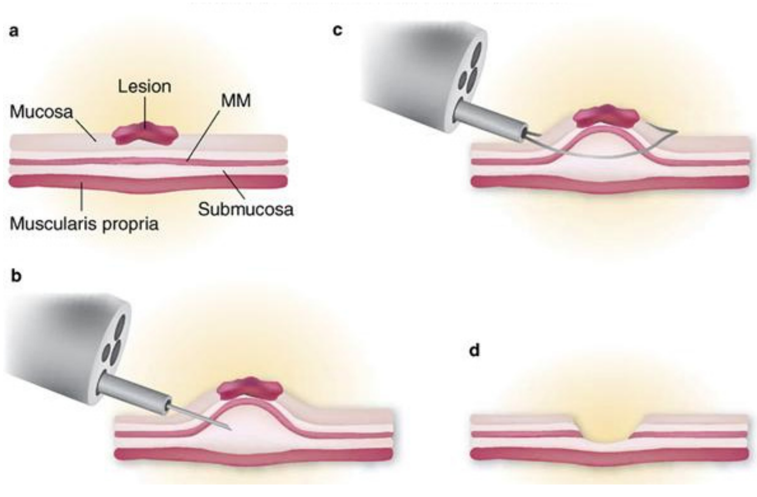

Figure 13: Endoscopic mucosal resection (EMR): a: early tumour located in the mucosal layer. B: injection of saline in the submucosal layer to lift the lesion from the tissue underneath. C: resection by means of a snare using electric cautery. D: radical resection of early lesion with preservation of deeper tissue layers. Reprinted by permission from Springer Nature.

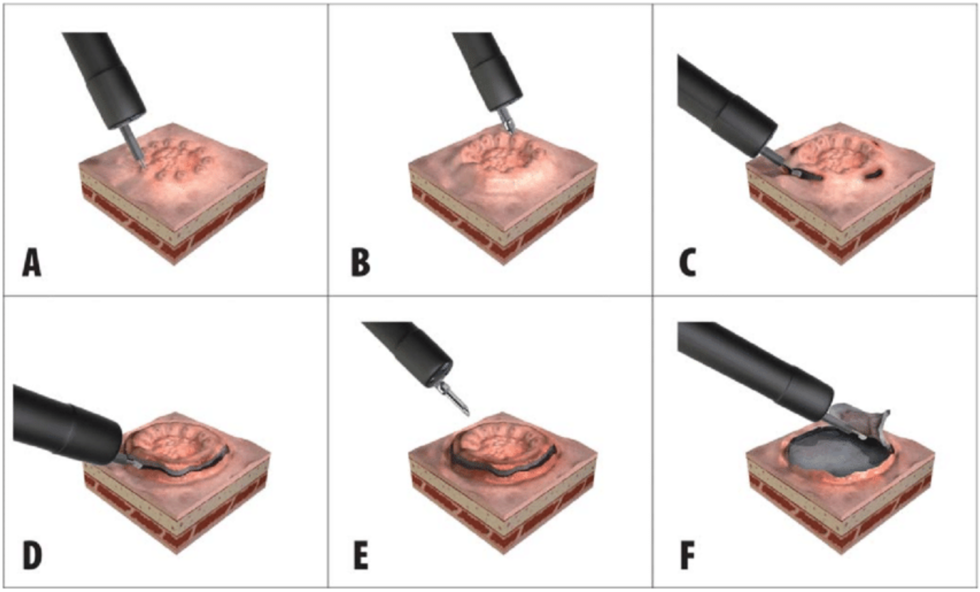

Figure 14: Endoscopic submucosal dissection (ESD): A: delineation of lateral resection edge with argon plasma coagulation (APC). B: submucosal injection of saline. C-D: cutting the surrounding mucosa with an endoscopic knife. E: additional fluid injection. F: dissection of the submucosal layer and removal of resected specimen. Reprinted by permission from Springer Nature.

The advantages of the EMR technique include a steep learning curve, easy removal of lesions < 20 mm *en bloc* and low risk of procedural/post-operative complications, such as bleeding, perforation and stenosis. While having the potential for *en bloc* resection of lesions ≥ 20 mm, the ESD technique comes with a longer learning curve, it is more time-consuming and carries a higher risk of serious adverse events. Based on current scientific evidence the European Society of Gastrointestinal Endoscopy (ESGE) (91) recommends ESD for superficial SCCs in the oesophagus based on a meta-analysis showing a higher rate of curative resections and lower rates of recurrence compared to EMR (92). EMR is an alternative in lesions < 10 mm where *en bloc* resection can be achieved. In early Barrett's lesions, ESD does not offer the same advantages as in early SCC which is why the ESGE recommends EMR for resection in these patients. For early gastric lesions the ESGE recommends the ESD technique, but EMR is an acceptable option for smaller lesions.

Oncological treatment of oesophageal and gastric cancer

Oncological treatment with curative intent in OG cancer can be administered either as the only tumour treatment (definitive oncological treatment) or as a combination therapy together with surgery (neoadjuvant or perioperative treatment).

Definitive oncological therapy in OG cancer is primarily indicated in cervical SCC as the first line of treatment or as an alternative to surgery of SCCs located in the thoracic/abdominal part of the oesophagus. A retrospective study by Gkika et al (93) showed long term survival up to 24% after combined CRT for cervical cancers. Radiation therapy with 50.4 Gy with 1.8-2.0 Gy/fraction is recommended. Higher doses have been tried but with a total dose of > 55 Gy the risks of salvage surgery increase. Cisplatinum with 5-FU is the most widely used concurrent chemotherapy (94) but both oxaliplatin/5-FU and carboplatin/paclitaxel are valid alternatives (66). In general, the same radiation doses and chemotherapy schemes that are relevant for cervical tumours are applicable in the treatment of tumours located in the thoracic/abdominal part of the oesophagus.

In 2012 van Hagen et al published the results of the CROSS study (5). Patients with oesophageal or GOJ cancer who were treated with neoadjuvant CRT with carboplatin/paclitaxel and 41.4 Gy in 23 fractions, had significantly better 5-year survival (47 vs. 33%) compared to patients who had surgery alone. Sub-group analysis showed that the therapeutic response was more pronounced in patients with SCC compared to adenocarcinomas (complete pathological response 49 vs. 23%). R0-resections were also more frequent among patients who received CRT.

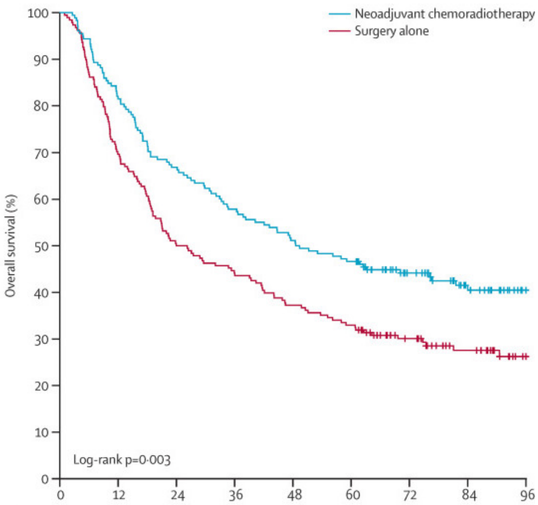

Figure 15: Kaplan-Meier curves of overall survival in the CROSS study. Repinted by permission from van Hagen P et al, N Engl J Med. 2012 May 31;366(22):2074-84, Copyright Massachusetts Medical Society.

Chemotherapy as an additional treatment to surgery in OG cancers is mainly administered in a peri-operative regimen. Evidence from the MAGIC study showed improved survival for patients who received a perioperative combination of epirubicin, cisplatin and fluorouracil for adenocarcinoma of the distal oesophagus and the GOJ, compared to patients who had surgery alone (4). The German FLOT-study later showed improved outcomes for patients with adenocarcinoma of the GOJ or stomach using a combination of perioperative 5-Fu, leucovorin, oxaliplatin and docetaxel compared to patients with a MAGIC-like regimen in addition to surgery (95).

Staging

Tumour stage is a strong prognostic factor for predicting outcome in oesophageal and gastric cancer (96). Tumours are staged using the UICC/AJCC TNM Classification combining T (depth of primary tumour), N (lymph node status) and M (presence of distant metastasis). The classification system is updated regularly, hence Papers I-III in this thesis used the 7th edition (97) whereas Paper IV used the 8th edition (98).

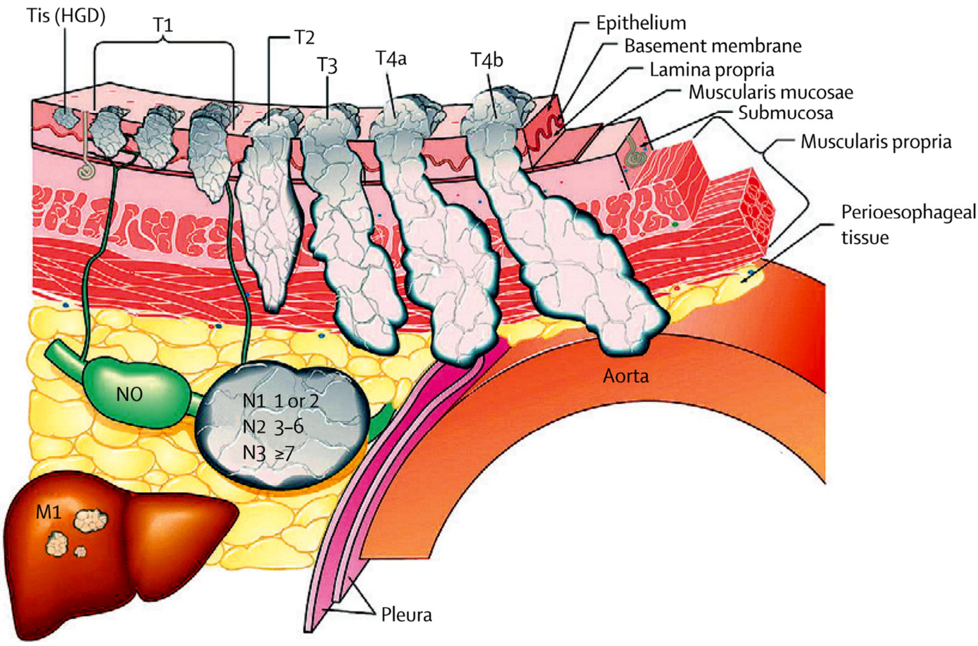

Figure 16: TNM classification of oesophageal tumours. In gastic cancer the adventitia is exchanged for serosa. Reprinted by permission from Elsevier.

