Investigating new biomarkers and therapy options in oesophagogastric cancer

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CHAPTER 1

General introduction

Gastric cancer

Incidence

Worldwide, gastric cancer is among the most frequently diagnosed cancers. In 2020, with more than 1 million cases and around 769.000 deaths, gastric cancer ranked fifth in terms of incidence rates and fourth in terms of mortality rates.[1] There are major regional differences. For instance, in 2017, in the high-income Asia Pacific region and East Asia the incidence was 29.5 and 28.6 per 100.000 population, respectively, whereas in high-income North America and Western Europe these rates were 6.5 and 10.5 per 100.000, respectively.[2] In the Netherlands, gastric cancer incidence has steadily declined over the past decades, from 1899 cases in 1990 to 984 cases in 2020 (**Figure 1**).[3] The most common types of gastric cancer are adenocarcinomas, accounting for 90% of the gastric tumours. Other less common tumours are gastrointestinal stromal tumours, lymphomas and sarcomas.

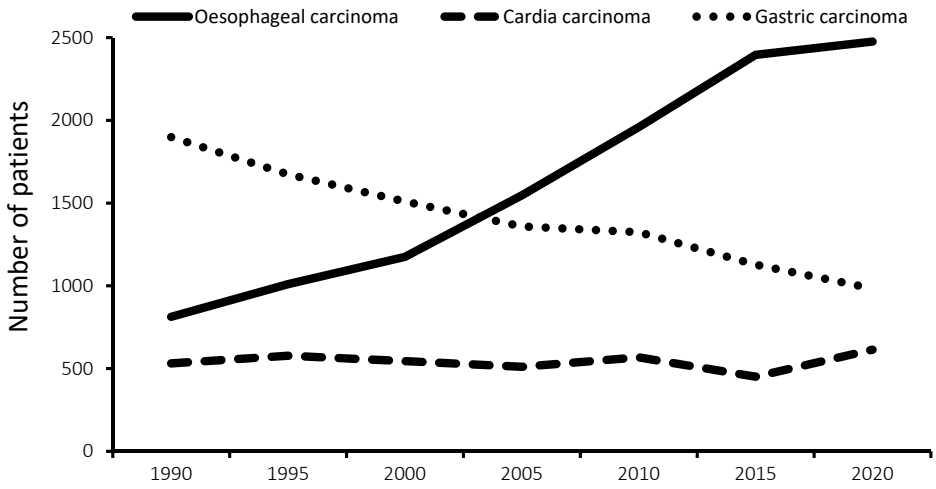

Figure 1. Incidence of oesophageal, cardia and gastric carcinoma in the Netherlands in 1990, 1995, 2000, 2005, 2010, 2015 and 2020.[3]

Risk factors

There are a few risk factors contributing to a high incidence of gastric cancer. *Helicobacter Pylori* infection is a major risk factor for non-cardia gastric cancer.[4] The relationship between the infection and gastric adenocarcinomas has been established in the 1980s. Since then, improved hygienic practices and effective treatment of the infection have lowered the infection rates and thereby the relative gastric cancer incidence. Other risk factors include a high salt diet, alcohol consumption and smoking.[5] About 1% to 3% of gastric adenocarcinomas is hereditary. It is caused by an inactivating germline mutation in the CDH1 tumour suppressor gene or mutations in other closely related genes.[6]

Classification

The tumour-node-metastasis (TNM) classification provides a standardised system to classify tumours in a uniform manner. Currently, for gastric carcinoma, the eight edition is used.[7] For histological subtyping the Lauren classification has been used since 1965.[8] The Lauren classification recognises three main types of gastric adenocarcinoma: the intestinal type, the diffuse type and the mixed type. In the intestinal subtype, the tumour cells are arranged in a tubular or glandular formation. In contrast, the diffuse type cells lack adhesion and infiltrate as single cells or small groups. To date, histological subtyping according to Lauren has no implications for clinical decision making.

Molecular characterisation

In the past decades, the lowering costs of next-generation sequencing have made genetic analysis of cancers more widely available. Several groups have identified frequently altered genes in gastric adenocarcinomas (e.g., TP53, ARID1A and CDH1, FGFR2 and ERBB2).[9,10] One of the largest world-wide projects is The Cancer Genome Atlas (TCGA). TCGA analysis of 295 gastric adenocarcinomas identified four subtypes: the Epstein-Barr virus positive tumours, the microsatellite instable tumours, the genomically stable tumours and the tumours with chromosomal instability.[11] The subtypes displayed different genomic characteristics. Furthermore, an association between the Lauren classification and the subtypes was seen. Most of the diffuse type tumours were of the genomically stable subtype and displayed frequent mutations in the RHOA gene. Mutations in the RHOA gene were not found in the intestinal type.[10] At current times, overexpression of Human Epidermal growth factor Receptor 2 (HER2) is the only genetic aberration that affects treatment choices. Generally, patients with HER2 overexpression have a poorer prognosis.[12] However, the addition of trastuzumab to the systemic chemotherapy regimen has a beneficial effect on overall survival, making it the first targeted treatment option for gastric cancer patients.[13] Microsatellite instability (MSI) is a marker for an inactive or defective mismatch repair system, which is associated with an increased mutation rate. In the MAGIC trial, patients with high MSI had a better survival compared to those with low MSI or microsatellite stable tumours when treated with surgery, but a worse survival when treated with perioperative chemotherapy plus surgery.[14] Results of the KEYNOTE-012 trial suggest that a major part of tumours with high MSI responds well to immunotherapy.[15] Therefore, in the near future, MSI status might be used to select patients for perioperative chemotherapy or immunotherapy.

Metastatic spread

Around 40% of patients present with metastatic disease at diagnosis, which is a major reason for the dismal overall survival of gastric adenocarcinoma patients.[16] Median overall survival for gastric adenocarcinoma patients presenting with metastatic disease was 4-5 months in a population-based study over the years 1990-2011.[16] The peritoneum is a predilection site for gastric cancer metastases.

Around 14% of the newly diagnosed gastric adenocarcinoma patients present with peritoneal metastases.[17] This percentage is even higher if patients who have tumour positive cytology of peritoneal fluid are also considered to have peritoneal dissemination. Between 9% and 24% of patients without visible metastatic disease on the peritoneum have tumour positive cytology.[18,19] Current national guidelines in the Netherlands dictate that stage IV gastric adenocarcinoma patients are treated with palliative systemic chemotherapy or best supportive care.

Treatment and survival

In the Netherlands, perioperative chemotherapy and surgical resection is considered the standard treatment option for resectable, nonmetastatic locally advanced gastric cancer.[20] This is largely based on the British MAGIC trial demonstrating survival benefit of perioperative chemotherapy with epirubicine, cisplatinum and fluorouracil (ECF) compared to surgery alone.[21,22] More recently, in the German FLOT4 trial, the docetaxel-based regimen FLOT (fluorouracil plus leucovorin, oxaliplatin and docetaxel) proved superior to ECF as a perioperative therapy in terms of overall survival.[23] In the per-protocol analysis of the Dutch CRITICS trial, patients who were treated with adjuvant chemotherapy had a better overall survival than those who were treated with adjuvant chemoradiotherapy.[24,25] Even with a multimodality treatment strategy survival of gastric adenocarcinoma patients remains dismal. Median survival in the FLOT arm of the FLOT4 trial was 50 months and in the CRITICS 1 trial median survival was around 40 months.[23,24] Overall, gastric cancer survival differs between TNM stages. Following the data of the Netherlands Cancer Registry (2010-2016; TNM seventh edition), 5-year survival was 66%, 48%, 16% and 2% for stage I, II, III, and IV non-cardia gastric cancer patients, respectively (**Figure 2**).[3]

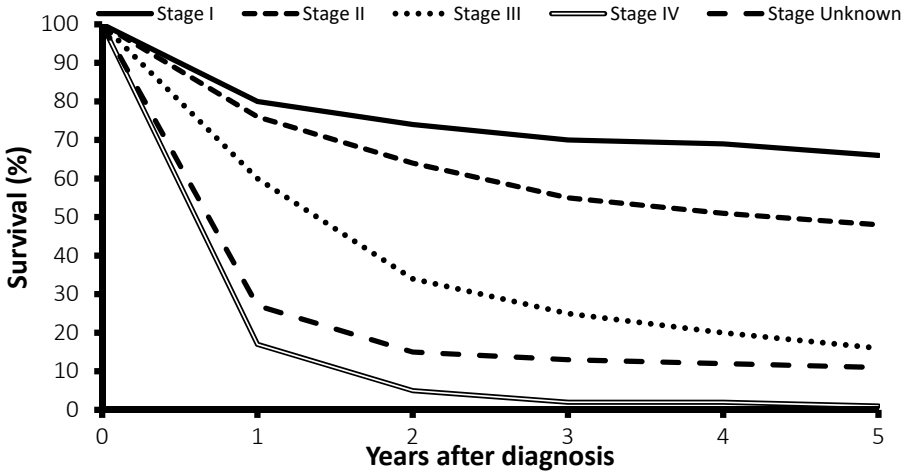

Figure 2. Survival of gastric cancer patients per TNM stage at diagnosis (TNM 7th edition) in the Netherlands between 2010 and 2016.[3]

HIPEC therapy

For patients with peritoneal metastasis of colorectal origin, a combination of cytoreductive surgery and Hyperthermic Intraperitoneal Chemotherapy (HIPEC) is a treatment option.[26] Long-term follow-up data of a randomised study comparing systemic chemotherapy alone with cytoreduction followed by HIPEC and systemic chemotherapy showed a median disease-specific survival of 13 months in the control arm versus 22 months in the HIPEC arm. In patients with advanced stage (stage III) ovarian cancer, a randomised trial demonstrated a survival benefit of HIPEC in addition to cytoreductive surgery as compared to surgery alone following neoadjuvant chemotherapy.[27] HIPEC has some advantages over systemic chemotherapy for the treatment of peritoneal metastases. Systemic chemotherapy does not penetrate peritoneal lesions as well as intraperitoneal chemotherapy.[28] Furthermore, intraperitoneally administered cytostatics barely invade the systemic circulation due to the peritoneal-plasma barrier, making it possible to give higher doses intraperitoneally.[29] Also, heated cytostatic agents can be used, possibly leading to enhanced peritoneal tissue concentration which has been demonstrated for e.g. intraperitoneal oxaliplatin.[30]

HIPEC for gastric cancer

In Asia, gastric cancer patients with peritoneal dissemination are commonly treated with cytoreductive surgery and HIPEC.[31] Numerous studies, primarily performed in Asian institutions, have suggested a beneficial effect of a HIPEC procedure in selected gastric cancer patients.[32] From the available literature, it is clear that the completeness of the cytoreduction is a critical prognostic factor for survival.[31] Recently, three Western national cohort studies including gastric cancer patients with peritoneal carcinomatosis treated with cytoreductive surgery and HIPEC were published.[33-35] Median overall survival was 13, 18.8 and 21.2 months in a German, French and Spanish study cohort, respectively. These results are promising, especially when compared to the dismal survival of gastric cancer patients with peritoneal metastasis treated with systemic chemotherapy alone.[36] However, the results of Asian studies cannot be extrapolated to the Western gastric cancer population as there is a difference in outcome after treatment between Eastern and Western gastric cancer patients.[37] This is probably related to tumour biology. Therefore, a Western randomised controlled trial is needed to study the role of HIPEC in a Western gastric cancer population with peritoneal dissemination.

PERISCOPE studies

To investigate the efficacy of a combination treatment of a gastrectomy, cytoreductive surgery and HIPEC for gastric cancer patients with peritoneal metastasis the PERISCOPE (*Treatment of PERItoneal dissemination in Stomach Cancer patients with cytOreductive surgery and hyperthermic intraPEritoneal chemotherapy*) study was initiated in the Netherlands.[38] Following a review of the literature, oxaliplatin and docetaxel were selected for the HIPEC procedure in the PERISCOPE study.[39] There is extensive

experience with the intraperitoneal use of oxaliplatin for various cancer types. In contrast, the experience with the use of intraperitoneal docetaxel is very limited and a combination of both had never been investigated.[40,41] The primary aim of the first PERISCOPE study was to investigate the safety and feasibility of a HIPEC procedure with oxaliplatin and docetaxel in gastric cancer patients with peritoneal dissemination following systemic chemotherapy. A dose-escalation schedule for intraperitoneal docetaxel was part of the study. A combination of 460 mg/m^2 hyperthermic oxaliplatin and 50 mg/m^2 normothermic docetaxel appeared safe and feasible.[42] The results and experiences in the first PERISCOPE study have formed the basis for the design of the PERISCOPE II trial.

Oesophageal cancer

Incidence and risk factors

In 2020, oesophageal carcinoma was responsible for about 604.000 new cases worldwide, ranking it seventh in terms of incidence, and for about 544.000 deaths, ranking it sixth in mortality.[1] The two most common histological subtypes, squamous cell carcinoma and adenocarcinoma, differ in etiology and geographic distribution.[43] In lower-income countries, squamous cell carcinoma comprises over 90% of all oesophageal cancers, whereas adenocarcinoma represents the majority of oesophageal cancers in high-income countries. In these countries, incidence rates of oesophageal cancer are increasing rapidly. In the Netherlands, the incidence of oesophageal cancer increased from 814 cases in 1990 to 2476 cases in 2020 (**Figure 1**).[3] This striking increase is completely the result of the rising incidence of oesophageal adenocarcinoma, while the incidence of oesophageal squamous carcinoma has remained stable.[44] The main risk factors for oesophageal adenocarcinoma are obesity and gastroesophageal reflux disease.[45] These factors are related to the occurrence of Barrett oesophagus, a condition in which the squamous epithelium is replaced by columnar epithelium as a response to acid and bile reflux. Barrett mucosa is a precursor of oesophageal adenocarcinoma.[45] Risk factors for oesophageal squamous cell carcinoma are smoking, alcohol consumption, nutritional deficiencies and possibly other dietary components.[46]

Molecular characterisation

TCGA analysed a group of 164 oesophageal carcinomas and discovered molecular features that differentiate oesophageal squamous cell carcinomas from oesophageal adenocarcinomas.[47] Squamous cell carcinomas had frequent genomic amplifications of the CCND1 and SOX2 genes, whereas adenocarcinomas frequently showed amplifications in the ERBB2, VEGFA and GATA4 genes. Other genes associated with oesophageal adenocarcinomas are TP53, CDKN2A, SMAD4, ARID1A and PIK3CA.[48] Interestingly, oesophageal squamous cell carcinomas resembled more closely squamous cell tumours from other origins than adenocarcinomas of the oesophagus. Furthermore, oesophageal

adenocarcinomas had stronger resemblance to a subtype of gastric adenocarcinomas (with chromosomal instability) than to oesophageal squamous cell carcinomas.[47]

Treatment and survival

Multimodality treatment consisting of neoadjuvant chemotherapy or chemoradiotherapy followed by surgery has become the standard treatment option for resectable, nonmetastatic locally advanced oesophageal cancer in Western countries.[49] In the Netherlands, chemoradiotherapy according to the CROSS study is the neoadjuvant therapy of preference.[50] In the randomised CROSS study, patients in the neoadjuvant chemoradiotherapy plus surgery group had a median overall survival of 48.6 months, compared to a median overall survival of 24.0 months in the surgery-alone group.[51] The survival difference was far more substantial for patients with squamous cell carcinoma (81.6 months versus 21.1 months) than for adenocarcinoma patients (43.2 months versus 27.1 months). A pathological complete response to neoadjuvant therapy is a known predictor of improved survival after treatment.[52] Unfortunately, in most patients (70-80%), a pathological complete response is not achieved after neoadjuvant chemoradiotherapy for oesophageal cancer.[52] For those with a pathological limited or no response to neoadjuvant chemoradiotherapy (around 35% of patients), overall survival was similar as it was in a matched group of patients who underwent primary oesophagectomy.[53] This calls for further research to find predictors of (non-)response to chemoradiotherapy. Overall, oesophageal cancer survival differs between TNM stages. Following the data of the Netherlands Cancer Registry (2010-2016; TNM seventh edition), 5-year survival was 59%, 37%, 26% and 2% for stage I, II, III, and IV oesophageal cancer patients, respectively (**Figure 3**).[3]

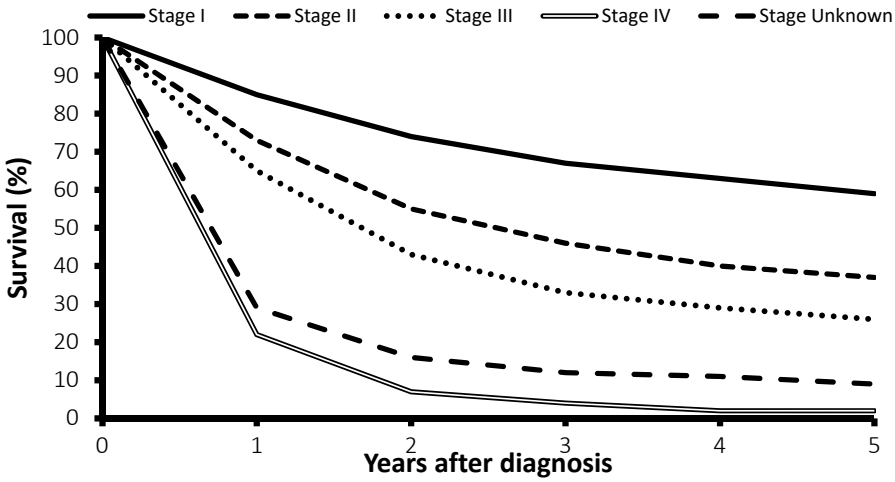

Figure 3. Survival of oesophageal cancer patients per TNM stage at diagnosis (TNM 7th edition) in the Netherlands between 2010 and 2016.[3]

Immunotherapy

In general, a promising new treatment option for cancer patients is immunotherapy. Immune checkpoint inhibitors have become standard therapy for patients with advanced stages of melanoma and non-small cell lung cancer.[54,55] These agents are directed against the programmed death-1/programmed death ligand-1 (PD-1/PD-L1) pathway. Cancer cells use immune checkpoint pathways to avoid elimination by the immune system. The PD-1/PD-L1 immune inhibitory axis protects tissues from damage during inflammation and diminishes the possibility of autoimmune reactions. In cancer, upon stimulation by certain pro-inflammatory cytokines or due to genetic alterations and activation of oncogenic signalling pathways, PD-L1 becomes expressed on tumour cells and on tumour associated immune cells, leading to an ineffective immune response.[56] By suppressing this mechanism, theoretically the host immune systems should be able to eliminate cancer cells. The current challenge is to identify those tumours that will respond to immunotherapy.

Tumour microenvironment

There is emerging evidence that immune cells and the tumour microenvironment have a role in the response of the tumour to conventional therapies such as chemotherapy and chemoradiotherapy and that they influence the prognosis of cancer patients, but the data are conflicting. Rectal adenocarcinomas with a high pre-treatment amount of CD8+ cells in the tumour microenvironment had a better response to neoadjuvant chemoradiotherapy than those with a low amount of CD8+ cells.[57] Further, patients with 'immunoscore'-high tumours with a high density of CD3+ and CD8+ cells in the tumour core and in the invasive margin had a better prognosis. In contrast, in another recent study, a subgroup of colorectal cancer patients with a high intratumoral CD8+ T-cell infiltration exhibited a poor prognosis after primary resection.[56] Less is known about the role of the immune system and the tumour microenvironment in the response to chemo(radio)therapy in oesophageal adenocarcinoma. Recently, it was shown that a DNA damage immune response (DDIR) positive signature was predictive for the response to chemotherapy and survival in oesophageal adenocarcinoma.[58] DDIR+ patients had higher pathological response rates and a better survival compared to DDIR- patients. DDIR+ tumours were associated with the presence of CD8+ lymphocytes as well as PD-L1 expression. However, in another study, involving gastric and oesophagogastric junction adenocarcinomas, high CD8+ levels and PD-L1 positivity were associated with a worse progression-free and overall survival.[59] These conflicting results ask for further studies on the PD-1/PD-L1 involvement in oesophagogastric cancer.

Scope of the thesis

The histological subtypes of oesophagogastric adenocarcinoma differ biologically. Moreover, there are differences in epidemiology and in prognosis. These differences have never been studied in a large national cohort. In **Chapter 2** a Dutch national cohort study was carried out to investigate the incidence and survival of intestinal and diffuse type adenocarcinoma of the oesophagus and stomach. It is hypothesised that intestinal and diffuse type gastric carcinomas have a different disease etiology and might have a different metastatic pattern. To test this hypothesis, another Dutch national cohort study was carried out to analyse the metastatic patterns of intestinal and diffuse type gastric carcinoma (**Chapter 3**).

One of the most common sites of gastric cancer metastases is the peritoneum. It is not known how the decreasing incidence of gastric cancer and the increasing proportion of diffuse type gastric tumours affect the incidence of gastric cancer patients with peritoneal metastases in the Netherlands. For that reason, we performed a Dutch national cohort study, presented in **Chapter 4**, that focusses on the incidence, treatment and survival of patients with synchronous peritoneal metastases of gastric cancer origin.

As yet, there is no conclusive evidence that HIPEC therapy offers a survival benefit in Western gastric cancer patients with peritoneal metastases. In the Dutch PERISCOPE I study, HIPEC treatment was safe and feasible in a selected patient group with gastric cancer and peritoneal dissemination. The PERISCOPE I study was not designed to establish the efficacy of a HIPEC procedure in gastric cancer patients. Nonetheless, clinical and pathological outcomes in this phase I-II study are relevant as background for the randomised controlled trial. **Chapter 5** reports these results of the PERISCOPE I study. It is known that HIPEC procedures are associated with considerable morbidity. This was also seen in the PERISCOPE I study and demanded specific perioperative management. **Chapter 6** provides insight in the perioperative management of the patients in the PERISCOPE I study. The intraperitoneal cytostatic regimen in the PERISCOPE I study consisted of oxaliplatin followed by docetaxel. Oxaliplatin is often used intraperitoneally, but there is almost no experience with the use of intraperitoneal docetaxel. In **Chapter 7**, the pharmacological behaviour of intraperitoneal docetaxel was studied in the patients treated in the PERISCOPE I study. The current standard treatment for gastric cancer patients with peritoneal metastases in the Netherlands is palliative systemic chemotherapy. The PERISCOPE II study is a multicentre randomised controlled trial to investigate whether cytoreductive surgery and HIPEC provides a survival benefit compared to systemic chemotherapy alone in gastric cancer patients with limited peritoneal dissemination. The background, rationale and study protocol are presented in **Chapter 8.**

Immunotherapy is an exciting new treatment option for cancer patients. However, for oesophageal cancer it is not yet clear which patients will respond to immunotherapy. PD-L1 positivity has been suggested as a predictor for tumour response in other types of carcinomas. In **Chapter 9** a review is presented that describes if PD-L1 expression can act as a valuable biomarker to predict response to immunotherapy in oesophageal cancer. Current standard therapy for locally advanced non-metastatic oesophageal cancer is neoadjuvant chemoradiotherapy followed by surgery. Differences in pathological tumour response are not understood. It is hypothesised that the tumour microenvironment has a role in the response to neoadjuvant therapy. In the final chapter of this thesis, **Chapter 10,** the tumour microenvironment of oesophageal adenocarcinoma was investigated in patients who underwent neoadjuvant chemoradiotherapy followed by surgical resection.

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CHAPTER 2

A population-based study on intestinal and diffuse type adenocarcinoma of the oesophagus and stomach in the Netherlands between 1989 and 2015

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Abstract

Aim | To investigate the nationwide time trends in the incidence and survival of oesophageal and gastric adenocarcinomas according to the Lauren classification (intestinal, diffuse and mixed type).

Methods | All patients diagnosed in the Netherlands with oesophageal or gastric adenocarcinoma between 1989 and 2015 were included. A syntax was developed to determine the histological subtype based on pathology reports as archived in the Dutch pathology registry. These reports were linked to individual data from the Netherlands Cancer Registry. Relative survival was used to assess survival.

Results | The histological subtype could be determined in 18.691 (84.1%) oesophageal and in 32.312 (83.5%) gastric adenocarcinomas. Among these, 79% were intestinal and 21% diffuse type in oesophageal cancers, compared to 55% intestinal and 44% diffuse type in gastric cancers. Relative median survival of intestinal type tumours was longer than that of diffuse type tumours, i.e. 12.1 versus 9.4 months for oesophageal carcinomas, and 10.1 versus 7.6 months for gastric carcinomas, respectively. Between 1989-2015 the relative median survival of non-metastatic intestinal and diffuse type oesophageal adenocarcinoma improved from 12.0 to 30.0 months, and from 12.0 to 19.2 months, respectively. The same was true for intestinal type gastric carcinoma (from 22.8 to 27.6 months) but for diffuse type gastric carcinoma the increase was less (from 16.8 to 18.0 months).

Conclusion | In this nationwide study, histological subtypes of oesophageal and gastric adenocarcinomas differed in frequencies and survival times. These findings may call for a differentiated treatment approach.

Introduction

In 2017, approximately 2500 patients were diagnosed with oesophageal carcinoma and 1200 with gastric carcinoma in the Netherlands.[1] The prognosis of these patients is poor with 5-year survival rates around 25-30% when diagnosed without metastases.[2,3] Standard treatment for these patients consists of surgical resection combined with (neo)adjuvant chemo(radio)therapy.[4,5] Since the introduction of these multimodal treatment strategies about 10-12 years ago, survival has improved.[6,7] Multiple small-scale patient cohort studies have shown that some oesophageal and gastric cancer subtypes respond better to treatments than others, indicating that there are subgroups of patients for whom different treatment strategies might be warranted.[8]

For gastric adenocarcinomas, the Lauren classification is a well-known histopathological classification system with prognostic value.[9] According to that system carcinomas are classified into intestinal type tumours which form glands and resemble adenocarcinomas of the large intestine, diffuse type tumours which consist of poorly cohesive cells with little or no gland formation (often containing signet ring cells) and mixed type tumours.[9,10] These subtypes differ with regard to epidemiological trends, molecular aspects and pathogenesis.[8,11] The Lauren classification was found to have both prognostic and predictive value in a single centre patient cohort with oesophageal adenocarcinomas.[8] In that study, it was demonstrated that, similar to what is known for gastric cancer, patients with diffuse type carcinomas of the oesophagus had a significantly worse prognosis than patients with intestinal type carcinomas of the oesophagus. And, intestinal type carcinomas showed a better response to neoadjuvant chemo- or chemoradiotherapy than diffuse type carcinomas.

Over the past decades, the incidence of oesophageal adenocarcinoma has rapidly increased in all Western countries while gastric adenocarcinoma incidence rates have declined.[12,13] Epidemiological data linked to histopathological data provide insight into the relative contribution of each subtype in these changing incidence figures. That may be a starting point for unravelling the underlying aetiological features. Furthermore, knowledge of survival trends per subtype potentially directs towards subtype-specific treatment strategies.

The aim of this study was to assess the incidence and survival trends of oesophageal and gastric adenocarcinomas in a Western population per histological subtype according to Lauren.

Methods

Data

Data were obtained from The Netherlands Cancer Registry (NCR). The NCR is a nationwide registery including all newly diagnosed malignancies within the total Dutch population. Patient, tumour and

treatment characteristics are routinely obtained from medical records by specifically trained data managers. Data on vital status are annually obtained through a linkage with the municipal personal records database that keeps record of all births, deaths and emigrations in the Dutch population. For this study, data on vital status was available until 1 February 2017. Tumour location and morphology are coded according to the International Classification of Diseases for Oncology (ICD-O).[14] Subsite distribution for oesophageal cancer was as follows: upper (C15.0, C15.3), middle (C15.4) lower (C15.5), and overlapping or not otherwise specified (C15.8, C15.9). oesophagogastric junction (C16.0) carcinomas were considered a separate entity, and were not included in this study. For gastric carcinomas, subsite distribution involved: fundus (C16.1), corpus (C16.2), antrum (C16.3), pylorus (C16.4), small curvature (C16.5), greater curvature (C16.6) and overlapping or not otherwise specified (C16.8, C16.9). In the NCR, tumours are staged at diagnosis according to the most recent International Union Against Cancer (UICC) TNM classification at time of registration. Edition 4 was used from 1989 until 1992, edition 4.2 from 1993 until 1998, edition 5 from 1999 until 2002, edition 6 from 2003 until 2009, and edition 7 from 2010 until 2015. To establish uniformity in tumour staging without losing data, all TNM stages were recoded to the TNM-6 classification.[15]

Patients

All patients diagnosed with oesophageal or gastric adenocarcinoma between 1 January 1989 and 31 December 2015 were selected from the NCR.

Histology

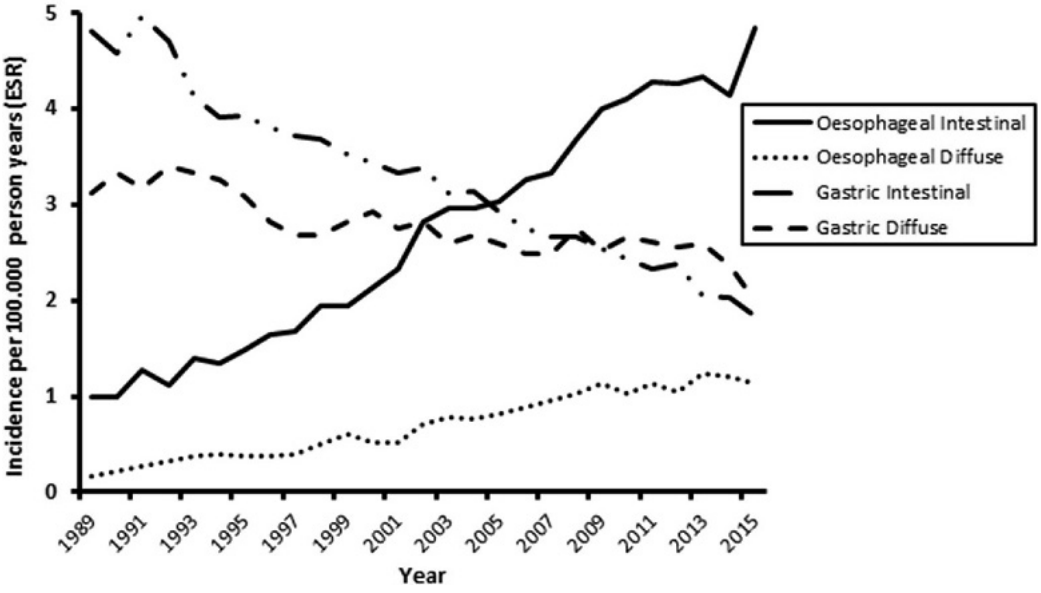
As histological subtyping according to Lauren is not part of the routine data collection in the NCR, nor part of the ICD-O coding, NCR patient data were linked to the corresponding pathology data collected in the nationwide registry of histo- and cytopathology in the Netherlands (PALGA). At first, a pilot was performed with 463 randomly selected adenocarcinoma cases from three different time periods (1989 – 1999 – 2009). A dedicated gastrointestinal pathologist (PS) and one of the other authors (RK) determined the histological subtype (reference subtype) of all 463 cases by manually reviewing the complete pathology reports of the biopsies available from PALGA. Cases were classified in 4 subtypes: intestinal type, diffuse type, mixed type and unknown type. Secondly, based on this pilot a syntax was designed in SAS (version 9.4 for Windows) to automatically determine the histological subtype from the pathology reports of all pilot cases. The outcome of the syntax was compared case-by-case with the reference subtype. With the final syntax, 94% of the pilot cases was correctly categorised. Thirdly, the NCR supplied the (pseudonymised) patient data of all registered oesophageal and gastric adenocarcinoma patients without the oesophagogastric junction carcinoma patients (N=62.843, 1989-2015), which were linked to the individual pathology reports from PALGA. NCR patient data were only linked to a PALGA report if the pseudonyms and a few additional variables (such as zip code or

pathology laboratory) matched in both data sets (NCR and PALGA). Based on the number of matching criteria that was fulfilled, the reliability of the match between NCR and PALGA was scored from 1 (very reliable) to 3 (least reliable; no complete match between matching criteria in NCR and PALGA, i.e., the NCR and PALGA data might be from different patients). All cases with a score of 3 were removed. Patients with a score of 2 were included if gender and age at diagnosis were matched. For 97.1% (61.021) of the patients a matching pathology report was found in the PALGA database.

Statistics

Subgroup comparisons (intestinal/ diffuse/ mixed/ unknown) were done using Fisher's exact test (for nominal variables) or the Kruskal–Wallis test (for continuous variables). Annual incidences were described per 100.000 person-years and standardised according to the European Standard Population, resulting in the European Standardised Rates (ESR). Relative survival was calculated for the different subgroups as the ratio of the survival observed in the study population to the survival that would have been expected based on age, gender and general population mortality in the corresponding year (Pohar Perme method).[16] The relative survival analyses were performed according to disease entity (oesophageal/gastric cancer) and presence of metastasis. Of 126 patients follow-up data for vital status was not correctly entered in the database. These patients were excluded from the relative survival analyses. All data were analysed using SPSS (version 22.0 for Windows) and STATA (version 13.0, Statcorp LP, College Station, TX).

Figure 1. Incidence of oesophageal and gastric adenocarcinoma per 100.000 person years (ESR) according to Lauren classification in the Netherlands between 1989 and 2015.



Results

The NCR data of 61.021 patients, registered in the NCR between 1 January 1989 and 31 December 2015 with oesophageal or gastric carcinoma, were matched to PALGA pathology reports. A total of 84 patient records were excluded because histology turned out to be other than adenocarcinoma (neuro-endocrine carcinomas, squamous cell carcinomas). In all, 22.217 Dutch patients were diagnosed with an oesophageal adenocarcinoma and 38.720 patients with a gastric adenocarcinoma. Patient and tumour characteristics are given in **Table 1**. The histological subtype according to Lauren could be determined in 18.691 (84%) oesophageal and in 32.312 (83%) gastric cancer patients. Over the years, the proportion of patients with an unknown histological subtype decreased for both oesophageal cancer (from 29% to 9%) as well as for gastric cancer (from 26% to 6%). Out of all oesophageal adenocarcinomas with a known histological subtype, 79% were of the intestinal type, 21% of the diffuse type and 1% of the mixed type. In gastric adenocarcinomas with a known subtype, 55% were of the intestinal type, 44% of the diffuse type and 1% of the mixed type. In the oesophageal adenocarcinoma group, age and gender distribution were comparable between intestinal and diffuse subtypes. In the gastric adenocarcinoma group, patients with a diffuse type carcinoma were younger and more likely to be female than those with an intestinal type.

The oesophageal adenocarcinoma incidence per 100.000 person years increased between 1989 and 2015 from 0.99 and 0.16 to 4.84 and 1.14 for the intestinal and the diffuse type, respectively (**Figure 1**). In contrast, gastric adenocarcinoma incidence decreased from 4.81 and 3.12 in 1989 to 1.83 and 1.99 in 2015 per 100.000 person years for the intestinal and the diffuse type, respectively. Of note, for gastric adenocarcinomas, the proportion of diffuse type carcinomas increased and eventually surpassed the proportion of intestinal type carcinomas (from 59% intestinal type and 40% diffuse type in 1989-1995 to 48% intestinal type and 50% diffuse type in 2011-2015).

Relative median survival rates of patients with intestinal and diffuse type carcinoma are given in **Table 2**, and survival curves are shown in **Figure 2**. Overall, relative median survival of patients with intestinal type carcinoma was better than that of patients with diffuse type carcinoma, both in the oesophageal cancer group (12.1 versus 9.4 months, respectively) as well as in the gastric cancer group (10.1 versus 7.6 months, respectively) (**Figure 2A and 2B**).

Since 1989 relative median survival of patients with M0 oesophageal adenocarcinomas increased from 12.0 to 30.0 months for the intestinal type, and from 12.0 to 19.2 months for the diffuse type. Survival of patients with M0 gastric adenocarcinoma increased only for patients with intestinal type carcinoma (from 22.8 to 27.6 months). For patients with diffuse type M0 gastric carcinoma relative median survival differed barely over the years (from 16.8 to 18.0 months) (**Table 2**).

Figure 2. Relative survival in the Netherlands between 1989 and 2015 according to the Lauren classification of patients with oesophageal or gastric adenocarcinoma. In panels A+B survival curves for all patients (both M0 and M1) with oesophageal (A) or gastric (B) adenocarcinomas are depicted. Panels C+D represent survival percentages at 1 year, 3 years and 5 years after diagnosis for patients with M0 disease per time period.

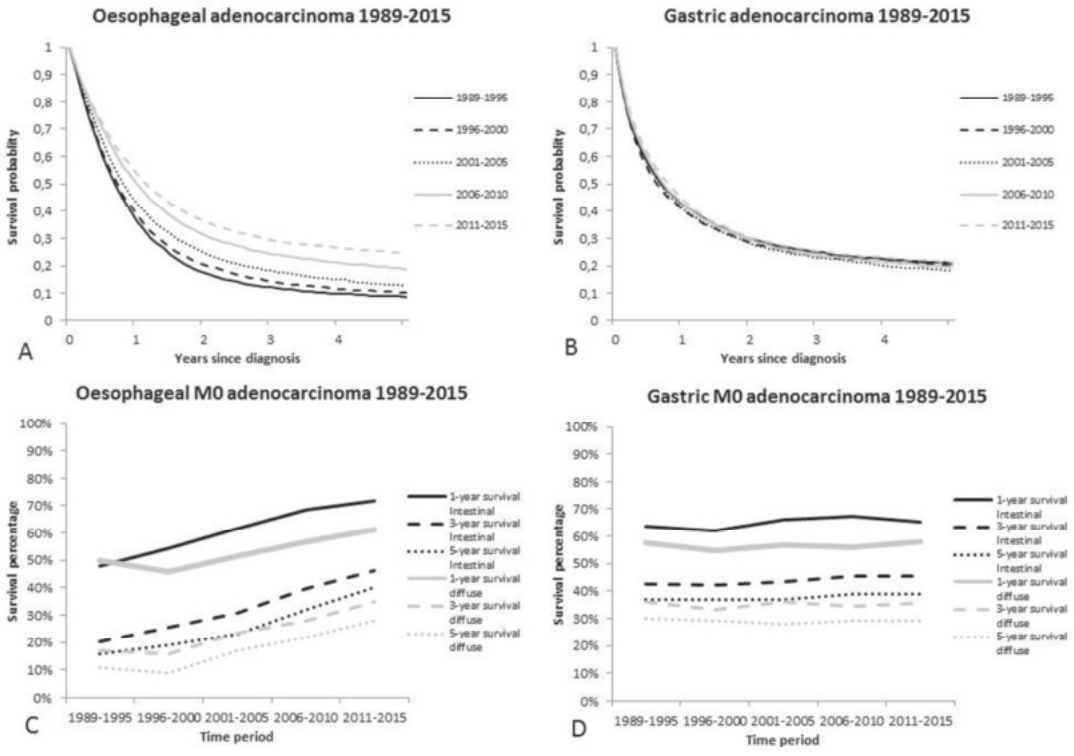


Table 1. Patient and tumour characteristics of all patients with oesophageal or gastric adenocarcinoma in the Netherlands between 1989 and 2015 according to the Lauren classification.

