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The Performance of Circulating Biomarkers in the Prediction of Response to Neoadjuvant Therapy in Patients with Oesophago-gastric Cancer

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A thesis submitted to Plymouth University
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Copyright statement

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by

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A thesis submitted to Plymouth University
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In collaboration with
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Abstract

Author: David Mark Bunting

Title: The performance of circulating biomarkers in the prediction of response to neoadjuvant therapy in patients with oesophago-gastric cancer

Introduction

The prognosis in oesophago-gastric cancer is poor with less than 15% patients surviving beyond 5 years after diagnosis. The addition of neoadjuvant therapy has been shown to increase survival in patients suitable for curative surgery. However, the additional gains are modest and the majority of patients do not respond sufficiently from therapy to gain any benefit. There is an urgent need to identify markers that can predict response to neoadjuvant therapy in order provide safer, more effective, individualised treatment regimes.

Methods

A prospective, multi-centre, collaborative study was undertaken in patients with oesophago-gastric cancer undergoing neoadjuvant therapy and potentially curative surgery. Levels of circulating biomarkers M2-Pyruvate kinase, alkaline phosphatase, CA19-9, CEA and CA 72-4 were measured in patients before and after administering the first cycle of chemotherapy. Binary logistic regression analysis was performed to assess the ability of biomarkers to predict histological response to therapy.

Results

165 patients were recruited to the main study. 105 patients had complete histopathological data for analysis. There were 27 responders and 78 non-responders to neoadjuvant therapy. There were no differences in pre-therapy demographic, pathological or treatment factors between the two groups. Responders had less post-operative lymphovascular invasion (P= 0.004) and higher R0 resection rates (P=0.03). Pre-therapy M2-Pyruvate kinase levels were lower in responders compared to non-responders (P=0.037) and levels were able to predict response with each unit increase in the biomarker level being associated with a 4.1% decrease in the likelihood of response (P=0.027). M2-PK levels were not associated with any pre-operative demographic, clinical or pathological factors.

Conclusions

Pre-therapy dimeric M2-PK levels can predict response to neoadjuvant therapy in patients with oesophago-gastric cancer. The test could be of clinical value for 1 in every 8 patients undergoing the test.

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Acknowledgements

This study was undertaken in response to the urgent need for biomarkers that are able to predict response to neoadjuvant therapy in patients with oesophago-gastric cancer in an attempt to improve the prognosis in patients suffering from this lethal disease.

By the very nature of this multi-centre, multi-disciplinary, collaborative study, a large number of people have been involved in some way. I have had the great fortune of working with colleagues who have given up their expertise and time, often working outside their usual duties to support this project. Any task is made more rewarding in the company of enthusiastic and kind-hearted colleagues, many of whom have become friends. I cannot name them all but I would like thank the following in particular for their individual and valuable contributions. The research project was supported by a grant from the Plymouth Hospitals NHS Trust Charitable Funds Small Grants Scheme without which it could not have been undertaken.

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Rachel, my wife has had her home invaded by journal articles and her husband on loan to biomarker research but all the while and despite this, it has been Rachel who has provided the support necessary to complete this work.

Declaration

At no time during the registration for the degree of Doctor of Medicine has the author been registered for any other University award without prior agreement of the Graduate Committee.

Work submitted for this research degree at the Plymouth University has not formed part of any other degree either at Plymouth University or at another establishment

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Publications:

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Bunting D, Lai W, Wheatley T, Berrisford R, Sanders G. Positron emission tomography in oesophageal cancer staging: a tailored approach. ASGBI International Surgical Congress, Harrogate, April 2014

Bunting D. Can we predict the response to neoadjuvant therapy in upper GI cancer? A systematic review of candidate biomarkers. ASiT Annual Conference, Glasgow, February 2015

Bunting D, Hornby S, Ball S, Vincent Z, Ayling R, Wheatley T, Sanders G. Redefining response to neoadjuvant chemotherapy in patients with oesophago-gastric cancer. Association of Upper Gastrointestinal Surgeons of Great Britain and Ireland Annual Conference, Brighton, September 2014