The Swedish National Register for Oesophageal and Gastric Cancer (NREV)

In 2005, The Swedish Association for Upper Gastrointestinal Surgery (SFÖAK), with financial support from the Swedish Board of Welfare, decided to merge two previous registers for upper gastrointestinal cancers, the Swedish Oesophageal and Cardia Cancer Register (SECC) and the Swedish Gastrointestinal Register (SWEGIR). NREV was initiated 2006 with the purpose of describing and registering important aspects of the care of patients with oesophageal and gastric cancer. It also supports research and development of evidence-based treatments of these diseases. The Steering Committee of NREV consists of surgeons, oncologists, pathologist, nurses, a patient representative and statisticians representing university, regional and county hospitals. The Steering Committee of NREV is responsible for establishing the national guidelines for the care of patients with oesophageal and gastric cancer. These guidelines are updated biennially. The annual NREV report is available to the public and other interest groups.

NREV allows for registration of all patients diagnosed with oesophageal and gastric cancer, including patients receiving palliative treatment. Though recommended strongly, registration of data into NREV is not mandatory for the participating hospitals. NREV data are acquired in three surveys. The individual hospital responsible for the diagnosis and treatment of the patients reports data directly to the register. The surveys are further processed and validated by trained staff at six regional cancer centres before data are finally filed into the register. Data are validated against the National Cancer Register annually, which has close to 100% coverage, and reminders are sent to hospitals if data are lacking. Data can be analyzed at both a regional and a national level.

The first survey consists of the clinical work-up and treatment recommendations for all patients presenting with a new diagnosis of oesophageal or gastric cancer. The second survey is only used for patients planned for resection and includes details about the surgical procedure. Since 2009, two standardized QoL forms are sent to those patients alive one year after diagnosis. Since 2010 the register has incorporated data on endoscopic mucosal resections and submucosal dissections. The third survey is completed at the postoperative follow-up, but no earlier than 30 days after surgery. This survey also contains information on the pathology report. An additional oncological treatment survey was initiated in 2017. The NREV database has recently been validated documenting a high grade of completeness, accuracy and concordance (8). A new feature (Open NREV) was introduced in 2019 and gives health care professionals online up-to-date information on a subset of indicators for quality of care for OG cancer patients.

The Dutch Upper GI Cancer Audit (DUCA)

The Dutch Institute for Clinical Auditing (DICA) was founded in 2011 with the objective of facilitating and organizing nationwide audits in a uniform format. The DUCA group, founded in the same year, started nationwide registration of all patients undergoing surgery with the intention of resection for oesophageal or gastric cancer. Hence patients with the intention of curative oncological or palliative treatment are not included in the DUCA. The parameters included in the DUCA dataset are derived from the Swedish NREV, the British National Oesophago-Gastric Cancer Audit (NOGCA) as well as from Dutch evidence-based guidelines. The content of the data set is evaluated on an annual basis. Participation in the DUCA has been a mandatory quality standard since 2012. No private institutions are involved in OG cancer surgery as healthcare in the Netherlands is based on a public healthcare system. Similar to NREV, the DUCA has a directional board and a scientific committee including surgeons, gastroenterologists, medical/radiation oncologists and pathologists. Participating hospitals can monitor their results online and compare them to those of other hospitals. These results are updated on a weekly basis.

Gender differences in OG cancer

Previous studies have shown that men have a higher risk of developing both oesophageal and gastric cancer compared to women (99). Some argue that higher exposure to known risk factors among men (100) accounts for the higher incidence of SCC which is roughly 3:1 (101). The generally higher incidence of oesophageal adenocarcinoma in men globally has considerable regional differences with the male:female ratio ranging from 1.03 in Africa to 7.64 in Northern America during 2003-2007 (102). These differences cannot solely be explained by differences in exposure to known risk factors (103). The male:female ratio for gastric cancer incidence is roughly 2:1 in the Western hemisphere (1). A protective role of oestrogen has been proposed for pre-menopausal women for both oesophageal and gastric cancer (101, 104).

Prognosis after OG cancer surgery might also be affected by gender. Kauppila et al found improved survival for women with oesophageal SCC compared to men whereas no gender difference was evident for oesophageal adenocarcinoma (105). Based on data from the EUROCARE-4, the authors concluded that women had higher survival rates compared to men after both oesophageal and gastric cancer (106). Luo et al showed that female gender was an independent prognostic factor for better survival in patients with oesophageal SCC who were treated with definitive CRT (107).

The immune system

The main function of the immune system is to identify foreign pathogens, mount an immune reaction and eliminate them. Any host immune response can broadly be divided into the adaptive or innate (non-adaptive) response. The adaptive immune response is highly specific for a certain pathogen and possesses a memory function. It means that any subsequent presentation of a pathogen/foreign material to the adaptive immune system will generate a response from the immune system. This is the basis for many vaccines against, for example, measles and diphtheria. B- and T-lymphocytes are key components of the adaptive immune response (108).

The innate immune system uses non-specific recognition of pathogens. It acts as the first line of defence against foreign pathogens and is mainly mediated via phagocytic cells such as monocytes and macrophages. Considerable interaction exists between the innate and adaptive immune responses (108).

The tumour microenvironment (TME) consists of extracellular matrix, fibroblasts, endothelial and adipose cells. Another integral component of the TME is the immune system. The immune system is thought to recognize and eliminate mutated cells to prevent carcinogenesis. If the immune system fails to do so, chronic inflammation will follow, which creates a favourable microenvironment in which tumour cells can proliferate (109). Successful tumorigenesis relies upon evasion of the immune system by expressing low immunogenic antigens on the surface of tumour cells (immunoselection), and also by suppressing the host response (immunosubversion) (110).

The mononuclear phagocyte lineage is derived from myeloid bone marrow stem cells and its primary function is to engulf foreign pathogens followed by internalization of the pathogens and subsequent elimination. Differentiation of myeloid progenitors in the bone marrow into promonocytes and then further into circulating blood monocytes prepares the monocytes for subsequent migration through the vessel wall into tissues and final differentiation into macrophages.

Tumour-associated macrophages (TAMs) are key components of the TME where they function as immune regulators. TAMs may have both anti- and pro-tumoral effects related to two different polarizations, M1 (classically activated) and M2 (alternatively activated) TAMs (111). M1 macrophages function to promote responses with tumoricidal and microbicidal effects, whereas activation of M2 macrophages promotes tissue repair, angiogenesis, immune suppression and tumour progression (111). In reality this polarization of M1/M2 macrophages is a continuum and the considerable plasticity (112) in the macrophage lineage enables them to carry out functions attributable to both ends of this spectrum depending on the microenvironment in which they exist.

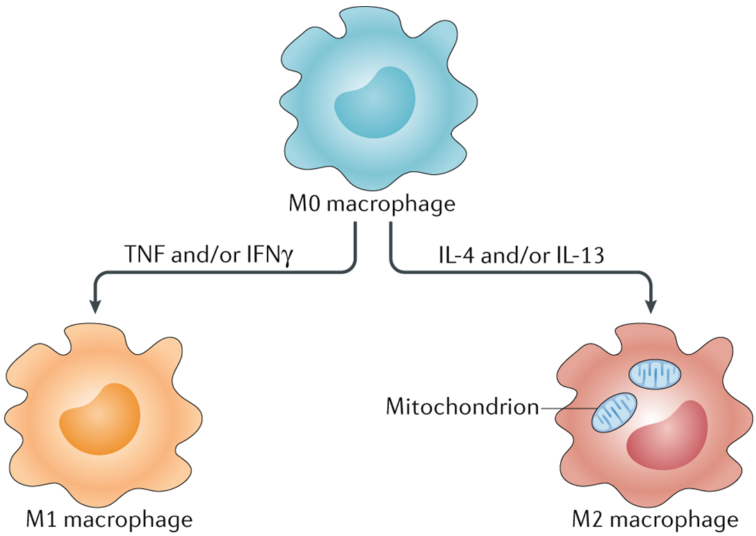

Figure 17: Polarization of tumour-associated macrophages (TAMs) into M1 or M2 macrophages. Reprinted by permission from Springer Nature.

One study showed an association between high M2/M1-ratio and poor survival and a higher rate of lymph node metastasis in patients with adenocarcinoma of the oesophagus (113). In patients with SCC of the oesophagus, a high infiltration with M2 macrophages correlated with worse survival and poor response to chemotherapy (114). In gastric cancer, high infiltration of M2-macrophages was associated with poor prognosis (115).

Tissue microarray technique

The tissue microarray (TMA) technique was first described in 1998 (116). It is a high-throughput method for *in situ* detection of DNA or protein expression in paraffin blocks containing multiple donor tissue cores. The cores are typically 0.6-2 mm in diameter and punched out from donor blocks of formalin-fixed paraffin embedded tissue and then inserted into a receiver block. The receiver block containing cores from multiple donor blocks are then cut in thin layers of 4 µm thickness and subjected to immunohistochemical staining and analysis. Figure 18 illustrates the construction of a TMA.