	Oesophageal adenocarcinoma (n=22.217)					Gastric adenocarcinoma (n=38.720)				
	Intestinal	**Diffuse**	**Mixed**	**Unknown**		**Intestinal**	**Diffuse**	**Mixed**	**Unknown**	
	N=14696	**N=3850**	**N=145**	**N=3526**		**N=17732**	**N=14326**	**N=254**	**N=6408**	
	N (%)	*N (%)*	*N (%)*	*N (%)*	*p-value*	*N (%)*	*N (%)*	*N (%)*	*N (%)*	*p-value*
Age at diagnosis, median (IQR)	68 (60-77)	68 (60-77)	64 (58-73)	70 (61-79)	<0.0001	74 (66-80)	69 (59-78)	69 (59-77)	74 (65-81)	<0.0001
Age at diagnosis					<0.0001					<0.0001
<50 years	883 (6.0%)	234 (6.1%)	12 (8.3%)	207 (5.9%)		590 (3.3%)	1661 (11.6%)	27 (10.6%)	308 (4.8%)	
50 - 59 years	2631 (17.9%)	675 (17.5%)	33 (22.8%)	560 (15.9)		1513 (8.5%)	2102 (14.7%)	37 (14.6%)	598 (9.3%)	
60 - 69 years	4409 (30.0%)	1189 (30.9%)	49 (33.8%)	928 (26.3%)		3888 (21.9%)	3528 (24.6%)	69 (27.2%)	1426 (22.3%)	
≥70 years	6773 (46.1%)	1752 (45.5%)	51 (35.2%)	1831 (51.9%)		11741 (66.2%)	7035 (49.1%)	121 (47.6%)	4076 (63.6%)	
Gender					<0.0001					<0.0001
Male	11726 (79.8%)	3130 (81.3%)	125 (86.2%)	2708 (76.8%)		11448 (64.6%)	7648 (53.4%)	163 (64.2%)	3881 (60.6%)	
Female	2970 (20.2%)	720 (18.7%)	20 (13.8%)	818 (23.2%)		6284 (35.4%)	6678 (46.6%)	91 (35.8%)	2527 (39.4%)	
Year of diagnosis					<0.0001					<0.0001
1989 - 1995	1414 (9.6%)	353 (9.2%)	17 (11.7%)	745 (21.1%)		5364 (30.3%)	3706 (25.9%)	84 (33.1%)	3166 (49.4%)	
1996 - 2000	1649 (11.2%)	417 (10.8%)	12 (8.3%)	677 (19.2%)		3459 (19.5%)	2481 (17.3%)	46 (18.1%)	1403 (21.9%)	
2001 - 2005	2698 (18.4%)	689 (17.9%)	32 (22.1%)	739 (21.0%)		3240 (18.3%)	2595 (18.1%)	26 (10.2%)	883 (13.8%)	
2006 - 2010	3877 (26.4%)	1046 (27.2%)	40 (27.6%)	735 (20.8%)		2973 (16.8%)	2730 (19.1%)	31 (12.2%)	585 (9.1%)	
2011 - 2015	5058 (34.4%)	1345 (34.9%)	44 (30.3%)	630 (17.9%)		2696 (15.2%)	2814 (19.6%)	67 (26.4%)	371 (5.8%)	
Tumour location					<0.0001					<0.0001
Cervical oesophagus	35 (0.2%)	12 (0.3%)	0 (0.0%)	19 (0.5%)						
Upper 1/3 oesophagus	153 (1.0%)	33 (0.9%)	0 (0.0%)	81 (2.3%)						
Middle 1/3 oesophagus	939 (6.4%)	212 (5.5%)	7 (4.8%)	373 (10.6%)						
Lower 1/3 oesophagus	12769 (86.9%)	3353 (87.1%)	133 (91.7%)	2795 (79.3%)						
Overlapping	478 (3.3%)	156 (4.1%)	5 (3.4%)	160 (4.5%)		4105 (23.2%)	4619 (32.2%)	69 (27.2%)	1835 (28.6%)	
NOS	322 (2.2%)	84 (2.2%)	0 (0.0%)	98 (2.8%)		1437 (8.1%)	1108 (7.7%)	16 (6.3%)	779 (12.2%)	
Fundus						647 (3.6%)	318 (2.2%)	10 (3.9%)	240 (3.7%)	
Corpus						2818 (15.9%)	2093 (14.6%)	32 (12.6%)	853 (13.3%)	
Antrum						5336 (30.1%)	3999 (27.9%)	75 (29.5%)	1518 (23.7%)	
Pylorus						1309 (7.4%)	966 (6.7%)	27 (10.6%)	444 (6.9%)	
Small curvature						1479 (8.3%)	895 (6.2%)	23 (9.1%)	502 (7.8%)	
Greater curvature						601 (3.4%)	328 (2.3%)	2 (0.8%)	237 (3.7%)	

cT stage*: *n*(%)					<0.0001				<0.0001
cTis	97 (0.7%)	2 (0.1%)	0 (0.0%)	4 (0.1%)		41 (0.2%)	3 (0.0%)	0 (0.0%)	12 (0.2%)	
cT1	850 (5.8%)	122 (3.2%)	11 (7.6%)	137 (3.9%)		619 (3.5%)	335 (2.3%)	7 (2.8%)	154 (2.4%)	
cT2	2391 (16.3%)	615 (16.0%)	21 (14.5%)	435 (12.3%)		2102 (11.9%)	2049 (14.3%)	41 (16.1%)	444 (6.9%)	
cT3	4258 (29.0%)	1269 (33.0%)	68 (46.9%)	716 (20.3%)		854 (4.8%)	753 (5.3%)	13 (5.1%)	316 (4.9%)	
cT4	754 (5.1%)	255 (6.6%)	4 (2.8%)	284 (8.1%)		2311 (13.0%)	2136 (14.9%)	38 (15.0%)	1095 (17.1%)	
Unknown	6346 (43.2%)	1587 (41.2%)	41 (28.3%)	1950 (55.3%)		11805 (66.6%)	9050 (63.2%)	155 (61.0%)	4387 (68.5%)	
cN stage*: *n*(%)					<0.0001				<0.0001
cN0	4651 (31.6%)	1118 (29.0%)	51 (35.2%)	866 (24.6%)		4853 (27.4%)	4038 (28.2%)	68 (26.8%)	1051 (16.4%)	
cN+	6972 (47.4%)	2001 (52.0%)	79 (54.5%)	1584 (44.9%)		4787 (27.0%)	3853 (26.9%)	91 (35.8%)	1509 (23.5%)	
Unknown	3073 (20.9%)	731 (19.0%)	15 (10.3%)	1076 (30.5%)		8092 (45.6%)	6435 (44.9%)	95 (37.4%)	3848 (60.0%)	
cM stage*: *n*(%)					<0.0001				<0.0001
cM0	8911 (60.6%)	2373 (61.6%)	105 (72.4%)	1702 (48.3%)		10207 (57.6%)	7915 (55.2%)	167 (65.7%)	2689 (42.0%)	
cM+	4631 (31.5%)	1189 (30.9%)	26 (17.9%)	1347 (38.2%)		5274 (29.7%)	4683 (32.7%)	59 (23.2%)	2573 (40.2%)	
Unknown	1154 (7.9%)	288 (7.5%)	14 (9.7%)	477 (13.5%)		2251 (12.7%)	1728 (12.1%)	28 (11.0%)	1146 (17.9%)	
Clinical stage*					<0.0001				<0.0001
I†	1333 (9.1%)	230 (6.0%)	10 (6.9%)	150 (4.3%)		1350 (7.6%)	1034 (7.2%)	20 (7.9%)	283 (4.4%)	
II	2163 (14.7%)	545 (14.2%)	36 (24.8%)	356 (10.1%)		896 (5.1%)	781 (5.5%)	19 (7.5%)	175 (2.7%)	
III	2844 (19.4%)	933 (24.2%)	45 (31.0%)	554 (15.7%)		1337 (7.5%)	1203 (8.4%)	23 (9.1%)	524 (8.2%)	
IV	4631 (31.5%)	1189 (30.9%)	26 (17.9%)	1347 (38.2%)		5643 (31.8%)	4981 (34.8%)	65 (25.6%)	2708 (42.3%)	
Unknown	3725 (25.3%)	953 (24.8%)	28 (19.3%)	1119 (31.7%)		8506 (48.0%)	6327 (44.2%)	127 (50.0%)	2718 (42.4%)	

IQR, Inter Quartile Range. *Based on the 6th edition of the TNM classification. †Including carcinomas diagnosed as cTis.

Discussion

This study is the first in which nationwide incidence and survival figures for oesophageal and gastric adenocarcinomas have been assessed by histological subtype according to Lauren. Out of all subtyped adenocarcinoma cases, diffuse type tumours accounted for 21% of oesophageal adenocarcinomas and for 44% of gastric adenocarcinomas. Patients with intestinal type carcinoma of the oesophagus or stomach had better survival rates than those with diffuse type carcinoma. In the past decades, prognosis has mainly improved for patients with M0 oesophageal adenocarcinoma. Especially, survival of patients with M0 intestinal type oesophageal carcinoma markedly increased (from 12.0 to 30.0 months), whereas survival of patients with diffuse type gastric carcinoma remained almost unchanged. These results show that both in oesophageal and in gastric adenocarcinoma, histological subtyping identifies a subgroup of patients with a poor prognosis for whom exploration of additional or alternative treatment strategies might be warranted.

Patients with diffuse type gastric carcinomas were younger and more likely to be female than those with intestinal type gastric carcinoma. This has been described previously and underlines a difference in aetiology.[17–19] Intestinal type gastric carcinomas typically originate in a background of chronic inflammatory mucosal damage (reflux disease or *H. Pylori* induced gastritis), which, encouraged by multiple stimuli, progresses into cancer.[19,20] Diffuse type gastric carcinomas are most likely the result of genomic aberrations in genes related to cell-matrix interaction.[18,19,21–23]

Table 2. Relative median survival in months of patients with oesophageal or gastric adenocarcinoma according to the Lauren classification and according to clinical M stage in the Netherlands between 1989 until 2015. 126 Patients were excluded from the analysis due to incomplete follow-up data.

		All tumour stages				
		1989-1995	1996-2000	2001-2005	2006-2010	2011-2015
Oesophageal adenocarcinoma	**n=22.197**	7.1	8.4	9.6	12.0	13.2
Gastric adenocarcinoma	**n=38.614**	7.2	7.2	7.2	8.4	9.3
		M0 oesophageal adenocarcinoma				
Intestinal type	**n= 8911**	12.0	14.4	18.0	24.0	30.0
Diffuse type	**n= 2371**	12.0	10.8	13.2	16.8	19.2
		M0 gastric adenocarcinoma				
Intestinal type	**n=10.201**	22.8	24.0	26.4	28.8	27.6
Diffuse type	**n=7909**	16.8	15.6	16.8	18.0	18.0
		M1 oesophageal adenocarcinoma				
Intestinal type	**n=4629**	3.6	4.8	4.8	4.8	4.8
Diffuse type	**n=1189**	3.6	2.4	3.6	3.6	3.6
		M1 gastric adenocarcinoma				
Intestinal type	**n=5264**	2.4	2.4	2.4	3.6	3.6
Diffuse type	**n=4676**	2.4	2.4	2.4	2.4	3.6

In gastric cancer, the proportion of diffuse type carcinoma increased during the study period and exceeded the proportion of intestinal type carcinoma from 2011 onwards. In oesophageal cancer, the proportion of diffuse type carcinoma increased as well, but the majority remained of the intestinal type. These time trends should be interpreted with caution. In gastric cancer trials, stratification for histological subtype has become common practice over the years [24,25], while in oesophageal cancer the potential value of the Lauren classification was suggested only recently.[8] The observed trends might partly reflect the increasing documentation of the Lauren subtypes in gastric cancer, rather than a true shift.[26,27] Of note, a declining incidence of intestinal type gastric carcinoma with a corresponding (relative) increase in diffuse type gastric carcinoma has been described previously in Japan (1975-1989) and in the United States (1978-2005).[11,17] Therefore, there may be a genuine shift in the incidence of diffuse type gastric adenocarcinoma.

For oesophageal adenocarcinoma patients, overall survival increased from 7.1 to 13.2 months between 1989-2015. In this period, changes in oesophageal cancer treatment included the expanding use of neoadjuvant chemoradiotherapy from 2007 onwards, and centralisation of oesophageal cancer surgery (as of 2006).[4,7,28] Survival benefit was mainly seen in patients with M0 intestinal type oesophageal adenocarcinoma. However, a relevant survival improvement was also seen for patients with M0 diffuse type oesophageal adenocarcinoma. Both prolonged survival and differences in survival between intestinal and diffuse carcinomas became more apparent in the latest time cohorts (from 2006 onwards). The introduction of multimodality treatment is a possible explanation. This is supported by the observation that diffuse type tumours respond worse to neoadjuvant chemo- and chemoradiotherapy than intestinal type tumours.[8,29,30] It is most likely that survival differences between patients with intestinal and diffuse type carcinomas primarily originate from differences in natural tumour behaviour, supplemented with differences in treatment response in this era of multimodality therapy.

Despite significant changes in treatment, relative survival of patients with gastric adenocarcinoma improved only slightly in the Netherlands between 1989-2015 (from 7.2 to 9.3 months). For M0 gastric adenocarcinoma, multimodality treatment was increasingly implemented from 2006 onwards.[28] Furthermore, gastric cancer surgery was centralised in 2012 and D2 lymph node dissection became standard practice.[31,32] These developments seem to have had most impact on the prognosis of patients with M0 intestinal type gastric cancer, for whom relative survival increased from 22.8 to 27.6 months. Possibly, better staging and improved general health have played an additional role in the observed survival improvements. The prognosis of patients with diffuse type gastric cancer has remained largely unchanged.

As part of The Cancer Genome Atlas (TCGA) project 295 primary gastric adenocarcinomas were analysed, identifying four different subtypes of gastric cancer (Epstein-Barr virus positive tumours, microsatellite instable tumours, genomically stable tumours and chromosomal instable tumours).[23] Although diffuse type gastric carcinomas were found in all four TCGA subtypes, the majority of the diffuse type carcinomas was of

the genomically stable subtype and the intestinal type tumours were mostly classified as chromosomally instable. In a similar TCGA effort to classify oesophageal adenocarcinomas, only 1.4% of oesophageal adenocarcinomas was classified as genomically stable and the rest as chromosomally instable.[33] This contrasts the 21% classified as diffuse type oesophageal carcinoma in the present study. The reason for these discrepancy is unknown. It can be speculated that oesophageal carcinomas, that are morphologically classifiable as diffuse type, may actually most often be a dedifferentiated poorly cohesive form of intestinal type carcinomas. This, however, remains to be determined in future research. The actual clinical impact of the TCGA classifications is yet uncertain. In the future, it might help to select appropriate targeted therapies.[34]

In conclusion, it is for the first time that nationwide epidemiological data have been linked to the histopathological subtyping of oesophageal and gastric adenocarcinoma. The prognosis of patients with diffuse type cancers was significantly worse than that of patients with intestinal type cancers. Patients with M0 intestinal type oesophageal adenocarcinoma seem to have benefitted most from the widespread use of multimodality therapy. Among M0 patients, patients with diffuse type gastric adenocarcinoma improved to the least extent in their survival time during the study period. These findings may call for a differentiated treatment approach towards patients with adenocarcinoma of the oesophagus or stomach according to the histological subtypes of the Lauren classification.

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CHAPTER 3

The metastatic pattern of intestinal and diffuse type gastric carcinoma – a Dutch national cohort study.

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Abstract

Aim | The Lauren classification of gastric adenocarcinoma describes three histological subtypes, the intestinal, the diffuse and the mixed type carcinoma. The metastatic pattern of gastric adenocarcinoma by histological subtype has not been studied.

Methods | Gastric adenocarcinoma patients with metastatic disease at the time of diagnosis between 1999 and 2017 were identified through the Netherlands Cancer Registry. The Lauren classification was determined based on pathology reports archived in the Dutch Pathology Registry and was linked to individual cases in the Netherlands Cancer Registry.

Results | Among 8 231 newly diagnosed, metastatic and evaluable gastric adenocarcinoma patients, 57% had an intestinal type carcinoma, 38% patients had a diffuse type carcinoma and 5% had a mixed type carcinoma. Intestinal type carcinomas more often metastasized to the liver (57% versus 21%, $p<0.0001$) and lungs (13% versus 7%, $p<0.0001$), whereas diffuse type carcinomas more often metastasized to the peritoneum (58% versus 29%, $p<0.0001$) and bones (9% versus 6%, $p<0.0001$). Patients with a diffuse type carcinoma had a worse survival perspective regardless of the number or the location of the metastases.

Conclusion | In this national cohort study, metastatic gastric adenocarcinoma of the intestinal type had a predilection for the liver and that of the diffuse type for the peritoneum.

Introduction

In 2018, over 1 million patients were diagnosed with gastric carcinoma worldwide accounting for 5.7% of all cancer patients.[1] Gastric carcinoma is aggressive and up to 40% of the patients has metastatic disease at diagnosis.[2] Predilection sites for gastric carcinoma metastases are the peritoneum, liver, lungs and bones.[3] The survival of patients with metastatic gastric carcinoma is poor. Even with the administration of palliative systemic chemotherapy median survival remains around 7-8 months.[2,4]

The Lauren classification for gastric adenocarcinoma was introduced in 1965 and is still widely used to group gastric adenocarcinomas into subtypes based on histological characteristics.[5] The three subtypes are the intestinal type, the diffuse type and a combination of the two, the mixed type. Intestinal type carcinomas are arranged in glands and resemble adenocarcinomas of the large intestine whereas diffuse type carcinomas consist of poorly cohesive cells with little or no gland formation, that often, but not always, contain various proportions of signet ring cells.[5] The subtypes differ in epidemiology, tumour biology and survival perspective.[6,7] In a previous study, using NCR data it was documented that the diffuse type histology accounted for 44% of all gastric adenocarcinomas in the Netherlands.[8]

It has been hypothesised that the histological subtypes according to the Lauren classification have different metastatic patterns. Riihimäki et al. identified a difference in metastatic pattern between signet ring cell carcinomas and intestinal type carcinomas.[3] In that study, only 11% of the patients had a signet ring cell carcinoma. It is unknown whether their metastatic pattern can be extrapolated to the entire group of patients with diffuse type gastric adenocarcinomas. The aim of this study was to describe the metastatic pattern of gastric carcinoma by histological subtype according to Lauren. Next to that, the survival of patients with metastatic gastric carcinoma was studied in relation to the Lauren classification.

Methods

Study population

A nationwide population-based cohort study with data from the Netherlands Cancer Registry (NCR) was conducted. For the purpose of the study, all gastric adenocarcinoma patients with metastatic disease at diagnosis between 1999 and 2017 were identified. The NCR registers all newly diagnosed tumours in the Netherlands, by using the data of the nationwide network and registry of histo- and cytopathology (PALGA). Specifically trained data managers collect patient, tumour and treatment characteristics. Through linkage with the Municipal Administrative Database data on vital status were obtained. Dutch gastric cancer incidence figures are publicly available from the NCR.[9] Before 2008, the NCR consisted of several regional databases. The presence of metastatic disease at diagnosis was registered by all regions, but not all regions registered the location of the metastasis. Thus, due to regional registration differences, the location of the metastasis was

not known in all patients. These patients were excluded. This is not expected to introduce a selection bias in the cohort of study patients prior to 2008. No ethics approval was required according to the Central Committee on Research involving Human Subjects. However, the study was approved by the Privacy Review Board of the Netherlands Cancer Registry, the scientific committees of the Dutch Upper-GI Cancer Group (DUCG) and nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA).

Topography and morphology in the NCR are coded according to the *International Classification of Diseases for Oncology* (ICD-O)[10]. Patients with a primary tumour in the stomach were selected. The tumour location was categorised as follows: oesophagogastric junction (OGJ) and cardia (C16.0), proximal/middle stomach (fundus, corpus, and lesser and greater curvature) (C16.1, C16.2, C16.5, C16.6), distal stomach (antrum and pyloric region) (C16.3, C16.4), overlapping regions (C16.8), and not otherwise specified (C16.9). The distant metastatic locations involved: peritoneum (C17.0- C19.9, C20.9, C21.8, C2.9, C26.9, C48.1-C48.8, C49.4, C49.5 C52.9, C53.9-C53.3, C54.8, C54.9, C55.9, C56.9-C57.4, C57.8, C66.9-C67.1, C67.8, C67.9, C76.2, C76.3), liver (C22-C22.1), lung (C34-C34.9), extra-regional lymph nodes (C77-C77.9), bones (C40-C41.9), adrenal gland (C74-C74.9) and other. Additional variables used included: year of diagnosis, sex, age, clinical TNM stage and pathological TNM stage. The TNM classification changed over the years. From 1999 till 2002 the fifth edition was used, from 2003-2009 the sixth edition was used, from 2010-2016 the seventh edition was used and for the year 2017 the eight edition was used. The four TNM classifications were re-coded into a single uniform code for all included gastric carcinomas (**supplementary table S1**).[11]

For the years 1999-2015, the histological subtyping according to the Lauren classification was neither part of the NCR, nor part of the ICD-O coding. Through the use of a specifically designed algorithm, previously described by van der Kaaij *et al,* the Lauren classification for each tumour was defined based on the individual pathology reports from PALGA.[8] These PALGA data were linked to the corresponding NCR patient data. For the years 2016-2017 the PALGA data was updated and the same algorithm was used to define the Lauren classification.

Statistical analyses

Differences between groups were analysed using a chi-square test. A multivariable logistic regression analysis was performed in order to study differences in metastatic spread between groups, adjusted for year of diagnosis, sex, age, tumour location, clinical tumour (cT) stage, clinical node (cN) stage, number of metastatic locations and Lauren classification. Patients registered with a T0 tumour were removed from the analyses. Odds ratios (ORs) were calculated and presented with 95% confidence intervals (CIs). Kaplan-Meier curves were generated and compared by means of the log rank test. Survival time was defined as time from diagnosis to death or until February 2019. P-values of <0.05 were considered statistically significant. All statistical analyses were performed using SPSS v25 (IBM, Armonk, United States).

Results

In total, 34 943 patients were registered with gastric carcinoma between 1999 and 2017 in the NCR. Among them, 12 759 (37%) patients were documented to have metastatic disease at time of diagnosis. Over the years, the incidence of gastric carcinoma steadily declined from 2 101 patients in 1999 to 1 521 patients in 2017. The percentage of patients registered with metastatic disease at diagnosis increased from 31% in 1999 to 44% in 2017.

The location of the metastases was known in 10 631 patients. In this group of patients, the Lauren classification could be determined in 8 231 patients (77%). Among these patients, 4 724 (57%) had an intestinal type carcinoma, 3 112 (38%) had a diffuse type carcinoma and 395 (5%) had a mixed type carcinoma. Patient and tumour characteristics are presented in **Table 1**. Patients with a diffuse type carcinoma were more likely to be female and younger of age compared to patients with an intestinal type carcinoma. Intestinal type carcinomas were more likely to be located at the EGJ or cardia, whereas diffuse type carcinomas more often involved overlapping regions of the stomach.

Metastatic pattern

The metastatic pattern varied significantly by histological subtype (**Table 1**). Intestinal type carcinomas more often metastasised to the liver (57% versus 21%, $p<0.001$) and lungs (13% versus 7%, $p<0.001$), whereas diffuse type carcinomas metastasised more often to the peritoneum (58% versus 29%, $p<0.001$) and bones (9% versus 6%, $p<0.001$).

A multivariable logistic regression analysis showed that being diagnosed with a diffuse type carcinoma was associated with a higher risk for peritoneal metastases than being diagnosed with an intestinal type carcinoma (OR=2.76, 95%CI 2.48-3.06) (**Table 2**). Patients with a diffuse type carcinoma were significantly less prone to have liver (OR=0.22, 95%CI 0.20-0.25) and lung metastases (OR=0.68, 95%CI 0.58-0.81) compared to patients with intestinal type carcinomas. There was no significant difference in the risk for extra-regional lymph metastases between the different histological subtypes.

In all, 32% of the patients had metastatic disease in more than one location (**Table 1**). The risk of multiple metastatic locations did not differ between the histological subtypes. However, the risk of additional metastatic locations was different for the various metastatic sites. The majority of patients (73%-80%) with lung metastases had another metastatic location, whereas the majority of patients (59%-69%) with peritoneal metastases had no additional metastatic locations. An overview of metastatic locations per histological subtype is shown in **Table 3.**

Table 1: Baseline characteristics of the study population categorised by histological subtype according to the Lauren classification of gastric adenocarcinoma.

	Total N=8231 n (%)	**Intestinal N=4724** n (%)	**Diffuse N=3112** n (%)	**Mixed N=395** n (%)	p-value*
Year of diagnosis					<0.001
1999-2002	880 (11)	577 (12)	286 (9)	17 (4)	
2003-2007	1818 (22)	1109 (23)	642 (21)	67 (17)	
2008-2012	2924 (36)	1644 (35)	1134 (36)	146 (37)	
2013-2017	2609 (32)	1394 (30)	1050 (34)	165 (42)	
Sex					<0.001
Male	5433 (66)	3353 (71)	1829 (59)	251 (64)	
Female	2798 (34)	1371 (29)	1283 (41)	144 (36)	
Age at diagnosis					<0.001
<45 years	413 (5)	143 (3)	244 (8)	26 (7)	
46-60 years	1610 (20)	806 (17)	721 (23)	83 (21)	
61-75 years	3766 (46)	2186 (46)	1413 (45)	167 (42)	
>75 years	2442 (30)	1589 (34)	734 (24)	119 (30)	
Tumour location					<0.001
OGJ/cardia	2503 (30)	1775 (38)	634 (20)	94 (24)	
Proximal/Middle stomach	1698 (21)	962 (20)	654 (21)	82 (21)	
Distal stomach	1658 (20)	925 (20)	642 (21)	91 (23)	
Overlapping	1960 (24)	826 (17)	1023 (33)	111 (28)	
NOS	412 (5)	236 (5)	159 (5)	17 (4)	
cT stage^					<0.001
T0	2 (0)	0 (0)	2 (0)	0 (0)	
T1	109 (1)	62 (1)	39 (1)	8 (2)	
T2-3	2481 (30)	1357 (29)	982 (32)	142 (36)	
T4	1269 (15)	666 (14)	531 (17)	72 (18)	
Tx	4370 (53)	2639 (56)	1558 (50)	173 (44)	
cN stage^					<0.001
N0	1345 (16)	655 (14)	610 (20)	80 (20)	
N1-2	4074 (49)	2447 (52)	1423 (46)	204 (52)	
N3	326 (4)	185 (4)	128 (4)	13 (3)	
Nx	2486 (30)	1437 (30)	951 (31)	98 (25)	
Number of metastatic locations					0.42
1	5623 (68)	3200 (68)	2148 (69)	275 (70)	
>1	2608 (32)	1524 (32)	964 (31)	120 (30)	
Metastatic location#					
Peritoneum	3368 (41)	1347 (29)	1817 (58)	204 (52)	<0.001
Liver	3422 (42)	2674 (57)	642 (21)	106 (27)	<0.001
Lung	858 (10)	594 (13)	230 (7)	34 (9)	<0.001
Extra-regional lymph nodes	2361 (29)	1381 (29)	860 (28)	120 (30)	0.231
Bones	591 (7)	265 (6)	292 (9)	34 (9)	<0.001
Adrenal gland	210 (3)	140 (3)	58 (2)	12 (3)	<0.009
Other	477 (6)	221 (5)	226 (7)	30 (8)	<0.001

OGJ, oesophagastric junction; NOS, not otherwise specified; cT, clinical Tumour stage; cN, clinical Nodal stage
*Chi-squared; ^after re-coding of four different TNM classifications (as depicted in Table S1)
#percentages within groups do not add up to 100% because of patients with more than one metastatic location.

Survival

Median overall survival of all patients was 4.1 months and was different for patients with an intestinal type carcinoma than for patients with a diffuse type carcinoma: 4.3 months versus 3.9 months, respectively (*p<0.001*) (**Figure 1**). In patients with metastatic disease at a single location, diffuse type carcinomas conferred a significantly worse overall survival compared to intestinal type carcinomas (median 4.4 versus 4.9 months, *p<0.001*). Location specific survival for patients with metastatic disease at a single location differed by histological subtype (diffuse versus intestinal) for peritoneal (median 4.6 versus 5.1 months, *p=0.001*), liver (median 3.3 versus 4.0 months, *p=0.02*), lung (median 5.1 versus 6.7 months, *p=0.026*) and extra-regional lymph node metastases (median 5.1 versus 8.1 months, *p<0.001*), respectively.

Table 2: Multivariable regression analysis for different locations of metastatic disease.

	Peritoneal metastases			Liver metastases			Lung metastases			Extra-regional lymph node metastases		
	OR	(95% CI)	p-value	OR	(95% CI)	p-value	OR	(95% CI)	p-value	OR	(95% CI)	p-value
Year of diagnosis												
1999-2002	1.00			1.00			1.00			1.00		
2003-2007	0.98	(0.81-1.18)	0.84	0,9	(0.71-1.02)	0.08	1.15	(0.84-1.58)	0.41	1.24	(0.99-1.55)	0.06
2008-2012	1.13	(0.94-1.35)	0.18	0.71	(0.59-0.84)	<0.001	1.20	(0.88-1.62)	0.26	1.46	(1.18-1.80)	<0.01
2013-2017	1.39	(1.15-1.68)	0.001	0.75	(0.63-0.91)	0.003	1.05	(0.76-1.45)	0.77	1.37	(1.10-1.71)	<0.01
Sex												
Male	1.00			1.00			1.00			1.00		
Female	1.48	(1.33-1.64)	<0.001	0.67	(0.60-0.74)	<0.001	0.03	(0.70-0.99)	0.06	0.84	(0.74-0.94)	<0.01
Age at diagnosis												
<45 years	1.00			1.00			1.00			1.00		
46-60 years	0.74	(0.58-0.95)	0.02	1.13	(0.87-1.47)	0.38	1.12	(0.74-1.70)	0.52	1.10	(0.85-1.43)	0.48
61-75 years	0.62	(0.49-0.78)	<0.001	1.47	(1.14-1.88)	0.003	1.56	(1.05-2.31)	0.02	0.96	(0.76-1.23)	0.76
>75 years	0.52	(0.41-0.66)	<0.001	1.64	(1.27-2.12)	<0.001	1.96	(1.31-2.94)	0.001	0.99	(0.76-1.27)	0.91
Tumour location												
OGJ/cardia	1.00			1.00			1.00			1.00		
Proximal/Middle stomach	2.38	(2.06-2.76)	<0.001	0.83	(0.72-0.96)	0.009	0.58	(0.47-0.72)	<0.001	0.62	(0.53-0.72)	<0.001
Distal stomach	2.65	(2.28-3.06)	<0.001	0.70	(0.61-0.81)	<0.001	0.54	(0.43-0.68)	<0.001	0.72	(0.61-0.84)	<0.001
Overlapping	3.56	(3.09-4.11)	<0.001	0.54	(0.47-0.63)	<0.001	0.45	(0.35-0.56)	<0.001	0.65	(0.55-0.75)	<0.001
NOS	2.49	(1.97-3.16)	<0.001	0.70	(0.55-0.88)	0.003	0.76	(0.52-1.09)	0.14	0.58	(0.43-0.77)	<0.001
cT stage												
T1	1.00			1.00			1.00			1.00		
T2-3	2.06	(1.31-3.24)	0.001	0.58	(0.37-0.87)	0.013	0.93	(0.44-1.96)	0.85	1.10	(0.68-1.78)	0.71
T4	2.99	(1.89-4.73)	<0.001	0.55	(0.35-0.83)	0.001	0.92	(0.43-1.96)	0.83	0.74	(0.45-1.22)	0.23
Tx	1.17	(0.75-1.83)	0.49	0.90	(0.57-1.32)	0.62	1.19	(0.57-2.47)	0.65	1.05	(0.65-1.70)	0.84
cN stage												
N0	1.00			1.00			1.00			1.00		
N1-2	0.37	(0.32-0.43)	<0.001	1.48	(1.27-1.71)	<0.001	1.01	(0.79-1.28)	0.95	3.16	(2.64-3.77)	<0.001
N3	0.26	(0.19-0.34)	<0.001	1.06	(0.80-1.41)	0.40	0.83	(0.54-1.25)	0.37	6.53	(4.88-8,74)	<0.001
Nx	0.75	(0.64-0.87)	<0.001	1.41	(1.20-1.66)	<0.001	0.76	(0.58-0.99)	0.05	1.19	(0.97-1.46)	0.09

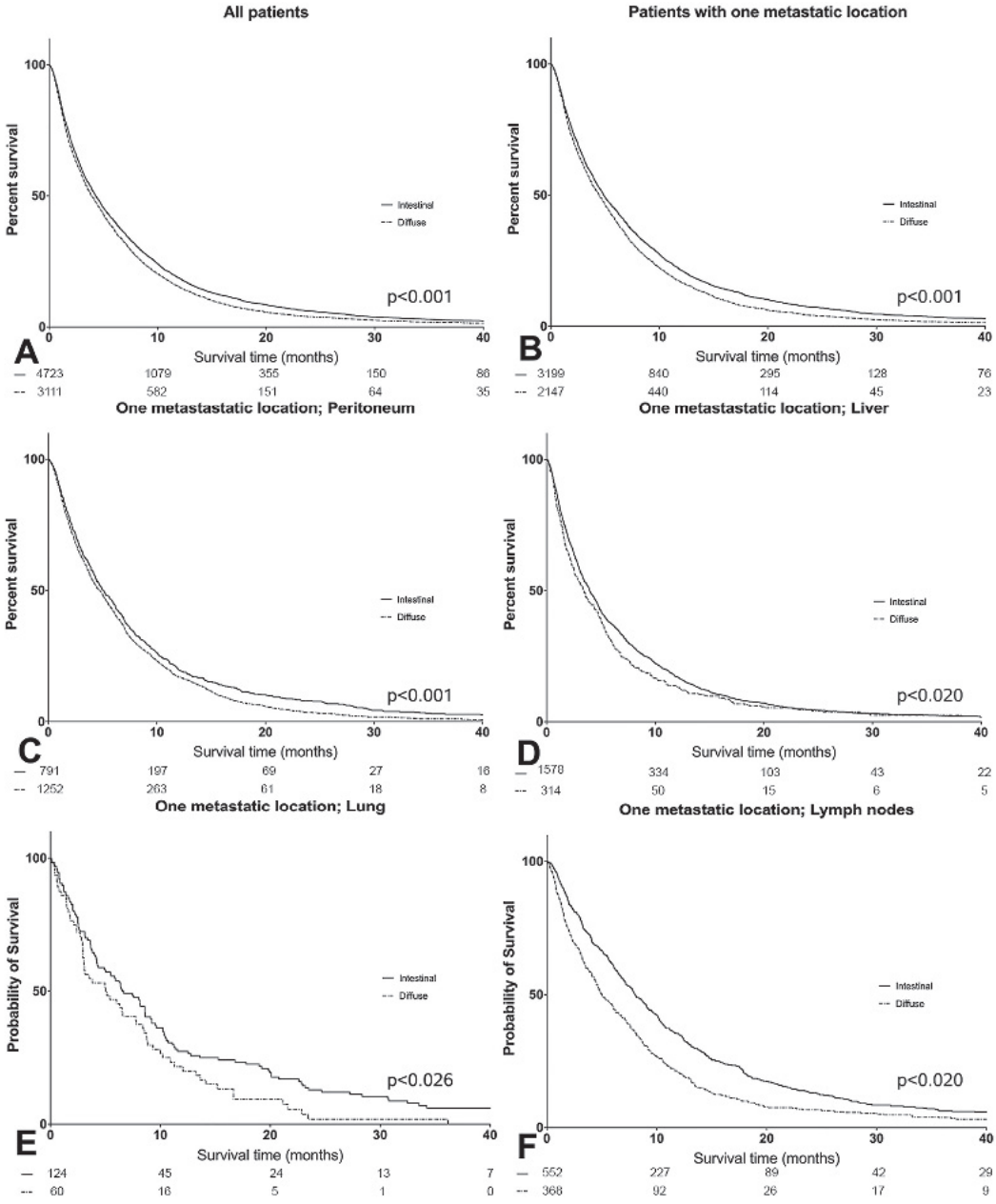
Number of metastatic locations												
1	1.00			1.00			1.00			1		
>1	1.62	(1.46-1.81)	<0.001	2.85	(2.55-3.17)	<0.001	9.7	(8.16-11.55)	<0.001	5.36	(4.80-5.99)	<0.001
Lauren classification												
Intestinal	1.00			1.00			1.00			1.00		
Diffuse	2.76	(2.48-3.06)	<0.001	0.22	(0.20-0.25)	<0.001	0.68	(0.57-0.81)	<0.001	1.09	(0.97-1.22)	0.17
Mixed	2.14	(1.71-2.67)	<0.001	0.32	(0.25-0.41)	<0.001	0.79	(0.53-1.16)	0.79	1.20	(0.93-1.54)	0.16

HR, Hazard ratio; 95% CI, confidence interval; OGJ, oesophagogastric junction; NOS, not otherwise specified; cT stage, clinical Tumour stage; cN stage, clinical Node stage.
Results of metastases in the bones, adrenal gland and other locations not shown due to low incidence numbers.

Table 3: Distribution of metastatic locations per histological subtype according to the Lauren classification of gastric adenocarcinoma.

	Peritoneal metastases			**Liver metastases**			**Lung metastases**			**Extra-regional lymph node metastases**		
	Intestinal	Diffuse	p-value	Intestinal	Diffuse	p-value	Intestinal	Diffuse	p-value	Intestinal	Diffuse	p-value
Number of metastatic locations												
1	792 (59%)	1253 (69%)	<0.001	1579 (59%)	315 (49%)	<0.001	125 (20%)	61 (27%)	0.09	552 (40%)	368 (43%)	0.19
>1	555 (41%)	564 (31%)		1095 (41%)	327 (51%)		499 (80%)	169 (73%)		829 (60%)	492 (57%)	
Additional metastatic sites											(0%)	
Peritoneum	**x**	**x**		336 (13%)	130 (20%)	<0.001	66 (11%)	48 (21%)	<0.001	194 (14%)	214 (25%)	<0.001
Extra-regional lymph nodes	194 (14%)	214 (12%)	0.03	542 (20%)	156 (24%)	0.03	201 (32%)	75 (33%)	0.74	**x**	**x**	
Liver	336 (25%)	130 (7%)	<0.001	**x**	**x**		355 (57%)	75 (33%)	<0.001	542 (39%)	156 (18%)	<0.001
Lung	66 (5%)	48 (3%)	0.001	355 (13%)	75 (12%)	0.28	**x**	**x**		201 (15%)	75 (9%)	<0.001
Bones	35 (3%)	57 (3%)	0.37	115 (4%)	43 (7%)	0.1	42 (7%)	29 (13%)	0.01	96 (7%)	102 (12%)	<0.001
Adrenal gland	18 (1%)	15 (1%)	0.16	67 (3%)	13 (2%)	0.48	27 (4%)	10 (4%)	0.90	54 (4%)	23 (3%)	0.12
Other	52 (4%)	95 (5%)	0.07	84 (3%)	27 (4%)	0.18	36 (6%)	20 (9%)	0.18	58 (4%)	65 (8%)	<0.01

Figure 1: Kaplan-Meier survival curves by histological subtype according to the Lauren classification of gastric adenocarcinoma for (**A**) all patients, (**B**) patients with metastases at a single location, (**C**) patients with peritoneal metastases only, (**D**) patients with liver metastases only, (**E**) patients with lung metastases only and (**F**) patients with extra-regional lymph node metastasis only. The number at risk at 0, 10, 20, 30 and 40 months is displayed below every survival curve.



Discussion

The Lauren classification has been used for decades to classify gastric adenocarcinomas into three subtypes based on histological characteristics, the intestinal, the diffuse and the mixed type.[5] In this nationwide cohort study of gastric cancer patients with metastatic disease at time of diagnosis, it was shown that the histological subtypes differed in metastatic pattern and in survival. Metastatic gastric adenocarcinoma of the intestinal type had a predilection for the liver and that of the diffuse type for the peritoneum. This resembles the metastatic pattern of gastric signet ring cell carcinomas.[3]

Over the years, the gastric cancer incidence decreased, a trend that is seen in various Western countries.[12] This has been related to *H. pylori* eradication and improvement in lifestyle.[13] Following the present study results, the percentage of patients with metastatic disease at time of diagnosis increased over the years. This can be related to an increased usage of (improved) diagnostic tools such as the CT-scan, PET-scan and staging laparoscopy. The latter has been recommended in the Dutch guidelines for patients with cT3-T4 gastric cancer since 2016.[14]

The clinical implication of the observed differences in metastatic patterns between intestinal and diffuse type gastric carcinoma remains to be established. However, treatment of oligometastatic disease in gastric carcinoma is widely studied, for example, hepatic resection for liver metastases and HIPEC therapy for peritoneal metastases.[15-19] Even a small case series of the surgical management of pulmonary metastases from gastric cancer origin was published.[20] If these oligometastatic treatment options appear to be of value in the treatment of metastatic gastric cancer, knowledge of the metastatic patterns becomes clinically more relevant and the Lauren classification could help to fine-tune follow-up protocols and additional treatment strategies. For instance, it could be hypothesised that prophylactic HIPEC therapy might be indicated for patients with diffuse type carcinomas due to the high risk of peritoneal metastases, whereas for patients with intestinal type carcinomas this might not have additional value.[21] Individualised treatment of gastric cancer based on the Lauren classification is not new; it was established before that the Lauren classification can help to select which chemotherapy regimen will most likely benefit the patient.[6,22,23]

Since the publication of the Cancer Genome Atlas (TCGA) data for gastric adenocarcinoma, it is known that the intestinal and diffuse type carcinomas are inherently different.[7] The diffuse type carcinomas are most frequently genomically stable, whereas the intestinal type carcinomas are chromosomally unstable. The differences in metastatic pattern underline that, although originating from the same organ, the diffuse type and intestinal type gastric adenocarcinomas are different disease entities and should, perhaps, be treated differently. Of note, there is growing evidence that the micro-satellite unstable tumours, which are associated with the intestinal type histology, respond well to

immunotherapy (e.g. anti-PD-L1 and anti-CTLA4 antibodies), but poorly to chemotherapy.[24-26] A differentiated treatment approach to intestinal and diffuse type gastric adenocarcinoma might be the future. It is therefore important that clinical trials involving (metastatic) gastric cancer patients stratify for histological subtype.