Bunting D, Lai W, Sanders G. Positron emission tomography in oesophageal cancer staging: a tailored approach. Association of Surgeons in Training Annual Conference, Belfast 2014

Bunting D, Lai W, Tanase A, Sanders G. Staging laparoscopy in oesophago-gastric cancer: a tailored approach. Association of Surgeons in Training Annual Conference, Belfast 2014

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CHAPTER 1 - INTRODUCTION

Oesophageal and Gastric Cancer

Background

In the UK, oesophageal and gastric cancer together represent the 4th and 8th most common cancers in men and women respectively, see Figure 1 and Figure 2[1]. Over the last decade, the incidence of oesophageal cancer has increased by 11% in males and decreased by 9% in females, Figure 3[2]. The incidence of gastric cancer is on the decrease in Europe and the UK (Figure 4). Survival in oesophago-gastric cancer is poor. The 5-year survival for oesophageal cancer in England is 13.1% in men and 14.4% in women, Figure 5[3]. The corresponding rates in gastric cancer are 17.8% and 19.9% in men and women respectively[3]. Disease-free survival rates are even lower. As a result, oesophago-gastric cancers are the 4th and 5th leading UK cause of cancer deaths in men and women respectively[4].

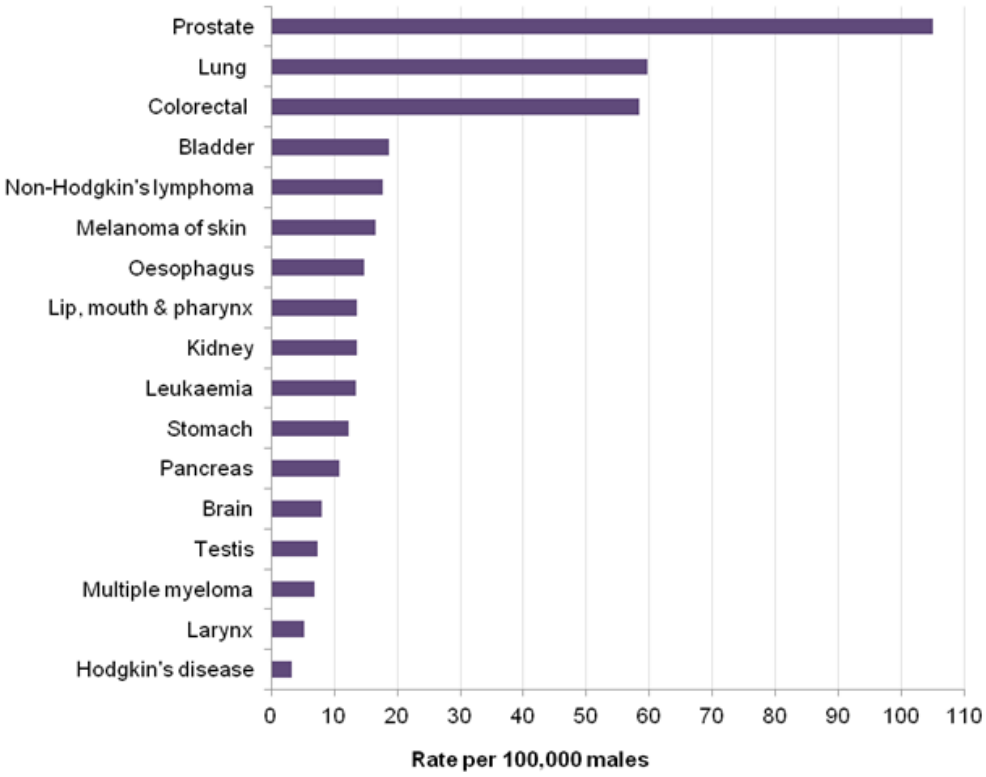

Figure 1 Male Cancer Incidence in the United Kingdom, 2008-10. *Office for National Statistics* 2012[1]