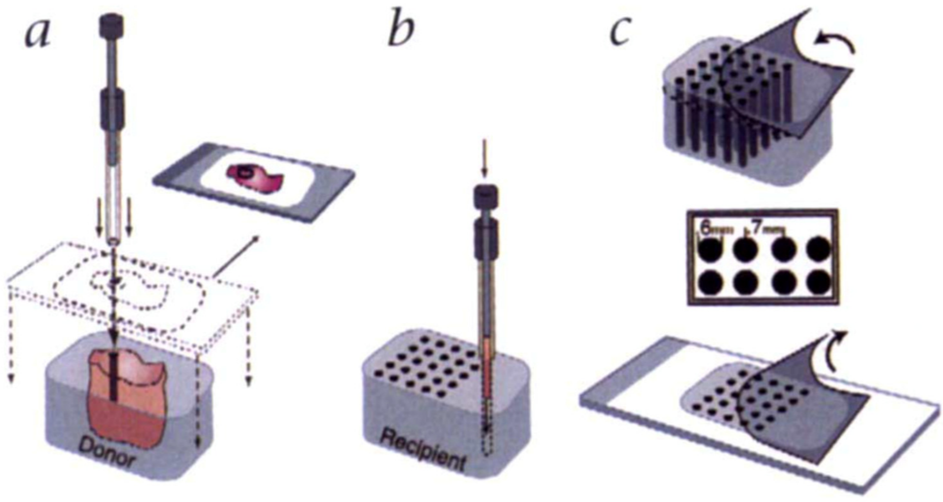

Figure 18: The tissue microarray (TMA) technique. Reprinted by permission from Springer Nature.

Benefits of the TMA technique include the ease of analyzing cores from multiple tumours simultaneously, less consumption of donor tissue due to the small size of the tissue cores and also less use of antibody for immunohistochemical analysis compared to full-face tissue sections (117). A potential pitfall of the TMA technique is the issue of tumour heterogeneity where immunohistochemical analysis of a tissue core of 2 mm might not reflect the properties of the whole tumour (118). Analyzing duplicate tissue cores from different tumour locations in the donor block, or preferably different donor blocks, might partly offset this problem.

Immunohistochemistry

The technique of immunohistochemistry (IHC) was first described by Coons in 1942 (119). By means of fluorescence microscopy he used colour-tagged antibodies to localize antigens in tissues. The technique was further improved by Nakane in the 1960s who made it possible to localize antigens using normal light microscopy (120). IHC represents an important diagnostic, prognostic and predictive tool in modern medicine being used for several different purposes such as KIT-detection in gastrointestinal stromal tumours (121) and HER2-testing in OG cancer (122).

In order to analyze tumour tissue with IHC, several important steps must be followed in a logical sequence. First, the fixation process of tumour tissue must be started promptly after removal from the patient in order to avoid autolysis. Formalin is the most commonly used agent for this purpose, and it binds to proteins of the tissue, forming methylene bridges, which stabilizes the tissue. This is followed by dehydration and embedding in paraffin.

De-masking of the epitopes of the tumour tissue is required to make it possible for antibodies to bind to them again. This is done by a multistep process of cooling and heating in a variety of buffers at different pH-values. Application of an antibody follows, either as a directly labelled primary antibody or with the use of a labelled secondary antibody, with a chromogenic or fluorescent detection method (123). The choice of antibody and interpretation of the reaction are the most pivotal steps in using IHC (124). Validation of sensitivity and specificity of antibodies is essential to ensure accurate interpretation of the results.

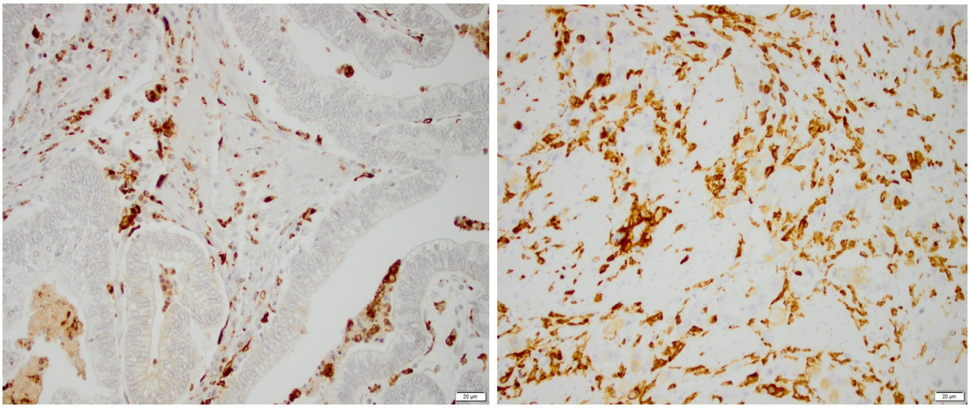

Figure 19: Immunohistochemistry of $CD68^{+}$ and $CD163^{+}$ macrophages. © Karin Jirström.

Investigative biomarkers

CD68 (Cluster of Differentiation 68) is a transmembrane glycoprotein with high expression in monocytes and tissue macrophages. The protein is a member of the scavenger receptor family and its functions are to promote phagocytosis, mediate the recruitment and activation of macrophages and to clear cellular debris. In IHC CD68 is useful as a panmacrophage marker for cell types such as monocytes, histiocytes and Kupfer cells. Though contradictory results exist (125), a high expression of CD68 in the tumour stroma is in general correlated with tumour aggressiveness, higher tumour grade and lymph node metastasis (126, 127). High infiltration of $CD68^+$ TAM-s was also shown to correlate with clinical stage and poorer surgical outcomes in gastric cancer (128).

The CD163 protein is a member of the scavenger receptor cysteine-rich superfamily and is highly expressed on resident tissue macrophages. The receptor may function as an innate immune sensor for bacteria and induces local inflammation. CD163 serves as a selective marker for macrophages that differentiate into the alternatively activated path of M2-macrophages (129). A study by Medrek and co-workers showed that $CD163^+$ macrophages in the tumour stroma of breast cancer patients correlated with higher grade, larger tumour size and triple negative tumours (130). In another study, $CD163^+$ macrophages in the tumour stroma and tumour margins of gastric cancer patients were significantly correlated with tumour size, depth of invasion and poor survival (131).

MARCO (Macrophage receptor with collagenous structure) is a member of the class A scavenger receptor family and is also a part of the innate antimicrobial immune system. Expression of MARCO is found on dendritic cells and a subset of tissue macrophages, wherein it acts by sensing and clearing pathogens through the recognition of pathogen-associated molecular patterns (PAMPs) (132). Lundgren et al showed a correlation between high density of $MARCO^+$ macrophages and poor survival in patients with intestinal-type tumours of the periampullary region (133). To the best of our knowledge, no study has yet examined the expression and prognostic impact of MARCO in OG cancer.

Aims

The overall aim of this thesis was to describe different aspects and trends of oesophageal and gastric cancer care on a national and international level as well as to evaluate short- and long-term outcome and prognostic biomarkers after major OG cancer surgery in patients operated on at Skåne University Hospital.

Specific aims were:

- To describe how trends in oesophageal and gastric cancer treatment have changed in Sweden during 2007-2016.
- To evaluate potential differences in OG cancer care and if they affect short-term outcome after surgery in Sweden compared to the Netherlands.
- To evaluate the potential benefit of THX-ABD in patients with large Siewert II & III tumours of the GOJ.
- To investigate the prognostic value of infiltration of $CD68^+$, $CD163^+$ and $MARCO^+$ macrophages in patients with OG cancer.

Patients and Methods

Papers I & II

Both Paper I and II were retrospective studies based on register data from the national quality registers for OG cancer in Sweden (NREV) in Papers I and II and the Netherlands (DUCA) in Paper II. The study cohort of Paper I was comprised of 12,242 patients registered in NREV between 2007-2016. Of those, 6926 patients were diagnosed with oesophageal or GOJ (Siewert I-III) cancer and 5316 patients were diagnosed with gastric cancer according to TNM 7 (97). In Paper II the study cohorts consisted of 2509 patients who underwent oesophagectomy (Sweden n=475 and the Netherlands n = 2034) and 1930 patients who underwent gastrectomy (Sweden n = 554 and Netherlands n = 1376) between 2012-2014.

The NREV data used in these studies were acquired in three surveys (as described in the Background chapter about NREV) in which the individual hospital responsible for the diagnosis and treatment of the patients reported data directly to the register. The DUCA data were acquired in a similar fashion and entered into a generic, internet-based program to enable data entry to a secure online environment. NREV contains information on all patients with OG cancer, including those patients for whom curative oncological treatment is recommended and those who are recommended palliative treatment, whereas the DUCA only contains information on patients who had surgical resections.

In Paper I, data were analyzed on a group basis for each variable with no access to individual register data. In Paper II we had access to individual register data on all variables included in the study.

In both papers, patient, tumour, and treatment characteristics were presented in frequency tables. Categorical variables were compared using Chi-squared tests. Changes over time were compared using Chi-squared tests for trend. Statistical significance was set at a threshold of 0.05, with p-values calculated by two-sided tests. In Paper I, survival after resectional surgery was illustrated using Kaplan-Meier curves and the log-rank test. In Paper II, uni- and multivariable analyses were performed to determine the impact of different covariables on short-term survival.

Paper III

This was a retrospective descriptive single institution study based on a cohort of 83 patients (70 men and 13 women) with Siewert II & III tumours at the GOJ operated on with the THX-ABD at Skåne University Hospital between 1986-2011. Of those 83 patients, 65 had a Siewert type II tumour and 18 had a Siewert type III tumour of the GOJ. Of those 83 patients, four patients had *ad hoc* palliative resections. No patient had any neoadjuvant treatment before surgery. Data on comorbidity (ASA-score), diagnostic work-up, details of the surgical procedure and the post-operative course were retrieved from individual patient charts. Survival status of the patients was determined by follow-up date (16 February 2016) or the date of death according to the Population Register of Sweden.

The THX-ABD started with an upper abdominal midline incision and initially followed the principles of a total gastrectomy with an *en bloc* D1+ or D2 lymph node dissection. The preparation of the long Roux loop is technically very demanding in order to reach the oesophagus at the level of the azygos vein. The preparation of the Roux loop can begin 15 to 20 cm distal to the ligament of Treitz. After identification of a vessel with a strong pulsatile flow, we isolated one to three of the supporting vessels proximal to the selected vessel and clamped them temporarily to evaluate the small bowel for signs of ischemia. If we saw no signs of ischemia, we proceeded and ligated the temporarily closed vessels until an approximately 50-cm long Roux loop was prepared. The abdomen was thereafter temporarily closed, and the patient was repositioned for a posterolateral thoracotomy. Continued proximal dissection of the oesophagus and adjacent lymph nodes including the paraoesophageal nodes of the lower and middle part of mediastinum, subcarinal and the lymph nodes along the right and left main bronchus were also included in the *en bloc* resection. After removal of the specimen, a circular stapled anastomosis to the oesophagus at the level of the azygos vein was performed. The thoracic incision was closed, and a during the second laparotomy, the long Roux loop was stretched and secured to the crus of the diaphragm to facilitate emptying of food and drink and to avoid herniation of the conduit. A stapled entero-entero anastomosis re-established the gastrointestinal continuity.