Median survival of all patients in this study was 4.1 months, comparable to published survival rates for metastatic gastric cancer patients.[27] Between the patients with an intestinal type carcinoma and those with a diffuse type carcinoma a small but significant difference in survival was found. This was seen for the whole study population as well as for patients with metastatic disease at a single location. A worse survival for patients with diffuse type gastric cancer as compared to those with intestinal type gastric cancer has been shown before.[23] These results emphasise that the Lauren classification can be used as a prognostic factor for metastatic gastric cancer patients.[27]

Strengths of the present study include its nation-wide, population-based design and its large number of included patients. The linkage of individual NCR records with pathology reports of the Dutch Pathology Registry (PALGA) is unique. A limitation of this study is that the Lauren classification in the years 1999-2015 was created by a syntax.[8] Although this syntax has been validated, it can be expected that it is not entirely accurate. However, classifying mistakes are most likely made in all directions and due to the large study population these wrongly classified patients will not have influenced the results.

In conclusion, in this national cohort study, the metastatic pattern of gastric adenocarcinoma differed by histological subtype according to the Lauren classification. Intestinal type carcinomas more often metastasised to the liver, whereas diffuse type carcinomas more often metastasised to the peritoneum. Moreover, the Lauren classification was prognostic for overall survival. Patients with intestinal type carcinomas had a slightly better overall survival compared to patients with diffuse type carcinomas. In the future, these differences in metastatic pattern and in survival could become clinically relevant when personalised therapy is introduced for the different gastric cancer disease entities.

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Supplementary table

Table S1: Re-coding of four different TNM classifications (editions) into a single uniform code for all included gastric carcinomas (1999-2017) from the Netherlands Cancer Registry.

Year of registration in the NCR	**1999-2002**	**2003-2009**	**2010-2016**	**2017**	**1999-2017**	
Edition of TNM classification	5	6	7	8	**Uniform code**	**Description**
T stage	T0	T0	T0	T0	**T0**	No evidence of tumor
	Tis	Tis	Tis	Tis	**Tis**	Carcinoma in situ
	T1	T1	T1, T1a, T1b	T1, T1a, T1b	**T1**	Tumor invades lamina propria, muscularis mucosea, submucosa
	T2	T2, T2a, T2b	T2, T3	T2, T3	**T2-3**	Tumor invades muscularis propria, subserosal connective tissue, gastrocolic or hepatic ligaments, omentum
	T3, T4	T3, T4	T4, T4a, T4b	T4, T4a, T4b	**T4**	Tumor invades the serosa (visceral peritoneum) or adjacent structures
N stage	N0	N0	N0	N0	**N0**	Regional nodes not involved
	N1	N1	N1, N2	N1, N2	**N1-2**	1-6 regional nodes involved
	N2, N3	N2, N3	N3, N3a, N3b	N3, N3a, N3b	**N3**	>6 regional nodes involved
	Nx	Nx	Nx	Nx	**Nx**	Regional node status unknown

TNM, Tumor Nodes Metastasis; NCR, Netherlands Cancer Registration

CHAPTER 4

Synchronous peritoneal metastases of gastric cancer origin: incidence, treatment and survival of a nationwide Dutch cohort.

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Abstract

Introduction | The peritoneum is a predilection site for gastric cancer metastases. Current standard treatment for gastric cancer patients with synchronous peritoneal metastases is palliative systemic therapy. However, its efficacy is largely unknown. The aim of this study was to investigate the incidence, treatment and survival patterns of gastric cancer patients with synchronous peritoneal metastases in the Netherlands.

Methods | All newly diagnosed gastric adenocarcinoma patients with synchronous peritoneal metastases between 1999 and 2017 were selected from the Netherlands Cancer Registry (NCR). Incidence, treatment and survival patterns were analysed.

Results | In total, 3.773 patients were identified from the NCR. The incidence of synchronous peritoneal metastases in gastric cancer patients increased from 18% in 2008 to 27% in 2017. The use of systemic therapy increased from 15% in 1999-2002 to 43% in 2013-2017 ($p<0.001$). The median survival of the entire cohort did not significantly increase over time. Median survival of patients treated with systemic therapy increased from 7.4 months in 1999-2002 to 9.4 months in 2013-2017 ($p=0.005$). In contrast, median survival of patients *not* treated with systemic therapy decreased from 3.3 months in 1999-2002 to 2.1 months in 2013-2017 ($p<0.001$). Some clinical and pathological data such as the extent of the peritoneal metastases were not available.

Conclusion | Synchronous peritoneal metastases are increasingly diagnosed in gastric cancer patients. In recent years, more patients were treated with systemic treatment and survival of these patients increased. However, as survival of the entire group did not improve over time, the effect of systemic therapy remains unknown.

Introduction

Worldwide, the incidence of gastric cancer has steadily declined over the last 50 years.[1] This has been linked to a decline in Helicobacter pylori infections and their treatment, and to dietary changes.[1,2] In the Netherlands, the incidence of gastric cancer has decreased from 2054 patients in 1999 to 1535 in 2017.[3] Multimodality treatment consisting of a surgical resection with perioperative systemic therapy has become standard therapy for patients treated with curative intent.[4,5] However, survival outcomes of gastric cancer patients remain poor with a 5-year overall survival of 18-25 percent for all stages.[6] A major reason for this dismal survival is the high percentage of patients presenting with metastatic disease at diagnosis. A Dutch population-based study showed that about 40% of patients presented with synchronous metastatic disease.[7] Most common sites for gastric cancer metastases are the liver, the peritoneum, the lung, and the bones.[8]

Median survival of all gastric cancer patients with synchronous metastases is around 4 months.[9,10] For patients presenting with peritoneal metastases, median survival is 3 to 4 months.[11] Currently, the only treatment option for these patients in the Netherlands is palliative systemic therapy. Its efficacy in improving survival is subject of debate.[11,12]

In the past decade, new diagnostic tools and treatment options were introduced for gastric cancer patients. For example, a diagnostic laparoscopy has become part of the standard diagnostic work-up of newly diagnosed locally advanced gastric cancer patients as it was added to the Dutch guideline in 2016.[13] It is unclear whether these changes in diagnostic work-up and treatment options have affected incidence and outcome of patients with synchronous peritoneal metastases of gastric cancer origin. This study aimed to analyse the incidence, treatment strategies and survival of gastric cancer patients with synchronous peritoneal metastases in the Netherlands over the past two decades.

Methods

Data collection

A nationwide population-based cohort study with data from the Netherlands Cancer Registry (NCR) was conducted. The NCR registers all newly diagnosed malignancies in the Netherlands through notification by the pathological anatomical national automated archive (PALGA) and administrative hospital data. Specially trained data managers collect patient, tumour and treatment characteristics. Through linkage with the Municipal Administrative Database, in which all records of births, deaths and emigrations in the Dutch population are registered, data on vital status were obtained of all patients until February 2019.

Patient selection

All patients who were diagnosed with a gastric adenocarcinoma (non-cardia only) and synchronous peritoneal metastases between 1999 and 2017 were included. Before 2008, there were regional differences in the registration of certain items, such as the location of the metastases which was not registered in all regions. As a result, the incidence numbers of peritoneal metastases in gastric cancer patients before 2008 are not complete for the entire nation and are therefore not reported. As this is only due to regional registration differences, it is not expected to introduce a selection bias in the analyses regarding treatment and survival data of patients with synchronous peritoneal metastases prior to 2008.

Until 2015, the diagnostic methods used to detect peritoneal metastases were not registered in the NCR; this information is therefore not included. Topography, morphology and metastatic locations are coded in the NCR according to the third edition of the International Classification of Disease for Oncology (ICD-O3).[14] The tumour location was categorised as follows: proximal/middle stomach (fundus, corpus, and lesser and greater curvature) (C16.1, C16.2, C16.5, C16.6), distal stomach (antrum and pyloric region) (C16.3, C16.4), overlapping regions (C16.8), and not otherwise specified (C16.9). The following ICD-O codes for metastatic locations were categorised as peritoneal metastases: C48.1-C48.8. Additional variables that were collected: age, sex, clinical TNM stage, pathological TNM stage, year of diagnosis, Lauren classification and the administered therapy (gastric cancer resection, systemic therapy, cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC), and 'other', the latter mostly consisting of radiotherapy or local treatment of metastases (by surgery or radiotherapy).

TNM classification

Over the years, the subsequent UICC TNM classifications have been used: the fifth (1999-2002), the sixth (2003-2009), the seventh (2010-2016), and the eighth (since 2017). All TNM classifications were re-coded to establish uniformity (table S1).

Statistical analysis

European Standardised Rate (ESR) incidence per 100.000 person-years was calculated according to the European standard population. Categorical variables were compared using a Chi-square test. Kaplan-Meier overall survival curves were compared by the log rank test. Overall survival was defined as time from diagnosis to death or until February 2019. A p-value $p < 0.05$ was considered statistically significant. Multivariable logistic regression analyses were performed in order to investigate an association between clinical characteristics and the administration of systemic therapy. Adjustments were made for: year of diagnosis, sex, age, tumour location, clinical tumour (cT) and clinical nodal (cN) stage, Lauren classification and number of metastatic locations. Due to the low number, patients

registered with a cT0 tumour were removed from the multivariable analyses. A multivariable Cox-regression analysis was performed to identify prognostic factors for overall survival stratified for systemic therapy and for patients with metastatic disease confined to the peritoneum or at multiple localisations adjusted for year of diagnosis, sex, age, tumour location, Lauren classification, cT stage and cN stage. All statistical analyses were performed using SPSS v25 (IBM, Armonk, United States) or SAS 9.4 (SAS Institute, North Carolina, United States).

Results

Incidence

Between 1999 and 2017, 3773 patients were registered in the NCR with gastric cancer and synchronous peritoneal metastases. In 2437 (65%) patients, the peritoneum was the only metastatic location. Most frequently affected other locations were the liver (n=656, 41%) and extra regional lymph nodes (n=558, 35%). While the ESR of all gastric cancer diagnoses decreased over time, the ESR of gastric cancer patients with synchronous peritoneal metastases remained stable from 2008 (1.19/100.000 person-years) to 2017 (1.10/100.000 person-years), resulting in an increased proportion of gastric cancer patients with synchronous peritoneal metastases over the years; from 18% (n=244) in 2008 to 27% (n=276) in 2017. (**Table 1**)

Table 1: Incidence of gastric cancer patients and gastric cancer patients with synchronous peritoneal metastases as registered in the Netherlands Cancer Registry (NCR).

	2008	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**
Gastric cancer patients *(n)*	1358	1333	1324	1317	1322	1275	1219	1128	1186	1042
ESR gastric cancer	6.48	6.11	5.99	5.82	5.73	5.47	5.02	4.54	4.63	3.98
Peritoneal metastases *(n)*	244	250	260	236	269	265	261	262	284	276
ESR gastric cancer with synchronous peritoneal metastases	1.19	1.17	1.20	1.07	1.20	1.17	1.11	1.10	1.18	1.10
Proportion *(%)*	18,0	18,8	19,6	17,9	20,3	20,8	21,4	23,2	23,9	26,5

ESR, European Standardised Rate

Baseline characteristics

Baseline characteristics stratified for time-period are summarised in **table 2**. The majority of patients was male (54%) and the median age was 68 years. The primary tumour was mainly located in the proximal/middle stomach (26%), distal stomach (26%), or overlapping regions (39%). In most cases, a clinical tumour stage of cT2-3 (30%) or cT4 (23%) and a clinical nodal stage of cN0 (24%) or cN1-2 (36%) was found (**Table 2**). Of note, the proportion of patients with diffuse type gastric cancer increased over time (34% from 1999-2002 to 45% from 2013-2017, p<0.001).

Table 2: Baseline characteristics of gastric cancer patients with synchronous peritoneal metastases.

Time period	1999-2002 n=413[#]	2003-2007 n=753[#]	2008-2012 n=1259	2013-2017 n=1348	p-value
Median age, years (range)	67 (22-91)	68 (16-92)	68 (19-94)	69 (25-100)	<0.001
Sex *n(%)*					0.005
Male	230 (56)	370 (49)	658 (52)	765 (57)	
Female	183 (44)	383 (51)	601 (48)	583 (43)	
Tumour location					<0.001
Proximal/middle stomach	99 (24)	159 (21)	316 (25)	388 (29)	
Distal stomach	118 (29)	186 (25)	332 (26)	352 (26)	
Overlapping	146 (35)	324 (43)	521 (41)	496 (37)	
NOS	50 (12)	84 (11)	90 (7)	112 (8)	
cT stage^ *n(%)*					<0.001
T0	1 (0)	3 (0)	2 (0)	1 (0)	
T1	4 (1)	11 (1)	17 (1)	2 (0)	
T2-3	22 (5)	113 (15)	318 (25)	674 (50)	
T4	146 (35)	174 (23)	276 (22)	253 (19)	
Tx	240 (58)	452 (60)	646 (51)	418 (31)	
cN stage^ *n(%)*					<0.001
N0	28 (7)	91 (12)	305 (24)	476 (35)	
N1-2	121 (29)	224 (30)	446 (35)	576 (43)	
N3	4 (1)	21 (3)	27 (2)	46 (3)	
Nx	260 (63)	417 (55)	481 (38)	250 (19)	
Lauren classification					<0.001
Intestinal type	129 (31)	209 (28)	373 (30)	346 (26)	
Diffuse type	139 (34)	295 (39)	567 (45)	601 (45)	
Mixed type	6 (1)	26 (3)	60 (5)	76 (6)	
Unknown	139 (34)	223 (30)	259 (21)	325 (24)	
Metastatic locations *n(%)*					<0.001
Peritoneal metastases only	288 (70)	508 (67)	809 (64)	832 (62)	
Peritoneal metastases and others	125 (30)	245 (33)	450 (36)	516 (38)	

NOS, not otherwise specified; cT, clinical tumour stage; cN, clinical nodal stage
[#]Incomplete numbers due to regional registry differences before 2008; ^after re-coding of four different TNM classifications (as depicted in Table S1)

Table 3: Treatment of gastric cancer patients with synchronous peritoneal metastases.

	Systemic chemotherapy	Primary tumour resection	CRS and HIPEC	Other*	None
	n (%)	*n (%)*	*n (%)*	*n (%)*	*n (%)*
Time period					
1999-2002	61 (15)	76 (18)	0 (0)	134 (32)	228 (55)
2003-2007	203 (27)	124 (16)	0 (0)	234 (31)	375 (50)
2008-2012	527 (42)	171 (14)	4 (0)	329 (26)	555 (44)
2013-2017	580 (43)	157 (12)	29 (2)	392 (29)	616 (46)
p-value	*<0.001*	*0.001*	*<0.001*	*0.002*	*<0.001*
Metastatic locations					
Peritoneal metastases only	887 (36)	443 (18)	27 (1)	776 (32)	1102 (45)
Peritoneal metastases and others	484 (36)	85 (6)	6 (<1)	307 (23)	664 (50)

CRS, cytoreductdive surgery; HIPEC, hyperthermic intraperitoneal chemotherapy *therepies registered as other, radiotherapy, metastasectomy

Treatment

The use of systemic treatment in gastric cancer patients with peritoneal metastases increased over time. In the period 1999-2002, 15% (n=61) of patients were treated with systemic therapy, whereas in the period 2013-2017, it was administered to 43% (n=580) of patients (p<0.001) (**Table 3**). Multivariable regression analysis showed that patients diagnosed in the more recent time cohorts (2003-2017), patients of younger age, patients with a primary tumour in the proximal/middle stomach, and patients with metastases confined to the peritoneum were more likely to undergo systemic therapy (**Table 4).**

Table 4: Multivariable logistic-regression analysis for the administration of systemic therapy in gastric cancer patients with synchronous peritoneal metastases.

	OR	(95% CI)	*p-value*
Year of diagnosis			
1999-2002	1.00		
2003-2007	2.45	(1.75-3.43)	*<0.001*
2008-2012	5.16	(3.75-7.12)	*<0.001*
2013-2017	4.81	(3.46-6.69)	*<0.001*
Sex			
Male	1.00		
Female	0.92	(0.79-1.07)	*0.25*
Age			
<45 years	1.00		
46-60 years	0.56	(0.41-0.75)	*<0.001*
61-75 years	0.23	(0.18-0.31)	*<0.001*
>75 years	0.05	(0.04-0.07)	*<0.001*
Tumour location			
Proximal/middle stomach	1.00		
Distal stomach	0.77	(0.63-0.95)	*0.02*
Overlapping	0.80	(0.67-0.97)	*0.02*
NOS	0.58	(0.43-0.79)	*0.001*
cT stage^			
T1	1.00		
T2-3	1.51	(0.67-3.42)	*0.32*
T4	1.10	(0.49-2.49)	*0.82*
Tx	1.10	(0.49-2.47)	*0.82*
cN stage^			
N0	1.00		
N1-2	1.21	(0.99-1.47)	*0.06*
N3	0.76	(0.47-1.21)	*0.24*
Nx	0.73	(0.59-0.9)	*0.003*
Lauren classification			
Intestinal type	1.00		
Diffuse type	0.94	(0.78-1.14)	*0.55*
Mixed type	1.11	(0.76-1.62)	*0.58*
Unknown	1.09	(0.88-1.35)	*0.46*
Metastatic locations			
Peritoneal metastases only	1.00		
Peritoneal metastases and others	0.87	(0.74-1.02)	*0.08*

NOS, not otherwise specified; cT, clinical Tumour stage; cN, clinical Nodal stage
^after re-coding of four different TNM classifications (as depicted in Table S1)

The proportion of patients undergoing primary tumour resection decreased over time, from 18% (n=76) in 1999-2002 to 12% (n=157) in 2013-2017 (p=0.001). CRS and HIPEC was only performed in the most recent time cohorts, on a very limited scale. The proportion of patients that received no treatment decreased from 55% (n=228) in 1999-2002 to 46% (n=616) in 2013-2017 (p<0.001).

Survival

Median overall survival of all gastric cancer patients with synchronous peritoneal metastases did not change significantly over time (**Table 5,** *p=0.065*). Also, no improved overall survival was seen in patients with metastases confined to the peritoneum (*p=0.051*). Finally, overall survival remained stable in patients with metastases at multiple locations (*p=0.633*).

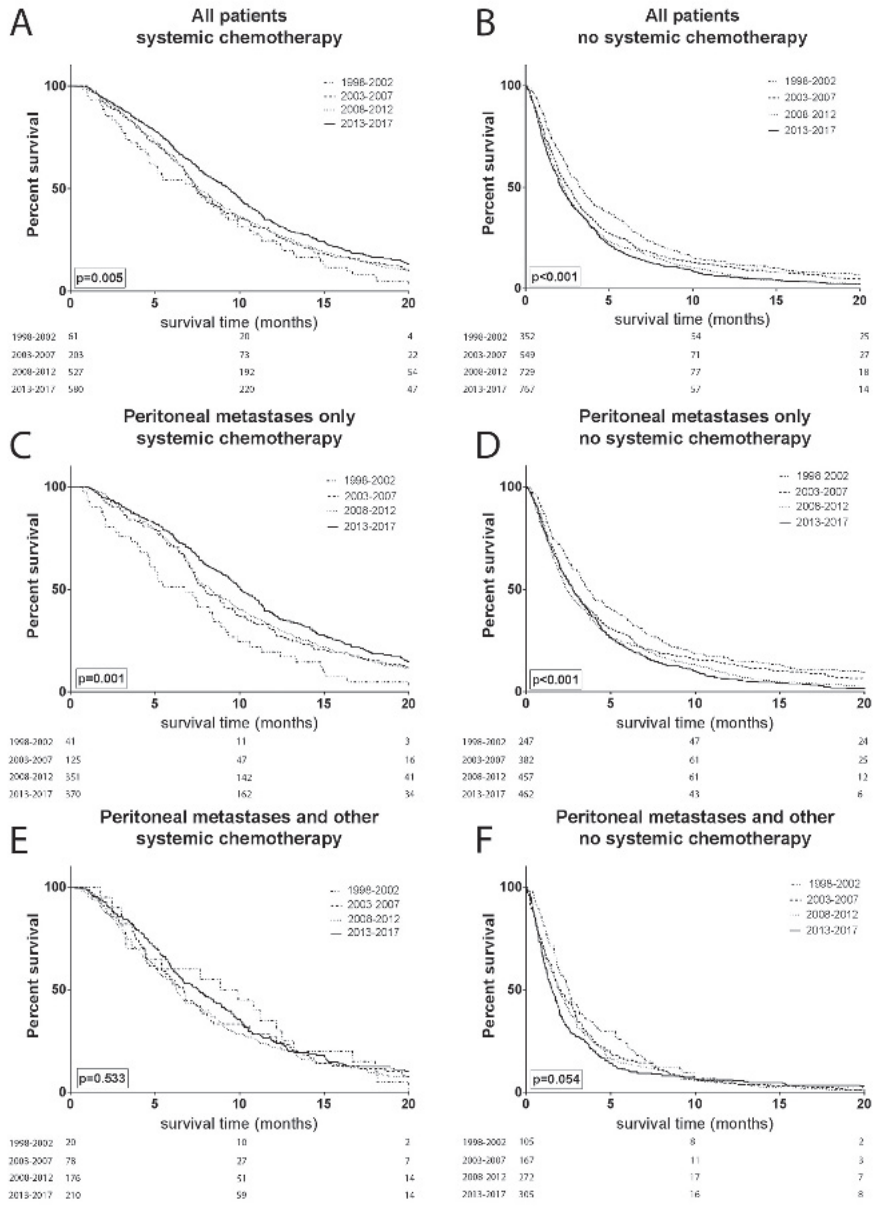
Table 5: Overall survival of gastric cancer patients with synchronous peritoneal metastases.

	1999-2002				**2003-2007**				**2008-2012**				**2013-2017**				***p-value#***
	n	(%)	MS	HR	n	(%)	MS	HR	n	(%)	MS	HR	n	(%)	MS	HR	
All patients	413		3.6		753		3.6		1259		4.1		1348		4.4		*p=0.065*
Systemic chemotherapy	61	(15)	7.4	Ref.	203	(27)	7.5	0.81	527	(42)	7.6	0.84	580	(43)	9.4	0.77	*p=0.005*
No systemic chemotherapy	352	(85)	3.3	Ref.	550	(73)	2.6	1.26^	732	(58)	2.2	1.45^	768	(57)	2.1	1.67^	*p<0.001*
*p-value**			*p=0.002*				*p<0.001*				*p<0.001*				*p<0.001*		
	n	(%)	MS	HR	n	(%)	MS	HR	n	(%)	MS	HR	n	(%)	MS	HR	
Peritoneal metastases only	288		3.9		508		3.8		809		4.9		832		5.3		*p=0.051*
Systemic chemotherapy	41	(14)	6.7	Ref.	125	(25)	8.0	0.61^	351	(43)	8.3	0.65^	370	(44)	10.0	0.62^	*p=0.001*
No systemic chemotherapy	247	(86)	3.6	Ref.	383	(75)	2.8	1.34^	458	(57)	2.4	1.60^	462	(56)	2.8	1.79^	*p<0.001*
*p-value**			*p=0.37*				*p<0.001*				*p<0.001*				*p<0.001*		
	n	(%)	MS	HR	n	(%)	MS	HR	n	(%)	MS	HR	n	(%)	MS	HR	
Peritoneal metastases and other	125		3.0		245		3.0		450		3.1		516		3.1		*p=0.633*
Systemic chemotherapy	20	(16)	8.9	Ref.	78	(32)	6.7	1.19	176	(39)	6.3	1.23	210	(41)	7.6	1.00	*p=0.533*
No systemic chemotherapy	105	(84)	2.5	Ref.	167	(68)	2.0	1.18	274	(61)	1.9	1.37^	306	(59)	1.5	1.61^	*p=0.054*
*p-value**			*p<0.001*				*p<0.001*				*p<0.001*				*p<0.001*		

MS,Median overall survival in months HR, Hazard ratio between time periods after multivariable adjustment for sex, age, tumour location, cT stage, cN stage and Lauren classification ^ Significant hazard ratio; *systemic chemotherapy versus no systemic chemotherapy; # median survival between time periods

Median overall survival increased over time in patients treated with systemic therapy (**Figure 1A,** *p=0.005*). Remarkably, the increase in overall survival was most evident between the latest two time cohorts, where an increase was observed from 7.6 months in 2008-2012 to 9.4 months in 2013-2017, whereas the proportion of patients treated with systemic therapy did not increase concordantly, from 42% to 43%. The same trend was observed in patients with metastases confined to the peritoneum (**Figure 1C,** *p=0.001*), but not in patients with peritoneal metastases and metastases at other locations (**Figure 1E,** *p=0.533*). In patients who did not undergo systemic therapy, the median overall survival decreased over time (**Figure 1B**, p<0.001). This trend was also seen in patients with metastases confined to the peritoneum (**Figure 1D**, p<0.001), but the trend was not significant in the group of patients with peritoneal metastases and metastases at other locations (**Figure 1F**, p=0.054)

Figure 1: Kaplan-Meier survival curves by time period for (**A**) all patients treated with systemic chemotherapy, (**B**) all patients not treated with systemic chemotherapy, (**C**) patients with peritoneal metastases only treated with systemic chemotherapy, (**D**) patients with peritoneal metastases only not treated with systemic chemotherapy (**E**) patients with peritoneal metastases and metastases at other locations treated with systemic chemotherapy, (**F**) patients with peritoneal metastases and metastases at other locations not treated with systemic chemotherapy.



After multivariable adjustment for sex, age, tumour location, cT and cN stage, the survival of patients with metastases confined to the peritoneum who were treated with systemic therapy improved over time, while the survival of patients with both peritoneal metastases and systemic metastases who were treated with systemic therapy did not significantly change. In patients who did not undergo systemic therapy, survival decreased over time, both in patients with peritoneal metastases only and in patients with metastases at multiple locations.

Discussion

In this nationwide cohort study, it was found that the proportion of gastric cancer patients diagnosed with synchronous peritoneal metastases increased over time. That is, there was a yearly increase in the absolute number of gastric cancer patients with synchronous peritoneal metastases while the incidence of gastric cancer itself decreased. At the end of the study period, in 2017, 27% of the newly diagnosed gastric cancer patients had synchronous peritoneal metastases, revealing the peritoneal cavity to be a clinically relevant and challenging metastatic site. In addition, an increase in the use of systemic therapy over time was observed. However, this did not result in a significant increase in the overall survival of gastric cancer patients with synchronous peritoneal metastases. A relatively high proportion of patients was documented to have a primary tumour without serosal involvement (<T4). However, it should be noted that stage grouping in this study was almost invariably based on clinical staging, which is known for its inaccuracy in gastric cancer.[15] In addition, T-stage was often unknown (**Table 2**). From other studies, it is known that advanced tumour stage is associated with the presence of peritoneal metastases.[16]

Recently, a shift in the distribution of histological subtypes of gastric adenocarcinoma was described with the diffuse type now being the predominant subtype.[9,17] This might partially explain the increase in patients with peritoneal metastases, as the diffuse type gastric cancer is more prone to metastasise to the peritoneum than the intestinal type.[18,19] Additional explanations for the increased frequency of detecting peritoneal metastases can be found in the diagnostic work-up of gastric cancer. The accuracy of Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) has improved over the years.[20,21] Furthermore, the diagnostic laparoscopy has been added to the Dutch national guideline for staging gastric cancer patients with locally advanced disease in 2016.[13] The diagnostic laparoscopy is essential for the evaluation of the peritoneum in gastric cancer and it avoids unnecessary laparotomies.[22] The efficacy of the diagnostic laparoscopy in gastric cancer staging is currently under investigation.[23]

With the introduction of new systemic therapies, such as taxanes, trastuzumab, ramucirumab, and trifluoride/tipiracil, the armamentarium of the medical oncologist expanded during the last decades.[24-26] Over the years, the proportion of patients with gastric cancer and synchronous peritoneal metastases who were treated with systemic treatment increased from 15% in 1999-2002 to 42% in 2008-2012 and remained stable

thereafter. Nevertheless, the overall survival of all gastric cancer patients with peritoneal metastases did not improve in this time period. This finding questions the benefit of systemic therapy in this patient group. The survival increase in patients treated with systemic therapy is most likely the result of lead time bias. That is, by improved diagnostic modalities and more use of the diagnostic laparoscopy, peritoneal metastases have been diagnosed at an earlier stage, leading to an apparently longer survival time. Furthermore, the poor prognosis of patients not treated with systemic therapy can in part be explained by immortal-time bias.

Thus, systemic therapy alone is not the optimal palliative treatment strategy for peritoneal metastases. Intra-abdominal chemotherapy might be a better option as it has a few advantages over systemic therapy. Firstly, it provides superior penetration into the peritoneal lesions; secondly, the peritoneum-blood barrier allows for a higher intra-abdominal concentration of cytostatic drugs without systemic toxicity; and lastly, the chemotherapeutic agents can be heated which potentially improves the cytotoxic effects.[27-29] The combination of CRS and HIPEC has been used to treat patients with peritoneal metastases of colorectal and ovarian origin.[30,31] Similarly, recent nationwide cohort data have suggested a survival benefit for gastric cancer patients with synchronous peritoneal metastases treated with CRS and HIPEC.[32-34] At current times, in the Netherlands, a HIPEC procedure for gastric cancer is only performed within the context of the PERISCOPE II trial.[35] There are other ways to apply intra-abdominal chemotherapy with palliative intent. A few studies showed effect of a catheter-based approach in gastric cancer patients with peritoneal metastases.[36] Also, pressurised intraperitoneal aerosol chemotherapy (PIPAC) was recently introduced as a new technique, and is practiced in a growing amount of hospitals worldwide.[37] A feasibility study showed that PIPAC is safe and well tolerated in gastric cancer patients with peritoneal metastases.[38] Nevertheless, PIPAC is not yet practiced for gastric cancer in the Netherlands. Although catheter-based intra-abdominal chemotherapy and PIPAC have theoretical advantages over systemic therapy, their efficacy has not yet been proven. Therefore, these techniques should only be used in a study setting.

The median overall survival in our nationwide cohort (3.6 to 4.4 months) was lower than in other studies, with median overall survival rates ranging from 4.8 to 17.0 months.[39-43] This can be explained by the fact that we reported on the entire population of patients with peritoneal metastases from gastric origin, including 47% of patients who did not receive any anti-cancer treatment at all. All other studies reported on patients who underwent treatment, such as palliative systemic therapy or a primary tumour resection. Although the effects of these treatments on overall survival are unclear, patients selected for treatment are likely to have a more favorable prognosis than patients considered unsuitable for treatment. Furthermore, selecting a patient for treatment inevitably creates immortal-time bias.

The strengths of this study are the nationwide population-based study design and the large number of included patients. Before 2008, the NCR consisted of several regional databases, which all registered the presence of metastatic disease, but not invariably its location. This led to an underestimation of the proportion

of patients with synchronous peritoneal metastases in the years 1999-2007. Therefore, the incidence rates in these years were not reported in this study. Even nowadays, peritoneal metastases may be missed during the initial staging process, thus there still is an underestimation of the actual number of patients with synchronous peritoneal metastases.[44,45] Another limitation of the study is the lack of information on the extent of peritoneal disease. The peritoneal carcinomatosis index is known to affect overall survival, but was not registered by the NCR during the study years.[46] The peritoneal carcinomatosis index has been integrated in the NCR nowadays, but still for many patients with (widespread) peritoneal metastases, its exact extent is irrelevant. Finally, another limitation of the study is the high proportion of unknown clinical tumour and clinical nodal stage which may have impeded the interpretation of these factors in multivariate analyses.

In conclusion, this population-based study showed that the absolute and relative incidence of gastric cancer patients with synchronous peritoneal metastases increased in the Netherlands. Although the use of systemic treatment increased significantly, there was no improvement of overall survival for the total group of patients. Therefore, it is important to study alternative treatment strategies, such as CRS and HIPEC, catheter-based intra-abdominal chemotherapy or repetitive PIPAC to treat peritoneal metastases of gastric cancer origin.

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CHAPTER 5

Tumour characteristics and clinical outcome of peritoneal metastasis of gastric origin treated with a hyperthermic intraperitoneal chemotherapy (HIPEC) procedure in the PERISCOPE I trial.

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Abstract

Introduction | The PERISCOPE I study was conducted to investigate safety and feasibility of Hyperthermic Intraperitoneal Chemotherapy (HIPEC) in gastric cancer patients with limited peritoneal dissemination. In the current study, tumour characteristics and clinical outcome of the patients treated in the PERISCOPE I trial were investigated.

Methods | Patients who had undergone the full study protocol were selected; i.e., preoperative systemic chemotherapy, followed by a surgical procedure consisting of a (sub)total gastrectomy, cytoreductive surgery and HIPEC with oxaliplatin (460 mg/m^2) and docetaxel (in escalating doses).

Results | Twenty-five PERISCOPE I patients underwent the full study protocol. Most patients had an ypT3-4 tumour (96%) and the diffuse type histology was predominant (64%). Seven patients (28%) had a microscopically irradical (R1) resection. In all patients, a complete cytoreduction was achieved. Median follow-up was 37 (95% CI 34-39) months. Disease recurrence was detected in 17 patients (68%). Median disease-free and overall survival were 12 and 15 months, respectively.

Conclusion | In this series of gastric cancer patients with limited peritoneal dissemination who underwent HIPEC surgery, unfavourable tumour characteristics were common. Survival might be encouraging but disease recurrence was frequent. The efficacy of a HIPEC procedure in improving prognosis is currently being investigated in the PERISCOPE II trial.

Introduction

Peritoneal dissemination is common in gastric cancer. About 14-24% of the newly diagnosed patients have peritoneal metastases at the time of diagnosis.[1-3] In about 9% of the newly diagnosed patients with gastric cancer, the peritoneum is the only location for metastases. In the Netherlands, standard treatment for these patients is palliative systemic chemotherapy. Despite this treatment, their life expectancy is still only about 4 months.[4]

In patients with peritoneal metastases from colorectal or ovarian cancer, cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) have shown therapeutic efficacy in terms of survival in randomised controlled trials.[5,6] In Asia, a HIPEC procedure is used in treating gastric cancer patients with peritoneal metastases.[7] However, results obtained in Asian patients cannot be extrapolated to Western patients due to differences in gastric cancer behaviour.[8] Moreover, available Asian and Western data almost invariably come from retrospective studies which vary immensely concerning patient selection, HIPEC technique and chemotherapeutic agents used.[9-12] Prospective randomised data on patient outcome and disease recurrence are needed before implementation of CRS and HIPEC as a standard treatment option can be considered.

The PERISCOPE I (Treatment of PERItoneal dissemination in Stomach Cancer patients with cytOreductive surgery and hyPErthermic intraperitoneal chemotherapy) study has been conducted in the run-up to the initiation of a randomised controlled trial, the PERISCOPE II trial.[13,14] The primary outcome of the dose-finding PERISCOPE I study involved the safety and feasibility of a HIPEC procedure with oxaliplatin and docetaxel in patients with gastric cancer and limited peritoneal dissemination.[14,15] The aim of the current analysis was to investigate the pathological tumour characteristics and the clinical outcome of the patients in the PERISCOPE I study.

Methods

Patients

The PERISCOPE I study was conducted to investigate safety and feasibility of a HIPEC procedure in gastric cancer patients with limited peritoneal dissemination. It was a multicentre, phase I-II dose-escalation study to determine the maximum tolerated dose of intraperitoneal docetaxel in combination with a fixed dose of intraperitoneal oxaliplatin. The primary endpoint was treatment –related toxicity. Patient selection criteria for inclusion in the PERISCOPE I study were previously described[15]. All patients had a potentially resectable locally advanced gastric adenocarcinoma with limited peritoneal metastases and/or tumour positive peritoneal cytology. Peritoneal metastases, confirmed by diagnostic laparoscopy or laparotomy, had to be limited to the upper abdominal cavity (above the transverse colon) with at most one location in the lower abdominal cavity

(e.g., Douglas' pouch, ovarian metastasis, Sister Mary Joseph nodule). Small bowel (or mesenterial) dissemination and distant metastases were not allowed. Provided that disease progression during systemic chemotherapy was absent, surgery was planned. In total 37 patients were included in the PERISCOPE I study. Twelve patients did not undergo the complete study protocol due to disease progression (n=2), toxicity of the systemic treatment (n=2), an irresectable primary tumour (n=2) or gross peritoneal tumour dissemination (n=6). For the current analyses, only patients who completed the full surgical protocol were selected. The study protocol was approved by the Medical Ethics Committee (METC) of the Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital. All patients gave written informed consent.

Surgery

The surgical procedure consisted of a (sub)total gastrectomy with D2 lymphadenectomy, CRS and HIPEC with 460 mg/m^2 hyperthermic (41-42^{o}C) oxaliplatin for 30 minutes followed by normothermic (37^{o}C) docetaxel for 90 minutes in a dose level escalation scheme (0, 50, 75 mg/m^2).[14] After midline laparotomy cytological samples were obtained from ascites (if present) and from rinsing the left and right subphrenic space and Douglas' cavity. The extent of peritoneal metastases was assessed according to the PCI (Peritoneal Cancer Index).[16] Only if a complete cytoreduction could be achieved, the HIPEC procedure was performed. If necessary for peroperative decision making, biopsies of peritoneal lesions and/or resection margins of the gastrectomy specimen were sent for frozen section analysis.

Histopathology

Resection specimens were assessed according to standard protocols. The following parameters were determined; pathological T stage, pathological N stage, histological subtype according to Lauren, tumour regression grade (TRG) according to Mandard, radicality of the gastrectomy specimen and tumour involvement of resected peritoneal lesions[17-19]. All frozen section specimens were re-assessed as formalin-fixed, paraffin-embedded (FFPE) tissue samples. In doubtful cases, immunohistochemistry (CAM5.2) was used. Resected lymph nodes were classified according to the Japanese classification of gastric carcinoma as N1 (station 1-6) or N2 (station 7-12) nodes.[20]

Adjuvant chemotherapy

Adjuvant chemotherapy was not part of the PERISCOPE I study protocol. It was administered at the discretion of the treating medical oncologist following the multidisciplinary tumour board meeting.

Follow-up

All patients were seen at the outpatient clinic once every 3 months for the first year and every 6 months thereafter. Follow-up included blood sampling for tumour markers (CEA, CA19.9 and CA125) at 3, 6, 12 and 18 months after the procedure and computed tomography (CT) scans at 3, 6, 12 and 24 months after the

procedure. Additional diagnostic investigations were performed on indication. Time, location and treatment of recurrent disease were documented. Follow-up data were collected until December 2019.

Survival and statistics

Follow-up time was determined using the Reverse Kaplan-Meier method. Overall survival was measured from the time of surgery to the time of death, including post-operative mortality. Disease-free survival was measured from the time of surgery to the time of first tumour recurrence detection (clinical or radiological). Survival analyses were performed using the Kaplan-Meier method. The log-rank test was used to compare survival distributions between subgroups. A p-value of <0.05 was considered significant. All analyses were carried out with SPSS 25.0 (IBM, Armok, United States).

Results

Between January 2014 and November 2017, 25 patients underwent the complete study protocol. Baseline characteristics of all patients are given in **Table 1**. All patients underwent systemic chemotherapy prior to the operation. Given regimens were: epirubicin + cisplatin + capecitabine (n=10), docetaxel + oxaliplatin + capecitabine (n=7), capecitabine + oxaliplatin (n=3), oxaliplatin +5-fluoruracil (n=2) and epirubicin or epiadriamycin + cisplatin or oxaliplatin + 5-fluoruracil or capecitabine (n=3). In one patient, HER2 was amplified in the pre-treatment biopsy and transtuzumab was added to the regimen. The median number of cycles given was 3.