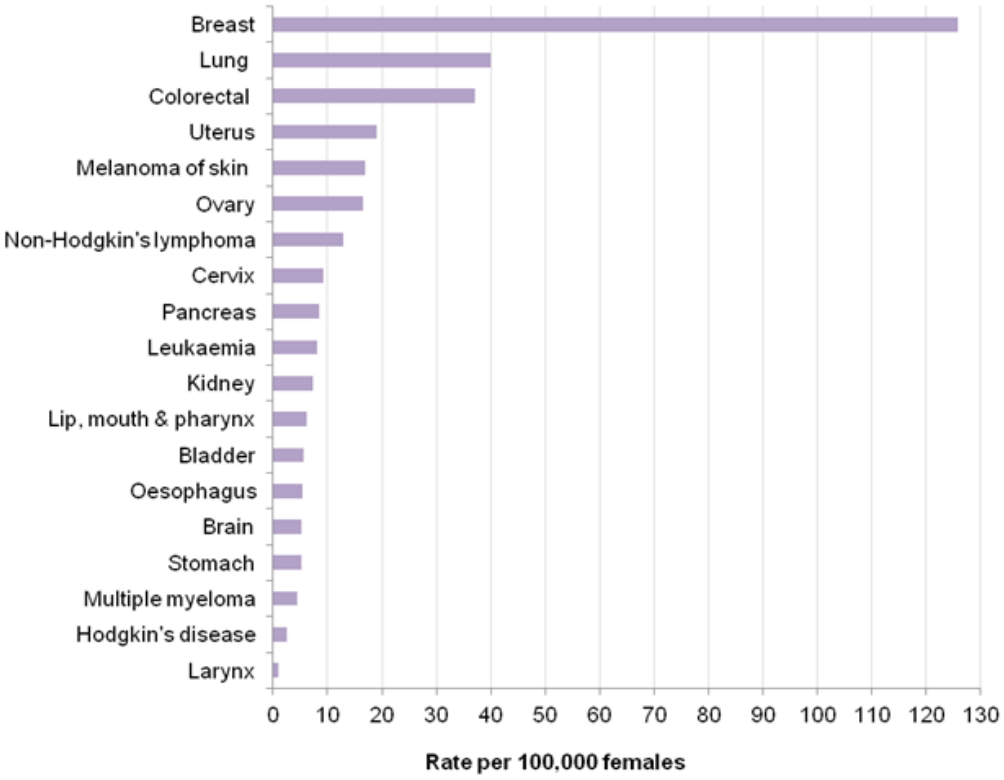

Figure 2 Female Cancer Incidence and in the United Kingdom, 2008-10. *Office for National Statistics* 2012[1]

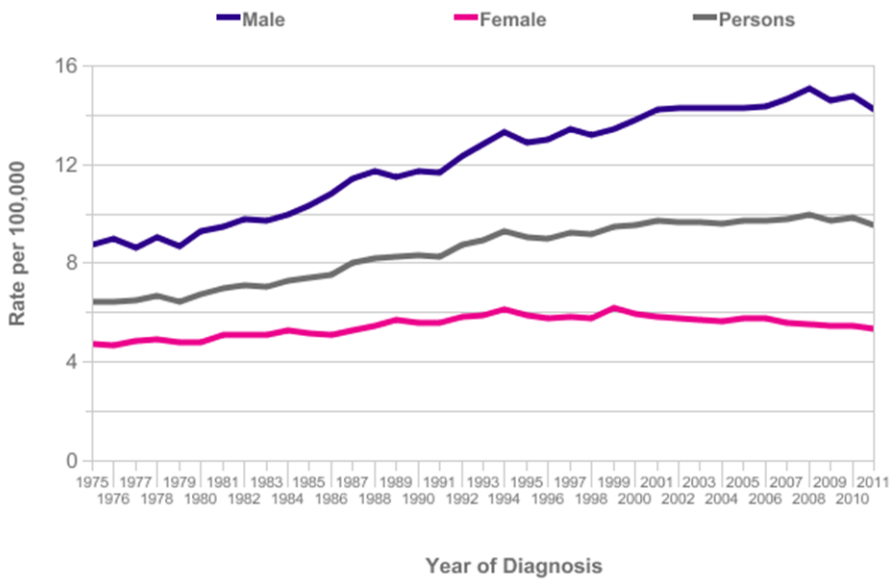

Figure 3 European Age-Standardised incidence rates of oesophageal cancer by sex, Great Britain. Cancer Research UK, http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/oesophageal-cancer/incidence#heading-Two, Accessed October 2015.