The index of estimated benefit from lymph node dissection (IEBLD) was calculated by multiplying the incidence of metastases by the percentage of 5-year survival rate of patients with positive lymph nodes at that station (134). The overall cumulative 5-year survival rate of patients with metastases at each nodal station was calculated, irrespective of the presence/absence of metastases at other nodal stations.

Survival rates were shown graphically as Kaplan-Meier plots and compared using the log-rank test. A multivariable Cox regression model was performed using all significant univariable impact factors. A p-value of < 0.05 was considered significant.

Paper IV

This was a retrospective study based on a cohort of 174 patients (oesophageal cancer n = 99 and gastric cancer n = 75 according to TNM 8) consecutively operated on at Skåne University Hospital between 1 January 2006 to 31 December 2010. None of the patients received any neoadjuvant treatment before surgery. Information on clinical data, and cause of death was obtained via medical charts. Last follow up of survival status was conducted in March 2016 from the Population Register of Sweden.

TMAs were constructed using a semi-automated arraying device. Duplicate tissue cores were obtained from primary tumours, each from a separate donor block. IHC analysis of expression of the pan-macrophage marker CD68, the M2 macrophage marker CD163 and the scavenger receptor marker MARCO was performed. All stainings were evaluated in light microscopy by two independent observers, one being a senior pathologist. The total infiltration as well as infiltration into TN, defined as being juxtaposed to a tumour cell or in the direct vicinity of a tumour cell, was denoted as 0 (none/sparse), 1 (intermediate) or 2 (high). In cases with different expression between the two cores the one with the highest score was used.

Differences in the distribution of CD68, CD163 and MARCO expression, according to clinicopathological parameters, were analyzed using the non-parametric Mann-Whitney U test for continuous variables and the Chi-squared test for categorical variables. Kaplan-Meier analysis and the log-rank test were used to compare OS in patients with IHC staining 0-1 vs. 2. Unadjusted and adjusted hazard ratios (HR) for OS were calculated using Cox regression proportional hazard modelling. The adjusted model only included variables that were significant in the unadjusted model. The Backward conditional model according to Wald was used in the adjusted model.

Ethics

Paper I

This study was approved by the Regional Ethics Committee, Stockholm, Sweden (Dnr 2013/1091-31/2 and 2016/1486-32).

Paper II

Ethical permission to conduct this study using Swedish register data was approved by Regional Ethics Committee, Stockholm, Sweden (Dnr 2013/596-31/3 and 2016/891-32). Under Dutch law, no ethical approval or informed consent was required for the present study.

Paper III

The study was approved by the Regional Ethics Committee, Lund University, Sweden (Dnr 2013/587).

Paper IV

The study was approved by the Regional Ethics Committee, Lund University, Sweden (ref no 445/07), whereby no need for consent other than the option to opt out was waived.

Results

Paper I

Survey 1 had an average annual coverage grade of 95.3%. The corresponding figures were 93.0% and 89.2% for Surveys 2 and 3, respectively. The proportion of patients with oesophageal/GOJ cancer presented at a multi-disciplinary conference (MDC), rose from 58.1% in 2007 to 91.9% in 2016. A similar increase was seen for patients with gastric cancer, though starting from a lower level (from 29.3% to 85.9%).

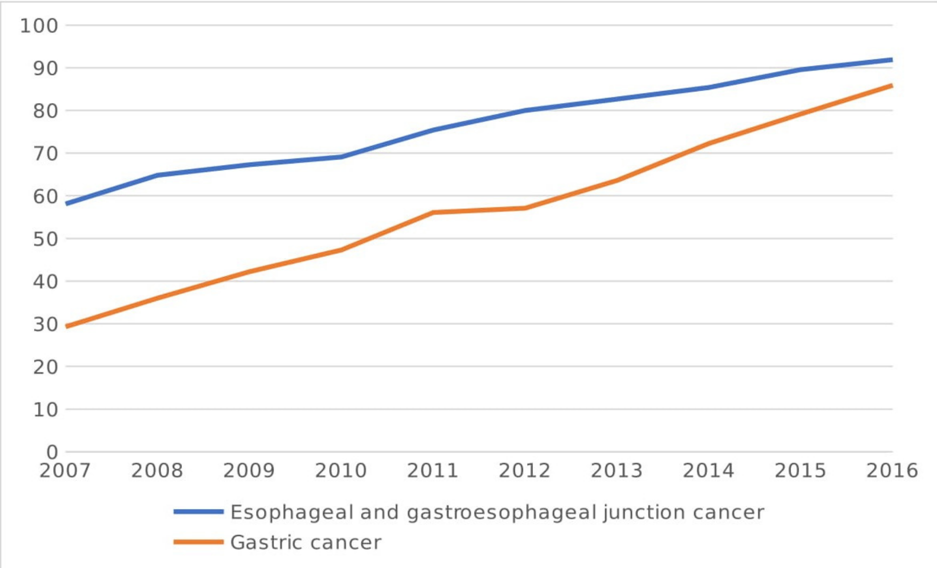

Figure 20: Percentage of patients presented at a multi-disciplinary conference during 2007-2016. Reprinted by permission from Oxford University Press.

Fewer hospitals performed resectional surgery for oesophageal/GOJ and gastric cancer at the end of the study period. Only four hospitals performed 20 or more oesophageal resections and five hospitals more than 20 gastrectomies in 2016, all of which were university hospitals.

Minimally invasive surgery for oesophageal and gastric cancer was widely introduced in Sweden in 2012. The technique has gained popularity, and in 2016, 65% of the oesophageal and 20% of the gastric resections were performed using minimally invasive techniques.

Adenocarcinoma was the predominant subtype of oesophageal tumour representing 65.2% of all cases diagnosed and 75.2% of all resected patients. Resection rates varied markedly between the Swedish geographical regions (Figure 21).

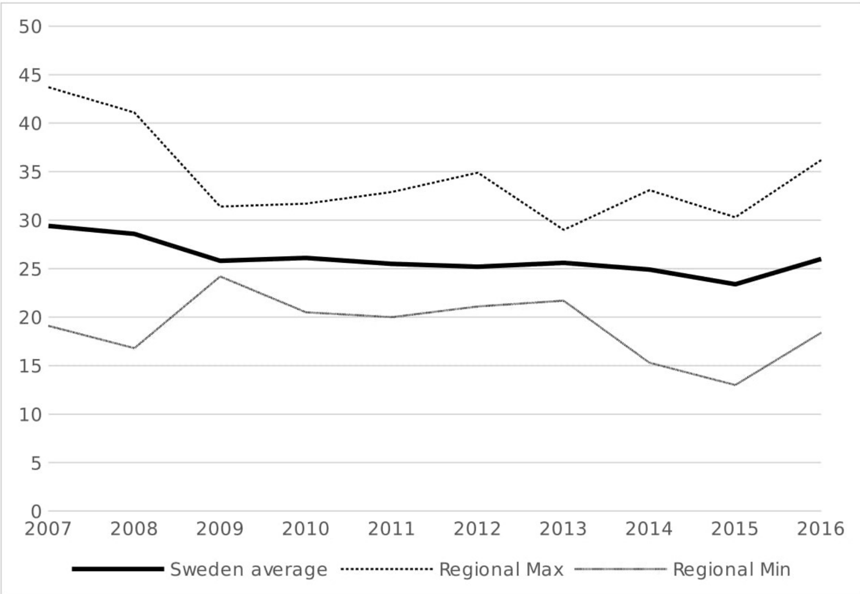

Figure 21: Resection rates for oesophageal and gastrooesophageal junction cancer in Sweden 2007-2016. Reprinted by permission from Oxford University Press.

Of all patients presenting with oesophageal/GOJ cancer, a larger proportion of males eventually underwent resection (male:female ratio for all patients being 74.0:26.0% vs. resected patients 78.9:21.1%; $p < 0.001$). Overall 5-year survival for all patients with oesophageal/GOJ cancer was 15.7% with no gender difference (females 16.3 vs. males 15.5%; $p = 0.26$). The overall 5-year survival after resectional surgery for oesophageal/GOJ cancer was 38.5%. It was significantly higher in females compared to males (47.1% vs. 36.2%; $p < 0.001$).

Some 42.6% of patients with oesophageal/GOJ cancer had neoadjuvant oncological therapy during the early years of the study period 2007-2010 compared to 76.4% in the latter years 2014-2016 ($p < 0.001$). The R0 resection rate was significantly higher at the end of the study period (91.3% vs. 86.7%; $p = 0.025$). No significant changes in the 30- and 90-day postoperative mortality rates were evident during the study period. Except for a peak at 18% in 2015, the anastomotic leakage rates ranged from 7.1% to 13.1%. An increase in the proportion of resections yielding ≥ 15 lymph nodes was also observed (58.2% vs. 75.3%) from 2007-2016 ($p < 0.001$).

Large regional differences in resection rates (from 20.7% in 2007 to 41.5% in 2016) were evident also in patients with gastric cancer. No significant gender differences were noted in terms of the proportion of patients having resectional surgery compared to all patients, overall 5-year survival (women 18.2% vs. men 16.4%; $p = 0.20$) and 5-year survival after resection (women 37.3% vs. men 34.8%; $p = 0.18$).

Some 20.4% of patients with gastric cancer received neoadjuvant treatment during 2007-2010 compared to 42.4% in 2014-2016 ($p < 0.001$). The 30-day mortality rate improved significantly from 4.2% to 1.6% ($p = 0.005$), but the decrease in 90-day mortality from 8.5% to 5.5% was not statistically significant ($p = 0.061$). An increase in the proportion of ≥ 15 lymph nodes resected was observed from 35.7% in 2006 to 74.0% in 2016 ($p < 0.001$).