Tumour characteristics

All patients but one (96%) had a pathological ypT3-T4 tumour after systemic chemotherapy (**Table 1**). Median number of removed lymph nodes was 30 (range 1-47). Seventeen patients (68%) had lymph node metastases. In 15 of these N+ patients (88%) the tumour positive lymph nodes were exclusively found in N1 lymph node stations; only two patients had tumour positive lymph nodes in N2 nodal stations (stations 9 and 12). Diffuse type histology was most common (64%) and a substantial proportion (40%) of the patients had a minimal (TRG 4) or no (TRG 5) response to systemic chemotherapy. Additional tumour characteristics are presented in (**Table 1**).

Radicality of the gastrectomy

In 8 patients (32%), the proximal resection margin was sent for frozen section analysis. In 7 out of 8 patients, the frozen section analysis and the FFPE assessment were concordant, while in one patient FFPE examination showed vital tumour cells that were not seen at frozen section analysis. In 3 patients, the proximal resection margin was tumour positive on frozen section assessment. Only one of them finally had a tumour free resection margin. In the other two patients, the additional final proximal resection margin was still tumour positive. For those two patients resection of another additional part of the oesophagus was technically not possible. In all, 7 of 25 patients (28%) had a microscopically irradical (R1) resection due to the presence of

tumour cells in the proximal resection margin. Six of these 7 patients had a diffuse type tumour, and in 4, the circumferential resection margin of the distal oesophagus was also tumour positive. In addition, one of these 7 patients also had a tumour positive distal resection margin.

Table 1. Baseline characteristics of patients who underwent the full study protocol in the PERISCOPE I study (n=25)

Patient characteristics		
Age at operation (years): median (range)	60	(32-75)
Gender: *n(%)*		
Male	16	64%
Female	9	36%
ASA score: *n(%)*		
I	11	44%
II	13	52%
III	1	4%
Tumour characteristics		
Tumour location: *n(%)*		
Cardia	2	8%
Corpus	3	12%
Antrum	6	24%
Linitis plastica	6	24%
Overlapping locations		
Corpus + antrum	3	12%
Cardia + fundus + corpus	4	16%
Fundus + corpus + antrum	1	4%
Pathological T stage*: *n(%)*		
T1b	1	4%
T3	10	40%
T4	14	56%
Pathological N stage*: *n(%)*		
N0	8	32%
N+	17	68%
Tumour histology^: *n(%)*		
Intestinal type	8	32%
Diffuse type	16	64%
Mixed type	1	4%
WHO tumour type: *n(%)*		
Mucinous adenocarcinoma	3	12%
Tubular adenocarcinoma	5	20%
Signet cell carcinoma	13	52%
Undifferentiated carcinoma	1	4%
Adenocarcinoma not otherwise specified	3	12%
Mandard classification: *n(%)*		
TRG 2	3	12%
TRG 3	12	48%
TRG 4	8	32%
TRG 5	2	8%
HER2 score[$]: *n(%)*		
0	16	64%
1	2	8%
2	2	8%
3	2	8%
not done	3	12%
Vascular invasion: *n(%)*		
Yes	9	36%
No	11	44%
Unknown	5	20%
Lymphatic invasion: *n(%)*		
Yes	13	52%
No	10	40%
Unknown	2	8%
Perineural invasion: *n(%)*		
Yes	14	56%
No	8	32%
Unknown	3	12%
Extent of peritoneal disease[#]		
Peritoneal tumour deposits: *n (%)*	16	64%
Tumour positive cytology only: *n (%)*	2	8%
PCI: median (range)	2	(0-9)

*According to the 7th TNM classification[18]
^According to the Lauren classification[16]
[#]At the time of the HIPEC surgery; i.e., after systemic chemotherapy.
[$]ImmunoHistoChemistry test
ASA, American Society for Anesthesiology
TRG, Tumor Regression Grade[17]
PCI, Peritoneal Cancer Index

Cytoreduction

In all patients, a complete cytoreduction was achieved. Median PCI was 2 (range 0-9). Frozen section analysis was performed on 12 peritoneal lesions in 5 patients, which was concordant with the final FFPE analysis in 9 of the 12 assessments. In two frozen section analyses, the pathologist had a strong suspicion of the presence of vital tumour cells, but these were not identifiable on frozen section nor on FFPE slides. However, using immunohistochemistry the presence of tumour cells was demonstrated (**Figure 1**). Lastly, one frozen section result was false negative, since vital tumour cells were detected with FFPE examination and immunohistochemistry.

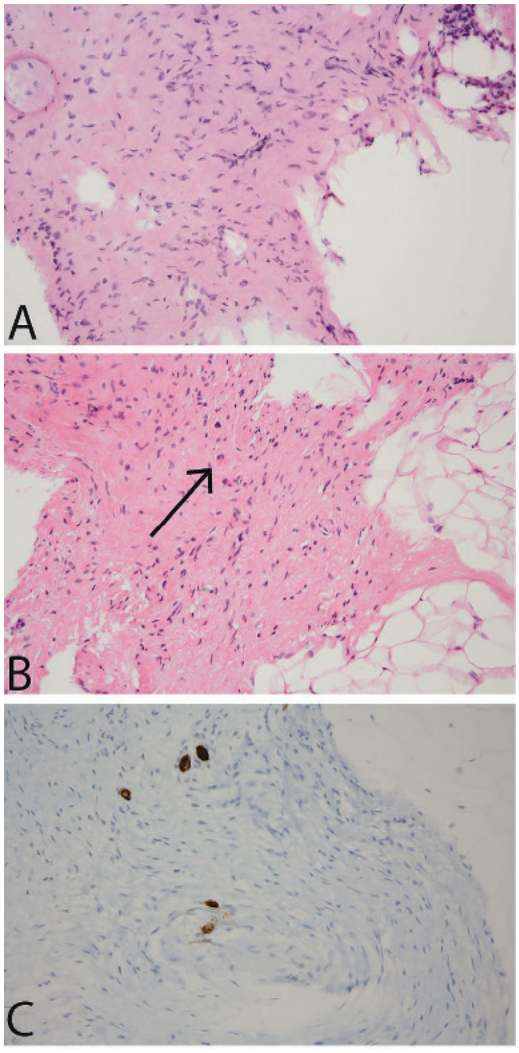

Figure 1. Frozen section analysis (A, x20, haematoxylin-eosin stain), corresponding formalin-fixed, paraffin embedded (FFPE) section (B, x20) and immunohistochemistry (C, x20) of a peritoneal lesion from the small bowel mesentery in the PERISCOPE I study. The patient had a diffuse type gastric cancer and underwent surgery after systemic chemotherapy. (A) There is fibrosis present in which there is increased cellularity. Diffuse type carcinoma cells were not seen. The result of the frozen section examination was reported to the surgeon as “difficult to assess because of fibrosis; tumour cells are not seen but their presence cannot be excluded”. (B) In the FFPE section there are a few cells that are suspicious of being cancer cells (arrow). (C) The keratin stain (CAM5.2) demonstrates sporadic positive cells within the fibrosis. The cells were heterogeneously positive in the CDX2 staining (not shown). This case demonstrates that fibrosis in a peritoneal lesion of a patient with gastric cancer who underwent systemic chemotherapy should alert the pathologist. Diffuse type gastric carcinoma generally has intrinsic fibrosis and fibrosis may also (partly) represent regression. The sparsity of tumour cells in this case is likely to reflect tumour regression.

Adjuvant therapy

Seven patients (28%) started adjuvant chemotherapy; all between 1 and 3 months after the HIPEC procedure. Administered regimens were docetaxel + oxaliplatin + capecitabine, epirubicin + oxaliplatin + capecitabine, 5-fluorouracil + oxaliplatin and 5-fluorouracil + oxaliplatin + epirubicine. In all but one of the patients these regimes were the same as the regimen given prior to the operation.

Disease recurrence

Median follow-up time was 37 months (95% CI 34-39 months). During follow-up disease recurrence was detected in 17 patients (68%), mostly at multiple locations. In 11 patients disease recurrence was detected due to clinical symptoms and subsequent investigations. In the other 6 patients recurrent disease was diagnosed on follow-up CT scans. Six patients had locoregional recurrence, i.e., in the stomach bed, at the anastomosis and/or in regional nodal basins. Ten patients presented with new peritoneal lesions or malignant ascites (in combination with tumour recurrence at another location in 7 patients) and 11 patients developed distant metastases, in the liver (n=3), lungs (n=3), mediastinal lymph nodes (n=2), abdominal wall (n=3), or bones (n=3). Six patients were treated with chemotherapy or radiotherapy. Median survival time after diagnosis of disease recurrence was 9 (range 0-28) months.

Survival

At the time of last follow-up, 20 patients (80%) had died. All but three patients died of tumour recurrence. Two patients died due to postoperative complications (one within 30 days and one within 60 days after surgery), and one patient died of a cardiac arrest during follow-up. None of these 3 patients had signs of tumour recurrence. Another patient died within 60 days after surgery due to early disease progression (malignant pleural effusion). Median overall survival time was 15 (0-53) months. Median disease-free survival time was 12 (range 0-29) months. No significant survival differences were found between groups according to histological subtype (intestinal versus diffuse/mixed tumours; p=0.92), radicality of the gastrectomy (R0 versus R1 resection; p=0.10), response to systemic therapy (TRG2/3 versus TRG 4/5, p=0.66), or extent of peritoneal disease (presence versus absence of macroscopic tumour deposits at the time of HIPEC surgery; p=0.43).

Discussion

The PERISCOPE I study was conducted to investigate safety and feasibility of a HIPEC procedure with oxaliplatin and docetaxel in gastric cancer patients with limited peritoneal metastases and/or tumour positive cytology.[15] Initial results have been published recently, i.e., the HIPEC procedure appeared safe and feasible with an intraperitoneal dose of 460 mg/m^2 oxaliplatin followed by 50 mg/m^2 docetaxel in a selected group of patients using a strict postoperative care protocol.[14] In the current study, histopathological data and clinical follow-up results of the PERISCOPE I patients were evaluated to increase our knowledge of gastric cancer with peritoneal dissemination.

In a large German series of gastric cancer patients without peritoneal dissemination treated with chemotherapy followed by surgery 21% of the patients had a complete or near-complete tumour response and only 25% had minimal or no response.[21] In the PERISCOPE I study, there was no patient who had a complete response and 40% had a minimal or no response. Moreover, the proportion of patients with ypT3-4 stage tumours was considerably higher (96%) than in other surgical gastric cancer study populations treated with neoadjuvant chemotherapy, for example, in the MAGIC trial cohort (48.5%).[22] This is most likely related to selection bias, as it was demonstrated previously that high T stage is a risk factor for peritoneal dissemination.[23] Nonetheless, these data underline the unfavourable tumour characteristics of gastric cancer with peritoneal metastases.

In the gastric cancer CRITICS trial, the percentage of patients with a microscopically irradical (R1) resection was 9%.[24] In earlier series, the R1 percentage of gastric cancer surgery after neoadjuvant chemotherapy was about 16%.[25,26] The R1 rate in the PERISCOPE I study was considerably higher (28%). This high R1 percentage may partly be explained by the advanced tumour stages included in this study (96% ypT3-T4 tumours). In addition, it may be related to the relatively high proportion of diffuse type tumours (64%). Diffuse type adenocarcinoma tumour cells are discohesive, tend to aggressively invade the entire stomach wall and do not rarely infiltrate the distal oesophagus if the proximal stomach is involved.[17] Normally, a frozen section analysis of the resection margin can help to prevent R1 resections. However, during the PERISCOPE I study frozen section assessment often appeared difficult. The distinction between small sporadic vital tumour cells and inflammatory cells or fibroblasts in the background of fibrosis may be impossible.[27] In our study, there was no significant difference in survival between patients with a R0 resection and those with a R1 resection. This is probably due to the small size of the study population. On the other hand, the survival of these patients is mostly determined by other sites of disease recurrence, so R status might not be crucial in this patient group.

One of the most important prognostic factors after a HIPEC procedure is the completeness of the cytoreduction. An incomplete cytoreduction is correlated with a dismal survival perspective.[7,10-12,28-30] In a French series of patients with gastric cancer and peritoneal metastasis treated with a HIPEC procedure, median survival of those patients in whom a complete cytoreduction had been achieved was 22.1 months, compared to 8.4 months for those in whom cytoreduction had been incomplete[28]. The authors concluded that a HIPEC procedure should only be considered if the surgeon expects to achieve a complete cytoreduction.[28] This principle was applied in the PERISCOPE I study, as the HIPEC procedure was only carried out if the surgeon considered a complete cytoreduction feasible. The likelihood of achieving a complete cytoreduction is clearly related to the extent of the peritoneal metastases.[7] To establish the PCI and the possibility of a complete cytoreduction frozen section analysis of peritoneal lesions can be necessary, as was done in 5 (20%) of the patients in the PERISCOPE I study. Again, frozen section assessment was difficult and false negative results occurred.

In previous studies evaluating HIPEC surgery for gastric cancer patients the procedure provided a survival benefit only in patients with limited peritoneal involvement (PCI<7).[7,11,12,28] For this reason, in the successor study (the PERISCOPE II study), only patients with a PCI<7 as assessed during diagnostic laparoscopy or laparotomy are included.[13] In a meta-analysis published in 2017, median survival of patients with gastric cancer and peritoneal metastasis treated with HIPEC was 11.1 months.[9] For patients with limited peritoneal disease (PCI<7) median survival rates between 18 and 33.6 months have been reported.[7,11,12,28] In the present study, median survival was 15 months. However, this was a dose finding feasibility study, not designed to assess the therapeutic efficacy of the HIPEC procedure.

In more than half of the patients in this series (68%) disease recurrence was detected. Peritoneal recurrences were most frequently seen, followed by recurrent disease at the primary tumour site and/or nodal basins. Distant metastasis were seen at various locations (e.g. lungs, liver and bones). Thus, the abdominal cavity was at highest risk for disease recurrence in our patients who underwent HIPEC for gastric cancer. This is in line with the findings of other investigators.[31]

The PERISCOPE I study paved the way for the initiation of the PERISCOPE II study, the first randomised controlled trial in which a HIPEC procedure is compared to the current standard treatment with palliative systemic chemotherapy in a Western population with gastric cancer and peritoneal dissemination.[13,32] The PERISCOPE I study was essential as it provided information on patient selection, perioperative care and the safety of the HIPEC treatment with oxaliplatin and docetaxel. The maximum tolerated dose of intraperitoneal docetaxel was determined at 50 mg/m.[2,14] Moreover, from the current analyses it is learned that the underlying gastric tumours exhibit a number of unfavourable features (high T stage, diffuse type histology, limited response to systemic chemotherapy). Frozen section analysis of the proximal resection margin appeared to have its shortcomings in this patient group. The same was true for frozen section analysis of peritoneal lesions. Disease recurrence was common, especially in the peritoneal cavity. These results stress the importance to compare this intensive treatment protocol to the current standard (palliative systemic chemotherapy) in a randomised study, with survival as primary endpoint.

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CHAPTER 6

Perioperative management of gastric cancer patients treated with (sub)total gastrectomy, cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (HIPEC): lessons learned.

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Abstract

Introduction | The PERISCOPE I study was designed to assess the safety and feasibility of a (sub)total gastrectomy, cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) with oxaliplatin and docetaxel in gastric cancer patients with limited peritoneal dissemination. In the current analysis, changes in perioperative management were investigated together with their impact on postoperative outcomes.

Methods | Patients with resectable gastric cancer and limited peritoneal dissemination were treated with (sub)total gastrectomy, CRS and HIPEC with oxaliplatin (460 mg/m^2) and docetaxel (escalating scheme: 0, 50, 75 mg/m2). Of the 25 patients who completed the study protocol, 14 patients were treated in the dose-escalation-cohort and 11 patients in the expansion-cohort (to optimise perioperative management).

Results | A significant proportion of patients in the dose escalation-cohort (n=7; 50%) had ileus-related complications. In this cohort, enteral nutrition was started immediately after surgery at 20 ml/hour which was increased on day 1 to meet nutritional needs. In the expansion-cohort, enteral nutrition was started at 10 ml/hour until day 3 and restricted to 20 ml/hour until day 6, supplemented with total parenteral nutrition to meet nutritional needs. Ileus-related complications occurred in 2 (18%) patients of the expansion-cohort. ICU readmission rate decreased from 50% (n=7) to 9% (n=1; p=0.04).

Conclusion | The implementation of a strict nutritional protocol during the PERISCOPE I study was associated with a decrease in postoperative complications. Following these results, a perioperative care path has been described for the gastric cancer HIPEC patients in the PERISCOPE II study.

Introduction

A combination of cytoreductive surgery (CRS) and Hyperthermic Intraperitoneal Chemotherapy (HIPEC) is increasingly used for the treatment of peritoneal dissemination of various cancer types.[1-3] For peritoneal dissemination of gastric cancer origin, HIPEC surgery is subject of investigation.[4,5] Recently, several nationwide database studies reported a survival benefit of HIPEC treatment in selected gastric cancer patients.[6-8] As yet, results from a randomised controlled trial to assess the role of CRS and HIPEC in the treatment of gastric cancer patients with peritoneal dissemination are lacking.

HIPEC surgery has been associated with considerable morbidity and mortality rates.[9,10] Various reports have been published addressing the perioperative management of patients undergoing CRS and HIPEC, mostly for peritoneal dissemination of colorectal cancer.[11-14] The chemotherapeutic agents most commonly used in these HIPEC procedures include oxaliplatin, cisplatin and mitomycine C.[11] Careful postoperative start of enteral nutrition is recommended in most published papers.[15,16]

The dose-finding PERISCOPE I study (treatment of PERItoneal dissemination in Stomach Cancer patients with cytOreductive surgery and hyperthermic intraPEritoneal chemotherapy) was designed to assess the safety and feasibility of a CRS-HIPEC procedure with 460 mg/m^2 hyperthermic (41-42^{o}C) oxaliplatin followed by normothermic docetaxel in escalating dosages (0, 50, 75 mg/m^2) in gastric cancer patients with limited peritoneal dissemination.[17] A diverse spectrum of postoperative complications was encountered, with fairly high rates of intestinal complications.[18] During the PERISCOPE I study adaptations were made to the postoperative care path. The aim of the current analysis was to investigate the changes in the perioperative management of the PERISCOPE I patients over time, together with their impact on postoperative outcomes. Following this, the goal was to describe the postoperative care path to be used in the PERISCOPE II study.[5,19]

Methods

The PERISCOPE I study

All patients were treated in the PERISCOPE I study, a dose-finding phase I-II study, with treatment-related toxicity as primary outcome measure.[17] The trial was conducted in two Dutch centres experienced in HIPEC and gastric cancer surgery, the Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital in Amsterdam and the Sint Antonius Hospital in Nieuwegein. The study protocol has been published previously.[17] In short, gastric cancer patients with a resectable primary tumour and limited synchronous peritoneal metastasis and/or tumour positive peritoneal cytology were eligible for study inclusion provided that there was no disease progression during systemic chemotherapy. The PERISCOPE I study was approved by the Medical Ethics Committee of the Netherlands Cancer Institute and written informed consent was obtained from all patients.

For the current analysis, only patients who completed the entire study protocol were selected, i.e., all patients in this paper underwent systemic chemotherapy followed by an operative procedure consisting of a (sub)total gastrectomy with D2 lymph node dissection, CRS and HIPEC. An open HIPEC technique was used with a fixed dose (460 mg/m2) of hyperthermic (41-42^{o}C) oxaliplatin, followed by normothermic (37^{o}C) docetaxel in a dose-escalation scheme (0 mg/m^2, 50 mg/m^2, 75 mg/m^2) to establish the maximum tolerated dose of intraperitoneal docetaxel. At dose-level 3 (75 mg/m2 docetaxel) treatment-related toxicity was unacceptable. At that time, 14 patients were included in the study, the dose-escalation-cohort (**Table 1**). Dose-level 2 (50 mg/m2) was defined as the maximum tolerated dose of intraperitoneal docetaxel for this procedure. Eleven extra patients were treated at this dose-level (460 mg/m2 oxaliplatin followed by 50 mg/m2 docetaxel) to optimise perioperative care protocols. These patients were included in the expansion-cohort. In all patients, after HIPEC and BII or Roux-en-Y reconstruction, a feeding jejunostomy was inserted routinely.

Table 1. Dose-level assignment in the PERISCOPE I study.

	Dose-escalation-cohort			Expansion-cohort
Dose-level	1	2	3	2
Oxaliplatin dosage	460 mg/m^2	460 mg/m^2	460 mg/m^2	460 mg/m^2
Docetaxel dosage	0 mg/m^2	50 mg/m^2	75 mg/m^2	50 mg/m^2
Number of patients	n=4	n=6	n=4	n=11

Anaesthesiologic management

All patients received combined epidural anaesthesia and general anaesthesia. Standard anaesthesiologic monitoring plus hemodynamic monitoring using stroke volume variation and cardiac output measurements were used to assess the fluid status (EV1000, Edwards life science, Ivrine, CA). To strive for normovolemia and optimal oxygen delivery to the tissues, fluid support and vasopressors (noradrenaline) were given during the operation. In the majority of the patients (92%) Dexamethasone was given just prior to the docetaxel chemoperfusion to prevent a possible allergic reaction. Body temperature was measured continuously during the procedure. Peroperative blood gas analysis was carried out at regular intervals during the operation in 18 patients operated in the Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital. After the operation, all patients were extubated in the operating room and then transferred to the intensive care unit (ICU).

Data collection and statistics

Clinical data were derived from the prospective database of the PERISCOPE I study. Postoperative complications were recorded based on the National Cancer Institute Common Terminology Criteria for adverse events 4.03.[20] Ileus, abdominal infection, intestinal perforation, anastomotic leakage, duodenal leakage, wound infection and gastrointestinal fistula were grouped as abdominal complications whereas pneumonia, aspiration pneumonia, pneumothorax, respiratory failure and pleural effusion were grouped as respiratory complications. A subset of both categories (ileus, intestinal perforation, gastrointestinal fistula and aspiration

pneumonia) was seen as ileus-related complications. Additional data regarding preoperative nutritional status, peroperative fluid management, postoperative ICU stay and nutritional management were retrospectively derived from the following sources: anaesthesia protocols, ICU medical files (MetaVision, Essen-Kettwig, Germany) and electronic patient records. Differences between groups were analysed with the Fisher's exact test for categorical variables and the Mann-Whitney U test for continuous variables. Numbers are shown as medians and ranges. A P-value of <0.05 was considered statistically significant.

Results

Patient characteristics

Of the 25 included patients, 19 were operated in the Netherlands Cancer Institute – Antoni van Leeuwenhoek Hospital and 6 were operated in the Sint Antonius Hospital. Median (range) age of the patients was 61 (33-75) years and 16 patients (64%) were male.

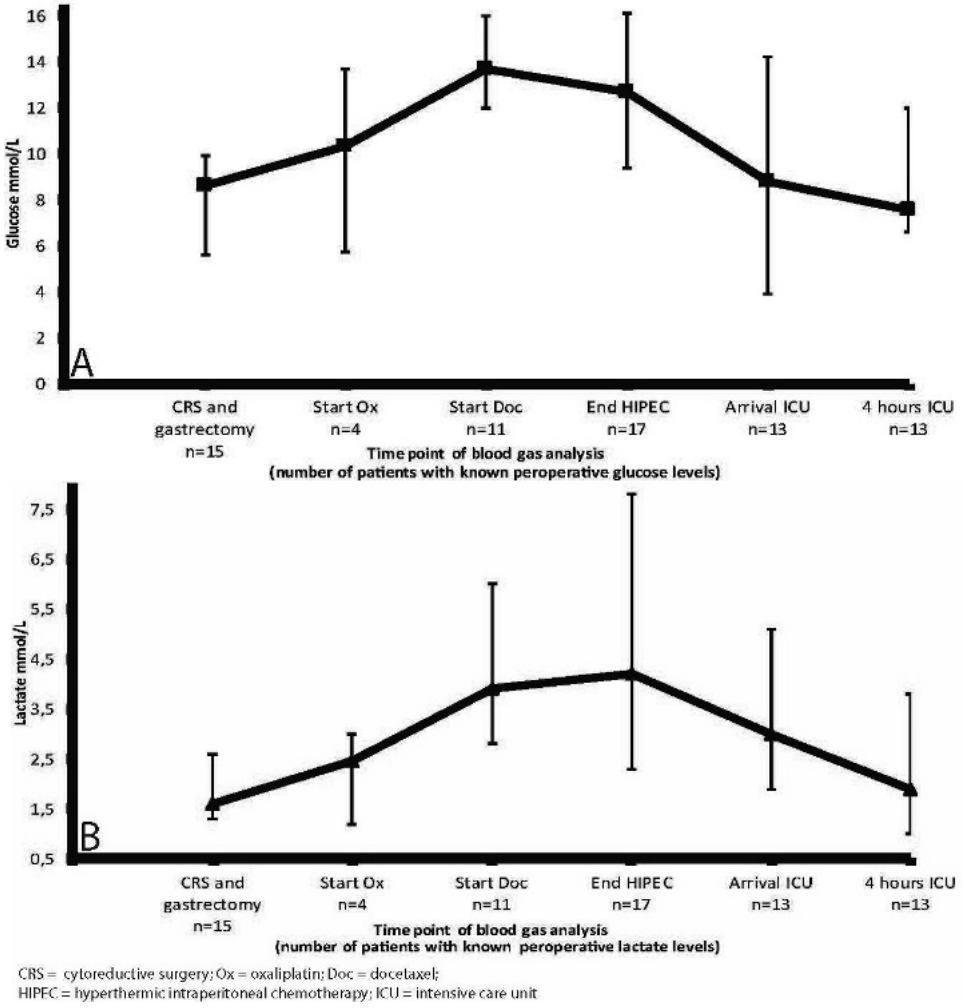
Preoperative nutritional details

The majority of the patients (n=20; 80%) had experienced weight loss at the time of gastric cancer diagnosis. Prior to the operation, 22 patients (88%) were seen by a dietician and in 17 patients (68%), nutritional support was given (via an enteral tube in 4 patients).

Peroperative details

Median duration of the operation (including HIPEC) was 7 (range 3-10) hours. Nineteen patients (76%) had a total gastrectomy and 6 patients (24%) had a subtotal gastrectomy. A median volume of 6.5 litres of intravenous fluids (range 3.6-10.5) was administered during the operation. Median blood loss was 610 (range 100-1810) ml. In 4 patients blood products (e.g., packed cells or fresh frozen plasma) were given. Peroperative glucose and lactate levels were known in 18 patients. In all patients glucose and lactate levels rose during the HIPEC phase of the procedure (**figure 1**). Plasma lactate peaked at the end of the intraperitoneal chemoperfusion, at a median value 4.2 (range 3.0-7.8) mmol/L. For 11 patients the intraoperative peak concentration had been 2.3 mmol/L or higher. In all patients body temperature increased during the hyperthermic part of the procedure. It peaked at the end of the oxaliplatin chemoperfusion at a median value of 38.1 (range 36.7-39.1)^{o}C.

Figure 1: Median (range) of (**A**) glucose and (**B**) lactate plasma levels during surgery in gastric cancer patients undergoing gastrectomy, CRS and HIPEC in the PERISCOPE I study.



CRS = cytoreductive surgery; Ox = oxaliplatin; Doc = docetaxel;
HIPEC = hyperthermic intraperitoneal chemotherapy; ICU = intensive care unit

Postoperative nutritional details

Median length of ICU stay (including readmissions) was 1 day (range 1-33) days. Enteral nutrition via the surgical jejunostomy was started immediately after arrival at the ICU at 20 ml/hour in the dose-escalation-cohort and at 10 ml/hour in the expansion-cohort. In the dose-escalation-cohort, enteral nutritional intake via the jejunostomy was increased every hour on postoperative day 1 until calculated nutritional needs were reached.[21] In the expansion-cohort, enteral nutrition was started at 10 ml/hour until day 3 and restricted to 20 ml/hour until day 6and total parenteral nutrition (TPN) was started routinely around day 3 to meet nutritional needs. After day 6 enteral nutrition was increased provided there were no ileus-related symptoms.

In the dose-escalation-cohort, TPN was given to 5 (33%) patients starting at median day 5 (range day 2 – day 8). In the dose-expansion-cohort, 10 (91%) patients received TPN starting at median day 3 (rang day 2 – day 8). Following the differences in postoperative nutritional management, the amounts of enteral nutrition per day differed significantly between the two groups in the early postoperative period (**Table 2**).

Table 2: Median (range) millilitres of enteral nutrition per day via the surgical jejunostomy after HIPEC surgery for gastric cancer.

	Dose-escalation-cohort	Expansion-cohort	p-value
Day 0	287 (139-434)	146 (110-174)	<0.01
Day 1	1364 (819-1768)	218 (184-240)	<0.01
Day 2	1503 (1000-2075)	220 (190-420)	<0.01
Day 3	1584 (1000-2081)	285 (170-429)	<0.01

Postoperative complications

Overall, 17 patients (68%) experienced one or more serious adverse events (SAEs). Patients in the dose-escalation-cohort had a more complicated postoperative course than patients in the expansion-cohort, although the difference did not reach statistical significance (86% versus 45%; p=0.081) (**Table 3**). The number of SAEs was significantly higher in the dose-escalation-cohort than in the expansion-cohort (p=0.021). In total, 25 abdominal complications occurred (abdominal infection 6; ileus 6; anastomotic leakage 5; intestinal perforation 3; wound infection 3; duodenal leakage 1 and gastrointestinal fistula 1) and 16 respiratory complications occurred (pneumonia 9; aspiration pneumonias 3; pneumothorax 2; pleural effusion 1 and respiratory failure 1). Ileus-related complications (defined as ileus, intestinal perforation, gastrointestinal fistula and aspiration pneumonia) occurred in 7 (50%) patients of the dose-escalation cohort versus 2 (18%) patients of the expansion-cohort (p=0.208). The proportion of patients re-admitted at the ICU was significantly higher in the dose-escalation-cohort than in the expansion-cohort (50% versus 9%; p=0.04). Three patients died within 60 days after surgery (one due to early disease progression and two as a result of postoperative complications); all three in the dose escalation-cohort. The intraoperative peak concertation of plasma lactate was associated with the re-intervention rate, i.e., 6 (55%) of 11 patients with a peak level of ≥4 mmol/L needed a re-intervention versus no patient in the group of patients with a peak level below 4 mmol/L (p=0.038).

Table 3: Post-operative complications after HIPEC surgery for gastric cancer.

	Dose-escalation-cohort (n=14)	Expansion-cohort (n=11)	p-value
Patients with a SAE	12 (86%)	5 (45%)	0.081*
Number of SAE's	25	6	0.021#
Number of respiratory complications	14	2	0.012#
Number of abdominal complications	16	9	0.533#
Number of ileus related complications	11	2	0.068#
Patients with Ileus-related complications	7 (50%)	2 (18%)	0.208*
Number of re-interventions^	12	6	0.183#
Number of re-operations	9	0	0.059#
ICU stay in days (mean; range)	6 (1-33)	2 (1-9)	0.494#
Patients re-admitted at the ICU	7 (50%)	1 (9%)	0.042*
Hospital stay in days (mean; range)	36 (9-185)	25 (12-53)	0.536#
90-day mortality	3	0	0.230*

*Two-sided Fisher's exact test. #Mann-Whitney U test (exact 2-tailed)
^Re-interventions included: endoscopic stent placement, percutaneous drainage of thorax/abdomen, radiological embolisation.
SAE= *Serious Adverse Event*; ICU= *Intensive Care Unit*

Discussion

The PERISCOPE I study was the first dose-finding feasibility study in gastric cancer patients undergoing HIPEC surgery with oxaliplatin and docetaxel. The two participating centres had extensive experience in both HIPEC treatment and gastric cancer surgery before the start of the study. Nevertheless, serious post-operative complications occurred more frequently than anticipated. The aim of the current analysis was to describe the changes in the perioperative management of the PERISCOPE I patients over time and their impact on postoperative outcomes. It led to the development of a perioperative care path for the gastric cancer HIPEC patients in the PERISCOPE II study (**Table 4**).[5]

A significant proportion of patients in the dose-escalation-cohort (50%) had ileus-related complications. Although ileus-related complications are common after HIPEC surgery, its sequelae in the PERISCOPE I cohort required a change in postoperative management.[22] It is hypothesised that these sequelae were caused by the loss of the stomach's reservoir function that normally helps to prevent ileus-related complications such as an aspiration pneumonia and intestinal perforations. In our study, gastrectomy patients with a paralytic ileus due to cytoreductive surgery and intraperitoneal chemotherapy in whom enteral nutrition via the jejunostomy was given in such amount that nutritional needs could be met were at an increased risk to develop one or more SAEs (86%). Alternatively, in those patients in whom enteral nutrition was restricted during the first postoperative days, the risk to develop one or more SAEs was lower (45%). To meet the nutritional needs and prevent a catabolic state, TPN was started. Previously, Shannon et al. suggested to start TPN after gastrectomy and HIPEC as early as postoperative day 1 or 2.[23] In our opinion, TPN should be started after day 3, i.e., after the initial systemic inflammatory response to the operation has faded away, to prevent metabolic complications.[24,25] To prevent small bowel atrophy and improve gut motility a small amount of enteral

nutrition was given via the jejustomy during the first week, to a maximum of 20 ml/hour. This strategy is contradictory to current recommendations in HIPEC literature, but in the PERISCOPE I study the implementation of this strict nutritional protocol was associated with a decrease in postoperative complications and ICU readmission rate (50% versus 9%).[11,12,26]

Following the results of the peroperative blood gas analyses, glucose and lactate levels rose during the HIPEC phase of the surgical procedure in the PERISCOPE I study. A rise in plasma lactate levels during HIPEC with oxaliplatin has been related to the use of dextrose 5% as carrier solution for oxaliplatin, causing hyperglycemia and the metabolic relation between glucose and lactate.[15] However, in the PERISCOPE I study, Dianeal PD04 (1.36% glucose) was used as carrier solution for oxaliplatin. Most likely, the rise in glucose and lactate levels was due to a combination of the 1.36% glucose in the Dianeal, inadequate tissue perfusion following blood and fluid loss, and the use of hyperthermic chemotherapeutics. The latter also explains the increase in body temperature and heart rate during the HIPEC phase.[27,28] A high peak lactate level has been associated with a worse surgical outcome.[29] Similarly, in the PERISCOPE I cohort, patients with an intraoperative peak lactate level of ≥ 4 mmol/L had a higher re-intervention rate than those with lactate levels below 4 mmol/L (55% versus 0%).

The small study population and the three different doses of intraperitoneal docetaxel limited the conclusions that can be drawn from the comparison between the dose-escalation-cohort and the expansion-cohort. Another limitation of the current analysis was its retrospective design, i.e., the two cohorts were formed after completion of the study. However, notwithstanding the relatively limited sample size, this study does show that HIPEC procedures in combination with gastric cancer surgery are complex and require a different postoperative management protocol than HIPEC procedures in other cancer patients.

In the PERISCOPE I study, it appeared feasible to treat gastric cancer patients, following systemic chemotherapy, with a combination of a (sub)total gastrectomy, cytoreductive surgery and HIPEC using 460 mg/m^2 hyperthermic oxaliplatin followed by 50 mg/m^2 normothermic docetaxel. Over time, a strict perioperative management protocol was adopted to counteract the predominantly ileus-related complications. This protocol has become part of the experimental arm in the randomised PERISCOPE II study.

Table 4: Perioperative care path following the lessons learned in the PERISCOPE I study.

	Timeline	Action
Prior to surgery		
Consult dietician	directly after diagnosis	Start nutritional support if necessary
Consult physiotherapist	directly after diagnosis	Stimulate physical activity
During surgery		
Maintain normovolemia		- Fluid administration + vasopression - Hemodynamic monitoring
Pain control		Thoracic epidural analgesia
Dexamethason		8 mg intravenously 30 minutes prior to intraperitoneal chemoperfusion of docetaxel (i.e., just after the oxaliplatin perfusion)
After surgery		
Admit to ICU		
Noradrenaline	day 0*	Reach aimed mean arterial pressure with fluids and vasopression
Ringer's lactate	day 0	Strive for normovolemia
Hydrocortisone	If SIRS continues after day 1	50-100 mg three times a day
Discharge to surgical ward		If hemodynamically stable (no vasopressor, no pain, adequate diuresis)
Drains		
Gastric tube	after day 3	Remove if production <100 cc/day for 3 consecutive days
Abdominal drains	after day 2	Remove if production <50 cc/day (serous fluid)
Nutrition		
Enteral feeding via	day 0 - 3	10 cc/hour
jejunostomy	day 3 - 6	20 cc/hour
	after day 6	Increase in absence of ileus-related symptoms
TPN	after day 3	Increase until calculated nutritional needs are reached
Oral feeding	after gastric tube removal	Start oral intake
Pain medication		
Epidural	day 0	Bupivacaine 0.05% 16-20 ml/uur, if needed additional 100 ug sufentanil or clonidine 300ug
Paracetamol	day 0	1000 mg four times a day
Other		
Thrombosis prophylaxis	day 0	Fraxiparine 5700 EH
Prokinetics	day 1	Magnesiumoxide 500 mg three times a day
Enema	after day 3	If no defecation
Anti-emetics	day 0	- Metoclopramide 10 mg three times a day - Granisteron 1 mg three times a day - Droperidol 0.625 mg three times a day
Antibiotics		Only on indication
Physiotherapy	day 1	Start mobilisation

*Day 0 = *day of surgery*; TPN = T*otal Parenteral Nutrition;* SIRS *= Systemic Inflammatory Response Syndrome;* HIPEC *= Hyperthermic Intraperitoneal Chemotherapy*
ICU= Intensive Care Unit

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CHAPTER 7

Systemic exposure of oxaliplatin and docetaxel in gastric cancer patients with peritonitis carcinomatosis treated with intraperitoneal hyperthermic chemotherapy.

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Abstract

In the PERISCOPE I study, gastric cancer patients with limited peritoneal dissemination were treated with systemic chemotherapy followed by (sub)total gastrectomy, cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (HIPEC) with 460 mg/m^2 hyperthermic oxaliplatin followed by normothermic docetaxel in escalating doses (0, 50, 75 mg/m^2). In total, 25 patients completed the study protocol. Plasma samples were collected before the start of the HIPEC procedure, after oxaliplatin washing, after docetaxel washing and the following morning. Median peak plasma concentrations were $5.5*10^{-3}$ mg/ml for oxaliplatin, $89*10^{-6}$ mg/ml for docetaxel (dose 50 mg/m^2) and $113*10^{-6}$ mg/ml for docetaxcel (dose 75 mg/m2). The following morning median plasma concentrations were 32% and 4% of the measured peak concentrations for oxaliplatin and docetaxel, respectively. For both cytostatic agents, no correlation was found between intraperitoneal fluid concentration and peak plasma concentration. High doses oxaliplatin and docetaxel can be given intraperitoneally without causing potentially toxic systemic concentrations.

Introduction

In gastric cancer, the peritoneum is a predilection site for tumour dissemination. In a Dutch cohort study, 14% of the patients diagnosed with gastric cancer had peritoneal metastasis at the time of diagnosis.[1] Prognosis for this group of patients is dismal and the effect of systemic chemotherapy on overall survival is questioned.[2] Possibly, selected patients might benefit from cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (HIPEC).[3-6] In Europe, various studies have been initiated to evaluate if a HIPEC procedure offers survival benefit to gastric cancer patients with peritoneal dissemination.[7,8]

HIPEC has a few advantages over systemic chemotherapy for the treatment of peritoneal metastases. Firstly, it penetrates peritoneal tumour lesions better.[9] Secondly, due to the peritoneum-blood barrier, intraperitoneally administered drugs barely invade the systemic circulation, allowing higher local drug concentrations without major systemic complications.[10] Lastly, the chemotherapeutic agents can be heated which might improve the cytotoxic effect.[11,12] The ideal drug for the use in HIPEC for gastric cancer has a few important attributes, e.g. proven efficacy in gastric cancer treatment, favourable pharmacokinetics such as adequate tissue penetration and acceptable toxicity.[13] Agents often used intraperitoneally in gastric cancer patients are oxaliplatin, cisplatin, and mitomycin C.[3-6,13]

The PERISCOPE I study, a multi-centre dose-escalation HIPEC trial, was conducted to investigate safety and feasibility of a HIPEC procedure with oxaliplatin and docetaxel in Western gastric cancer patients with limited peritoneal dissemination.[14,15] Oxaliplatin and docetaxel were chosen as the most promising agents for the treatment of peritoneal metastasis of gastric cancer origin based on an extensive literature review.[13] Intraperitoneal oxaliplatin is widely used in HIPEC procedures for various cancer types and its pharmacokinetics have been studied.[16,17] There is far less experience with the use of intraperitoneal docetaxel, and studies on pharmacokinetics of intraperitoneally administered docetaxel are very scarce.[18,19] In this analysis, the systemic pharmacokinetics of intraperitoneal oxaliplatin and docetaxel were investigated.