Paper II

Both countries experienced centralization of surgery with gradually fewer hospitals performing oesophagectomies and gastrectomies during the study period. In 2012, oesophagectomies and gastrectomies were performed in 10 and 33 hospitals, respectively, in Sweden versus 8 and 26 hospitals in 2014, whereas in the Netherlands, in 2012, oesophagectomies and gastrectomies were performed in 23 and 44 hospitals versus 22 and 27 hospitals in 2014. In both countries, the annual procedural hospital volume increased between 2012 and 2014 for oesophagectomy and gastrectomy but higher annual hospital volumes were observed in the Netherlands. Estimated resection rates for oesophageal and gastric cancer were not statistically different.

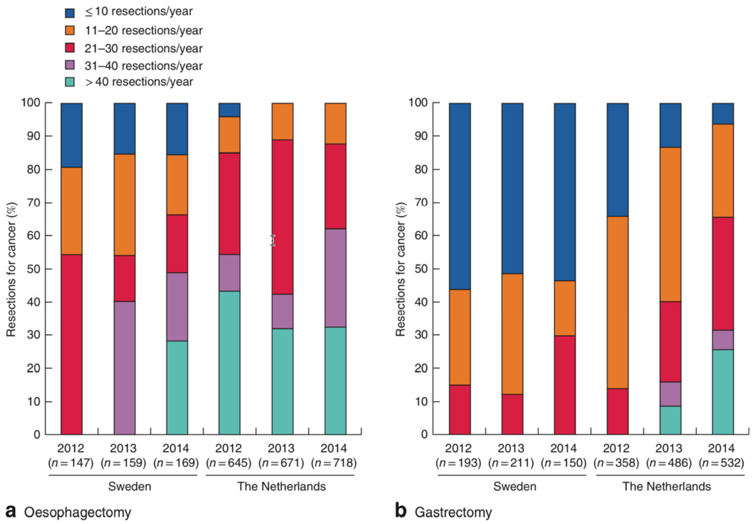

Figure 22: Annual procedural hospital volumes for oesophagectomies and gastrectomies in Sweden and the Netherlands 2012-2014. Published under the Creative Commons Attribution Non-Commercial No Derivatives License, Busweiler et al, BJS Open. 2018 Oct 19;3(1):62-73. © John Wiley & Sons Ltd.

Patients registered in the NREV had less co-morbidity but were older than the patients in the DUCA. Patients in the DUCA had a more advanced clinical tumour stage than those in the NREV.

Neoadjuvant treatment rates were higher for patients who underwent oesophagectomy and gastrectomy in the Netherlands (90.0 and 56.6%) than in Sweden (68.6 and 38.3%). In the Netherlands, CRT was the most commonly used neoadjuvant treatment modality in patients undergoing oesophagectomy. A transthoracic approach (94.7%) with an intrathoracic anastomosis (77.5%) was used in the majority of patients who had an oesophagectomy in Sweden. In the Netherlands, a transhiatal approach (35.8%) was also common, and a cervical anastomosis (70.4%) was preferred.

Complication rates were lower in Sweden than in the Netherlands for both oesophagectomy (42.5 versus 60.5% respectively; $p < 0.001$) and gastrectomy (30.0 versus 37.4%; $p < 0.001$).

Univariable analysis showed a difference in the 30-day mortality rate following gastrectomy (1.8% in Sweden versus 3.8% in the Netherlands; $p = 0.026$). After oesophagectomy, the 30-day mortality rate was 1.7 and 2.5% respectively ($p = 0.285$). In the multivariable model, the risk of 30-day and/or in-hospital mortality was lower for patients who underwent gastrectomy in Sweden than for those in the Netherlands (odds ratio 0.53, 95% CI: 0.29-0.95).

Paper III

The in-hospital mortality was 2/83 (2.4%). No in-hospital deaths were recorded after 2001. Anastomotic leakages were observed in eight patients: five of them required operative intervention including one patient in whom the Roux-loop had to be removed due to complete necrosis. The other three patients with anastomotic leakages were treated conservatively. Pulmonary complications were observed in 21 patients. In 18/83 patients (22%) we found Clavien Dindo 3b-5 complications.

A median of 22 lymph nodes (range 4-76) were harvested in patients (n=40) whose pathology reports included the exact number of examined lymph nodes. The right and left paracardial lymph node stations were the most common sites of metastatic nodes, being present in 29/83 and 27/83 of the patients, respectively. Nine patients had positive lymph nodes retrieved via right thoracotomy, all of whom had Stage IIIC or IV disease with zero 5-year survival.

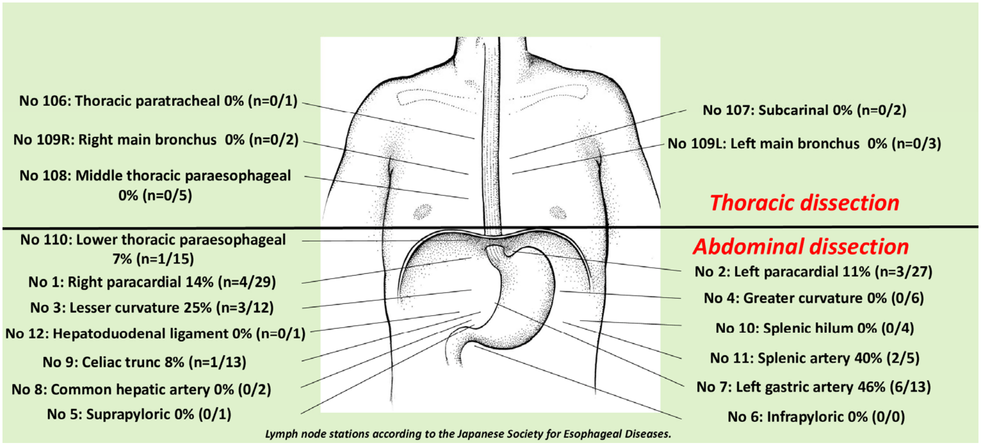

Figure 23: The 5-year survival for patients with metastatic lymphnodes in each specific nodal station. Reprinted by permission from Elsevier Inc.

The calculation of the therapeutic lymph node index showed the highest index for lymph nodes at the left gastric artery (7.2), followed by right paracardial (4.8), left paracardial and lesser curvature (3.6). The index was 0 for all nodal stations dissected via right thoracotomy, not reachable through an abdominal only approach.

The R0-rate was 70/83 (84%). Overall 5-year survival for the cohort was 22/83 (27%). Long-term survival in the unadjusted Cox regression analysis was best determined by tumour stage ($p < 0.005$), N0 vs. N+ ($p < 0.005$), R-status ($p = 0.04$) and T-stage (T1-T2 vs. T3-T4 ($p = 0.04$)). In the adjusted analysis including only significant impact factors from the univariable analysis, tumour stage ($p < 0.005$) was the only factor with a significant impact on long-term survival.

Paper IV

The expression of CD68 could be evaluated in 162/174 (93.1%) patients, the expression of CD163 in 165/174 (94.8%) patients and the expression of MARCO in 166/174 (95.4%) patients. Sample IHC images of staining for CD68, CD163 and MARCO are shown in Figure 24.

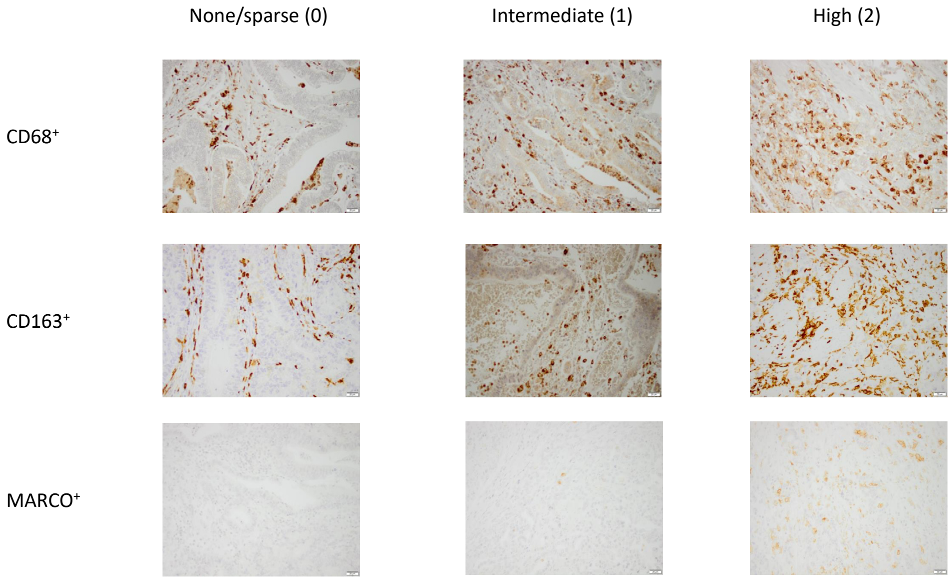

Figure 24: Sample IHC-stainngs for $CD68^+$, $CD163^+$ and $MARCO^+$ macrophages. © Karin Jirström.

Associations of $CD68^+$, $CD163^+$ and $MARCO^+$ macrophage infiltration in TN with patient and tumour characteristics indicated that high infiltration of $CD68^+$ and $CD163^+$ macrophages into TN correlated with higher pT-stage ($p = 0.02$ and $p = 0.009$, respectively), low differentiation grade ($p < 0.001$ for both) and diffuse tumour type ($p < 0.001$ for both variables). High infiltration of $CD68^+$, but not $CD163^+$, macrophages correlated with higher pN-stage ($p = 0.031$). There were no significant associations between $MARCO^+$ macrophages and any clinicopathological factors.

Kaplan-Meier analyses of OS in relation to total and TN infiltration of CD68+, CD163+ and MARCO+ macrophages, respectively, showed that infiltration in TN had a stronger prognostic value (Figure 25). There was no significant association between the expression of MARCO and OS.