Methods

Patients

All patients participated in the dose-escalation PERISCOPE I study and completed the whole study protocol. The study was approved by the Medical Ethics Committee of the Netherlands Cancer Institute/Antoni van Leeuwenhoek hospital. Patient selection criteria and a detailed study protocol were published earlier.[14]

Study procedure

All patients underwent neoadjuvant systemic chemotherapy with a combination of cytotoxic agents. Three or four cycles of neoadjuvant chemotherapy were recommended, though no requirements were set for the number of cycles nor for the cytostatic drugs. Provided that there were no clinical and radiological signs of

tumour progression, patients underwent an operative procedure consisting of a (sub)total gastrectomy, cytoreductive surgery and HIPEC. All patients received a fixed dose of hyperthermic intraperitoneal oxaliplatin (460 mg/m^2, 41-42^{o}C) for 30 minutes. To determine the maximum tolerable dose (MTD) of intraperitoneal docetaxel a dose-escalation scheme was set: level 1, 0 mg/m^2 (no docetaxel perfusion); level 2, 50 mg/m^2 and level 3, 75 mg/m^2. Docetaxel perfusion was normothermic (37^{o}C) for 90 minutes. If less than 2 out of at least 3 patients had a dose limiting toxicity, the level was considered safe and the next level was opened.

Sample collection and analysis

Blood samples were collected at the start of the HIPEC procedure (T=0), at the end of the oxaliplatin perfusion (T=oxaliplatin), at the end of the docetaxel perfusion (T=docetaxel) if docetaxel was administered, and the next morning at the Intensive Care Unit (T=morning). Plasma was separated, directly frozen and stored at -80^{o}C. Oxaliplatin and docetaxel plasma concentrations were measured according to in house developed and validated methods, which were previously described.[20,21]

Results

Neoadjuvant systemic chemotherapy generally consisted of a platinum derivate (oxaliplatin or cisplatin) in combination with a fluoropyrimidine. Last dose was given at a median of 47 (range 31-125) days before the HIPEC procedure. Overall, the median dose of oxaliplatin was 901 (range 708 - 1012) mg in a median of 5 (range 3.8-7.0) L of perfusion fluid leading to a median oxaliplatin concentration of 0.160 (range 0.121 – 0.253) mg/ml in the perfusion fluid. The median dose of docetaxel in dose level 2 was 94 (range 77 - 106) mg in a median of 5 (range 3.1 – 7.0) L of perfusion fluid leading to a median docetaxel concentration of 0.019 (range 0.015 – 0.031) mg/ml in the perfusion fluid. In dose level 3, the median dose of docetaxel was 150 (range 140 - 180) mg in a median of 5 (range 4.0 – 5.5) L of perfusion fluid, for a median concentration of 0.029 (range 0.027 – 0.045) mg/ml (**Table 1**).

Table 1. Doses (mg) and concentrations (mg/ml) of intraperitoneally administered oxaliplatin and docetaxel during HIPEC in gastric cancer patients.

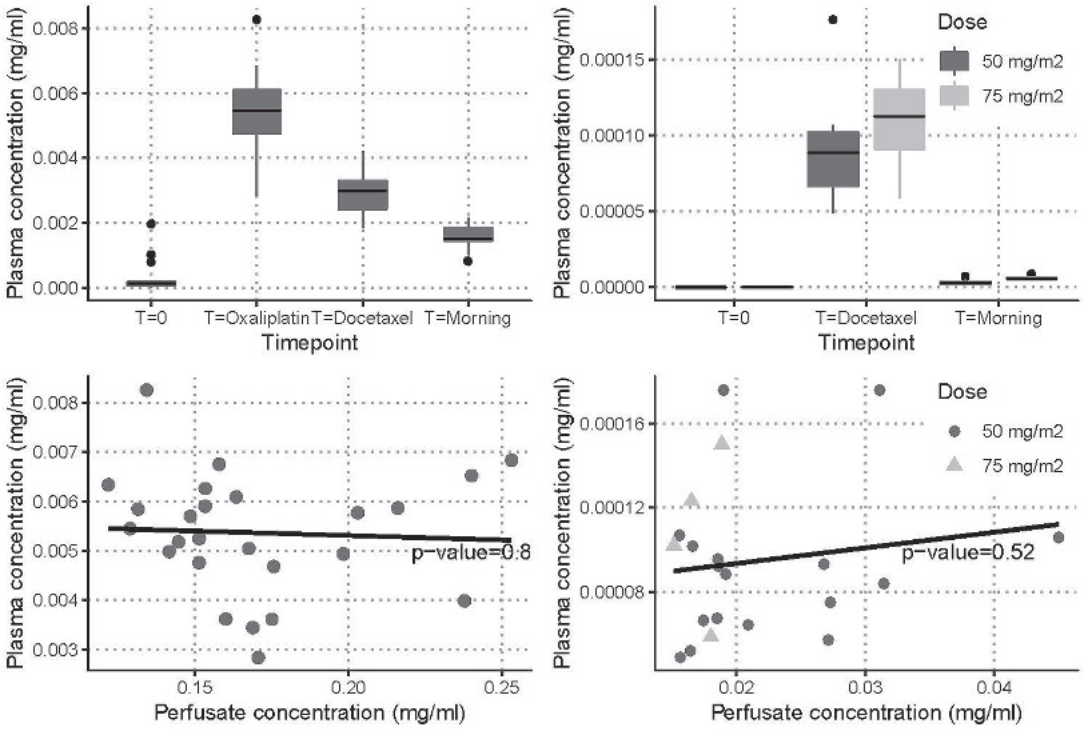
Dose level	Number of patients	Dose oxaliplatin	Dose docetaxel	Concentration oxaliplatin	Concentration docetaxel
Level 1	4	953 (908 – 1012)	-	0.149 (0.134 – 0.253)	-
Level 2	17	864 (708 – 920)	94 (77 – 106)	0.163 (0.121 – 0.216)	0.019 (0.015 – 0.031)
Level 3	4	916 (868 – 960)	150 (140 – 180)	0.178 (0.153 – 0.240)	0.029 (0.027 – 0.045)

Dose level 1 = 460 mg/m^2 oxaliplatin perfusion only; dose level 2 = 460 mg/m2 oxaliplatin and 50 mg/m^2 docetaxel; dose level 3 = 460 mg/m2 oxaliplatin and 75 mg/m^2 docetaxel. Numbers are given as medians (ranges).

All patients had a measurable plasma concentration of platinum (here presented as oxaliplatin concentration) at T=0 with a median value of 136 (range 15 - 1966)*10^{-6}mg/ml. In all but two patients, measured plasma levels of oxaliplatin were highest at the end of the oxaliplatin perfusion (T=oxaliplatin), with a median peak plasma oxaliplatin concentration (Cmax) of 5.5 (range 2.8 – 8.3)*10^{-3}mg/ml. At T= morning, 32% of the

oxaliplatin concentration measured at T=oxaliplatin was still measurable. Docetaxel was measured only for patients in dose levels 2 and 3 (N=21). None of the patients had a measurable concentration of docetaxel at T=0. The median Cmax of docetaxel in dose level 2 was 89 (range 77 – 176)*10^{-6}mg/ml and the median Cmax of docetaxel in dose level 3 was 113 (range 59 – 150)*10^{-6}mg/ml. The following morning 4% of the docetaxel concentration measured at T=docetaxel was measurable in the plasma. For both oxaliplatin and docetaxel, there was no correlation between intraperitoneal chemotherapy concentration and plasma chemotherapy concentration (**Figure 1**).

Figure 1. Plasma concentrations of oxaliplatin (A) and docetaxel (B) before the start of the HIPEC procedure (T=0), at the end of the oxaliplatin perfusion (T=oxaliplatin), at the end of the docetaxel perfusion (T=docetaxel), and the following morning (T=morning); and correlation between perfusate concentration and peak plasma concentration for oxaliplatin (C) and docetaxel (D) in gastric cancer patients undergoing HIPEC in the PERISCOPE I study.



Discussion

The dose-escalation PERISCOPE I study gave us the opportunity to study the pharmacokinetics of (heated) intraperitoneal oxaliplatin and docetaxel. In all patients, the intraperitoneal dose of hyperthermic oxaliplatin was 460 mg/m^2.[14,15] Median Cmax of oxaliplatin in the plasma was measured at 5.5*10^{-3}mg/ml. Reported values of oxaliplatin Cmax after intravenous administration of 130 mg/m^2 ranged from 4.8 to 9.5 *10^{-3}mg/ml.[22] Previous studies of intra-abdominal oxaliplatin (460 mg/m2) reported Cmax plasma oxaliplatin concentrations above 21 *10^{-3}mg/ml.[17,23] In the current study, the highest plasma oxaliplatin Cmax was 8.3 *10^{-3}mg/ml. With the analysis method used in this study all platinum, bound and unbound, is measured. Only unbound platinum has a cytostatic effect. Oxaliplatin degrades into active platinum species which bind covalently to proteins. It is very likely that most of the platinum that entered the systemic circulation was bound platinum in an inactive form.[22] Therefore, although the systemic platinum concentration in the current study was comparable to the reported platinum concentrations after intravenous administration, the systemic fraction of unbound platinum is probably much lower after intraperitoneal use than after intraveneous use. Following this, the intraperitoneal use of 460 mg/m^2 oxaliplatin is not expected to cause general systemic toxicity.

Intraperitoneal docetaxel was given in escalating doses.[14,15] After 50 mg/m^2 intraperitoneal docetaxel, median Cmax of docetaxel in the plasma was measured at 89*10^{-6}mg/ml, and after 75 mg/m^2 intraperitoneal docetaxel, this was 113 *10^{-6}mg/ml. Ten Tije et al described a Cmax of 4060 *10^{-6}mg/ml and Goey et al reported a Cmax of over 1000 *10^{-6}mg/ml after intravenous administration of 75 mg/m^2 and 100 mg/m^2 docetaxel, respectively.[24,25] In a Greek study, patients with peritoneal metastases from gynaecological origin underwent HIPEC with 75 mg/m^2 docetaxel (41-43^{o}C, 2 hour perfusion); plasma Cmax was documented at 230 *10^{-6}mg/ml.[19] And, in a Japanese study, HIPEC was performed with 40 mg/m^2 docetaxel.[18] Plasma Cmax was reached at the end of the 40 minutes perfusion with a mean value of 113.4 *10^{-6}mg/ml. Cmax levels of docetaxel in the plasma are low after its intraperitoneal use. Next to that, the plasma concentrations decline fast overnight. Thus, it is not expected that systemic toxicities occur when docetaxel is used for intraperitoneal perfusion.

No correlations were found between perfusate concentration and plasma concentration. This suggests that the concentration of intra-abdominal chemotherapy does not influence its transition into the systemic circulation. Our hypothesis is that wound surface and damaged peritoneum (e.g. by cytoreductive surgery or surgical hands) are more relevant for the penetration of cytostatic drugs into the systemic circulation than their concentration.

This is one of the first studies wherein the pharmacokinetics of intraperitoneal docetaxel were studied. A limitation of this study is that it might be possible that peak concentrations were actually higher, since samples were only collected at the end of the perfusion period. However, in the literature, peak plasma concentrations

have invariably been described at the end of the perfusion.[23] It is therefore most likely that the peak concentration measured in this study represents the actual peak concentration. Furthermore, the peritoneal cavity is extensively washed after the procedure, so the remainings of the cytostatic drugs after the HIPEC procedure will be low.[17,23]

Peak plasma concentrations of oxaliplatin and docetaxel after their intraperitoneal use are low and decline overnight. This is reasuring for the use of oxaliplatin and docetaxel in HIPEC surgery, since systemic complications from their intraperitoneal administration are unlikely. However, uncontrolled use of higher intraperitoneal concentrations should be avoided, as intraperitoneal complications may occur.[15]

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CHAPTER 8

Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy versus palliative systemic chemotherapy in stomach cancer patients with peritoneal dissemination, the study protocol of a multicentre randomised controlled trial (PERISCOPE II)

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Abstract

Background | At present, palliative systemic chemotherapy is the standard treatment in the Netherlands for gastric cancer patients with peritoneal dissemination. In contrast to lymphatic and haematogenous dissemination, peritoneal dissemination may be regarded as locoregional spread of disease. Administering cytotoxic drugs directly into the peritoneal cavity has an advantage over systemic chemotherapy since high concentrations can be delivered directly into the peritoneal cavity with limited systemic toxicity. The combination of a radical gastrectomy with cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) has shown promising results in patients with gastric cancer in Asia. However, the results obtained in Asian patients cannot be extrapolated to Western patients.

Aim | to compare the overall survival between patients with gastric cancer with limited peritoneal dissemination and/or tumour positive peritoneal cytology treated with palliative systemic chemotherapy, and those treated with gastrectomy, CRS and HIPEC after neoadjuvant systemic chemotherapy.

Methods | In this multicentre randomised controlled two-armed phase III trial, 106 patients will be randomised (1:1) between palliative systemic chemotherapy only (standard treatment) and gastrectomy, CRS and HIPEC (experimental treatment) after 3-4 cycles of systemic chemotherapy. Patients with gastric cancer are eligible for inclusion if (1) the primary cT3-cT4 gastric tumour including regional lymph nodes is considered to be resectable, (2) limited peritoneal dissemination (Peritoneal Cancer Index <7) and/or tumour positive peritoneal cytology are confirmed by laparoscopy or laparotomy, and (3) systemic chemotherapy was given (prior to inclusion) without disease progression.

Discussion | The PERISCOPE II study will determine whether gastric cancer patients with limited peritoneal dissemination and/or tumour positive peritoneal cytology treated with systemic chemotherapy, gastrectomy, CRS and HIPEC have a survival benefit over patients treated with palliative systemic chemotherapy only.

Trial registration | clinicaltrials.gov NCT03348150; registration date November 2017; first enrolment November 2017; expected end date December 2022; trial status: Ongoing.

Background

Gastric cancer has an aggressive natural behaviour, with 40% of the patients having metastatic disease at the time of diagnosis.[1] The peritoneum is a predilection site for tumour dissemination and is synchronously affected in 14% of all patients. Around 9% of the patients have peritoneal dissemination without other metastatic localisations. The prognosis of patients with peritoneal dissemination is dismal, with a median overall survival of only 3-4 months.[1,2]

Systemic therapy is less effective in patients with peritoneal dissemination compared to patients with metastases in other locations.[3-5] Administering cytotoxic drugs into the peritoneal cavity offers several advantages over systemic chemotherapy. Firstly, high concentrations can be delivered directly into the peritoneal cavity with limited systemic toxicity.[6] Secondly, heating enhances the cytotoxicity of some agents (e.g. cisplatin and oxaliplatin).[7,8] Rat models have shown enhanced tumour penetration in intraperitoneal tumour deposits if chemotherapeutic agents are administered intraperitoneally compared to intravenously.[9] Hyperthermic Intraperitoneal Chemotherapy (HIPEC) has proven its therapeutic efficacy in patients with peritoneal dissemination from several cancer types, e.g. colon cancer and ovarian cancer.[10-12] For patients with peritoneal dissemination from gastric cancer there is data, primarily Asian, suggesting that intraperitoneal chemotherapy combined with gastrectomy and cytoreductive surgery (CRS) may improve survival.[13-15]

Previously, our study group conducted a phase I-II dose-escalation trial (PERISCOPE I) to study safety and feasibility of a procedure combining gastrectomy, CRS and HIPEC with oxaliplatin (41-42^{o}C) followed by docetaxel (37^{o}C).[16] In a strictly selected group of patients, the treatment was safe and feasible with an intraperitoneal dose of 460mg/m^2 oxaliplatin followed by 50mg/m^2 docetaxel after the evolvement of a stringent post-operative care protocol.[17] In the Netherlands, the Ministry of Health appointed this novel approach as highly innovative, having led to participation in the Coverage with Evidence Development (CED) program. Within this program, the gastric HIPEC procedure is currently conditionally reimbursed by health insurance.

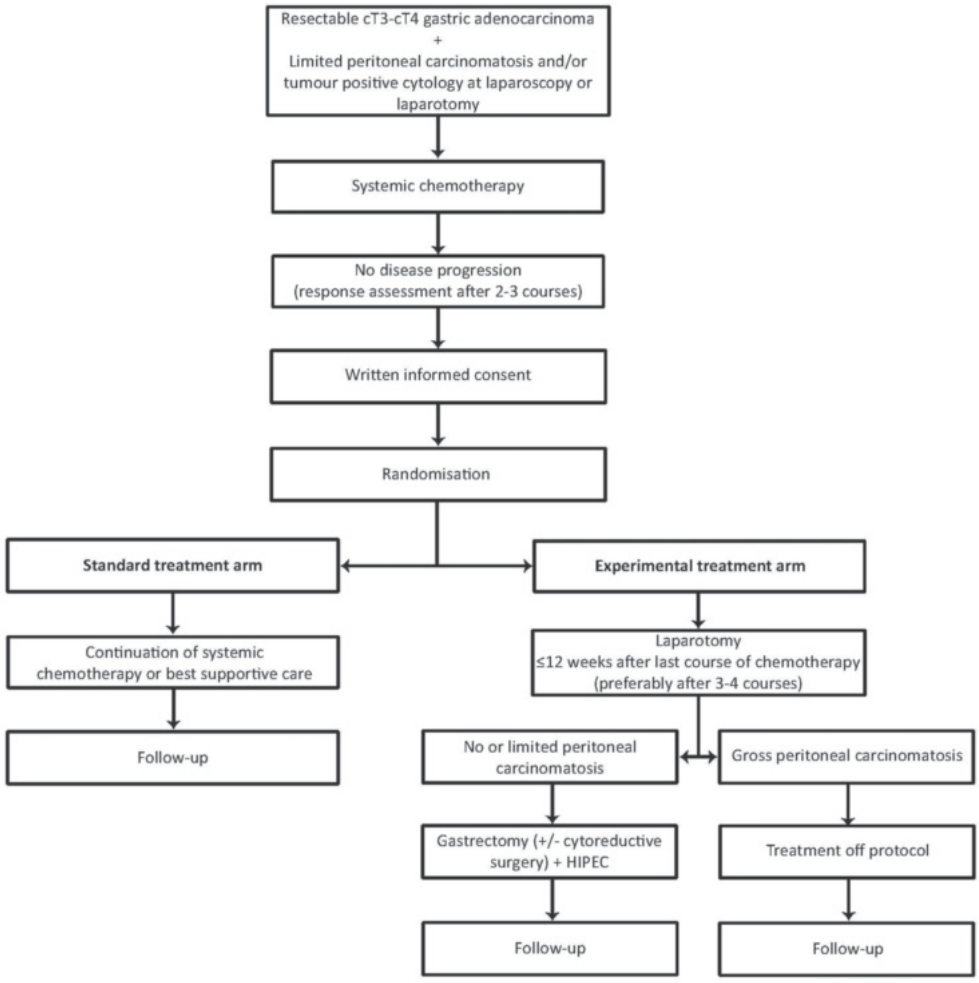
The primary objective of the present study is to compare overall survival between gastric cancer patients with limited peritoneal dissemination and/or tumour positive peritoneal cytology treated with the current standard treatment, i.e. palliative systemic chemotherapy, and those treated with gastrectomy, CRS and HIPEC after neoadjuvant systemic chemotherapy. Within the CED program, the second objective of this study is to calculate cost-effectiveness. If the experimental treatment provides a survival benefit over the standard treatment, health insurance coverage will be made unconditional.

Methods

Study design

The PERISCOPE II study is a multicentre randomised controlled two-armed phase III trial (**Figure 1**). After 3-4 cycles of systemic chemotherapy, patients are randomly allocated (1:1) to the standard treatment arm (continuing palliative systemic chemotherapy) or to the experimental treatment arm (gastrectomy, CRS and HIPEC). The study protocol has been approved by the medical ethical committee of the Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital. A research grant has been provided by The Netherlands Organisation for Health Research and Development (ZonMW).

Figure 1: PERISCOPE II study flowchart.



Study Population

Adult patients (18 years or older), with histologically proven locally advanced (cT3-cT4, any N) adenocarcinoma or undifferentiated carcinoma of the stomach with limited peritoneal dissemination and/or tumour positive peritoneal cytology are eligible for participation. In this trial, limited peritoneal dissemination is defined as a Peritoneal Cancer Index (PCI) below 7.[18-20] At first, patients have to be treated with systemic chemotherapy. Study candidates are included provided that the primary gastric tumour is considered resectable, there is no disease progression during systemic chemotherapy and distant metastases are absent. A detailed list of the inclusion and exclusion criteria can be found in the Additional file.

Sample size

Previous data have indicated that the median survival time of patients with gastric cancer with peritoneal metastasis is about 3-4 months.[1] It is expected that in the experimental arm 75% of patients will receive protocol treatment and 25% of patients will be treated off protocol due to gross peritoneal dissemination (PCI≥7) at the time of the laparotomy. It is hypothesised that the median overall survival among the 75% of patients in the experimental arm who actually undergo CRS and HIPEC will be 12 months, while the other 25% of patients in the experimental arm will have a median overall survival of only 3 months.

A total of 106 patients, 53 in each arm, will be included and followed until a total of 80 deaths is observed. Assuming exponential survival with medians as described above in each of the three groups, this will yield 90% power to detect a difference in overall survival at the two-sided 95% confidence level (intention-to-treat analysis).

In the Netherlands, around 200 patients per year are diagnosed with gastric cancer and synchronous peritoneal carcinomatosis without distant metastases.[1] At least 60% of these patients will not be eligible for the study because of co-existing diseases, poor condition, irresectability of the gastric tumour and/or gross peritoneal tumour involvement. This leaves an estimated number of 80 patients per year eligible for inclusion. Next to that, around 5% of all newly diagnosed gastric cancer patients who are found suitable for treatment with curative intent has tumour positive peritoneal cytology [21,22]. This group of patients, around 50 per year, is also eligible for inclusion. It adds up to a total of 130 potential study candidates per year in the Netherlands.

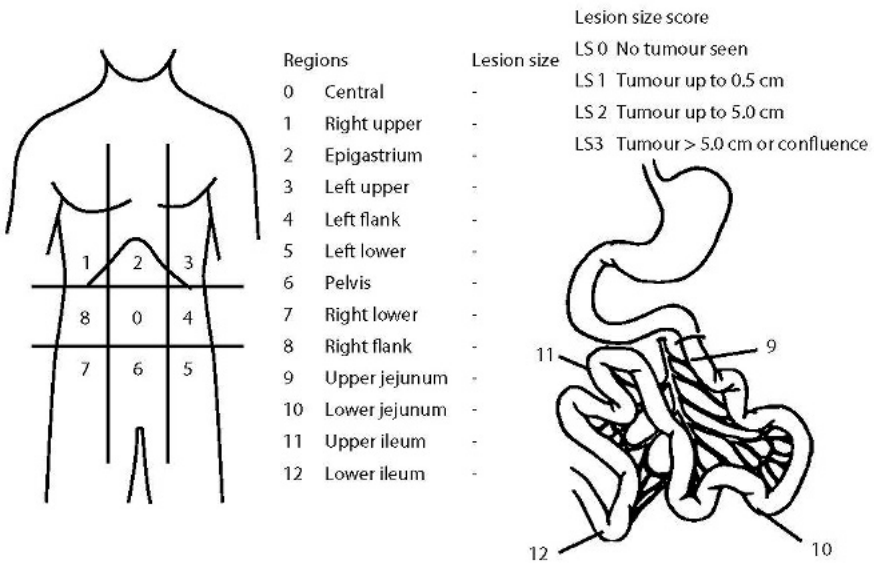
The expected accrual is around 35 patients per year. Via the Dutch Upper GI Cancer Group (DUCG) all medical oncologists and surgeons who treat patients with gastric cancer are being informed on a regularly basis about the study progress and referral issues. Patient inclusion will take about three years. Thereafter, there will be an additional follow-up period of two years, for a total study period of five years.

Study procedures

Prior to inclusion: laparoscopy or laparotomy

Prior to inclusion all patients undergo a diagnostic laparoscopy or laparotomy. During this procedure the extent of peritoneal dissemination is assessed. The presence and number of macroscopic tumour deposits are recorded according to the PCI (**Figure 2**).[18]

Figure 2: Peritoneal cancer index.[18]



Prior to inclusion: systemic chemotherapy and response assessment

Patients are treated with systemic chemotherapy prior to inclusion. Accepted chemotherapy regimens generally consist of a platinum-drug combined with a fluoropyrimidine. Additionally, an anthracycline or taxane can be added according to the local protocol. Examples of accepted chemotherapy regimens are: docetaxel + oxaliplatin + 5-FU, docetaxel + cisplatin + 5-FU, epirucibin + cisplatin + 5-FU, epirucibin + oxaliplatin + 5-FU. In patients with a Her2 positive gastric tumour, trastuzumab can be added to the combination of chemotherapeutic drugs.

Response assessment is done by a Computed Tomography (CT)-scan after 2-3 courses. In the absence of disease progression, patients can be included. Response evaluation and patient's study inclusion are discussed in (local or regional) multidisciplinary tumour board meetings.

Inclusion and randomisation

After written informed consent is obtained, the patient is registered and randomised. Patients are randomised centrally by computer and are stratified for centre (name of hospital), main histological subtype (intestinal versus diffuse) and for the extent of peritoneal dissemination (macroscopic peritoneal tumour deposits versus tumour positive peritoneal cytology only).

Treatment

Standard arm

After randomisation, patients included in the standard arm continue treatment with systemic chemotherapy. The treating physician determines which chemotherapeutic regimen is used and the duration of the treatment. Surgery in this arm is only performed to relieve severe symptoms, such as a gastric outlet obstruction.

Experimental arm

If allocated to the experimental treatment arm, preferably 3-4 courses of systemic chemotherapy are given prior to surgery, as is usual in the potentially curative setting for gastric cancer. Within 4 weeks before the planned operation an additional CT-scan is made. If there are still no signs of tumour progression patients proceed to surgery.

Laparotomy

Surgical approach is via a midline laparotomy. A thorough inspection of the peritoneal cavity is performed. If ascites is found, representative samples are obtained for cytological assessment. The presence and number of macroscopic tumour deposits are recorded to score the PCI (**Figure 2**).

Gross peritoneal dissemination (PCI ≥7), small bowel dissemination and/or an irresectable primary gastric tumour preclude further study treatment. In these instances, HIPEC is not performed and it is up to the surgeon to decide whether a palliative surgical intervention is indicated.

Gastrectomy, cytoreductive surgery and HIPEC

If a potentially curative gastric cancer resection is possible and the PCI is below 7, a (sub)total gastrectomy with D2 lymphadenectomy is performed. Patients with macroscopic peritoneal tumour deposits undergo CRS to leave no macroscopic disease behind. Gastrointestinal continuity is restored by either a Billroth II or Roux-en-Y reconstruction.

HIPEC is performed via 3 inflow and 2 outflow catheters using an open abdominal technique under continuous circulation. The peritoneal cavity is perfused with 460 mg/m^2 oxaliplatin (max 920mg) at an intraperitoneal temperature of 41°C to 42°C. After 30 minutes, the perfusion fluid is drained from the abdomen and the peritoneal cavity is perfused with 50mg/m^2 docetaxel (max 100mg) at an intraperitoneal temperature of 37°C,

for 90 minutes. A feeding jejunostomy catheter is inserted and will remain in situ until oral intake is adequate. The three inflow catheters are left in situ for postoperative drainage.

Postoperative care

After surgery, all patients are admitted to the Intensive Care Unit. Postoperative enteral feeding via the jejunostomy catheter can start on the day of surgery at a very low dosage (maximum 10cc/hour). Besides that, total parenteral nutrition is started on postoperative day 3. When there are no (more) signs of a postoperative ileus, oral feeding is introduced and enteral feeding via the jejunostomy catheter is gradually increased.

Adjuvant treatment

Adjuvant treatment is not part of the standard study protocol but will be discussed in the multidisciplinary tumour board meeting for all patients included in the study. The decision is based upon the patient's individual intraoperative and pathological results, the response to and toxicity from neoadjuvant systemic therapy, as well as the postoperative recovery.

Follow-up

All patients, including those patients whose treatment has deviated from the study protocol, are seen at the outpatient clinic every 3 months for 1.5 years and every 6 months thereafter until 3 years after randomisation. Survival status and disease recurrence/progression are assessed until death. Follow-up consists of physical examination, diagnostic investigations (tumour markers in blood samples and CT-scans) and registration of hospital re-admission details (if applicable). Quality of life (QoL) questionnaires are sent to the patient at 3, 9, 15, 24 and 36 months after randomisation.

Safety

All adverse events and serious adverse events are recorded until 100 days after randomisation (standard arm) or surgery (experimental arm). To ensure quality of data, study integrity and compliance with the protocol and the various applicable regulations and guidelines, a data monitor of the Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital has been appointed to conduct site visits to the participating centres and randomly check patient data. Data from all patients are also checked at the central data centre of the Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital. An independent safety monitoring board including a statistician, surgeon and medical oncologist has been installed. After the first 20 patients have completed 90 days of follow-up the safety monitoring board will advise on the continuation of the study. This procedure will be repeated after the inclusion of 40 patients with 90 days of follow-up.

Analysis

Study outcome parameters will be analysed using descriptive statistical methods. Overall and disease-free survival analyses will be performed by the Kaplan-Meier method for all patients following the intention-to-treat principle. A per-protocol analysis will be performed. In these analyses, survival will be measured from

the date of randomisation to the date of disease recurrence and/or death. An interim analysis for efficacy will be performed when 40 deaths (i.e. half of the required number of events) have been observed.

Cost-effectiveness Analysis

The cost-effectiveness analysis will compare the costs and health benefits of the standard treatment (palliative systemic chemotherapy) to those of the experimental treatment (including the HIPEC procedure). This analysis will include direct costs (surgery, HIPEC, diagnostic work-up, treatment of recurrences, follow-up visits and palliative care) and indirect costs such as productivity losses. The primary outcome for health effects will be quality adjusted life years, measured by means of the EuroQol 5D, being part of the study QoL questionnaires.

Responsibilities

Protocol modifications will be submitted as amendment to the central medical ethical committee by the study coordinator. Communication between the study centres, the independent safety monitoring board and the data monitor is coordinated by the study coordinator. Participating study centres are responsible for patient inclusion, patient treatment, patient follow-up and data collection in the central data portal. At least twice a year a meeting will be organised for all relevant parties, i.e., the principle and local investigators, the trial sponsors, the data monitor, and the study coordinator, to discuss progress, problems and possible protocol modifications. The study coordinator – together with the principle investigator - will have access to the final dataset and is responsible for publishing study results. The results will be submitted to a peer-reviewed journal.

Discussion

Study rationale

The primary objective of the PERISCOPE II study is to compare overall survival between patients with gastric cancer with limited peritoneal dissemination and/or tumour positive peritoneal cytology treated with the current standard treatment, i.e. palliative systemic chemotherapy and those treated with gastrectomy, CRS and HIPEC, following systemic chemotherapy. In the dose-escalation PERISCOPE I study the combination of gastrectomy, CRS and HIPEC with oxaliplatin and docetaxel following systemic chemotherapy appeared safe and feasible provided that the following aspects were acknowledged.[16,17] Firstly, the maximum dose of intraperitoneal docetaxel should not exceed 50mg/m^2.[17] Secondly, patients were selected according to strict in- and exclusion criteria.[16] And, thirdly, to counteract the frequent occurrence of ileus-related postoperative complications, a stringent postoperative care protocol has been implemented.[23]

Patient selection

Complete cytoreduction is a key element in successful HIPEC surgery. There is a clear relationship between the probability to reach a complete cytoreduction and the extent of peritoneal disease, i.e. the PCI.[19]. In several studies of patients with gastric cancer with peritoneal metastasis treated with a HIPEC procedure, a PCI of 7 emerged as a cut-off value between patients with long-term survival and those without.[19,20,24] Therefore, in the PERISCOPE II study, a PCI below 7 has been defined as inclusion criterion. It can be expected that strict PCI criteria improve homogeneity of the included study population.

Choice of intraperitoneal chemotherapy

Based on a comprehensive literature review a combination of a platinum-based agent and a taxane was considered to be the most promising for the intraperitoneal treatment of peritoneal dissemination of gastric cancer origin.[25] Cisplatin and oxaliplatin are both platinum-based chemotherapeutic agents that are often used in HIPEC procedures. For gastric cancer patients oxaliplatin seems favourable for a number of reasons. Firstly, gastric cancer cell lines are more sensitive to oxaliplatin than to cisplatin.[26] Secondly, systemic oxaliplatin appears to be superior, or at least equal, in terms of overall and disease-free survival in patients with gastric cancer.[27,28] And, lastly, in contrast to cisplatin, oxaliplatin is not nephrotoxic.

The taxane docetaxel was chosen as second agent as it is widely used in the systemic treatment of gastric cancer.[29,30] It can be administered intraperitoneally, as shown in Asian studies wherein catheter-based-intraperitoneal docetaxel had clinical efficacy with acceptable safety.[13,29]

Learning curve

HIPEC procedures in general have a steep learning curve.[31-33] In the current trial, no more than 5 centres will participate in the experimental treatment arm. These 5 centres were selected based on their experience in gastric cancer surgery and in HIPEC procedures for other indications as well as on their geographic location in

the Netherlands. During the PERISCOPE I study, strict guidelines for per-operative and postoperative care were defined. Considering the extensive experience of the participating centres together with the strict guidelines, the learning curve in the PERISCOPE II is expected to be negligible.

Cost effectiveness

Based on the costs and the quality-adjusted life years a model can be drafted to estimate the financial impact of the experimental treatment. This will provide governments with a potential basis to draft legislation regarding cost authorisation for the HIPEC procedure as a possible treatment option in the management of gastric cancer patients.

Other HIPEC trials for gastric cancer

In the German GASTRIPEC trial (NCT02158988) gastrectomy and CRS are compared to gastrectomy, CRS and HIPEC with mitomycin C and cisplatin in patients with gastric cancer and synchronous peritoneal dissemination. In the French GASTRCHIP trial (NCT01882933) gastrectomy and HIPEC with oxaliplatin (250mg/m^2) are compared to gastrectomy only in patients with locally advanced gastric cancer defined as cT3-cT4 with either serosal invasion, tumour perforation, lymph node invasion or tumour positive peritoneal cytology [34]. The presence of macroscopic peritoneal lesions is an exclusion criterion in the GASTRICHIP trial. At present, the PERISCOPE II trial is unique in comparing gastrectomy, CRS and HIPEC with palliative systemic chemotherapy, which is the current standard treatment for patients with gastric cancer with peritoneal dissemination in the Netherlands.

Conclusion

The PERISCOPE II trial will determine whether patients with gastric cancer with limited peritoneal dissemination (PCI<7) and/or tumour positive peritoneal cytology treated with systemic chemotherapy followed by gastrectomy, CRS and HIPEC have a survival benefit over those treated with palliative systemic chemotherapy alone. The study will provide data on survival, toxicity, cost-effectiveness and quality of life in patients with gastric cancer undergoing HIPEC surgery. The ultimate goal is to define whether the HIPEC procedure can be used as a standard treatment option for patients with gastric cancer with limited peritoneal dissemination and/or tumour positive peritoneal cytology, provided that there was no disease progression during neoadjuvant systemic chemotherapy.

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Additional file

Inclusion criteria

In order to be eligible for participation in this study, a patient must meet all following criteria:

- Age ≥ 18 years.
- Biopsy proven primary adenocarcinoma (or undifferentiated carcinoma) of the stomach. Including tumours at the oesophagogastric junction provided that the bulk of the tumour is located in the stomach, and, the intended surgical treatment is a gastric resection and not an oesophagectomy. A high intra-thoracic anastomosis is allowed, but not if a thoracotomy is necessary.
- cT3-cT4 tumour (TNM classification, 7th edition) considered to be resectable (including lymph nodes).
- Limited peritoneal carcinomatosis (PCI <7) and/or tumour positive peritoneal cytology confirmed by laparoscopy or laparotomy and proven by pathological examination.
- Treatment with systemic chemotherapy, with the latest course ending within 8 weeks prior to inclusion. All currently standard chemotherapy regimens are acceptable.
- Absence of disease progression during systemic chemotherapy (prior to inclusion).
- World Health Organisation performance status 0-2.
- Adequate bone marrow, hepatic and renal function. Minimally acceptable laboratory values at start of the study inclusion:
    - White blood cell count (WBC) ≥3.0 x 10^9 /L
    - Platelet count ≥ 100 x 10^9 /L
    - Serum bilirubin ≤ 1.5 x ULN, and ALAT and ASAT ≤ 2.5 x ULN
    - Creatinine clearance ≥ 50 ml/min (measured or calculated by Cockcroft-Gault formula)
- For female patients who are not sterilised or in menopause (i.e., amenorrhea ≥1 year if age ≥60 years, or ≥2 years if age <60 years):
    - negative pregnancy test (urine/serum)
    - no breast feeding or active pregnancy ambition
    - reliable contraceptive methods
- Signed informed consent.

Exclusion criteria

A patient who meets any of the following criteria will be excluded from participation in this study:

- Distant metastases (e.g., liver, lung, para-aortic lymph nodes; i.e., stations 14 and 16) or small bowel dissemination.
- Recurrent gastric cancer.
- Prior resection of the primary gastric tumour.
- Non-synchronous peritoneal carcinomatosis.
- Current other malignancy (other than cervix carcinoma and basalioma).
- Uncontrolled infectious disease or known infection with Human Immunodeficiency Virus type -1 or -2.
- A known history of hepatitis B or C with active viral replication.
- Recent myocardial infarction (< 6 months) or unstable angina.
- Any medical condition not yet specified above that is considered to interfere with study procedures, including adequate follow-up and compliance and/or would jeopardise safe treatment.
- Known hypersensitivity for any of the applied chemotherapeutic agents and/or their solvents.

CHAPTER 9

Beyond the PD-L1 horizon: in search for a good biomarker to predict success of immunotherapy in gastric and oesophageal adenocarcinoma.

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Abstract

Gastric adenocarcinoma and oesophageal adenocarcinoma are aggressive cancers with a poor prognosis. Therefore, new therapeutic strategies are needed, especially for patients refractory to conventional treatment. Cancer immunotherapy (CIT), is a promising new treatment option and is effective in a proportion of patients with gastroesophageal malignancies. Biomarkers for selecting patients likely to benefit from CIT in gastroesophageal malignancies remain unproven. Programmed cell death ligand-1 (PD-L1), which is a validated biomarker in non-small cell lung cancer (NSCLC), is often also used to select patients for CIT in the context of gastroesophageal cancer, although this marker has not been validated for this purpose. We question the use of PD-L1 as a biomarker in gastroesophageal cancers, as there are fundamental differences in PD-L1 expression between NSCLC and gastroesophageal cancers. This review discusses the value of PD-L1 in selecting patients for CIT in oesophageal and gastric cancer. Potential alternatives, especially microsatellite instability and Epstein-Barr virus positivity, are discussed.

Introduction

Gastric adenocarcinoma (GA) and oesophageal adenocarcinoma (EAC) are aggressive cancers with a variable response to neo-adjuvant therapy and poor overall prognosis. Despite significantly improved survival since the introduction of perioperative chemotherapy and neo-adjuvant chemoradiotherapy, the 5-year overall survival after treatment with curative intent is 35% for patients with stage >II GA, and 47% in oesophageal cancer.[1,2] Therefore, there is a high clinical need for novel and more effective treatment options for these patient groups, especially for patients refractory to standard therapies.