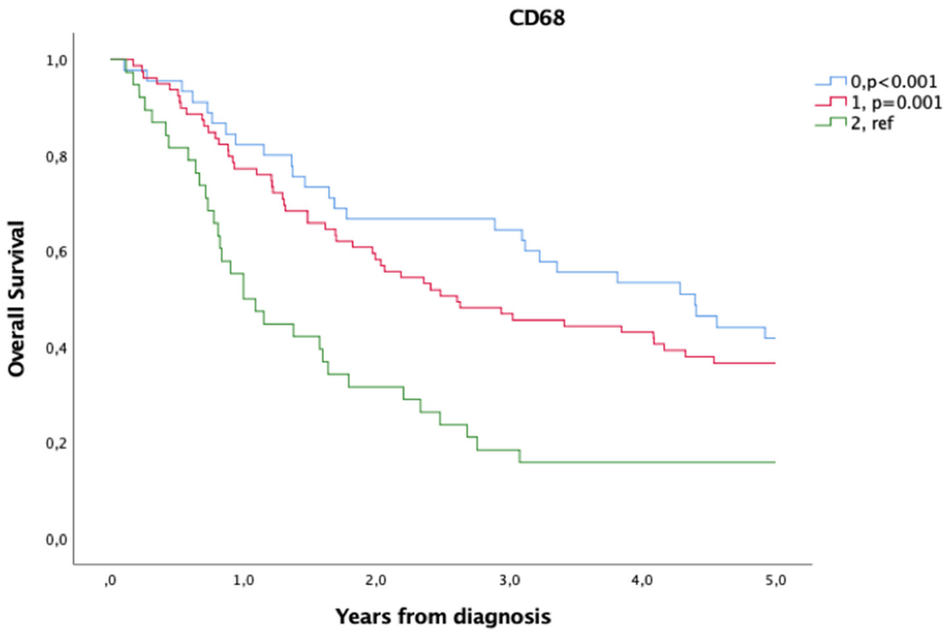

Figure 25: Infiltration of CD68+ macrophages in TN of OG cancer in relation to overall survival (OS). 0 = none/sparse, 1 = intermediate and 2 = high.

CD68+, CD163+ and MARCO+ infiltration was dichotomized into none/sparse and intermediate (0-1) versus high expression (2). Associations between high infiltration of CD68+ and CD163+ macrophages in TN and a reduced OS were shown in unadjusted Cox regression analysis (HR 2.27; 95% CI: 1.49-3.44, $p < 0.001$ and HR 2.49; 95% CI: 1.61-3.85, $p < 0.001$). In the adjusted model, only using significant variables from the unadjusted model (age, pStage, differentiation grade, R-status, CD68 and CD 163), these associations were confirmed for CD68+ (HR 1.61; 95% CI: 1.02-2.55, $p = 0.041$) but not for CD163+ macrophages (HR 0.68; 95% CI: 0.25-1.87, $p = 0.46$). Subgroup Cox regression analysis in patients with oesophageal cancer showed that high infiltration of CD68+ macrophages was prognostic in the unadjusted model, whereas high infiltration of both CD68+ and CD163+ macrophages were prognostic in gastric cancer. None of these associations was confirmed in the adjusted model. MARCO was not prognostic in any of the subgroup analyses.

Discussion

This thesis gives a broad perspective on cancer of the oesophagus and stomach. The papers presented herein cover a wide spectrum of topics related to these diagnoses with the common theme of how we as clinical researchers use data on patients gathered in our daily clinical practice to optimize future treatment strategies. This is exemplified by the data used for the different studies in this thesis, whereby two of them were based on validated high-quality national registers and the other two studies are based on well-defined local cohorts of patients with strict inclusion criteria.

The historical perspective of oesophageal and gastric cancer surgery is presented in the Background chapter of this thesis. We have come a long way from the days when mortality rates above 30% after oesophageal surgery were commonplace or the pre-antibiotic era when the slightest post-operative complication or infection was life-threatening to the patient. Today, advances in technology, oncological medications and patient care have cut mortality rates after surgery to below 2-6%, as exemplified in this thesis (3, 68, 135). Consequently, other measures for cancer care have been developed to monitor the quality of treatment in OG cancer. Resection rates, number of harvested lymph nodes during surgery and anastomotic leakages rates are all examples of such outcome measures discussed in this thesis.

Still, as this thesis shows, more extensive surgery does not necessarily yield better outcomes. Higher hospital volumes of OG cancer procedures is not the only factor influencing short-term outcomes after surgery and patient selection might impact upon post-operative outcomes in several ways.

Patient selection is closely related to individualized treatment wherein patient- and tumour-specific factors are considered for each patient in order to give the best possible tailored treatment. In modern OG cancer care, these treatment recommendations are made at the MDC. As clinicians, we base our recommendations on a mix of histological, endoscopic and radiological information. To date, no prognostic or predictive biomarker is yet in clinical use in OG cancer care. Finding such markers for risk stratification and for prediction of response to neoadjuvant oncological therapies is essential for tailoring individual treatment strategies in the future.

Aspects of methodology

The register-based studies

Studies based on data from national quality registers, assuming adequate coverage and high validity of data in registers, can be viewed as complementary to well-designed and adequately powered randomized controlled trials (RCTs) (7). Even though it is generally regarded that the highest level of scientific evidence is obtained from RCTs, there are limitations in the generalizability of outcomes of such trials. Hence, confirmatory population-based studies with data retrieved from national registers can reflect general clinical practice and generate evidence when certain methods or strategies are implemented on a nationwide basis.

Both NREV and the DUCA have validated their data recently (8, 136). To maintain this high level of validity, coverage and accuracy also in the future, it is essential that those responsible for supplying patient data to the registers continue to do so in the same meticulous manner. Reporting data to the DUCA is mandatory whereas reporting to NREV is not. Still, the same high level of validity of data can be maintained in both registers.

When the DUCA was initialized, variables from NREV and the NOGCA were used to put together a set of quality measures to compose a data set. This has allowed for easier comparisons between countries (68, 137). Further measures to facilitate international register studies and comparisons have been taken recently (138-140). Still differences in reporting several types of variables such as comorbidities, type of minimally invasive procedure and complications between NREV and the DUCA, forced us to omit some comparisons in Paper II.

With the Swedish personal identification number (PIN) (141), unique to every Swedish citizen, we can cross-link data from a wide range of different registers (142). In Papers I and II this allowed us to calculate both in hospital- and 90-day mortality for all patients in our Swedish cohorts. The latter could not be performed in the Netherlands due to legal constraints regarding transfer of data between different registers. More importantly, this might prevent a future study with the long-term follow-up and comparison of survival of the Swedish and Dutch cohort respectively.

Whereas the DUCA only includes patients who are planned for surgical treatment, NREV includes information on all patients with diagnoses of OG cancer, even those patients who after the MDC are treated with palliative intention. This harbours future potential for studies based on the NREV data on patients planned for palliative treatment who, by nature of the disease, represent the majority of patients in NREV.

The retrospective studies

A retrospective study design is well suited for studies running over a long period of time, studying rare events (surgical procedures) and the long-term effects of these events. Since the THX-ABD was a procedure only used when other less extensive procedures were not an option due to concerns about achieving R0, it took 25 years to accumulate 83 patients in Paper III. A prospective study running for that period of time would not be practically feasible. The long period of inclusion for this study also meant that several modalities in perioperative treatment such as anaesthesia, post-operative ICU care and physiotherapy training changed over time. On the other hand, one of the strengths of Paper III is the absolute uniformity in how the surgical procedure, the THX-ABD, was performed over the years. A limited group of skilled surgeons performed all of the 83 procedures included in this study.

Another complicating factor with the present study design in Paper III was the difficulty in finding a control group with which to compare our cohort. Since the THX-ABD was only used in the most advanced cases, there was an inherit selection bias of patients. Finding a matching cohort of patients with advanced tumours operated on with other techniques would require involving other surgical centres which was not deemed feasible. Hence the observational historical cohort design of this study.

Missing information and interpretation bias are potential risks encountered with the retrospective study design. In Paper III, the exact numbers of all dissected lymph nodes as well as the number of positive lymph nodes were only reported in the latter part of the study period. To estimate tumour stage, all patients with a note in the pathology report of ‘a positive lymph node’ in a specific position were judged to have one positive lymph node in that position. All patients with pathology reports including a passage of ‘positive lymph nodes’ in a specific location were interpreted as having only two positive nodes in that position. This probably led to an underestimation of N-status and staging of these patients.

To minimize selection bias in Paper IV, all patients included in the cohort were included consecutively. The cohort has previously been validated and characterized extensively by others (15, 143, 144). Of note, even though the TMA technique is a well-validated tool for biomarker studies, it comes with some limitations. The inherit risk of sampling bias was, however reduced by taking duplicate cores from two different blocks of the primary tumour, and it must also be pointed out that even the use of full-face sections comes with a risk of sampling bias, since these also only represent a small fraction of the tumour. Another limitation is that the present study was not powered primarily for subgroup analysis and the results from these analyses must therefore be validated in future studies.

Aspects of trends of OG cancer surgery in Sweden

The period of time during which NREV has been operational coincides with the implementation of several new treatment concepts such as neoadjuvant oncological therapy before surgery and centralization of major surgical procedures to fewer hospitals.

Neoadjuvant oncological treatment before surgery for both oesophageal and gastric cancer was used increasingly in Sweden during the study period in Paper I. This has probably been largely driven by the results of international RCTs arguing better outcomes for patients who received neoadjuvant treatment before surgery (4, 5). Even though the proportion of patients who receive neoadjuvant treatment in Sweden continues to rise, we do not reach the same levels as in the Netherlands as reflected in Paper II. The high proportion of patients receiving neoadjuvant CRT before oesophageal surgery in the Netherlands is probably due to the huge impact that the Dutch CROSS-study (5) has had on clinical practice in its own country. On the other hand, two Swedish studies from Klevebro and colleagues have shed light on the potential impact of neoadjuvant treatment in the short- and long term after surgery. The first study did not show a higher rate of complications in the group who received neoadjuvant therapy compared to the group who had surgery alone, though complications tended to be more severe in the group who had neoadjuvant CRT (145). The other study, based on data from NREV, indicated a potential survival benefit for patients with SCC who received neoadjuvant CRT but no survival benefit for any type of neoadjuvant treatment on patients with adenocarcinoma of the oesophagus and the GOJ (146). Apparently, the scientific evidence in favour of neoadjuvant treatment is not rock-solid, underlining the importance of confirmatory register-based studies on a nationwide basis to confirm or reject the results of RCTs.