Cancer immunotherapy (CIT) has emerged as a promising new treatment option for several cancer types. As cancer cells use pathways responsible for immune-tolerance to avoid elimination by the host immune system, so called "immune checkpoint pathways", targeted therapies against these pathways have emerged as a new powerful tool in the treatment of cancer patients. Most currently used immune checkpoint inhibitors are directed against the programmed cell death-1/programmed cell death ligand-1 (PD-1/PD-L1) pathway and against cytotoxic T lymphocyte associated antigen 4 (CTLA4). Immune checkpoint inhibitors have become standard therapy for, amongst others, melanoma [3], and non-small cell lung cancer (NSCLC).[4-7] For other malignancies, including GA and EAC, there are ongoing clinical trials investigating the effect of CIT. In 2017, the FDA approved pembrolizumab for patients with previously treated locally advanced or metastatic gastric or gastro-oesophageal junction (GEJ) cancer whose tumours express PD-L1.[8] At this moment our knowledge of patient selection criteria for CIT is very limited and enrichment strategies are urgently required to select those patients who are likely to benefit from immune checkpoint inhibitors. In many CIT clinical trials an effort was done to elucidate whether a biomarker can predict response to the therapy (summarised in **Table I**). Often these analyses were done retrospectively and the results are debatable.

Recent advances in basic, translational and clinical research in the field of cancer immunotherapy have resulted in the definition of a concept called the "cancer immunogram" which aims to describe the interaction between cancer and the immune system. Typically, such immunograms distinguish seven parameters that are considered likely to affect the antitumour immune response.[9] These parameters include: tumour PD-L1 expression, tumour mutational burden, the general immune status of the patient, presence of T cell immune infiltrates, sensitivity of tumour cells to T-cell killing, a myeloid cell-mediated inflammation, and high serum lactate dehydrogenase (LDH). Building on this 'cancer immunogram' concept, approaches for selecting the most promising biomarkers with respect to CIT can be explored and are thus being evaluated in various studies. The main directions in this respect are: PD-L1 expression, the amount of tumour infiltrating lymphocytes, mutational or neoantigen burden, peripheral blood markers (e.g. lymphocyte and neutrophil counts as well as the size of the different subcompatrments), immune gene expression signatures, or multiplex

immunohistochemistry to characterise the tumour infiltrating immune compartment.[10] At this moment PD-L1 is the most widely used biomarker for patient selection for CIT in clinical practice.

The role of PD-L1 in inflammation and tumour immune evasion

The role of PD-L1 in regulation of inflammatory response.

PD-L1 is a ligand expressed mainly by antigen-presenting macrophages and dendritic cells. PD-L1 binds to PD-1 expressed on activated T cells. This binding leads to downregulation and limitation of the T-cell response in inflammation. During the inflammatory response, naïve T-cells in the lymph nodes are exposed to antigens, expressed on MHC class I by antigen presenting cells (APC), especially the mature dendritic cells. If an antigen is immunogenic, antigen presentation leads to activation of the T-cell receptor (TCR) on T-cells. The resulting canonical TCR signal transduction provokes expression of TCR signalling-specific genes finally resulting in T-cell activation. In turn, T-cell activation provokes T cell proliferation, production of cytokines and differentiation towards cytotoxic T-cells or T helper cells. Activated T-cells leave lymph nodes and execute effector functions, for instance killing virus-infected cells. As such responses can result in exaggerated immunity and damage to healthy tissue, powerful mechanisms to regulate the extent of this immune response are involved. These control mechanisms are manifold and include immune-downregulation by the interaction between PD-L1 and PD-1. The latter provides a strong tolerogenic signal to the T cell and hence protects tissue from collateral damage during inflammation and diminishes the possibility of autoimmune reactions (**Figure 1**). The tolerogenic function of PD-L1 and PD-1 interaction is well illustrated by the fact that anti-PD-L1 treatment can result in auto-immune-like side-effects such as colitis, type 1 diabetes and hepatitis.[11]

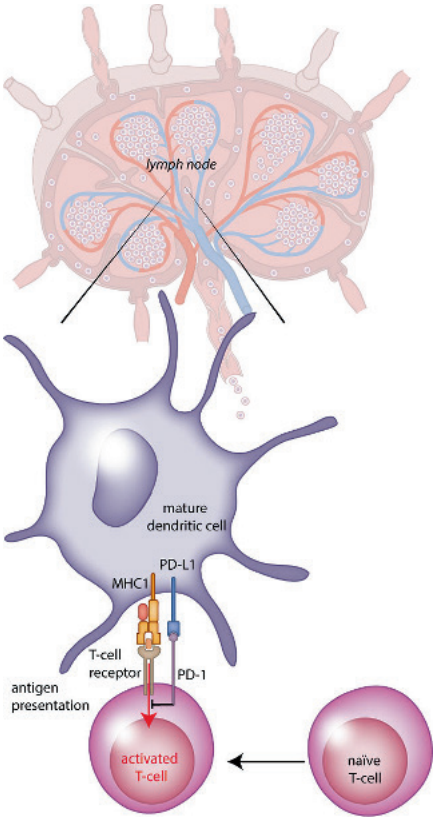


Figure 1: Mechanism of T-cell activation by antigen presenting cells. In the lymph nodes dendritic cells present antigens to naïve T-cells on MHC1 class I molecules (MHC1). Upon binding MHC1-antigen complex with a compatible T-cell receptor naïve T-cells become activated and further induce T-cell response during inflammation. This process can be downregulated/blocked by PD-L1/PD-1 interaction.

Anti-cancer immune response

Cancer cells produce cancer-specific peptides, so called neo-antigens, which are presented on the cancer cell's surface by MHC class I molecules. Without cross presentation of cancer-specific neo-antigens by APCs, the immune system is unable to activate T-cells and produce a sufficient response to eliminate the tumour. In general tumour cells do not have sufficient co-stimulatory activity to activate a naïve T cell. Nevertheless, neo-antigens are released to the extracellular medium due to natural tumour cell death and then are taken up by dendritic cells or macrophages, which can subsequently present these neo-antigens to T-cells with sufficient co-stimulatory activity, to activate TCR signalling in naïve T cells.[12] The activated T-cells produce anti-tumour cytokines, proliferate and differentiate into CD8+ positive cytotoxic T-cells, which participate in an effective immune response against the tumour. The resulting death of tumour cells and subsequent release of further neo-antigens provokes further activation of the immune system and, in an ideal situation complete tumour elimination (**Figure 2**).[13]

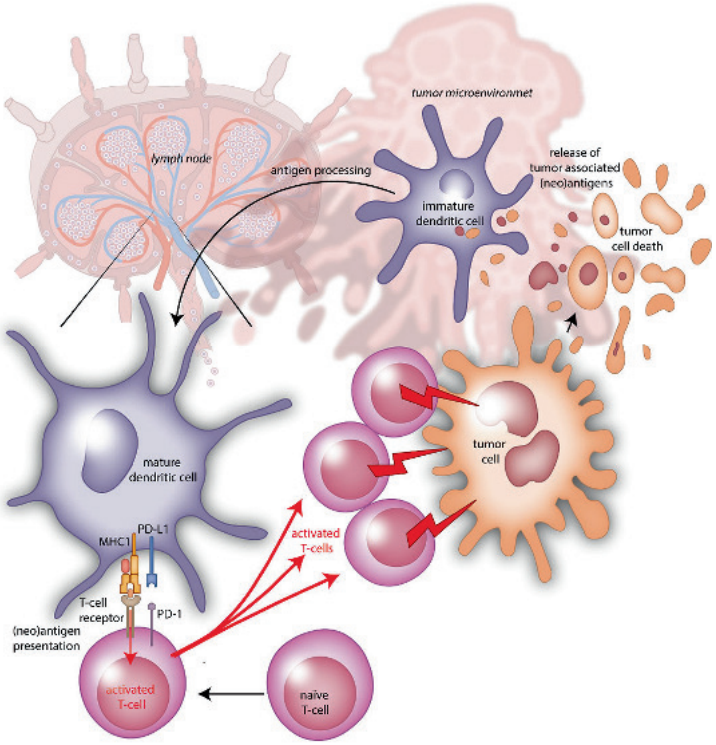
Mechanism of PD-1/PD-L1 induced tumour immune evasion

The PD-1/PD-L1 immune inhibitory axis is exploited by many tumours to evade T-cell-mediated anti-tumour immune response.[14] Tumour microenvironment plays an important role in this context where, for example

upon stimulation by certain pro-inflammatory cytokines, PD-L1 becomes expressed on cancer cells as well as on tumour-infiltrating immune cells, especially myeloid cells, such as macrophages and dendritic cells.[15] This PD-L1 upregulation is, amongst others, a consequence of IFN-y production by activated T-helper cells, CD8 positive cytotoxic T-cells and NK-cells, whose presence is common to the cancer microenvironment, especially at the periphery of the tumour.

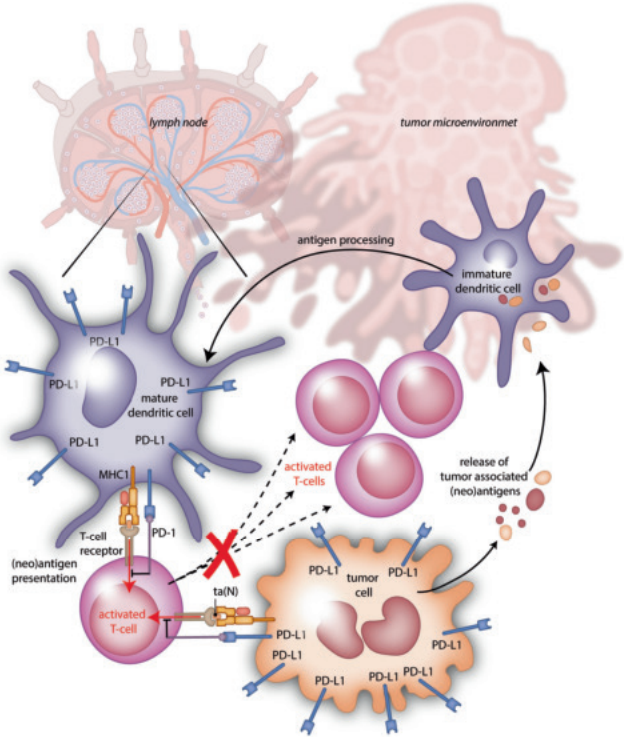
A correlation exists between PD-L1 expression, the amount of tumour infiltrating lymphocytes and IFN-y production in the tumour microenvironment.[16,17] The most well-known cytokine responsible for PD-L1 upregulation in the gastro-intestinal tract is the regulatory cytokine interleukin 10. Moreover, even independently of pro-inflammatory signals, cancer cells may show constitutive expression of

Figure 2: Immune response against cancer. Neoplastic cells release tumour associated (neo)antigens, which are taken up by immature dendritic cells. These (neo)antigens are then processed and are presented by mature dendritic cells on MHC class I molecules (MHC1) to naïve T-cells, leading to activation of the T-cell receptor (TCR) on T-cells. This results in an activation of the canonical TCR signal transduction, expression of TCR-signalling specific genes and T-cell activation. The activated T-cells produce anti-tumour cytokines, proliferate and differentiate into cytotoxic T-cells. This results in an active attack of the tumour by the immune system and tumour cell death with a subsequent release of large amount of tumour associated (neo)antigens and further activation of the immune system.



PD-L1 due to genetic alterations and activation of oncogenic signalling pathways, such as the AKT or STAT3, but this requires further investigation in the context of tumours of proximal tract tumours.[18] The extracellular binding of PD-L1 to PD-1 results in intracellular inhibition of TCR signalling. Following PD-L1 binding, PD-1 displays oligomerisation and this in turn allows the non-receptor tyrosine phosphatase SHP-2 to bind to the so-called PD-L1 immunoreceptor tyrosine inhibitory motif (ITIM) and immunoreceptor tyrosine switch motif (ITSM). This subsequently allows SHP-2 to exert its immune-inhibitory action on TCR signalling and also other Th1 immune signalling pathways.[19,20] This finally culminates in ineffective immune responses to tumour associated neo-antigens and finally to tumour immune evasion (**Figure 3**).[21] By use of immune checkpoint inhibitors this mechanism of tumour immune evasion can be combated. Therefore, assessing the expression of PD-L1 on tumour cells seems like a rational strategy for determining which patients are likely to benefit from therapy with immune checkpoint inhibitors.

Figure 3: PD-1/PD-L1 induced immune evasion in cancer. Under certain circumstances PD-L1 becomes expressed on cancer cells. Moreover, cancer cells produce cytokines that enhance expression of PD-L1 on antigen presenting cells (dendritic cells and macrophages). Binding of PD-L1 to PD-1 on T-cells results in inhibition of T-cell receptor signalling, unsuccessful response to tumour associated (neo) antigens (ta(N)) and downregulation of the anti-tumour immune response, thereby protecting the tumour from attack of the immune system.



Evidence for PD-L1 as a predictive biomarker for response to cancer immunotherapy

Clinical trials in melanoma and NSCLC.

A correlation between PD-L1 immunohistochemical positivity and response to CIT has been shown in a limited number of clinical trials. These studies have been performed almost invariably in patients with either metastatic melanoma or NSCLC. In a study by Topalian.[22] nivolumab was effective in 20-25% of patients with melanoma or NSCLC. Thirty-six percent of patients with PD-L1 positive tumours had an objective response, compared to none of the patients with PD-L1 negative tumours, suggesting a relationship between response and tumour cell PD-L1 expression. Also, in the POPLAR study, atezolizumab compared to docetaxel significantly improved survival in patients with previously treated NSCLC. The improvement was correlated to the levels of PD-L1 immunohistochemistry expression on tumour cells and tumour-infiltrating immune cells, suggesting that PD-L1 expression is predictive of atezolizumab benefit.[23] Also, in the OAK trial, the greatest survival benefit was seen in patients with tumours expressing high levels of PD-L1, although overall survival was also significantly improved in patients with less than 1% PD-L1 expression.[24]

So far, the most convincing evidence for correlation between PD-L1 expression and response to CIT was shown in the KEYNOTE-024 study.[25] In this study, with patients with advanced NSCLC and PD-L1 expression on at least 50% of the tumour cells, pembrolizumab was associated with significantly longer progression-free and overall survival than with platinum-based chemotherapy. Based on these and similar results, PD-L1-based selection of patients for CIT has gained a role in clinical management of several cancer types. However, even in NSCLC and melanoma, several challenges arise with using PD-L1 as a biomarker, including the dynamics and differential expression of PD-L1 within a tumour and the lack of a standard definition for overexpression. Detailed description of these and other limitations can be found in a previously published review by Topalian et al.).[26]

Clinical trials in gastroesophageal malignancies.

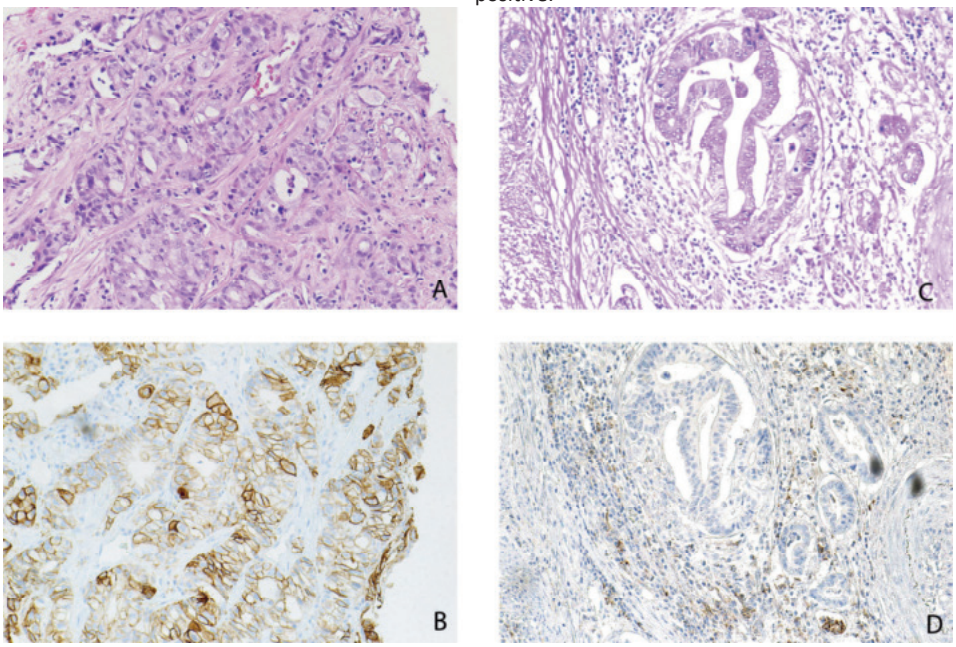
There is little data on CIT biomarkers in GA and EAC. It is tempting to draw parallels between lung and oesophageal and gastric adenocarcinoma, since these are all epithelial malignancies with many similarities in etiology, morphology and mutational profile. Extrapolating the correlations observed to EAC and GA, it would appear that PD-L1-based selection for CIT in EAC and GA is rational. Therefore, many clinical trials are already using PD-L1 expression as a selection criterion for CIT, either alone or in combination with chemotherapy. But, in the absence of compelling data in gastrointestinal cancers, the question arises whether it is reasonable to draw parallels between NSCLC and EAC and GA. For example, in the KEYNOTE-059 study responses to CIT were seen in both PD-L1 positive and negative gastroesophageal cancers, with objective response rates of 15.5% and 6.4% respectively. These data support the inadequacy of PD-L1 as a predictive a biomarker.[8,27] Additionally, in a comprehensive review about immunotherapy in gastroesophageal cancers, response rates

of single-agent checkpoint inhibitors in metastatic GA and EAC of approximately 22%-27% for PD-L1+ patients vs 10%-17% for unselected patients were described.[28] Moreover, in a recent issue of the Lancet, Kang et al. showed a survival benefit in patients with advanced gastroesophageal junction (GEJ) or GA who were treated with nivolumab.[29] Although this study was designed to investigate the effect of nivolumab in patients unselected for PD-L1 tumour expression, retrospective immunostaining for this biomarker showed that the effect of nivolumab was independent of PD-L1 positivity. In the KEYNOTE-061 study, pembrolizumab did not significantly improve overall survival compared with paclitaxel in advanced PD-L1 positive gastroesophageal cancers.[30] In all, the available data suggest that PD-L1 may not be the ideal biomarker to select patients with gastroesophageal cancer for immunotherapy.

Expression pattern of PD-L1 in gastroesophageal malignancies.

Muro et al. reported PD-L1 expression in around 40% of GEJ and GA. It is important to note that this expression is mainly observed in immune cells and not in the epithelial cancer cells, which is fundamentally different from what is seen in NSCLC.[31] In this study the tumours were considered positive, if at least 1% of assessable cells were positive, while membranous PD-L1 expression of more than 50% of only epithelial tumour cells was needed to demonstrate benefit of immunotherapy over chemotherapy in NCSLC.[32,33] Similar results were published by Thompson, reporting PD-L1 positivity in only 12% of tumour cells and 44% of immune stroma.[34] In gastric adenocarcinoma only 14% of tumours were positive for PD-L1 in >1% of epithelial tumour cells and 11% of tumours in >1% of stroma cells.[35] In the study of Kawazoe A et al, most gastric adenocarcinomas (61,4%) showed positivity in tumour infiltrating immune cells and only 22,8% in the epithelial tumour cells.[36] These data suggest that PD-L1 expression in EAC and GA cancers cannot be evaluated in the same manner as in NSCLC. In our experience we also observe a different pattern of PD-L1 expression in lung adenocarcinomas and gastric adenocarcinomas, unpublished data (**Figure 4**). In lung adenocarcinomas PD-L1 positive cells are observed in both compartments: the immune cells and in the epithelial cells. In contrast, most EAC and GA show PD-L1 expression (if positive) only in the immune cell compartment. To our opinion, future research should explore the association between PD-L1 expression in different tumour compartments in EAC and GA (epithelial cells versus immune stroma, with a particular focus on the immune stroma) and the associated response to CIT.

Figure 4. Differences in expression of PDL1 in lung adenocarcinoma and gastric adenocarcinoma. A, B: Histology (A) and PD-L1 immunohistochemistry (B) in lung adenocarcinoma. The tumour epithelial cells show moderate to strong expression of PD-L1. C,D: Histology and corresponding PD-L1 immunohistochemistry in gastric adenocarcinoma. The tumour epithelial cells are completely negative, and immune cells in stroma are positive.



Other biomarkers for patient selection in cancer immunotherapy

Microsatellite instability and EBV status.

If PD-L1 is not the best biomarker in gastroesophageal cancers, which other options do we have? In a large and comprehensive TCGA study including 295 primary GA, four subtypes of stomach cancer were identified: 9% of tumours showed positivity for Epstein-Barr virus (EBV subtype), 22% of tumours were classified as a microsatellite instability (MSI) subtype, 50% of tumours demonstrated high chromosomal instability (CIN subtype), and 20% were categorised as a genomically stable subtype.[37] Two of these subtypes, MSI and EBV tumours, are particularly interesting in regard to CIT. As such, a recent phase II trial described salvage treatment with pembrolizumab in metastatic gastric cancer patients. Patients with MSI-high (MSI-H) and EBV-positive tumours showed responses to pembrolizumab, with an overall response rate of 85,7% in MSI-high and 100% in EBV-positive gastric cancers.[38] These findings, which need to be validated prospectively, have the potential to substantially improve the treatment in a subset of gastric cancer patients and make MSI-H and EBV positivity reliable predictive biomarkers. Underlying mechanisms explaining the good response of these gastric cancer subtypes to CIT have not been elucidated yet, but such factors as high tumour mutational

burden, increased immune cell infiltration in the tumour microenvironment (TME) and a high neo-antigen load can play a role.

The role of tumour infiltrating lymphocytes in MSI and EBV tumours

The amount of tumour infiltrating lymphocytes (TILs) may be related to the efficacy of anti-cancer immune responses, a feature also appreciated by the "cancer immunogram". A recent meta-analysis showed that a high density of intratumoural CD8+ and CD3 T-cells is significantly associated with improved overall survival in gastric cancer patients.[39] In colorectal cancer patients, tumour infiltrating lymphocytes and the immunoscore are considered to be useful prognostic markers of survival.[40,41] The immunohistochemistry based assessment of T-cells in melanoma patients reveals the association between CIT efficacy and increased numbers of TILs.[42-44] At this moment there are no published data regarding the role of TILs as a separate biomarker in gastroesophageal cancers. However, tumours of both the EBV and MSI subtype exhibit high levels of tumour infiltrating lymphocytes. It is probable that such subtypes interact very differently with the immune system than other GA subtypes. Therefore, it is possible that the high level of TILs in the EBV and MSI subtypes of GA may correspond to increased success of CIT, and studies addressing this notion are currently under progress in our institution as well as elsewhere. Yet, TILs as a single marker is unlikely to be a successful strategy for selection of patients, as different subpopulations of lymphocytes exhibit different functions and can either potentiate or reduce tumour growth. Moreover, the interaction between the host immune system and tumour cells is extremely complex and depends on multiple factors, not only on the amount of TILs. Most likely, the amount of cytotoxic CD8-positive T-cells, in combination with other markers such as mutational load, PD-L1 expression, markers of IFN-γ pathway etc, will be more successful as a multifactorial biomarker in CIT.[26]

The role of high mutational load and neo-antigens in MSI tumours

MSI tumours are known to harbor high mutational loads, which in turn is associated with improved survival and response to immune checkpoint inhibitors.[45] This relationship could be explained by increases in tumour "foreignness" due to expression of deviant proteins/peptides as products of mutated genes (these represent the earlier-mentioned tumour-associated neo-antigens). It is probable that high numbers of TILs, characteristic of this GA subtype, is the result of this increased immunological "foreignness". This increased propensity to provoke an immunological response may indicate that immune-checkpoint targeted therapy will be relatively successful for such cancers. It is also encouraging in this respect that, in a recent issue of Nature, a neo-antigen-based stratification approach to predict tumour response to checkpoint blockade immunotherapy in pancreatic cancer was published.[46,47] The authors identified neo-antigens as a biomarker predicting immunogenic pancreatic tumours in patients prone to benefit from immunotherapy. This adds to the idea that MSI subtypes of GA are likely to respond to CIT. Based on these data one may hypothesise that the proposed TCGA subtyping of GA performs better in correlation to immune checkpoint inhibitor efficacy than the tumour PD-L1 expression currently favored in most clinical practice. Supporting this are the results

of a subgroup analysis (post-hoc analysis) of MSI high tumours in the KEYNOTE-061 study. Even though pembrolizumab as second-line therapy did not significantly improve overall survival compared with paclitaxel in patients with PD-L1 positive advanced GA or GEJ cancer, pembrolizumab was beneficial in the MSI-high subgroup.[30]

However, mutational load is also not a perfect predictor of response. Patients with NSCLC with a higher mutational load benefitted more from pembrolizumab than patients with lower mutational load, but there were clear outliers making it imperfect.[48] The same has been reported for ipilimumab and tremelimumab in melanoma patients, where mutational load correlated significantly to response to CIT but on its own was not enough to be used as a biomarker.[22,49]

Other possible biomarkers

The "cancer immunogram" suggests other biomarkers as general lymphocyte count, MHC expression and high serum LDH concentrations. Although some of them have been investigated in other cancer types, none of these additional biomarkers have been tested in EAC and GA, underlining the need for further research in this area. At this moment there are also no data available on specific mutations in tumour-suppressor genes or tumour-promoter genes in association with response to immunotherapy in EAC and GA. However, the recent systematic review and meta-analysis by Lee et al reported that in advanced NSCLC, checkpoint inhibitors improved overall survival compared to docetaxel and had a significantly improved overall survival for EGFR wild-type over EGFR mutant tumours.[50] Immunotherapy prolonged overall survival in EGFR wild-type patients (HR, 0.67; 95% CI, 0.60-0.75; P < .001), but not in EGFR mutant patients (HR, 1.11; 95% CI, 0.80-1.53; P = .54). Also, CIT prolonged overall survival in the KRAS mutant subgroup (HR, 0.65; 95% CI, 0.44-0.97; P = .03) but not in the KRAS wild-type subgroup (HR, 0.86; 95% CI, 0.67-1.11; P = .24). Whether specific mutations in GA and EAC are associated with response to CIT remains to be elucidated.

There is also a possible role for PD-L2 expression in gastric cancer as a biomarker. Though current research has only focused on it as a prognostic factor for survival. Whether PD-L2 expression can be used as biomarker for PD-L1 blockade therapy in gastroesophageal carcinomas should be further explored.[51-53]

Discussion

The currently available data imply that PD-L1 expression in cancer cells can be a good predictor of response to CIT in patients with NSCLC, but there is insufficient evidence to apply this approach for patients with gastroesophageal malignancies. The expression pattern of PD-L1 in gastroesophageal malignancies is different from the expression pattern in NSCLC. In gastroesophageal cancer, PD-L1 expression is mainly observed in immune stroma rather than in epithelial cells, in contrast to NSCLC. Therefore, an extrapolation of findings in NSCLC to gastroesophageal cancer does not seem reasonable. This topic needs to be addressed in future research.

As cancer-immune system interaction is complex and depends on many factors, it is likely that strategies to combine multiple markers may be more successful in comparison to a single marker with respect to prediction of clinical success of CIT. As such, it is plausible that tumours with a high expression of PD-L1 but with a low amount of tumour infiltrating lymphocytes respond less to CIT than tumours exhibiting both these features. Therefore, it seems to be important to study a combination of markers to get to an optimal prediction model for the response to CIT.

Presently, in gastroesophageal cancers, MSI and EBV status are the most promising predictive biomarkers for CIT. The exact biological mechanism of the striking effect of CIT in these tumours has not yet been fully elucidated, but a complex interplay between tumour cells and immune microenvironment, including tumour mutational load, TILs and PD-L1 expression, plays an important role. For an accurate selection of patients most likely to respond to CIT, future studies need to aim for a combination of improved biomarker strategies in conjunction with MSI status, EBV status, precise characterisation of the immune infiltrate and the neo-antigen burden in different types of gastroesophageal cancer separately.

Table 1. Overview of immunotherapy studies and researched biomarkers.

Author	Tumour type	Therapy	Biomarker	Reference
Chen et al.	Melanoma	CTLA-4 blockade + PD-L1 blockade	TILs	39
Fehrenbacher et al.	NSCLC	Atezolizumab	PD-L1	18
Fuchs et al.	Gastric + GEJ cancer	Pembrolizumab	PD-L1	8, 22
Garon et al.	NSCLC	Pembrolizumab	PD-L1	27
Hamid et al.	Melanoma	Ipilimumab	TILs	38
Herbst et al.	NSCLC	Pembrolizumab vs docetaxel	PD-L1	28
Kang et al.	GEJ cancer	Nivolumab	PD-L1	24
Kawazoe et al.	Gastric cancer	Pembrolizumab	MSI, EBV, PD-L1	31
Luksza et al.	Melanoma, lung cancer	CTLA-4 blockade or PD-L1 blockade	Neoantigens	42
Reck et al.	NSCLC	Pembrolizumab	PD-L1	20
Rizvi et al.	NSCLC	Pembrolizumab	Mutational load	43
Shitara et al.	Gastric + GEJ cancer	Pembrolizumab vs. paclitaxel	PD-L1	25
Snyder et al.	Melanoma	Ipilimumab and tremelimumab	Mutational load	44
Topalian et al.	Melanoma, prostate cancer, colon cancer, renal-cell cancer and NSCLC	Nivolumab	PD-L1	17
Tumeh et al.	Melanoma	Pembrolizumab	TILs	37
Rittmeyer et al.	NSCLC	Atezolizumab	PD-L1	19

NSCLC = non-small cell lung cancer; GEJ = gastro-esophageal junction; CTLA-4 = cytotoxic T lymphocyte associated antigen 4; PD-L1 = programmed cell death-1; TILs = tumor infiltrating lymphocytes; MSI = microsatellite instability; EBV = Epstein-Barr virus

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CHAPTER 10

High CD8+ tumour infiltrating lymphocyte density associates with unfavourable prognosis in oesophageal adenocarcinoma following poor response to neo-adjuvant chemoradiotherapy.

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Abstract

Introduction | Determining prognosis following poor response to neo-adjuvant chemoradiotherapy (nCRT) in oesophageal adenocarcinoma (OAC) remains challenging. An immunosuppressive tumour microenvironment (TME) as well as immune infiltrate density and composition are considered to play a critical role in the immune interaction between host and tumour and can predict therapy response and survival in many cancers, including gastro-intestinal malignancies. The aim of this study was to establish the TME characteristics associated with survival following a poor response to nCRT.

Methods | The prognostic significance of OAC-associated CD3+, CD4+, CD8+, FOXP3+ and PDL1 expression was studied by immunohistochemistry and quantified by automated image analysis in 123 patients who underwent nCRT and curative resection. Results from good and poor responders were contrasted and immune infiltration was related to disease course in both groups. Subsequently a cohort of 57 patients with a moderate response to nCRT was analysed in a similar fashion.

Results | Tumour cell percentage positively correlated to immune infiltration markers. In good responders and moderate responders, none of the immune infiltrate parameters was associated with survival, in poor responders CD8+ was an independent negative predictor of OS in univariate analysis (p=0.03) and high CD8+ infiltration was associated with worse OS (15 months vs 32 months, p=0.042).

Conclusion | A high CD8+ density is an independent biomarker of poor OS in poor responders to nCRT, but not in good and moderate responders. Our results suggest that patients with a poor response to nCRT but concomitant high CD8+ counts in the resection specimen require adjuvant therapy.

Introduction

Oesophageal adenocarcinoma (OAC) is the predominant type of oesophageal carcinoma in the Western world and its incidence is increasing rapidly.[1] Addition of neo-adjuvant therapies like chemotherapy or chemoradiotherapy (nCRT) to the surgical resection has improved clinical outcome of patients with oesophageal cancer, but overall survival (OS) still remains poor.[2,3] In the Netherlands and various other European countries, nCRT combined with oesophagectomy is the recommended treatment with curative intent for locally advanced oesophageal carcinoma.[4,5] Five-year survival of OAC patients after nCRT followed by surgery is around 45%. The pathological complete response (pCR) rate for OACs in the CROSS study was 23%.[5] Patients with a pCR have a better prognosis than patients without a pCR.[5,6] About one-third of the tumours (35%) in the CROSS study had a poor response to nCRT, with no or minimal tumour regression seen in the resection specimen. Patients with these tumours had a similar overall survival to patients treated with surgery alone. The factors that allow risk group stratification in the latter group remain largely obscure.

With the success of immunotherapy trials in melanoma patients it became clear that the immune infiltrate is an important component of the tumour microenvironment (TME). Also in gastro-intestinal malignancies the components of TME have been shown to have a prognostic value. Immunoscore assay, which is based on the total CD3+ T cells counts and CD8+ cytotoxic T cells counts, provided a reliable estimate of the risk of recurrence in colon carcinoma patients in a large multicentre international study.[7] As a rule of the thumb, tumours with an “immune-inflamed” phenotype are typically characterised by a high tumour mutational burden, high immune infiltrate density and programmed death ligand 1 (PD-L1) positivity, and have a better prognosis.[8] This probably relates to the activity of the CD8+ cytotoxic T lymphocyte compartment that can recognise tumour neo-antigens and kill the cancer cells involved. However, it is unknown how the TME in general and CD8+ infiltration in particular relates to prognosis in patients with an unfavourable response to nCRT.

Prompted by the above-mentioned consideration, we aimed to characterise the immune infiltrate and the PD-L1 expression in OAC patients treated with nCRT followed by surgery. We have mainly concentrated on CD3 and CD8 densities as these markers are components of the Immunoscore assay and have been shown to have prognostic relevance in gastro-intestinal malignancies. Additionally, we assessed different cell subsets in the immune infiltrate, like CD4+ T-helper cells, regulatory FOXP3+ T cells and CD20+ B-cells.

Methods

Patient selection

All OAC patients treated with nCRT according to the CROSS regimen followed by an oesophageal resection in the Netherlands Cancer Institute (NCI), Amsterdam or the Zuyderland Hospital, Heerlen between 2011 and 2018 were selected for this study.[5] Pathological response of the tumour to nCRT was assessed according to the Mandard-tumour regression grading (TRG)-system.[9] Such grading is hampered by subjective interpretation of the pathologist with respect to the resection specimens involved and especially TRG 3 is considered problematic in this respect and can furthermore not easily be dichotomously classified into poor or good responders.[10] Thus TRG3 patients were initially not included for analysis in this study. In addition TRG 1 patients have no tumour cells and can thus not be analysed for possible relations between TME and tumour cells, and hence were also not included. Hence, initially we only included patients with a near complete pathological response (TRG 2, categorised as good responders) and tumours with little to no response (TRG 4 or TRG 5, categorised as poor-responders).[9] Archived formalin fixed paraffin embedded tissue (FFPE) samples and Haematoxylin and Eosin (HE) slides of the pre-nCRT biopsies and surgical resection specimen were collected from the pathology archives. Clinical and survival data were derived from the patient records. Overall survival was calculated from the date of surgery to time of death. Following the initial analysis it was decided to include a TRG3 cohort of NCI patients (moderate responders) as well, for comparison. Work up for this cohort was identical to the poor responder and good responder groups. The study was approved by the Institutional Review Board (IRB) of the NCI and was registered as study CFMPB576.

Histopathological assessment and immunohistochemistry

All HE slides were reassessed by a specialised gastro-intestinal pathologist (LK) and for TRG 4/5 cases the FFPE tumour block of the resection specimen most concordant with this TRG grading was selected. For the TRG 2 cases mostly only one FFPE block that contained tumour cells was available. For the limited number of cases in which more than one FFPE block was available for a TRG 2 case, the block most concordant with TRG 2 grading was selected. Immunohistochemistry of the FFPE samples was performed on a BenchMark Ultra autostainer (Ventana Medical Systems). Briefly, paraffin sections were cut in 3 μm slides, heated at 75°C for 28 minutes and deparaffinised with EZ prep solution (Ventana Medical Systems). Heat-induced antigen retrieval was carried out using Cell Conditioning 1 (Ventana Medical Systems) for 32 minutes at 95^{o}C (CD3, CD8 and CD20), for 48 minutes at 95^{o}C (PD-L1) and for 64 minutes at 95^{o}C (FOXP3). CD3 was detected with clone SP7 (1/100 dilution, 32 minutes at 37^{o}C, ThermoScientific), CD8 with clone C8/144B (1/200 dilution, 32 minutes at 37^{o}C, DAKO /Agilent), CD20 with clone L26 (1/800 dilution, 32 minutes at 37^{o}C, DAKO), FOXP3 with clone 236A/E7 (1/200 dilution, 120 minutes at room temperature, AbCam) and PD-L1 with clone 22C3 (1/40 dilution, 60 minutes at room temperature, DAKO /Agilent). Bound antibody was detected using the OptiView

DAB Detection Kit, and slides were counterstained with Hematoxylin and Bluing Reagent (Ventana Medical Systems).

Digitalisation

HE and immunohistochemistry slides were scanned using the Aperio (Leica Biosystems, Buffalo Grove, IL, USA). To be able to compare different tumours with a large variation in tumour area (good responders versus poor responders), a uniform evaluation algorithm was applied. This algorithm consisted of identification of four hotspots of 0.5x0.5 mm^2 each, based on the highest apparent density of immune infiltrate in the presence of cancer cells. A preliminary exploratory analysis revealed that inclusion of more hotspots leads to inferior reproducibility as it forces the inclusion of more fibrotic areas not truly representative of tumour cell/immune infiltrate interaction, especially for the TRG 2 cases. For these TRG 2 cases the small tumour size necessitates selection of four hotspots relatively close to each other, whereas for TRG 3 and TRG 4/5 cases it was possible to select hotpots relatively distant from each other. The selection was performed without knowledge of clinical outcome or earlier pathological results. The same areas were analysed in the corresponding immunohistochemistry slides (**Figure 1**).

Image analysis and quantification

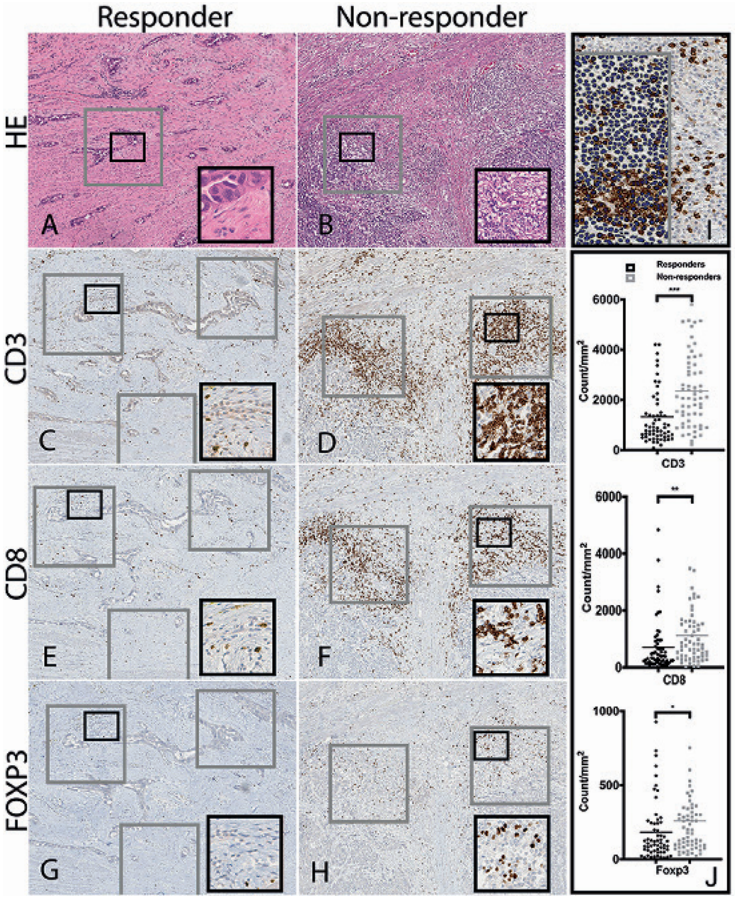
HALO imaging analysis software (Indica Labs, Corrales, New Mexico, USA) was used to quantify the amount of CD3+, CD4+, CD8+, CD20+ and FOXP3+cells in the hotspots. For each antibody, a software counting algorithm was first optimised and subsequently uniformly applied to all slides to provide the number of antibody positive cells per mm^2. Tumour percentage was scored by two specialised GI-pathologist (LK and PS); an interobserver Pearson correlation was performed and revealed acceptable concordance (R=0.89). PD-L1 positivity was scored manually by two researchers (LK and WK) who were blinded for clinical and pathological outcome. Tumours in which a PD-L1 positivity in at least 5% of the tumour immune stroma cells or tumour epithelial cells were classified as $PD\text{-}L1^{rich}$ for categorical analyses, based on the previous study of Turkington et al.[11]

Statistical analysis

Differences between groups (responders and non-responders) were analysed using the chi-square or Fisher's exact test (for clinical and pathological characteristics, and PD-L1 expression) or the Mann-Whitney U test (for the density of CD3, CD4, CD8, CD20 and FOXP3 positive cells density). Pearson correlation coefficient was used to establish a relation between tumour cell percentage and density of immune infiltrate. Association between parameters and patient survival was established through univariate analysis followed by multivariate analysis to correct for multiple testing. To this end, if appropriate, results of the image analysis were converted to numerical data by determining counts per mm^2 and then converted to ordinal data by grouping patients together based on the count/mm2 (0-250, 250-500, etc). Kaplan-Meier curves were generated and compared with the log-rank test. P-values of <0.05 were considered statistically significant. For dichotomal analysis of CD8+, a so-called rounded mean count was used for practical reasons. For all patients the true mean was 979

$CD8^+$ cells/mm^2, but counts in excess of 1000 $D8^+$ cells/mm^2 were used to classify patients as $CD8+^{high}$, likewise in good responders the true mean was 641 $CD8^+$ cells/mm^2, but counts in excess of 650 $CD8^+$ cells/mm^2 were used to classify patients as $CD8+^{high}$, whereas in poor responders the true mean was 1126 $CD8^+$ cells/mm^2, but counts in excess of 1100 cells/mm^2 were used to classify patients as $CD8+^{high}$. For moderate responders the true mean was 1177 $CD8^+$ cells/mm^2, but counts in excess of 1200 $CD8^+$ cells/mm^2 were used to classify patients as $CD8+^{high}$. All statistical analyses were performed using SPSS 22 (IBM, Armok, New York, United States).

Figure 1: Differences in immune infiltrate composition between responsive and non-responsive oesophageal adenocarcinomas treated with neo-adjuvant chemoradiotherapy. Grey squares (0.5*0.5 mm) represent the tumour areas selected for analysis, shown at x10. Black squares exhibit selected areas at x20. (A, B) H&E staining, (C, D) CD3+ antibody staining, (E, F) CD8+ antibody staining, (G, H) FOXP3+ antibody staining, (I) representative image of HALO automated analysis software. The number of brown cells (positive for specific antibody) was counted per mm^2 in the selected areas (grey squares), blue cells (negative for specific antibody) were not counted. (J) Quantification of CD3, CD8 and FOXP3 density in responders versus non-responders (horizontal lines represent the mean values).



Results

Patient and tumour characteristics

The initial study population consisted of 123 OAC patients treated with nCRT followed by surgical resection in the NCI (n=66, 54%) or in the Zuyderland Hospital (n=57, 46%). Median age was 64 (range 41-82) years. Patient and tumour characteristics are shown in **Table 1**. Because the study subjects hailed from two different centres, we compared characteristics of the patients from the two centres. For both poor responders as well as good responders no relevant differences were detected (**Supplementary table 1**). CD8 density was different between two cohorts in good responders and CD4 density in poor responders. Of the 123 patients, 62 (50%) were classified as good responders (Mandard TRG 2) and 61 (50%) were classified as poor responders (Mandard TRG 4 or 5). The distribution of pre-treatment T and N stages was similar between the good responders and poor responders. As expected, pathological stages differed significantly between the groups (**Table 1)**. Interestingly, poor responders had a significantly longer time between the start of nCRT and surgery as compared to good responders.

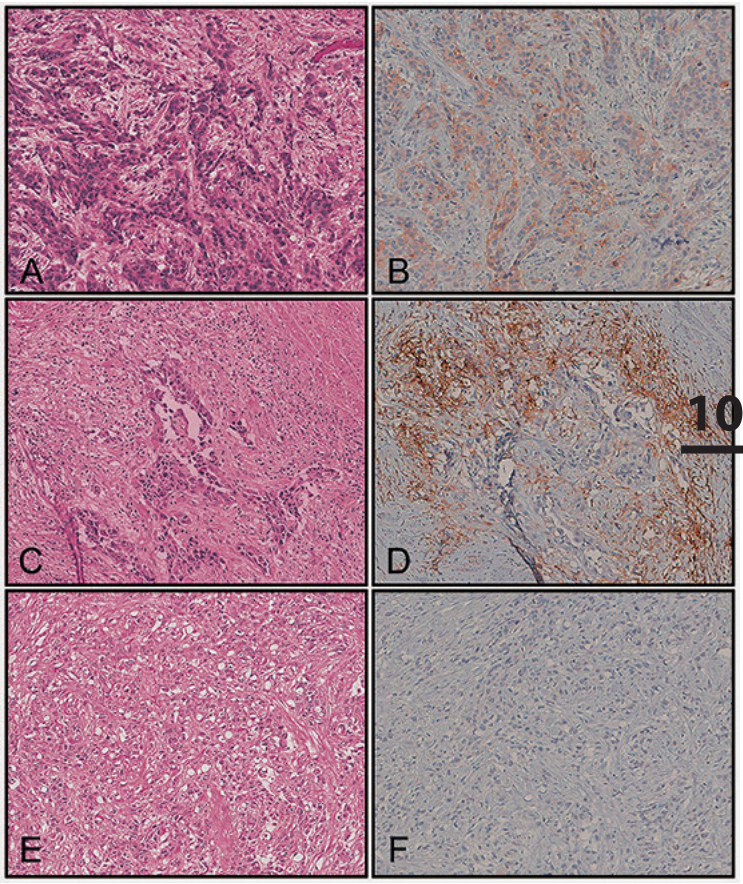


Figure 2: Patterns of PD-L1 expression in oesophageal adenocarcinomas. H&E (A) and PD-L1 expression in tumour cells (B). H&E (C) and PD-L1 positive expression in peritumoural immune infiltrate (D). H&E (E) and absence of PD-L1 expression in both tumour cells and in peritumoural (F). Shown at x100.

Table 1: Patient and tumour characteristics of oesophageal adenocarcinoma patients with good responders, moderate responders and poor response to neo-adjuvant chemoradiotherapy.

		All n=180	Good responders n=62	Moderate responders n=57	Poor responders n=61	p-value^ overall	p-value^ GR - MR	p-value^ MR - PR	p-value^ GR - PR
Median age (years, range)		65 (41-85)	65 (44-82)	67 (41-85)	63 (41-78)	0.678	0.498	0.414	0.892
gender	Male	144 *80%*	48 *77%*	47 *82%*	49 *80%*	0.788	0.648	0.816	0.693
	Female	36 *20%*	14 *23%*	10 *18%*	12 *20%*				
Median time nCRT and OK (days, range)		90 (63-298)	84 (69-123)	89 (64-111)	95 (63-298)	0.001	0.697	0.005	0.006
tumour location	Mid oesophagus	2 *1%*	1 *2%*	0 *0%*	1 *2%*	0.270	0.140	0.058	0.843
	Distal oesophagus	91 *51%*	28 *45%*	39 *68%*	24 *39%*				
	OGJ	65 *36%*	23 *37%*	18 *32%*	24 *39%*				
	Unknown	22 *12%*	15 *24%*	0 *0%*	23 *38%*				
Radical resection	Yes	149 *83%*	49 *79%*	55 *96%*	45 *74%*	0.498	1.00	0.412	0.432
	No	8 *4%*	2 *3%*	2 *4%*	4 *7%*				
	Unknown	23 *13%*	11 *18%*	0 *0%*	12 *20%*				
Operation technique	Trans-hiatic	104 *58%*	37 *60%*	39 *68%*	28 *46%*	0.497	0.238	0.581	0.586
	Trans-thoracic	38 *21%*	10 *16%*	18 *32%*	10 *16%*				
	Unknown	38 *21%*	15 *24%*	0 *0%*	23 *38%*				
cT stage	T1	1 *1%*	0 *0%*	0 *0%*	1 *2%*	0.248	0.427	0.511	0.183
	T2	48 *27%*	21 *34%*	15 *26%*	12 *20%*				
	T3	130 *72%*	41 *66%*	42 *74%*	47 *77%*				
	T4	1 *1%*	0 *0%*	0 *0%*	1 *2%*				
cN stage	N0	74 *41%*	27 *44%*	21 *37%*	26 *43%*	0.768	0.575	0.575	0.917
	N+	106 *59%*	35 *56%*	36 *63%*	35 *57%*				
pT stage	T0	1 *1%*	1 *2%*	0 *0%*	0 *0%*				
	T1	38 *21%*	17 *27%*	9 *16%*	5 *8%*	0.002	0.072	0.076	0.002
	T2	39 *22%*	22 *35%*	14 *25%*	11 *18%*				
	T3	100 *56%*	25 *40%*	32 *56%*	43 *70%*				
	T4	2 *1%*	0 *0%*	0 *0%*	2 *3%*				
pN stage	N0	107 *59%*	46 *74%*	32 *56%*	29 *48%*	0.008	0.053	0.364	0.002
	N+	73 *41%*	16 *26%*	25 *44%*	32 *52%*				

GEJ = gastro-esophageal junction, cT = clinical T stage, cN stage = clincal N stage, pT stage = pathological T stage, pN stage = pathological N stage, GR = good responders, MR = moderate responders, PR = poor responders
^Oneway Anova was used for continuous variables; chi-squared or fishers's exact test was used for catogorical variables (unknowns removed)

Relation between characteristics of the TME and response to nCRT in OAC

In order to characterise the immune infiltrate in good responders and poor responders, the density of CD3+ and, CD4+ lymphocytes as well as the density of cytotoxic CD8+ T-cells and FOXP3+ regulatory T-cells were assessed in both groups **(Table 2)**. In general, it is assumed that cancer-associated inflammation is driven by the presence of cancer cells and in the present study we also observe that the size of the immune T-cell compartment (as measured by CD3+ density and CD8+ density) strongly relates to tumour cell percentage when all patients were analysed (**Table 3**). The group of good responders shows also a significant association between tumour cell percentage and a total amount of T-cells (CD3+) and FOXP3+cells. Surprisingly, in poor responders tumour cell percentage was not related to the density of immune cell infiltration, either when taken overall or when analysed for specific immune cell subgroups (**Table 3**). Thus the relationship between tumour cell burden and immune cell infiltration is disturbed following an unsuccessful nCRT.

Relation between PD-L1 expression and response to nCRT

Overall, 40 tumours (33%) were PD-L1^{rich} and 83 tumours (67%) were PD-L1^{poor}. Of note, in all PD-L1^{rich} tumours, PD-L1 expression was seen in the tumour associated immune cells, whereas PD-L1 positivity in the tumour cells themselves was only seen in 5 tumours (4%) (**Figure 2**). There was no association between PD-L1 positivity in the tumour cells and the response to nCRT, with 3 PD-L1^{rich} tumours among the good responders and 2 PD-L1^{rich} tumours among the poor responders. However, the expression of PD-L1 in the tumour-associated immune cells was different between the two groups, with significantly more PD-L1^{rich} tumours among the poor responders than among the good responders (41% versus 24%, *p<0.001*) (**Table 2**). Thus in agreement with the results obtained with respect to immune infiltrate, PD-L1 expression correlated with poor response to nCRT and thus appeared related to the presence of tumour cell-driven immune infiltration. Interestingly, PD-L1 positivity did not correlate with survival (**Supplementary Figure 1** shows an analysis of the relation between PD-L1 positivity and survival of all patients included in the present study).

Table 2: Characteristics of the immune tumour microenvironment in oesophageal adenocarcinoma patients with good responders, moderate responders and poor response to neo-adjuvant chemoradiotherapy.

		Good responders n=62		Moderate responders[$] n=57		Poor responders n=61		p-value^ overall	p-value^ GR - MR	p-value^ MR - PR	p-value^ GR - PR
Mean tumour cell percentage[#]		8.6		23.3		37.2		<0.001	<0.001	<0.001	<0.001
CD3+ count/mm2		1324		2249		2370		<0.001	0.001	0.661	<0.001
CD4+ count/mm2		442				827					<0.001
CD8+ count/mm2		641		1177		1126		0.008	0.004	0.804	0.001
FOXP3+ count/mm2		182				261					0.024
CD20+ count/mm2		416				717					0.006
CD8+/CD3+ ratio		0.51				0.46					0.93
FOXP3+/CD3+ ratio		0.14				0.13					0.10
PD-L1 expression infiltrate	0%	44	*71%*	18	*32%*	28	*46%*	<0.001	<0.001	0.003	0.001
	1-5%	3	*5%*	23	*40%*	8	*13%*				
	6-9%	0	*0%*	0	*0%*	0	*0%*				
	10-29%	15	*24%*	14	*25%*	16	*26%*				
	>30	0	*0%*	2	*4%*	9	*15%*				
PD-L1 expression tumour	0%	59	*95%*	56	*98%*	58	*95%*	0.435	0.175	0.746	1.00
	1-5%	0	*0%*	1	*2%*	1	*2%*				
	6-9%	0	*0%*	0	*0%*	0	*0%*				
	10-29%	3	*5%*	0	*0%*	2	*3%*				
PD-L1 expression*	negative	47	*76%*	41	*72%*	36	*59%*	0.118	0.630	0.141	0.047
	positive	15	*24%*	16	*28%*	25	*41%*				

[#]mean of two observers, intra observer Pearson correlation 0.871 (p<0.001)

*Oesophageal tumours were designated as PD-L1 positive if >5% of immune tumour stroma cells/tumor epithelial cells demonstrated membranous staining.

^Oneway anova was used for continuous variables; Chi-squared or Fisher's exact test was used for catagorical variables.

[$]For moderate responders only Mean tumour cell percentage, CD3+, CD8+ counts and PD-L1 expression were analysed

Characteristics of moderate responders

Having observed that the relationship between tumour cell burden and immune cell infiltration breaks down following a poor response to nCRT, we wondered whether this effect was specific to the poor responder group or whether the relation between CD8+ density is more blurred and to a certain extent also present in the group of moderate responders (TRG3). Hence, we included a new group of 57 patients with TRG3, all from the NCI. When we analysed the difference in clinical characteristics of moderate responders compared to good responders and poor responders, only one significant difference was found. The time between the start of nCRT and surgery was again significantly different between moderate responders and poor responders. (**Table 1**). The tumour cell percentage and PD-L1 expression in moderate responders showed intermediate values when compared to good and poor responders (**Table 2**). Interestingly, CD3+ density and CD8+ density were significantly higher in moderate responders comparable to good responders, and the values were very similar to poor responders. Similarly to poor responders, no correlation was observed between tumour cell percentage and CD3 and CD8 densities in moderate responders (**Table 3**). Hence, moderate responders show high CD3+ and CD8+ densities similar to poor responders, whereas other characteristic are more intermediate when compared to poor and good responders.

Table 3: Pearson correlation coefficient between the tumour cell percentage and immune cell infiltration for all patients, good responders, moderate responders and poor responders.

	All		Good responders		Modere responders		Poor responders	
	Pearson	**p-value**	**Pearson**	**p-value**	**Pearson**	**p-value**	**Pearson**	**p-value**
CD3	0.264	<0.001	0.298	0.022	-0.33	0.812	0.103	0.435
CD4*	0.39	0.001	0.242	0.065			0.240	0.064
CD8	0.784	<0.001	0.094	0.488	-0.85	0.533	-0.068	0.605
FOXp3*	0.28	0.002	0.348	0.007			0.234	0.073

*Data for moderate responders not avialable

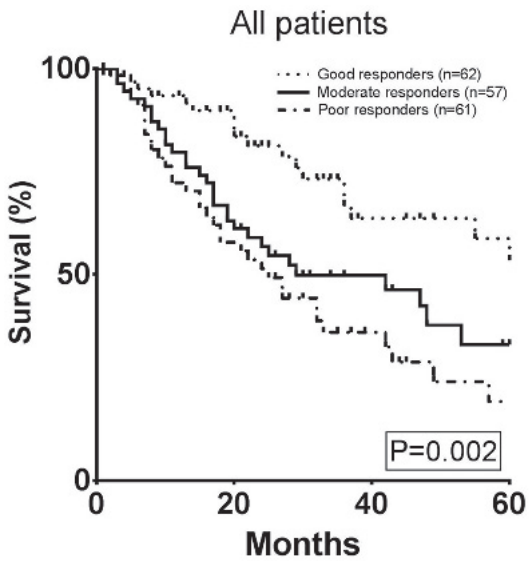


Figure 3: Survival curves for patients with oesophageal adenocarcinoma treated with neo-adjuvant chemoradiotherapy followed by surgical resection, according to response to nCRT.

Immune infiltrate density in relation to survival in OAC

As expected, response to nCRT was significantly associated with median overall survival, i.e., 64 months in responders versus 29 months and 24 months in moderate and non-responders, respectively (*p=0.002*) (**Figure 3**). In a univariate and in a multivariate analysis, clinical T stage and Mandard TRG were associated with OS (**Table 4**). In good responders and moderate responders to nCRT, no factors emerged as significantly associated with OS in a univariate analysis. Importantly, in poor responders to nCRT, only CD8+ density was significantly, but negatively, associated with OS (*p=0.023*) (**Table 4)**. A further analysis, involving categorisation of CD8+ count into the groups above and below the rounded mean count (see methods) for every group and relating CD8+ status to OS (**Figure 4**). For all patients this cut-off was set to 1000 cells/mm^2 (**Figure 4A)**, for good responders it was 650 cells/mm^2 (**Figure 4B)**, for moderate responders a cut-off of 1200 cells/mm^2 was used (**Figure 4C**) whereas for poor responders, we employed 1100 cells/mm^2 as a cut-off (**Figure 4D**). While for all patients, for patients with a good response to nCRT and for patients with a moderate response to nCRT, OS was not related to CD8+ density, in poor responders to nCRT, a high CD8+ density was associated with significantly worse overall survival (15 months versus 32 months, p=0.0042; **Figure 4D**). Thus we observe a highly specific negative predictive prognostic value of a CD8+ count of >1100/mm^2 in poor responders to nCRT of OAC.

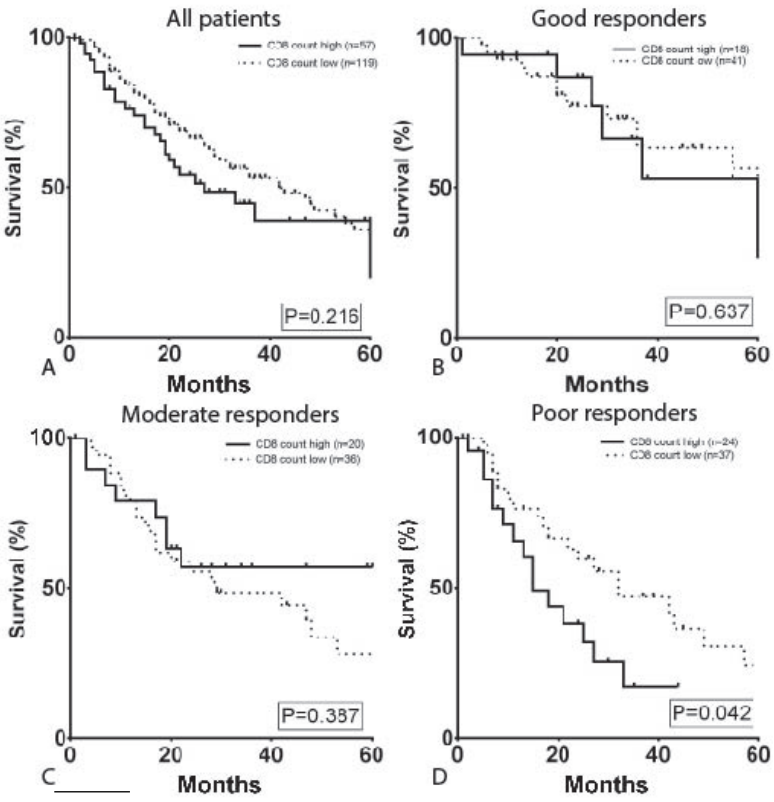


Figure 4: Survival curves for patients with oesophageal adenocarcinoma treated with neo-adjuvant chemoradiotherapy followed by surgical resection according to CD8+ density in all patients (A), good responders (B), moderate responders (C) and poor responders (D).

Discussion

This study shows that the TME provides insight into prognosis of poor responders to nCRT. Although generally our results are concordant with the canonical view that tumour cells drive immune cell infiltration and that immune infiltration is associated with better outcome, there is a disconnect in this relation following a poor response to nCRT. We propose that especially CD8+ density can identify patients who have bad prognosis and that may be candidates for more aggressive or alternative adjuvant therapy.

Earlier data on the role of CD8 density in OAC patient's survival are scarce. There is a paucity of high quality studies on immunohistochemistry-based TME characteristics as prognostic and predictive biomarkers, especially in correlation with nCRT. A meta-analysis failed to identify significant effects of CD3+ and CD8+ density in the TME prognosis of OAC patients.[12] Nevertheless, an association between CD8+ cells density and prognosis of oesophagogastric cancer has been suggested.[13] In a non-selected group of 34 gastric and oesophagogastric junction carcinomas CD8+ density was associated with poor survival, comparable with our results. In oesophageal adenocarcinoma DNA damage immune response activation was reported to be predictive for a benefit from neo-adjuvant chemotherapy followed by resection and it was associated with the presence of CD8+ lymphocytes.[11]

The disconnect between CD8+ infiltration and better survival as seen in the present study in poor responders to nCRT is discordant with the general behaviour of OAC patients. Better prognosis is generally associated with improved survival but previous studies have not stratified their results based on their response to nCRT.[14] Hence our results do not contradict previous work *per se*. Indeed, various observations suggest that for some specific groups of patients CD8+ density is inversely correlated to OS. A high profile study in colorectal carcinoma showed that a subgroup of patients (around 10%) displays a negative correlation between CD8+ immune infiltration and survival, resembling the situation observed in the present study.[15]

Why such infiltration may predict poor survival remains to be established but may relate to an increase in a specific subset of CD8+ cells, for example in CD8+Tc17 cells, which are known to be associated with poor survival in gastric cancers and head and neck cancers.[16,17] An ineffective cytotoxic action of CD8+ cells in these patients can also play a crucial role. An obvious mediator for CD8+ malfunction in this respect is PD-L1 expression, although in the present study PD-L1 expression was not significantly associated with poor survival in patients with an a bad response to nCRT. Many other immunomodulatory molecules exist and it would be interesting to investigate their association with OAC survival in this patient group.[18] For instance, staining for TIA-1 (cytotoxic granule-associated RNA binding protein), which marks activated CD8 cells rather as the overall compartment might prove very

helpful to understand the relation between CD8 counts and poor survival in the poor responder group and can clarify the difference between immune infiltrate in moderate responders and poor responders.[19] Such a study is currently being initiated in our institution.

Our study has various limitations. Apart from the parameters analysed, there are other histopathological characteristics that influence prognosis but were not analysed in relation to the findings made in this study. Lymphovascular invasion, for example, is an important predictor of outcome.[20] Unfortunately the pathology reports used for extraction of study data do not consistently report on this parameter thus making it impossible for us to refine our analysis in this respect. We do feel, however, that the magnitude of the CD8 infiltration effect in poor responders makes it unlikely that correction for this parameter would meaningfully influence conclusions of the present study. In addition the potential importance of MSI status was not taken into account. While MSI status is possibly relevant both with respect to prognosis and to immune infiltration, this status is not routinely assessed in the clinical setting of nCRT for OAC. Hence the importance of MSI in the context of CD8 infiltration in poor responders awaits further prospective studies. Until that time alternative possibilities should be kept in mind. A final limitation of the study is that CD8 infiltration is markedly different between poor responders, moderate responders, and good responders to nCRT, necessitating the use of different cut-off values for high and low CD8 counts between the different groups. This implies that the cut-off value of 1100 $CD8^+/mm^2$ only has clinical relevance for the TRG4/5 groups and should not be used for the TRG2 and TRG3 groups. As such patients are not likely to be selected for alternative therapy (*e.g.* immune checkpoint-directed therapy), we feel the impact of this qualifier relatively minor. In addition, we did not correct for time between the start of nCRT and surgery, thus patients may have survived longer. The difference in OS between patients with a high CD8+ count and a low CD8+ count, however, is very large (15 vs 32 months) and the lag time before surgery cannot possibly account for the differences observed. In this context, it is also important to consider the possibility that the longer lag time has influenced results. If true, this would have important implications for patient care but the present setup of the study does not allow substantiating or refuting this hypothesis and hence we aim to address this issue in future studies.

In conclusion, high CD8+ counts allow identification of patients with poor prognosis following an unsuccessful nCRT. These patients require more aggressive approach as compared to patients with a more favourable low CD8+ count and might be candidates for immune therapy and/or adjuvant chemotherapy.

Table 4: Cox regression analysis for overall survival for all patients, good responders, moderate responders and poor responders.

		All patients						Good responders#			Moderate responders#			Poor responders#		
		Univariate			Multivariate			Univariate			Univariate			Univariate		
		HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value
Age		1.009	0.985-1.034	0.445				1.014	0.963-1.068	0.599	0.996	0.960-1.034	0.847	1.025	0.983-1.068	0.254
gender	Male	1.00						1.00			1.00			1.00		
	Female	0.959	0.557-1.651	0.881				0.924	0.309-2.759	0.887	0.868	0.441-2.641	0.868	0.967	0.398-2.350	0.941
Time between nCRT and OK		0.997	0.987-1.006	0.459				0.992	0.959-1.027	0.653	0.974	0.940-1.009	0.143	0.994	0.985-1.004	0.261
tumour location	Mid oesophagus	*						*			*			*		
	Distal oesophagus	1.00						1.00			1.00			1.00		
	GEJ	1.249	0.804-1.941	0.323				0.968	0.360-2.597	0.948	1.659	0.811-3.395	0.166	0.986	0.492-1.976	0.969
	Unknown	0.925	0.393-2.177	0.857				0.871	0.191-3.983	0.859	*			1.037	0.340-3.167	0.949
Radical resection	Yes	1.00						*			1.00			*		
	No	2.955	0.951-9.179	0.061				*			2.389	0.319-19.923	0.397	*		
	Unknown	1.257	0.545-2.901	0.591				*			*			*		
cT stage	T1	*			*						*			*		
	T2	1.00			1.00			1.00			1.00			1.00		
	T3	**1.771**	**1.042-3.011**	**0.035**	1.672	0.981-2.848	0.059	1.143	0.441-2.963	0.783	2.056	0.842-5.020	0.113	1.813	0.697-4.716	0.222
	T4	**12.84**	**1.645-100.253**	**0.015**	**8.96**	**1.127-71.302**	**0.038**	*			*			*		
cN stage	N0	1.00						1.00			1.00			1.00		
	N+	1.468	0.952-2.265	0.083				1.077	0.448-2.591	0.868	2.183	0.972-4.900	0.058	1.207	0.625-2.331	0.576
Tumour cell percentage		1.010	0.999-1.022	0.086				0.976	0.928-1.027	0.297	0.988	0.961-1.016	0.413	1.000	0.979-1.022	0.992
TRG	Mandard 2	1.00			1.00											
	Mandard 3	**1.902**	**1.092-3.312**	**0.023**	**1.898**	**1.089-3.307**	**0.024**									
	Mandard 4 + 5	**2.588**	**1.511-4.431**	**0.001**	**2.384**	**1.382-4.114**	**0.002**									
CD3		1.019	0.983-1.056	0.305				0.980	0.950-1.060	0.611	1.000	0.928-1.076	0.992	1.013	0.955-1.075	0.665
CD8		1.049	0.998-1.102	0.062				0.987	0.850-1.147	0.868	0.997	0.911-1.091	0.949	**1.086**	**1.012-1.165**	**0.023**
PDL1	Negative	1.00						1.00			1.00			1.00		
	Positive	0.851	0.528-1.373	0.509				0.767	0.258-2.285	0.634	0.665	0.286-1.548	0.344	0.800	0.396-1.615	0.534

*not evalueted due to low number of patients, #multivariate analysis not performed

Inter immunoscores collinearity statisctics; all VIF's were <1.88

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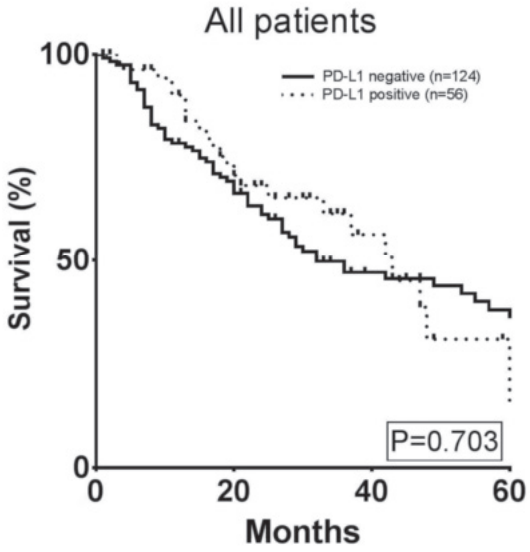

Figure S1: Survival curves for patients with oesophageal adenocarcinoma treated with neo-adjuvant chemoradiotherapy followed by surgical resection, according to response to PD-L1 status.

Supplementary table 1: Differences between the Netherlands Cancer Institute cohort and the Zudyerland cohort in characteristics of the immune tumour microenvironment in oesophageal adenocarcinoma patients with good and poor response to neo-adjuvant chemoradiotherapy.

		Netherlands Cancer Institute		Zuyderland		p-value^
Good responders		41		21		
Mean tumour cell percentage#		9.68%		6.45%		0.228
CD3+ count/mm2		1333		1307		0.938
CD4+ count/mm2		383		555		0.179
CD8+ count/mm2		498		945		0.029
FOXP3+ count/mm2		215		118		0.082
CD20+ count/mm2		519		223		0.450
PD-L1 expression*	negative	32	78%	15	71%	0.565
	positive	9	22%	6	29%	
Poor responders		25		36		
Mean tumour cell percentage#		37,81		36,81		0.825
CD3+ count/mm2		2332		2396		0.865
CD4+ count/mm2		586		998		0.008
CD8+ count/mm2		924		1265		0.231
FOXP3+ count/mm2		227		284		0.577
CD20+ count/mm2		502		866		0.256
PD-L1 expression*	negative	12		24		0.145
	positive	13		12		

#mean of two observers, intra observer Pearson correlation 0.871 (p<0.001)
*Oesophageal tumours were designated as PD-L1 positive if >5% of immune tumour stroma cells/tumor epithelial cells demonstrated membranous staining.
^Oneway anova was used for continuous variables; Chi-squared or Fisher's exact test was used for catagorical variables.

CHAPTER 11

General discussion

General discussion

Over the past decades, a multimodality treatment approach with surgery has become the standard of care for non-metastatic locally advanced resectable oesophageal and gastric carcinoma in the Netherlands.[1,2] After publication of the CROSS trial, neoadjuvant chemoradiotherapy followed by a surgical resection became the Dutch nationwide standard for oesophageal carcinoma, whereas perioperative chemotherapy is the standard of care for gastric carcinoma.[3,4] Furthermore, a national surgical audit was installed and centralisation of both oesophageal and gastric cancer surgery took place after which the quality of surgical care improved.[5]

Despite the above improvements, the survival perspective of oesophageal and gastric cancer patients remains dismal. Including all tumour stages, overall median survival for oesophageal and gastric adenocarcinoma patients diagnosed between 2011 and 2015 was 13.2 and 9.3 months, respectively (**Chapter 2**).[6] However, for non-metastatic oesophageal adenocarcinoma patients survival increased considerably between 1989 and 2015, especially for those with an intestinal type tumour (from 12.0 to 30.0 months) as compared to those with a diffuse type tumour (from 12.0 to 19.2 months). The survival perspective for non-metastatic gastric adenocarcinoma increased considerably less, that is from 22.8 to 27.6 months for patients with intestinal type cancer and from 16.8 to 18.0 months for those with diffuse type cancer (**Chapter 2**).[6] The survival difference between the intestinal and diffuse type tumours is most likely related to a difference in responsiveness to neoadjuvant therapy.[7] These differences call for a differentiated treatment approach. Current research should therefore stratify for the histological subtype, to investigate if new treatment options benefit either the intestinal type or the diffuse type, or both.

Although the number of patients with gastric adenocarcinoma declined over the past decades, the percentage of patients that presented with metastatic disease at diagnosis increased (**Chapter 3**).[8] This is possibly related to the increased use of diagnostic modalities as the CT-scan, PET-scan and diagnostic laparoscopy.[2] Interestingly, the metastatic pattern differed between the histological subtypes. Gastric adenocarcinoma of the intestinal type metastasised more often to the liver, whereas the diffuse type had a predilection for the peritoneum. As holds true for non-metastatic gastric adenocarcinoma, also in the metastatic setting, patients with a diffuse type tumour had a worse prognosis compared to patients with an intestinal type tumour. As yet, these differences have no clinical implications. It is possible that this will change in the near future. At current times, the treatment of oligometastatic disease from gastric cancer is being widely researched, i.e., the resection of hepatic or pulmonary metastases in combination with a gastrectomy or the combination of cytoreductive surgery, gastrectomy and HIPEC for peritoneal metastases.[9-12] If one or more of these therapies exhibit a survival benefit for patients with oligometastatic disease, differences in the metastatic pattern based on the Lauren classification become clinically relevant.

Gastric cancer research should unravel the differences in tumour behaviour between the intestinal and diffuse type tumours. It has already been shown that the two subtypes not only differ histologically but also differ in the molecular makeup. In the TCGA characterisation of gastric adenocarcinomas, the majority of the diffuse type tumours were from the genomically stable subtype, whereas the intestinal type tumours were most often classified as chromosomally instable.[13,14] These classifications provide a starting point for future research regarding genetic differences between the histological subtypes and possibly lead to new targets for treatment.

One of the most common sites for metastatic disease from gastric cancer origin is the peritoneum. While the number of gastric adenocarcinoma patients decreased over time, the proportion of patients that presented with peritoneal metastases has increased (**Chapter 4**).[15] In 2017, over a fourth (27%) of the newly diagnosed gastric adenocarcinoma patients presented with peritoneal metastases. This increase might be explained in part by the shift in the distribution of the histological subtypes, with the diffuse type now being the predominant subtype (**Chapter 2**).[6] Furthermore, it is most likely that the addition of diagnostic laparoscopy to the Dutch national guideline for staging gastric cancer patients also increased the incidence of synchronous peritoneal disease.[2] The value of diagnostic laparoscopy has been studied in the Dutch multicentre PLASTIC study of which the results will soon be published.[16] Interestingly, the percentage of gastric adenocarcinoma patients with peritoneal metastases treated with systemic chemotherapy increased dramatically in the past two decades. However, overall survival did not improve. This raises the question if systemic chemotherapy is the optimal treatment strategy for this patient group. Treatment with intra-peritoneal chemotherapy might be a more potent alternative. Some studies have shown a beneficial effect of catheter-based chemotherapy.[17] Another alternative is the use of a PIPAC (Pressurised Intraperitoneal Aerosol Chemotherapy) device.[18] Future research should provide data on the efficacy of these types of therapies.

The use of Hyperthermic Intraperitoneal Chemotherapy (HIPEC) for the treatment of peritoneal metastases from gastric cancer origin is subject of discussion. It is widely applied, especially, but not only, in Asian countries.[11] However, as yet, there is no conclusive evidence that a HIPEC procedure provides a survival benefit in addition to systemic chemotherapy in Western gastric cancer patients. To fill this knowledge gap, the PERISCOPE (Treatment of PERItoneal dissemination in Stomach Cancer patients with cytOreductive surgery and hyperthermic intraPEritoneal chemotherapy) studies were initiated. The dose-escalation PERISCOPE I study showed that a HIPEC procedure with 460 mg/m^2 hyperthermic oxaliplatin followed with 50 mg/m^2 normothermic docetaxel was feasible in gastric adenocarcinoma patients with limited peritoneal disease, following systemic therapy.[19] Overall survival of those patients who underwent the full study protocol was 15 months in the PERISCOPE I study (**Chapter 5**).[20] This is within the range of recently published results of national cohort studies from Germany, Spain and France.[21-23] Thereby, it is acknowledged that the PERISCOPE I was a dose-escalation study not designed to test the efficacy of the therapy. Perhaps not surprisingly, unfavourable

tumour characteristics were common in the PERISCOPE I study, as more patients had a high ypT3-T4 tumour stage and response to the neoadjuvant chemotherapy was considerably lower than in other surgical gastric cancer series.[4,24] Median disease-free survival was 12 months in the PERISCOPE I study, tumour recurrence in the abdominal cavity was most common, but distant metastases were also seen (e.g. in the liver, bones and lungs), comparable to recurrence patterns seen after a potentially curative gastric cancer surgery.[25]

Considerable morbidity and mortality rates have been associated with HIPEC procedures.[26,27] Postoperative complications occurred frequently in the PERISCOPE I study. Most were ileus-related complications possibly due to the loss of the reservoir function of the stomach (**Chapter 6**).[28] Postoperative management was adapted to counteract these complications. Most importantly, enteral feeding was restricted to very low amounts until day 6 and total parenteral nutrition was started to supplement nutritional needs. With this new care path, the rate of postoperative complications decreased. A similar approach has been suggested previously.[29]

A combination of intraperitoneal oxaliplatin and docetaxel appeared feasible in the PERISCOPE I study.[19] A major advantage of HIPEC therapy is the high concentration that can be given intraperitoneally, without systemic complications.[30] This was also seen in the pharmacokinetic study of the agents used in the PERISCOPE I trial (**Chapter 7**).[31] Systemic oxaliplatin concentrations after the HIPEC procedure were comparable to systemic concentrations after intraveneous administration.[32] However, it must be noted that platinum entering the systemic circulation after intraperitoneal use is expected to be bound, and therefore inactive platinum.[33] For docetaxel, systemic concentrations were much lower after the HIPEC procedure than after intravenous administration.[34,35] The combination of oxaliplatin and docetaxel can be used safely intraperitoneally. However, intraperitoneal concentrations must be regulated closely as it is a relatively new dual-agent HIPEC regimen.

In the PERISCOPE II study, gastric cancer patients with limited peritoneal disease are randomised between the standard treatment in the Netherlands, systemic chemotherapy alone, and an operative procedure including HIPEC, following systemic chemotherapy (**Chapter 8**).[36] The primary outcome is overall survival. An important inclusion criterion is a PCI below 7. One of the best predictors of survival after HIPEC is the completeness of the cytoreductive surgery.[37] It is advised to only perform a HIPEC procedure if a complete cytoredution can be achieved. The lower the PCI the higher the chance of a complete cytoreduction.[38] In various studies, a PCI value of 7 discriminated long term survivors from short term survivors after HIPEC in gastric cancer patients.[21,23,39]. Therefore, only patients with a PCI below 7 are included in the PERISCOPE II study. The PERISCOPE II study is not the only trial in Europe investigating a HIPEC procedure in gastric cancer patients. The French GASTRICHIP trial includes gastric cancer patients with serosal and/or lymph node involvement and/or tumour positive cytology and randomises between a gastric cancer resection with or without HIPEC with oxaliplatin.[40] The German GASTRIPEC study randomised gastric cancer patients with peritoneal metastases between a surgical resection with or without HIPEC with mitomycin C and cisplatin.[41]

Unfortunately, the GASTRIPEC stopped earlier than anticipated due to low accrual numbers. Hopefully, results of these trials will answer the question whether HIPEC surgery has additional value in the treatment of gastric cancer patients with peritoneal metastases.

As in all cancer types, the chance for cure is largely dependent on the response to treatment. In oesophageal cancer, especially oesophageal adenocarcinoma, response to neoadjuvant chemotherapy is usually partial and in a minority of the patients complete. This might be related to the fact that oesophageal adenocarcinomas have a high somatic mutation burden and high inter-tumour heterogeneity.[42,43] It has been suggested that tumour heterogeneity affects treatment efficacy. Highly heterogeneous tumours are more often therapy resistant.[44] This could explain the high percentage of oesophageal adenocarcinomas with limited response to neoadjuvant chemoradiotherapy.