A strong trend of centralization of major OG cancer surgery was evident in both Sweden and the Netherlands during the last decades, as shown in Papers I and II. In the Netherlands this process was driven by the Dutch Cancer Society who set minimum requirements for the number of OG cancer resections per year/hospital in 2007. In Sweden the process of centralization in the last decade was not driven by formal requirements until SKL (Sveriges Kommuner och Landsting), recommended that OG cancer surgery should be performed in only six hospitals from 2017 onwards.

The scientific support in favour of centralization of OG cancer and other major upper GI surgery is strong (147-150). Not only hospital but also surgeon volumes have been shown to impact upon outcome after surgery (151, 152). In line with the experience of others (136, 153), some of the presented quality improvements in Paper I, such as lower 30-day mortality after gastric surgery, a higher proportion of patients with more than 15 lymph nodes dissected during surgery and higher proportion of patients discussed at an MDC, might in part be attributed to centralization. Considering the drawbacks of centralization of complex surgical procedures (benign diagnoses receive lower priority in university hospitals and depletion of skilled surgeons in smaller hospitals), clearly evident in Sweden today, it is a matter of discussion how far the process of centralization shall be driven. Henneman et al argued that an increasing annual hospital volume with up to 40– 60 oesophagectomies/year was associated with a nonlinear decrease in mortality (154). Higher annual volumes yielded no further improvement. Similar results have been reported for gastric cancer (155). Sweden with approximately 170 oesophageal and 150 gastric resections per annum can probably strive for fewer hospitals performing these procedures. How many hospitals will be a matter of future debate.

Minimally invasive techniques for OG cancer surgery have gained wide acceptance in clinical practice in Sweden during the last decade. Learning curve errors are a well-known problem when introducing new surgical techniques (156). It is likely that the observed peak in anastomotic leakages after oesophageal cancer surgery in 2015 was in part due to learning curve errors when introducing thoracoscopic oesophagectomy. Measures must be taken in the future to keep learning curve errors to a minimum to decrease the associated patient morbidity.

Nationwide register studies offer insights into different aspects of patient selection. Swedish men have surgical resection for oesophageal and GOJ cancer proportionally more often than Swedish women. At the moment, the reasons for this are not clear. Considering the presented data with significantly better 5-year survival for women after oesophageal and GOJ cancer surgery, future studies addressing these issues are strongly indicated. The differences in regional resection rates in Sweden for both oesophageal and gastric cancer are also noteworthy. It may well be that the decision-making process behind those resection rates is correct and that patient-specific variables cause the observed differences. Therefore, it is of utmost importance for the future credibility of researchers and health care providers in Sweden to analyze these issues thoroughly.

Aspects of surgery at the gastrooesophageal junction

Historically, when multimodality treatment was not available, expanding the operating field as far as possible was one of the few options available in the quest for improving long-term outcome. One of the most radical approaches, the three-field lymphadenectomy, including cervical dissection, for oesophageal and GOJ cancer has been described by Lerut (70). Five-year survival in the whole cohort of patients was 42%, but for GOJ cancer patients with cervical lymph node metastasis

(18% of GOJ cancer patients) it was zero. Due to the increased morbidity of the procedure and failure to reproduce the results by others, it is not recommended for routine use today.

Our strategy with the THX-ABD was to incorporate the principles of abdominal gastrectomy (to achieve free distal resection margins and adequate abdominal lymph node clearance) with transthoracic oesophagectomy (to achieve free proximal resection margins and adequate thoracic lymph node clearance) into one procedure in order to maximize long-term outcome. Our results indicate that even though we achieved our intra-operative objectives with the THX-ABD, we did not cure more patients, as manifested by the long-term survival rate of 27%. This was not better compared to other studies of less extensive procedures for Siewert II and III tumours (11, 157) and highlights the need for multimodal treatment to improve survival.

The Siewert-classification only describes where the tumour has its epicentre, not where the proximal and distal margins of the tumour are located. Hence, a more pragmatic operative strategy might be to adapt the choice of surgical procedure in relation to what direction (proximal or distal) the tumour has as its main extension. This strategy is supported by data on lymph node spread from GOJ cancers where a recent prospective study from Kurokawa et al showed that if oesophageal involvement did not exceed 4 cm, there was only a weak recommendation to dissect lymph nodes retrieved from transthoracic dissection (158). Both the studies by Siewert (11) and Yamashita (159) indicated that the primary lymph node spread from Siewert II and III cancer was directed downwards towards the paracardial, the lesser curvature and left gastric nodes. Our study confirms the results of these studies and by means of the IEBLD-calculation, also shown by Hasegawa et al (160), emphasizes the importance of proper dissection of these lymph nodes to improve survival. No patient with metastatic lymph nodes retrieved from the transthoracic dissection (from middle mediastinum and upwards) in our study survived more than 5 years. This is in line with the results of Lagarde et al (77). Still, a study from Curtis et al showed that survival after surgery at the GOJ was significantly stepwise decreased for distal tumours (Type I<Type II<Type III) indicating that the Siewert classification has a prognostic significance (161). No such difference could be noted in our study, but due to the small number of patients in the subgroups, no firm conclusions can be drawn.

The in-hospital mortality rate of 2.4%, considering the historical perspective and the magnitude of surgery, is very low compared to international standards (11, 12, 76). The added morbidity due to the transthoracic dissection was, in part, manifested by a higher rate of pulmonary complications in our study compared to other studies with abdominal approach only for tumours in the same locations (157). On the other hand, almost 40% of patients had an uneventful post-operative course in our study, well in line with other reports of transthoracic surgery (76).

One of the few other studies on the THX-ABD was conducted by Ninomiya et al (162) who performed the thoracic dissection by means of thoracoscopy. In a small series of 10 patients with middle or lower oesophageal cancers they had no in-hospital mortality or anastomotic leakage. The thoracoscopic approach did not seem to prevent pulmonary complications.

The low number of studies on the THX-ABD can be interpreted on the basis of absent beneficial long-term results but also as a result of the complexity of the procedure. The construction of the long jejunal conduit with preservation of the vascular network to supply the Roux-loop is technically very demanding. Some prefer the colon interposition in those patients where the THX-ABD could have been an option (72, 163) while others argue the contrary (73, 164). Personal preferences clearly influence the choice of surgical procedure (165).

Future surgical management of patients with GOJ cancers will have a more individualized approach as described by Hölscher and Law (166). To lower the morbidity associated with these procedures, the gradual transition to minimally invasive technique will probably prove valuable. Increased use of intraoperative frozen sections (167) might prevent unnecessary proximal dissection of the oesophagus, further diminishing the role of the THX-ABD in the future.

Aspects of TAMs as prognostic biomarkers

High infiltration of $CD68^+$ and $CD163^+$ macrophages in tumour nest were negative prognostic factors for OS in Paper IV. The adjusted analysis showed that high infiltration of $CD68^+$ macrophages was an independent prognostic factor for a shorter OS. MARCO was not a prognostic factor for OS in our study. While a prognostic value of MARCO expression has previously been shown in pancreatic (133) and hepatocellular cancer (168), this study is, to the best of our knowledge, the first to describe the expression and prognostic impact of MARCO in OG cancer.

The prognostic value of TAMs in oesophageal cancer have mainly been studied in patients with SCC. High infiltration of TAMs, in particular $CD163^+$ macrophages, correlated with worse prognosis and poor response to neoadjuvant treatment in patients with oesophageal SCC (114), which is in line with another study showing a negative prognostic role for high infiltration of $CD163^+$ and $CD204^+$ macrophages in a cohort where over 90% had SCC (169). Patients with neoadjuvant treatment were, to various extents, included in these two studies.

In one of the few studies addressing the role of TAMs in oesophageal adenocarcinoma, Cao et al showed a correlation between the ratio of M2/M1-macrophages and lymph node metastasis and poor survival (113). Our present study, presented in Paper IV, represents to the best of our knowledge, the largest study to date including a well-defined consecutive series of CRT-naïve oesophageal adenocarcinoma. Our results are in line with the findings of Cao et al, even though our subgroup-adjusted analyses failed to show significant correlations with survival for $CD68^+$ and $CD163^+$ TAMs in patients with oesophageal and gastric cancer separately, probably due to the analyses being underpowered. In gastric cancer, two studies showed a correlation between high infiltration of $CD68^+$ macrophages with aggressive features and worse survival (170, 171). Only patients without neoadjuvant treatment were included in these studies.

Medrek et al (130) showed that high infiltration of $CD68^+$ macrophages in tumour stroma but not in TN correlated with reduced breast cancer-specific survival and Ohno et al (172) also stressed the importance of the histological location of infiltrating TAMs in endometrial cancer. Our results confirm these findings and highlight the importance of the compartmental localization of TAMs in the TME.

In clinical practice, high infiltration of $CD68^+$ and $CD163^+$ macrophages could possibly warrant intensified adjuvant treatment for patients who are at higher risk for recurrent disease after surgery without prior oncological treatment. Conversely, low infiltration of $CD68^+$ and $CD163^+$ macrophages could be an argument against adjuvant therapy in elderly and frail patients. Also, the results from this study support the hypothesis that high infiltration of $CD68^+$ and $CD163^+$ macrophages in pre-operative biopsies would signify more aggressive disease. Prospective studies with analysis of TAM-infiltration in pre-operative biopsies will have to address the relevance of this hypothesis.