It would be an enormous leap forward if one could identify patients who will respond - or will not respond – to neoadjuvant chemotherapy. Until now this has not been possible. In a study including 95 oesophageal adenocarcinoma patients, none of the copy number variation profiles was significantly associated with response to neoadjuvant chemoradiotherapy (own data, not published). This might be due to extreme heterogeneity of the oesophageal tumour and the relatively small cohort of 95 patients. In a larger cohort of over 500 oesophageal adenocarcinoma patients, 65 driver mutations were identified of which some (GAT4 and SMAD4) were predictive for survival.[45] Unfortunately, none of these patients were treated with neoadjuvant chemoradiotherapy. To find predictive biomarkers, future research should be conducted on very large numbers of high quality pre-treatment tumour samples. All patients should be treated similarly and the two histological subtypes (adenocarcinoma and squamous cell carcinoma) should be studied separately.

It is believed that the tumour microenvironment has a role in treatment response. Indeed, oesophageal adenocarcinomas unresponsive to neoadjuvant chemoradiotherapy more often displayed a tumour microenvironment with a high immune cell infiltration and programmed death ligand-1 (PD-L1) positivity, compared to responders (**Chapter 10**).[46] Furthermore, a high CD8 density was associated with poor survival in patients non-responsive to neoadjuvant chemoradiotherapy. A relationship between CD8 density and survival of oesophageal cancer patients has been suggested before, however, the exact role of CD8 cells in non-responsive tumours needs to be further investigated.[47,48]

Non-responsive oesophageal adenocarcinomas might be good candidates for immunotherapy. Immunotherapy has proven to be effective in metastatic gastric cancer especially in microsatellite instable gastric adenocarcinoma and in Epstein-Bar virus positive gastric carcinomas.[49] In a recent study, immunotherapy appeared extremely effective in the neo-adjuvant setting for patients with microsatellite instable colorectal carcinomas.[50] The efficacy of immunotherapy in oesophageal adenocarcinoma has not been established and biomarkers for selection of patients who are likely to benefit from immunotherapy are

not known yet. In non-small cell lung carcinoma PD-L1 expression was a good predictor of response to immunotherapy. This might not be the case in oesophageal adenocarcinoma (**Chapter 9**).[51-53] Contradictory to non-small cell lung carcinoma, PD-L1 expression in oesophagogastric cancer is mainly observed in stroma, not in tumour cells (**Chapter 10**).[48,54] For gastric cancer, the four TCGA subtypes might be the best predictors for immunotherapy response. However, for oesophageal carcinoma, a combination of markers might be a better predictor of response to immunotherapy. It can be hypothesised that oesophageal tumours with high PD-L1 expression and a high tumour infiltrating lymphocyte density are expected to respond well to checkpoint inhibition. Neo-adjuvant combination treatment of checkpoint inhibitors and chemotherapy in oesophagogastric cancer is currently being investigated in several clinical trials (e.g. PANDA trial; NCT03448835, ICONIC trial; NCT03399071). Further research is needed to determine which oesophageal adenocarcinomas will benefit from additional immunotherapy.

Future perspective

There is room for improvement in the treatment of oesophageal and gastric cancer patients. Although improvements have been made by the introduction of a multimodality treatment approach and better surgical care, still a majority of patients has a dismal survival perspective. Treatment strategies tailored to the individual patient should be able to improve survival and quality of life. With the introduction of immunotherapies the armamentarium of the medical oncologist expanded. However, the ultimate challenge remains to select patients who will benefit from certain types of treatment. A pathological complete response to the neoadjuvant treatment should be the ultimate goal. As it becomes less expensive to genetically characterise tumours, it is more feasible to start large biobanks and create study populations large enough to find similarities between tumours despite the extreme heterogeneity. However, the inter-tumour heterogeneity will still hamper treatment choices as some clones within a tumour might respond to the therapy whereas others might not. Next to that, response to therapy is probably not only related to the genetics of the tumour as the host immune system also plays a vital role. As yet, surgery remains the cornerstone in the treatment of oesophageal and gastric cancer. However, for patients with a clinical complete response an operation might not be necessary, as is currently assessed in the SANO trial.[55] Even for patients with oligometastatic disease the future might hold potentially curative treatment options, i.e. HIPEC surgery for peritoneal metastases and metastasectomy for lung and liver metastases. The success of such treatment strategies 'beyond the guidelines' will depend entirely on meticulous patient selection, multidisciplinary team involvement, and ongoing adaption of treatment options to new insights.

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11

CHAPTER 12

Summary

Samenvatting

Summary

The goal of this thesis was to investigate new biomarkers and therapy options for oesophagogastric cancer patients. At first, the epidemiology of oesophageal and gastric cancer was studied taking the different histological subtypes into account. Then, for gastric cancer, the focus was on the incidence and treatment of patients with metastatic disease, especially patients with peritoneal dissemination. Finally, translational research was done to find biomarkers that may predict response to immunotherapy. As for oesophageal cancer, the tumour microenvironment was studied in response to chemoradiotherapy.

Since more than five decades the Lauren classification has been used to subdivide gastric adenocarcinomas into intestinal, diffuse and mixed types. Nationwide time trends in the incidence and survival of oesophageal and gastric adenocarcinomas according to the Lauren classification were investigated in **Chapter 2**. Epidemiological data and histological data of patients diagnosed with oesophageal or gastric cancer in the Netherlands between 1989 and 2015 were linked. The Lauren classification was determined by a syntax. Median survival of patients with an intestinal type tumour was longer than that of patients with a diffuse type tumour in oesophageal cancer patients (12.1 versus 9.4 months, respectively) as well as in gastric cancer patients (10.1 versus 7.6 months, respectively). Between 1989 and 2015, median survival of non-metastatic intestinal type oesophageal and gastric cancer increased from 12.0 to 30.0 months and from 22.8 to 27.6 months, respectively. For the diffuse type non-metastatic oesophageal and gastric cancer, the increase in survival was less pronounced, from 12.0 to 19.2 months and from 16.8 to 18.0 months, respectively. These results underline the differences between the histological subtypes and suggest that patients with an intestinal type tumour experienced more benefit from the introduction of multimodality therapy than patients with a diffuse type tumour.

In **Chapter 3,** the metastatic pattern of gastric adenocarcinoma was studied by histological subtype in a national cohort study (1999-2017). Intestinal type carcinomas metastasised more often to the liver (57% versus 21%) and lungs (13% versus 7%), whereas diffuse type carcinomas metastasised more often to the peritoneum (58% versus 29%) and bones (9% versus 6%). As holds true for non-metastatic gastric cancer patients, metastatic gastric cancer patients with a diffuse type tumour had a worse survival perspective than those with an intestinal type tumour. These differences in metastatic pattern and in survival may guide differentiated treatment of the various gastric cancer disease entities.

The peritoneum is a predilection site for gastric cancer metastases, especially for the diffuse tumour type. Over the past decades (1999-2017), the proportion of gastric cancer patients diagnosed with peritoneal metastases increased (**Chapter 4)**. In 2017, 27% of the patients diagnosed with gastric cancer had synchronous peritoneal metastases, underlining the importance of the peritoneal cavity as a clinically relevant metastatic site. The treatment of patients with synchronous peritoneal metastases of gastric cancer origin changed over

time. Between 1999 and 2002, 15% of the patients was treated with systemic chemotherapy and 18% had a primary tumour resection. In contrast, between 2013 and 2017, 43% of the patients was treated with systemic chemotherapy and 12% had a primary tumour resection. Despite the staggering increase in the use of systemic chemotherapy, overall survival of gastric cancer patients with synchronous peritoneal metastases did not increase significantly over time, questioning the efficacy of systemic chemotherapy in this patient group.

As peritoneal dissemination is so common in gastric cancer patients and systemic chemotherapy might not be an effective treatment, other treatment options are being explored. Hyperthermic Intraperitoneal Chemotherapy (HIPEC) has been proven a successful treatment option for patients with peritoneal metastases of colorectal and ovarian cancer origin. There is no evidence, so far, that this is true for patients with peritoneal metastases of gastric cancer origin. The dose-finding PERISCOPE (Treatment of PERItoneal dissemination in Stomach Cancer patients with cytOreductive surgery and hyperthermic intraPEritoneal chemotherapy) I study showed that a combination of gastrectomy, cytoreductive surgery and HIPEC with oxaliplatin and docetaxel was feasible in gastric cancer patients with limited peritoneal dissemination, following neo-adjuvant treatment with systemic chemotherapy.

A total of 25 patients underwent the complete study protocol in the PERISCOPE I study (**Chapter 5**). The majority had an ypT3-4 tumour (96%) and the diffuse type histology was most frequent (64%), illustrating the unfavourable tumour characteristics of gastric carcinomas with peritoneal dissemination. Disease recurrence was detected in 17 (68%) patients and median disease-free and overall survival were 12 and 15 months, respectively. This survival perspective appears hopeful, but the PERISCOPE I was a phase I-II feasibility study not designed to assess the therapeutic efficacy of the HIPEC procedure.

In the PERISCOPE I study, 14 patients were treated in the dose-escalation-cohort and 11 patients were treated in the expansion-cohort (**Chapter 6**). The expansion-cohort was created to optimise perioperative management. In the dose-escalation-cohort a significant proportion of the patients (50%) had ileus-related complications. To counteract these complications the postoperative care path in the expansion-cohort involved a minimal enteral nutrition protocol, supplemented with total parenteral nutrition to meet nutritional needs. The frequency of ileus-related complications decreased (18%) and also the ICU readmission rate went down (from 50% to 9%).

Of all 25 patients who underwent a HIPEC procedure in the PERISCOPE I study, plasma samples were collected before the start of the HIPEC procedure, after oxaliplatin washing, after docetaxel washing and the following morning (**Chapter 7**). Median peak plasma concentration of oxaliplatin was $5.5*10^{-3}$ mg/ml, which is within the range of reported peak plasma concentrations after intravenous administration and below previously reported peak plasma concentrations after intra-abdominal use. Median peak plasma concentration of docetaxel was $89*10^{-6}$ mg/ml (for dose 50 mg/m^2) and $113*10^{-6}$ mg/ml (for dose 75 mg/m2), *i.e.,* well below

peak plasma concentrations after intravenous use and previously reported peak plasma concentrations after intra-abdominal use. For both agents, there was no correlation between the perfusate concentrations and plasma concentrations. These results are reasuring for the use of oxaliplatin and docetaxel in HIPEC surgery, since systemic complications from their intraperitoneal administration are unlikely.

In the PERISCOPE I study, safety and feasibility were established for a HIPEC procedure with oxaliplatin (hyperthermic, dose 460 mg/m^2) and docetaxel (normothermic, dose 50 mg/m^2) in gastric cancer patients with limited peritoneal dissemination following systemic chemotherapy. Survival rate and pharmacological data are encouraging and with a strict perioperative care path complication risk can be reduced. However, a randomised controlled trial is needed to study the efficacy of such procedure in a Western gastric cancer patient cohort with synchronous limited peritoneal dissemination. In **Chapter 8,** the rationale and the design of the PERISCOPE II study are described. The primary objective is to compare overall survival between patients with gastric cancer with limited peritoneal dissemination and/or tumour positive peritoneal cytology treated with the current standard treatment, *i.e.*, palliative systemic chemotherapy and those treated with gastrectomy, cytoreductive surgery and HIPEC, following systemic chemotherapy.

Oesophageal and gastric adenocarcinomas are aggressive cancers that are often non-responsive to neo-adjuvant therapy. Prognosis of patients with unresponsive oesophageal or gastric cancer is poor. Cancer immunotherapy is a promising new treatment option. However, only a small percentage of oesophageal and gastric cancer patients responds to immunotherapy. Therefore, biomarkers for patient selection are needed. In non-small cell lung cancer, programmed cell death ligand-1 (PD-L1) is a validated biomarker to select patients for immunotherapy. For oesophageal and gastric cancer, it is questioned if this biomarker is an adequate selection tool, as objective response rates are low in PD-L1 positive oesophagogastric cancers treated with immunotherapy (**Chapter 9**). There is a difference in PD-L1 expression pattern between non-small cell lung cancer and oesophagogastric cancer. In oesophagogastric cancer, expression of PD-L1 is mainly observed in the immune stroma, whereas in non-small cell lung carcinoma, expression is mainly observed in the epithelial cells. This might explain why PD-L1 is an adequate biomarker for non-small lung cell cancer but not for oesophagogastric cancer. Possibly, in oesophagogastric cancer, a combination of markers will qualify for an optimal prediction model. For an accurate selection of patients most likely to respond to immunotherapy, future studies need to aim for a combination of improved biomarker strategies in conjunction with MSI (Microsatellite Instability) status, EBV (Epstein Barr Virus) status, precise characterisation of the immune infiltrate and the neo-antigen burden in different types of oesophagogastric cancer separately.

Although the introduction of multi-modality therapy substantially improved the prognosis of oesophageal adenocarcinoma patients a substantial proportion of patients does not respond to neo-adjuvant chemoradiotherapy. Determining prognosis after a poor response to neo-adjuvant chemoradiotherapy remains challenging. An immunosuppressive tumour microenvironment (TME) as well as immune infiltrate

density and composition are considered to play a critical role in the immune interaction between host and tumour. It can predict therapy response and survival in many cancers, including gastro-intestinal malignancies. In oesophageal cancer patients with a poor response to neo-adjuvant chemoradiotherapy, a high CD8+ infiltration in the TME was associated with worse overall survival (**Chapter 10**). This suggests that patients with a poor response to neo-adjuvant chemoradiotherapy but concomitant high CD8+ counts in the resection specimen require adjuvant therapy.

Samenvatting

In dit proefschrift worden nieuwe biomarkers en behandelopties voor slokdarm- en maagkankerpatiënten onderzocht. Allereerst is de epidemiologie van de verschillende histologische subtypes van slokdarm- en maagkanker bestudeerd. Voor maagkanker lag vervolgens de focus op de incidentie en behandeling van patiënten met uitgezaaide ziekte, en in het bijzonder van patiënten met buikvliesuitzaaiingen. Tot slot is translationeel onderzoek verricht om biomarkers te vinden die de respons op immumotherapie kunnen voorspellen. Specifiek in slokdarmkanker is de tumor micro-omgeving onderzocht in relatie tot de respons op chemoradiotherapie.

Sinds meer dan vijftig jaar wordt de Lauren classificatie gebruikt om het adenocarcinoom van de maag in te delen in een intestinale type, een diffuus type en een gemengde type. Landelijke trends in de incidentie en overleving van de verschillende histologische subtypen van het slokdarm- en maagcarcinoom werden onderzocht in **Hoofdstuk 2**. Epidemiologische gegevens werden gekoppeld aan histologische gegevens van patiënten die tussen 1989 en 2015 in Nederland de diagnose slokdarm- of maagkanker kregen. De Lauren classificatie werd bepaald door een syntax. De mediane overleving van patiënten met een intestinaal type tumor was langer dan die van patiënten met een diffuus type tumor, zowel bij slokdarmkankerpatiënten (respectievelijk 12.1 versus 9.4 maanden) als bij maagkankerpatiënten (respectievelijk 10.1 versus 7.6 maanden). Tussen 1989 en 2015 nam de mediane overleving van het niet-gemetastaseerde *intestinaal type* slokdarmcarcinoom en maagcarcinoom toe, van respectievelijk 12.0 naar 30.0 maanden en van 22.8 naar 27.6 maanden. Voor het niet-gemetastaseerde *diffuus type* slokdarmcarcinoom en maagcarcinoom was de verbetering in overleving minder uitgesproken, van respectievelijk 12.0 naar 19.2 maanden en van 16.8 naar 18.0 maanden. Deze resultaten onderstrepen de verschillen tussen de histologische subtypes. Het is een aanwijzing dat patiënten met een intestinaal type tumor meer baat hebben gehad bij de introductie van multimodale behandeling dan patiënten met een diffuus type tumor.

In **Hoofdstuk 3** werd het metastaseringspatroon van de verschillende histologische subtypes van het maagcarcinoom bestudeerd in een landelijke cohortstudie (1999-2017). Intestinaal type tumoren metastaseerden vaker naar de lever (57% versus 21%) en de longen (13% versus 7%), terwijl diffuus type tumoren vaker metastaseerden naar het buikvlies (58% versus 29%) en de botten (9% versus 6%). De overleving van deze maagkanker patiënten met uitgezaaide ziekte was - net als bij maagkanker patiënten zonder uitzaaiingen - slechter in geval van een diffuus type maagcarcinoom dan in geval van een intestinaal type maagcarcinoom. De verschillen in metastaseringspatroon en overleving kunnen sturend zijn in een gedifferentieerde behandeling van de verschillende maagkanker entiteiten.

Het peritoneum is een voorkeursplaats voor uitzaaiingen van maagkanker, vooral bij het diffuus type maagkanker. In de afgelopen decennia (1999-2017) is het percentage maagkankerpatiënten dat

gediagnosticeerd werd met buikvliesuitzaaiingen toegenomen (**Hoofdstuk 4**). In 2017 had 27% van de patiënten met de diagnose maagkanker synchrone peritoneale metastasen. Dit onderstreept de klinische relevantie van het peritoneum als metastaseringsplaats. De behandeling van maagkanker patiënten met synchrone peritoneale metastasen is in de loop van de tijd veranderd. Tussen 1999 en 2002 werd 15% van de patiënten behandeld met systemische chemotherapie en 18% onderging een primaire tumor resectie. Terwijl, tussen 2013 en 2017, 43% van de patiënten behandeld werd met systemische chemotherapie en 12% van de patiënten een primaire tumor resectie onderging. Ondanks de toename van het gebruik van systemische chemotherapie nam de algehele overleving van maagkankerpatiënten met synchrone peritoneale metastasen niet significant toe. Dit plaatst vraagtekens bij de werkzaamheid van systemische chemotherapie in deze patiëntengroep.

Gezien het frequent voorkomen van buikvliesuitzaaiingen bij maagkankerpatiënten en de gebrekkige effectiviteit van systemische chemotherapie worden andere behandelopties onderzocht. Hypertherme Intraperitoneale Chemotherapie (HIPEC) is een bewezen succesvolle behandeling voor patiënten met buikvliesuitzaaiingen van dikke-darmkanker en eierstokkanker. Er is echter, tot op heden, geen bewijs dat dit ook zou gelden voor patiënten met buikvliesuitzaaiingen van maagkanker. De PERISCOPE (*Treatment of PERItoneal dissemination in Stomach Cancer patients with cytOreductive surgery and hyperthermic intraPEritoneal chemotherapy*) I studie was een dosisescalatie studie naar de haalbaarheid en veiligheid van een combinatie behandeling bestaande uit een maagresectie, cytoreductieve chirurgie en HIPEC met oxaliplatin en docetaxel bij maagkankerpatiënten met beperkte peritoneale ziekte na neo-adjuvante systemische chemotherapie.

In totaal ondergingen 25 patiënten in de PERISCOPE I studie het volledige studieprotocol (**Hoofdstuk 5**). De meerderheid had een *y*pT3-4 tumor (96%) en het diffuus type maagcarcinoom was meest voorkomend (64%); dit illustreert de ongunstige tumorkenmerken van maagkanker met peritoneale metastasen. Terugkeer van ziekte werd gevonden bij 17 (68%) patiënten, de mediane ziektevrije overleving was 12 maanden en de totale overleving was 15 maanden. Deze overlevingsdata lijken hoopgevend, maar de PERISCOPE I studie was een fase I-II haalbaarheidsstudie, niet ontworpen om de therapeutische effectiviteit van de HIPEC procedure op waarde te schatten.

In de PERISCOPE I studie werden 14 patiënten behandeld in het dosis-escalatie-cohort en 11 patiënten in het expansie-cohort (**Hoofdstuk 6**). Met het expansie-cohort werden de aanpassingen in de perioperatieve zorg getoetst. In het dosis-escalatie-cohort had een aanzienlijk deel van de patiënten (50%) ileus-gerelateerde complicaties. Om deze complicaties tegen te gaan werd het postoperatieve zorgpad in het expansie-cohort aangepast met een restrictief enteraal voedingsprotocol, aangevuld met totale parenterale voeding om aan de nodige voedingsbehoeften te voldoen. De frequentie van ileus-gerelateerde complicaties nam af (18%) en ook het percentage IC heropnames daalde (van 50% naar 9%).

Van alle 25 patiënten die een HIPEC procedure in de PERISCOPE I studie ondergingen werden plasmasamples afgenomen voorafgaand aan de HIPEC procedure, na de oxaliplatin spoeling, na de docetaxel spoeling en de volgende ochtend (**Hoofdstuk 7**). De mediane piekplasmaconcentratie van oxaliplatin was $5,5*10^{-3}$ mg/ml, binnen de waarden die in de literatuur beschreven zijn voor piekplasmaconcentraties na intraveneuze toediening en lager dan eerder beschreven piekplasmaconcentraties na intra-abdominale toepassing. De mediane piekplasmaconcentratie van docetaxel was $89*10^{-6}$ mg/ml (voor dosis 50 mg/m^2) en $113*10^{-6}$ mg/ml (voor dosis 75 mg/m^2), ruim lager dan in de literatuur beschreven piekplasmaconcentraties na intraveneus en intra-abdominaal gebruik. Voor beide cytostatica was er geen correlatie tussen de intra-abdominale perfusaatconcentraties en de plasmaconcentraties. Deze resultaten zijn geruststellend voor het gebruik van oxaliplatin en docetaxel bij HIPEC chirurgie, aangezien systemische complicaties van de intraperitoneale toediening onwaarschijnlijk zijn.

De PERISCOPE I studie heeft data gegenereerd over de veiligheid en haalbaarheid van een HIPEC procedure met oxaliplatin (hypertherm, dosis 460 mg/m^2) en docetaxel (normotherm, dosis 50 mg/m^2) bij maagkankerpatiënten met beperkte peritoneale ziekte na neo-adjuvante systemische chemotherapie. De overleving en farmacologische data zijn bemoedigend en met een strikt perioperatief zorgpad kan het complicatierisico worden verlaagd. Echter, een gerandomiseerde studie is nodig om de therapeutische effectiviteit te onderzoeken in een Westers maagkankerpatiënten cohort met synchrone beperkte peritoneale metastasering. In **Hoofdstuk 8** worden de rationale en het design van de PERISCOPE II studie beschreven. Het primaire doel is om te onderzoeken of de combinatiebehandeling van maagresectie, cytoreductieve chirurgie en HIPEC overlevingswinst biedt ten opzichte van de huidige standaardbehandeling, palliatieve systemische chemotherapie, bij maagkankerpatiënten met beperkte peritoneale metastasering en/of tumorpositieve peritoneale cytologie, na systemische chemotherapie.

Slokdarm- en maag-adenocarcinomen zijn agressieve vormen van kanker die regelmatig niet reageren op neo-adjuvante therapie. De prognose van patiënten bij wie de tumor geen respons op behandeling toont is slecht. Immunotherapie is een veelbelovende nieuwe behandeloptie. Echter, slechts een klein percentage van de slokdarm- en maagcarcinomen reageert op immunotherapie. Daarom zijn biomarkers voor patiënten-selectie nodig. Bij niet-kleincellig longcarcinoom is *programmed death-Ligand 1* (PD-L1) een gevalideerde biomarker om patiënten te selecteren voor immunotherapie. Voor slokdarm- en maagkanker is het echter de vraag of PD-L1 een adequate biomarker is, aangezien de tumorresponspercentages in PD-L1-positieve slokdarm- en maagcarcinomen die met immunotherapie worden behandeld laag zijn (**Hoofdstuk 9**). Er is een verschil in het PD-L1-expressiepatroon tussen niet-kleincellig longcarcinoom enerzijds en slokdarm- en maagcarcinomen anderzijds. Bij slokdarm- en maagcarcinomen wordt de expressie van PD-L1 voornamelijk waargenomen in het immuunstroma, terwijl bij niet-kleincellig longcarcinoom de expressie vooral wordt gezien op de epitheelcellen. Dit zou kunnen verklaren waarom PD-L1 een geschikte biomarker is voor niet-kleincellig

longcarcinoom, maar niet voor slokdarm- en maagcarcinomen. Het is mogelijk dat voor het slokdarm- en maagadenocarcinomen een combinatie van markers nodig is om te voldoen aan een optimaal predictiemodel. Voor een nauwkeurige selectie van patiënten die het best reageren op immunotherapie, moeten toekomstige studies streven naar een combinatie van biomarkerstrategieën waarin de MSI (microsatelliet instabiliteit) status, EBV (Epstein-Barr Virus) status, typering van het immuuninfiltraat en karakterisering van antigenen worden meegenomen in de beoordeling van de verschillende typen van het slokdarm- en maagadenocarcinoom afzonderlijk.

Hoewel de introductie van multimodale behandeling de prognose van patiënten met een slokdarmadenocarcinoom aanzienlijk heeft verbeterd, reageert een substantieel deel van de tumoren niet op de neo-adjuvante chemoradiotherapie. Het bepalen van de prognose na een slechte tumorrespons op neo-adjuvante chemoradiotherapie blijft een uitdaging. Een immunosuppressieve tumor micro-omgeving, evenals de dichtheid en samenstelling van het immuuninfiltraat, zou een rol kunnen spelen in de immuun interactie tussen gastheer en tumor. Hiermee kan de respons op behandeling en overleving bij veel type carcinomen, waaronder gastro-intestinale maligniteiten, worden voorspeld. Bij slokdarmkankerpatiënten met een slechte tumorrespons op neo-adjuvante chemoradiotherapie was een hoge CD8+-infiltratie in de tumor micro-omgeving geassocieerd met een slechtere overleving (**Hoofdstuk 10**). Dit suggereert dat patiënten met een slechte tumorrespons op neo-adjuvante chemoradiotherapie maar met een hoge CD8+-infiltratie in het resectiepreparaat adjuvante therapie nodig hebben.

Appendices

List of publications

Authors and affiliations

Author contributions per chapter

PhD portfolio

Dankwoord

Curriculum vitae

List of publications

Perioperative management of gastric cancer patients treated with (sub)total gastrectomy, cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (HIPEC): lessons learned.
W.J. Koemans, A. Houwink, R.T. van der Kaaij, E.C.E. Wassenaar, D. Boerma, C. Hahn, O. Imhof, M.G. Brandt, M.P. Ariëns, A.A.F.A. Veenhof, K.J. Hartemink, J.W. van Sandick
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Author contributions per chapter

A population-based study on intestinal and diffuse type adenocarcinoma of the oesophagus and stomach in the Netherlands between 1989 and 2015.
Study design| RK, JS
Data acquisition| RK, MP, PS, VL, RK, JL, WK
Data analysis and interpretation| RK, MP, PS, JD, AC, VL, RK, JS, WK, JL
Statistical analysis| RK, WK
Manuscript preparation| RK, WK
Manuscript editing and review| RK, MP, PS, JD, AC, VL, RK, JS, WK, JL

The metastatic pattern of intestinal and diffuse type gastric cancer – a Dutch national cohort study.
Study design| WK, JS, RK
Data acquisition| WK, JL, RK
Data analysis and interpretation| WK, JL, CG, PS, JS
Statistical analysis| WK, JL
Manuscript preparation| WK, JL
Manuscript editing and review| WK, JL, RT, CG, PS, RH, JS

Synchronous peritoneal metastases of gastric cancer origin: incidence, treatment and survival of a nationwide Dutch cohort.
Study design| WK, RL, IH, JS, RK
Data acquisition| WK, RL
Data analysis and interpretation| WK, RL, IH, JS, CG, RK
Statistical analysis| WK, RL, RK
Manuscript preparation| WK, RL
Manuscript editing and review| WK, RL, CG, RK, IH, JS

Tumor characteristics and clinical outcome of peritoneal metastasis of gastric origin treated with a hyperthermic intraperitoneal chemotherapy (HIPEC) procedure in the PERISCOPE I trial.
Study design| WK, JS, EW, DB
Data acquisition| WK, RK, EW, DB, JS, PS
Data analysis and interpretation| WK, JS, PS
Statistical analysis| WK
Manuscript preparation| WK, JS
Manuscript editing and review| WK, RK, EW, DB, HB, KS, ML, CG, KH, AV, LK, PS, JS

Perioperative management of gastric cancer patients treated with (sub)total gastrectomy, cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (HIPEC): lessons learned
Study design| WK, AH, JS
Data acquisition| WK
Data analysis and interpretation| WK, AH, CH
Statistical analysis| WK
Manuscript preparation| WK, AH, MB, MA
Manuscript editing and review| WK, AH, RK, EW, DB, CH, OI, MB, MA, AV, KH, JS

Systemic exposure of oxaliplatin and docetaxel in gastric cancer patients with peritonitis carcinomatosis treated with intraperitoneal hyperthermic chemotherapy.
Study design| WK, RK, AH, JS
Data acquisition| WK, RK, EW
Data analysis and interpretation| WK, HR, AH
Statistical analysis| WK, AH
Manuscript preparation| WK
Manuscript editing and review| WK, RK, EW, CG, HB, DB, ML, OI, JS, HR, AH

Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy versus palliative systemic chemotherapy in stomach cancer patients with peritoneal dissemination, the study protocol of a multicentre randomised controlled trial (PERISCOPE II).
Study design| RK, HB, VR, HT, SV, VN, AH, AA, GD, BE, BW, ML, DB, JS
Manuscript preparation| WK, RK
Manuscript editing and review| WK, RK, HB, TB, AV, KH, CG, PS, VR, HT, SV, VN, AH, CH, AH, ML, ML, PB, OI, AA, GD, BE, BW, ML, DB, JS

Beyond the PD-L1 horizon: In search for a good biomarker to predict success of immunotherapy in gastric and esophageal adenocarcinoma.
Study design| WK, LK
Data acquisition| WK, LK
Manuscript preparation| WK, LK
Manuscript editing and review| WK, MC, JS, JD, LK

High CD8+ tumour infiltrating lymphocyte density associates with unfavourable prognosis in oesophageal adenocarcinoma following poor response to neo-adjuvant chemoradiotherapy.
Study design| WK, LK, JS
Data acquisition| WK, LK, RR, MS, IH, AB, FL
Data analysis and interpretation| WK, LK, OK, IH, AB, MP
Statistical analysis| WK
Manuscript preparation| WK, LK
Manuscript editing and review| WK, JD, JB, GM, PS, MC, FL, RR, FV, MP, MS, JS, LK

PhD PORTFOLIO

Onderzoekschool Oncologie Amsterdam (OOA)

Name PhD student: W.J. Koemans

PhD period: July 2017 – November 2021

Name PhD supervisor: prof. dr. E.J.T. Rutgers

dr. J.W. van Sandick

PhD training

General courses	**Year**	**Hours/ECTS**
Introductory Course clinical & translational oncology	2018	1
How to become a successful grant applicant	2018	0,05
Getting your Manuscript out for review and your work the attention it deserves: a strategic approach	2018	0,4
Writing a scientific paper	2018	1
Basic medical statistics	2018	1,5
OOA retreat	2018	1
Presentations (oral/poster)		
Treatment of peritoneal dissemination in stomach cancer patients with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (HIPEC): first results of the PERISCOPE I study. (poster) *European Gastric Cancer Congress, Leiden, The Netherlands.*	2018	0,5
Postoperative complications after HIPEC with oxaliplatin and docetaxel in gastric cancer patients with peritoneal dissemination. (poster) *Peritoneal Surface Oncology Group Internationsal (PSOGI) Paris, France.*	2018	0,5
Hyperthermic Intraperitoneal Chemotherapy for peritoneal metastasis of gastric cancer origin. (oral) *Oncology Graduate School Amsterdam (OOA) Retreat, Renesse, the Netherlands.*	2018	0,5
Cutting edge in HIPEC land. (oral) *Refereer avond Heelkunde region II, Amsterdam, The Netherlands.*	2018	0,5
Survival after HIPEC with oxaliplatin and docetaxel for gastric cancer patients with limited peritoneal dissemination. (poster) *International Gastric Cancer Congress, Prague, Czech. Young researcher travel grant.*	2019	0,5

Behandeling van het peritoneaal gemetastaseerde maagcarcinoom met cytoreductie en hypertherme intraperitoneale chemotherapie (HIPEC): resultaten van de PERISCOPE I studie. (oral) *Nederlandse Vereniging voor Heelkunde (NVvH) chirurgendagen, Veldhoven, The Netherlands.*	2019	0,5
Peritoneal metastases of gastric cancer origin: incidence, treatment and survival of a nationwide cohort. (oral) *Nederlandse Vereniging voor Gastroenterologie (NVGE), Veldhoven, The Netherlands.*	2019	0,5
An immunosuppressive PD-L1 positive tumour microenvironment marks oesophageal adenocarcinomas refractory to neo-adjuvant chemoradiotherapy. (oral) *Nederlandse Vereniging voor Gastroenterologie (NVGE), Veldhoven, The Netherlands.*	2019	0,5
The metastatic pattern of intestinal and diffuse type gastric adenocarcinoma – a Dutch national cohort study. (poster) *European Society of Surgical Oncology, Rotterdam, the Netherlands.*	2019	0,5
An immunosuppressive PD-L1 positive tumour microenvironment marks oesophageal adenocarcinomas refractory to neo-adjuvant chemoradiotherapy. (poster) *United European Gastroenterlogy week, Barcelona, Spain. Travel grant.*	2019	0,5
An immunosuppressive PD-L1 positive tumour microenvironment marks oesophageal adenocarcinomas refractory to neo-adjuvant chemoradiotherapy. (oral) *Wetenschapsdag Heelkunde region I&II, Amsterdam, the Netherlands.*	2019	0,5
HIPEC bij het maagcarcinoom. *IKNL bijeenkomst werkgroep gastro-enterologische tumoren, Nijmegen, the Netherlands.*	2020	0,5

(Inter)national conferences attended

Dutch upper GI cancer group congress, Utrecht	2017	0,3
Digestive disease days, Veldhoven	2018	0,6
Chirurgendagen, Veldhoven	2018	0,6
European Gastric Cancer Congress, Leiden	2018	0,6
Pathsoc annual meeting, Maastricht	2019	0,6
Peritoneal surface malignancy group 11th international workshop, Paris	2019	0,9
International gastric cancer congress, Prague	2019	0,9
Chirurgendagen, Veldhoven	2019	0,6
Digestive disease days, Veldhoven	2019	0,6
39th congress of the European Society of Surgical Oncology, Rotterdam	2019	0,6
United European Gastroenterlogy week, Barcelona	2019	0,9

Other

Weekly department research meeting	2017-2020	4
Weekly multi-disciplinary upper-GI cancer tumour board	2017-2020	4
Research meetings Dutch Upper GI Cancer Group (DUCG)	2017-2020	2
Research meetings Dutch Peritoneal Oncology Group (DOPG)	2017-2020	2
Klankboard meeting for the PERISCOPE II study	2017-2020	4

Dankwoord

Ik ben enorm trots op het werk dat voor u ligt en dat heeft alleen tot stand kunnen komen met de hulp en steun van veel mensen.

Op de eerste plaats uiteraard mijn copromotor dr. J.W. van Sandick. Johanna! Aan jou ben ik het meeste dank verschuldigd. Je hebt me rechtstreeks uit de schoolbanken de kans gegeven om bij je te komen werken. Dit proefschrift was niet mogelijk geweest zonder jouw drive, ideeën en scherpe blik. Ik heb er de afgelopen jaren versteld van gestaan met hoeveel energie jij werkt, er is geen commissie waar je niet inzit en geen project waar je niet bij betrokken bent. Ik heb enorm veel geleerd van je talent voor schrijven en je oog voor detail. Dank voor alle kansen die ik van je heb gekregen, ik heb genoten van mijn tijd als onderzoeker.

Dan dr. L.L. Kodach. Liudmila, zonder jou waren de laatste 2 hoofdstukken in dit boekje niet verschenen. Je enthousiasme voor translationeel onderzoek is ongekend en aanstekelijk. Met jouw ideeën hebben we de eerste projecten in een mooie onderzoekslijn opgezet. Ik ben ervan overtuigd dat er nog veel meer komt. Ik wil je bedanken voor al je advies en de gezellige koppen koffie, je deur stond altijd voor me open.

Prof. Dr. E.J.T. Rutgers, ik vind het een eer dat ik onder u mag promoveren. Hoewel slokdarm en maagkanker niet uw aandachtsgebied is heb ik veel gehad aan onze evaluatiegesprekken. U was vanaf het eerste moment enthousiast over de projecten en u hebt me altijd het vertrouwen gegeven dat er een mooi proefschrift zou ontstaan.

Geachte leden van de promotiecommissie, prof. dr. V.E.P.P. Lemmens, prof. dr. M.I. van Berge Henegouwen, prof. dr. J.P. Medema, prof. dr. I.H.J.T. de Hingh, prof. dr. H.W.M. van Laarhoven en prof. dr. H.M.W. Verheul. Dank voor uw tijd voor het beoordelen van mijn proefschrift.

Alle medeauteurs, zonder jullie waardevolle inbreng en feedback waren de stukken nooit tot stand gekomen.

Alle leden van de slokdarm en maag onderzoeksgroep in het Antoni van Leeuwenhoek, dank voor jullie ideeën en feedback op mijn presentaties en stukken. Dr. C. Grootscholten, Cecile, dank voor het plaatsnemen in mijn OOA commissie. Sophie, Soof! We moeten de buik in! We waren de 2 jonkies van het SMO, maar we hebben gelachen. Je enorme relaxte houding heeft me niet alleen in het AvL maar ook in het OLVG veel goed gedaan! Dank voor het prachtige design van de cover.

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Promoveren in het Antoni van Leeuwenhoek was vaak een feest en dat kwam door de collega's uit het O gebouw, van de vrijdagmiddagborrel tot de ski reis het maakte de taaie promotie momenten een stuk dragelijker. Rebecca "Rebelse" Karsten, soms koffie, vaak bier, een paar weekenden weg en een hoop grappen. Je organiseert het allemaal en ik mocht meteen aanhaken. Zonder jou was mijn promotie tijd een stuk saaier geweest. Mijn kamer genoten. Arthur, ook jij was altijd in voor een goed verhaal en een berg afleiding van het echte werk, Judith, gelukkig bracht jij een beetje rust in de kooi. Alle andere O'ers, bedankt voor de lunches en de vrijmibo's.

Rosa, voor een grootdeel van dit proefschrift heb jij de eerste steen gelegd, dank dat ik het van je over heb mogen nemen.

Joe joe de echte crew, Julian, Marc, Maurits, Philippe, Sophie, Polo, Zarah, Raoul, Robert, Irene, Tim en Marjolein. Ik heb een zomer lang met jullie gegierd van het lachen in het artsenhok op de 5e en 6e.

De Ski-cie Maart en Hes, het is me nog steeds een raadsel waarom 45 mensen tegelijk stoppen met nadenken op een skiweekend. Gelukkig hebben we het met zijn 3e gemanaged! Het was een fantastische ervaring.

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(Oud) bewoners van de Club MP, Coen, Jur, Vic, Jesper, Luuk, Stan en Simon. Het was altijd goed thuiskomen!

Emma, twee artsen in de familie en straks ook nog twee doctoren, dat kunnen er maar weinig zeggen! Succes met je avontuur in Boston!

Opa Jaap, jou eerste en mijn 2e naam! Wat een eer naar je vernoemd te zijn. Altijd mocht ik mee op sleeptouw, altijd een verhaal en altijd wel iets om te doen. Je bent de beste opa die er is!

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Curriculum Vitae

Willem Jaap Koemans was born on 30 December 1992 in Prinsenbeek, The Netherlands. He attended the Mencia de Mendoza Lyceum in Breda. After graduation, in 2011, he started medical school in Maastricht. During his time in medical school he did an internship in South Africa. His final year was spent at the Department of Surgery of the Zuyderland hospital, where his interest in oesophagogastric cancer surgery was born. In collaboration with the Department of Pathology of the Maastricht University Medical Centre, he conducted research on lymph node metastasis in oesophageal cancer. In 2017, he obtained his medical degree.

Willem started working as a PhD candidate at the Department of Surgery of the Netherlands Cancer Institute/Antoni van Leeuwenhoek (NCI/AvL) in the summer of 2017 under the supervision of dr. J.W. van Sandick and prof. dr. E.J.T. Rutgers. He studied the epidemiology of metastatic gastric cancer with special focus on peritoneal metastasis. Furthermore, he was the study coordinator of the PERISCOPE II study, a multicentre randomised controlled trial that aims to answer the question if an operation involving gastrectomy, cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (HIPEC) provides a survival benefit for gastric cancer patients with limited synchronous peritoneal metastasis as compared to palliative systemic chemotherapy alone. Next to that, Willem performed translational research studying the tumour microenvironment in oesophageal cancer treated with neoadjuvant chemoradiotherapy. During his time at the NCI/AvL he also worked 6 months as a resident not in training at the various surgical wards.

In April 2020, he started working as a surgical resident not in training at the OLVG hospital in Amsterdam.