Even though a considerable proportion of patients with OG cancer is being operated on without prior neoadjuvant oncological treatment, trends are pointing towards increased use of oncological treatment before surgery (3). In analogy with the study of Sugimura on SCC (114), high infiltration of M2 macrophages in biopsies from patients undergoing neoadjuvant treatment for oesophageal and gastric adenocarcinomas could hypothetically signal weak therapeutic response to the oncological treatment, thus indicating a need for change of treatment strategy. Moreover, high infiltration of M2 macrophages was found to be associated with poorer response to neoadjuvant chemotherapy in patients with breast cancer (173). Finding biomarkers to predict response to oncological therapy is essential in the strive for more tailored and individual treatment regimens and has the potential of sparing cancer patients the harm of unnecessary side effects as well as being cost effective for the for the health care sector and society as a whole. The role of TAMs in this context is still investigational.

Conclusions

- The results from NREV 2007-2016 show significant improvements in several important quality indicators of care for patients with OG cancer in Sweden. Gender differences in oesophageal cancer care and regional differences in resection rates mandate further research.
- Significant differences in patient and treatment characteristics for patients with OG cancer were evident between Sweden and the Netherlands in 2012-2014. Prolonged follow-up is needed to evaluate if the differences observed in this study have an impact on long-term survival.
- The THX-ABD can be performed with high rates of R0-resections and low in-hospital mortality. Long-term prognosis after THX-ABD is not better than after other less extensive procedures for tumours in the same locations. Additional lymph node dissection in the thorax does not seem to improve survival. The THX-ABD should only be used when it is not possible to achieve R0 with other less extensive surgical procedures.
- Infiltrating TAMs in TN can be used to predict outcome after surgery for OG cancer. More studies are needed to investigate the potential role of TAMs in pre-operative biopsies in predicting outcome after surgery and their potential role in predicting response to neoadjuvant treatment.

Future perspectives

- Studies based on data from national quality registers will, assuming the same high validity and accuracy of data as in previous years, continue to be an important source of high-quality research in the future. Harmonizing individual national registers with other countries will provide researchers with even better opportunities for multinational register studies and international benchmarking. Linking of national quality registers to biobanks will allow for more translational studies on OG cancer in the future.
- It is clear from Paper III in this thesis that we as surgeons might have reached the far limits of when more extensive surgery only adds morbidity without any benefit for the patient's prognosis. The implementation of neoadjuvant therapy and minimally invasive surgery have changed OG cancer treatment during the last decade. A combination of oncological and modern surgical technique is imperative to improve survival and decrease morbidity after surgery for patients with large Siewert II and III tumours at the GOJ. Studies aimed at investigating a combination of these therapies, and possibly including patients with limited metastasized disease in strict study protocols, are probably the most efficient way of trying to improve long term prognosis for these patients.
- Modern health care will continue to pursue the concept of individualized treatment of OG cancer. The mounting scientific evidence of genetic and immunological heterogeneity of OG cancer implies that, depending on a wide array of tumour biological factors, each patient might have his/her personal treatment scheme in the future. Complementary biomarkers will have an increasingly important role in OG cancer in predicting prognosis and perhaps response to oncological therapy. Prospective studies with analyses of biopsies taken before the start of neoadjuvant therapy and perhaps during oncological therapy might give valuable information about the sensitivity and response to treatment, respectively. Considering the vast amount of information on prognostic biomarkers in OG cancer acquired by our group in the last few years, a multivariable analysis of all investigated biomarkers to elucidate the strongest predictors is indicated.

Populärvetenskaplig sammanfattning (Summary in Swedish)

Cancer i matstrupe och magsäck är ett globalt hälsoproblem som årligen drabbar miljoner människor världen runt. Cancer i matstrupen var 2018 den 7:e vanligaste cancerformen för nyinsjuknanden samt den 6:e vanligaste orsaken till cancerdöd i världen. Motsvarande siffror för cancer i magsäcken var 5 och 3 (1). I Sverige drabbades 2018 ungefär 800 respektive 400 personer av cancer i matstrupe respektive magsäck (2). Femårsöverlevnaden för patientgruppen som helhet överskrider inte 20%, främst på grund av att tumörsjukdomen ofta upptäcks för sent för att kunna erbjuda botande behandling.

Under de senaste decennierna har vi sett en utveckling av flera nya behandlingskoncept för patienter med cancer i matstrupe och magsäck. Välgjorda prospektiva randomiserade studier (RCT-s) har legat till grund för bland annat införandet av onkologisk tilläggsbehandling med strålning och/eller cellgifter i anslutning till kirurgi (4, 5). Införande av minimalinvasiv kirurgi (6) samt centralisering av kirurgiska ingrepp till färre sjukhus har också skett under denna period.

Studier som baseras på data från nationella kvalitetsregister kan ses som ett komplement till RCT-s när man vill utvärdera förändringar i behandlingsstrategier på nationell eller internationell nivå. Nationella registret för matstrups- och magsäckscancer i Sverige (NREV) startades 2006 och har sedan dess registrerat över 12 000 patienter med dessa diagnoser i databasen.

I delarbete I utvärderade vi trender för olika behandlingsstrategier i Sverige under 2007-2016 baserat på data från NREV. Vi fann att behandling med onkologisk terapi i tillägg till kirurgi ökade signifikant under denna period för patienter med kurativt syftande behandling av matstrups- och magsäckscancer. Det skedde en successiv centralisering av de avancerade kirurgiska ingrepp som syftar till att bota patienter med dessa tumörformer och man såg även en signifikant förbättring av korttidsöverlevnaden efter kirurgi för magsäckscancer. Delarbete I visade även för båda tumörformerna på stora regionala skillnader avseende ”resection rates”, det vill säga hur stor andel av de patienter som diagnosticeras med tumörsjukdom som senare opererades för densamma med botande intention. Dessutom hade kvinnor signifikant bättre femårsöverlevnad efter kirurgi för matstrupscancer jämfört med män. Orsakerna till de regionala skillnaderna i "resection rates" samt könsskillnaden i femårsöverlevnad är inte kända men föremål för pågående studier inom ramen för NREV-s verksamhet. I delarbete II jämförde vi resultat från NREV med Hollands motsvarighet the Dutch Upper GI Cancer Audit (DUCA) under 2012-2014. Sverige hade i jämförelse med Holland lägre årliga sjukhusvolymer av operativa ingrepp för matstrups- och magsäckscancer. Onkologisk tilläggsterapi vid kirurgi användes signifikant mindre i Sverige som dock hade lägre 30-dagars-/sjukhusmortalitet efter magsäcksoperationer för cancer jämfört med Holland.

Det råder inte konsensus bland kirurger med subspecialisering tumörer i övre magtarmkanalen hur cancer i övergången mellan matstrupe och magsäck, den gastroesofageala övergången (GOJ), ska opereras på ett optimalt sätt. GOJ indelas traditionellt sett i tre zoner enligt Siewert-klassifikationen. Vid stora tumörer utgångna från de nedersta två zonerna, Siewert II & III-tumörer, kan det ibland vara svårt att uppnå tumörfria marginaler ovan och nedom tumören med de mest vanliga operationsmetoderna. I Lund har vi hos dessa patienter använt en mer ovanlig operationsmetod som innefattar resektion av hela magsäcken samt 2/3 av matstrupen och rekonstruktion med ett långt segment av tunntarmen i ett kombinerat buk- och bröstkorgsingrepp (THX-ABD). Metoden medger vida resektions-marginaler ovan och nedom tumören med möjlighet till extensiv lymfkörtelutrymning i både buk och bröstkorg. Ingreppet är dock större och därmed behäftat med mer risk än konventionella standardingrepp för tumörer i GOJ. I delarbete III utvärderade vi denna metod på 83 patienter opererade på SUS Lund 1986–2011. Vi fann att den post-operativa sjukhusmortaliteten var låg (2.4%) och att ingreppet kunde genomföras med acceptabla nivåer av post-operativa komplikationer jämfört med andra kombinerade buk-bröstkorgsingrepp. Dock kunde vi inte visa på någon förbättrad långtidsöverlevnad med THX-ABD. Vi kunde dessutom inte visa att den utökade dissektionen av lymfkörtlar i bröstkorgen var patienten till nytta då spridning av dottertumörer till dessa lymfkörtlar indikerade dålig långtidsprognos med 0% femårsöverlevnad.

Biomarkörer är mätbara indikatorer av biologiska tillstånd som används i allt större utsträckning i modern sjukvård. Exempel på sådana är prostataspecifikt antigen (PSA) vid prostatacancer eller analys av östrogenreceptorer vid bröstcancer. Makrofager är celler vars primära uppgift är att delta i kroppens immunförsvar mot främmande organismer. Tidigare studier har visat att makrofager också har en viktig roll vid tumörutveckling och andra studier har visat att tumörassocierade makrofager (TAM-s) kan ha en prognostisk roll vid cancer i matstrupe- och magsäck. I delarbete IV analyserade vi med ljusmikroskopi infiltrationen av $CD68^+$, $CD163^+$ och $MARCO^+$ makrofager (subgrupper av TAM-s) i tumörvävnad från 174 patienter opererade för matstrups- eller magsäckscancer på SUS Lund/Malmö 2006-2010. Vi fann att hög infiltration av $CD68^+$ och $CD163^+$ makrofager i tumörvävnad korrelerade med signifikant sämre prognos. Inga samband mellan infiltration av $MARCO^+$ makrofager i tumörvävnad och prognos kunde ses.

Sammanfattningsvis visar resultaten av denna avhandling på en rad förbättringar av olika kvalitetsparametrar för vård och behandling av patienter med matstrups- och magsäckscancer i Sverige under åren 2007-2016. Det finns signifikanta skillnader i patienturval samt behandlingsstrategier mellan Sverige och Holland för denna patientgrupp. Uppföljande studier får utröna om de skillnader som beskrivs i delarbete 2 har en betydelse för långtidsöverlevnad. THX-ABD kan utföras med låg sjukhusmortalitet men med ringa påverkan på långtidsöverlevnad. Metoden bör bara användas då andra mindre extensiva metoder inte är alternativ för att uppnå kirurgisk radikalitet. Infiltration av TAM-s kan användas för att prognosticera överlevnad efter kirurgi för matstrups- och magsäckscancer.

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Oesophageal and Gastric Cancer

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