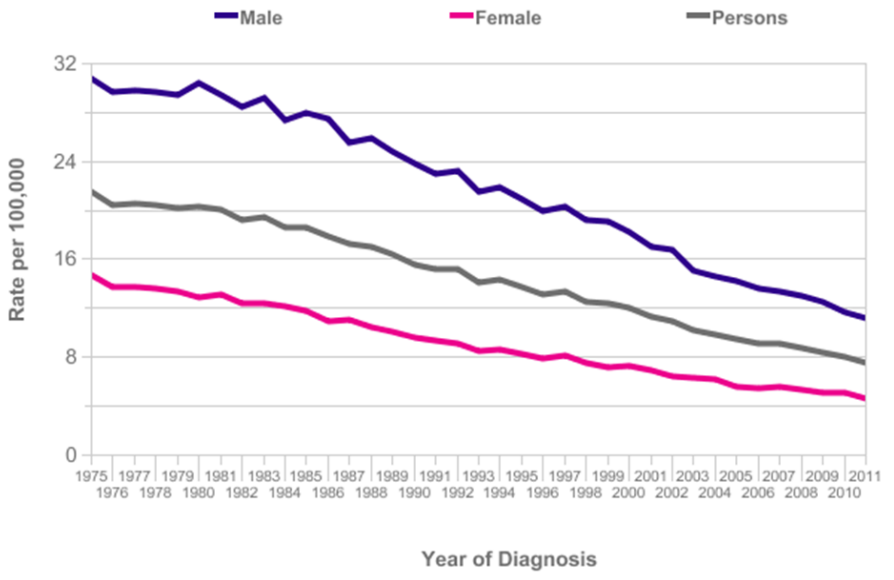

Figure 4 European Age-Standardised incidence rates of gastric cancer by sex, Great Britain. Cancer Research UK, http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/stomach-cancer/incidence#heading-Two, Accessed October 2015.

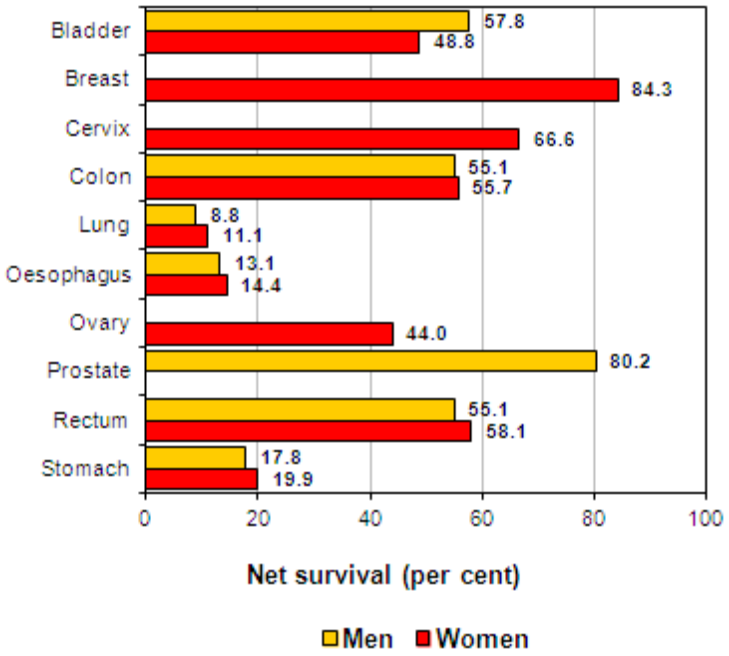

Figure 5 Five-year age-standardised net survival for adults diagnosed during 2006–2010 and followed up to 2011: England, 10 common cancers, by sex. Office for National Statistics. Cancer Survival in England: Patients Diagnosed 2006–2010 and Followed up to 2011, Summary 23-Oct-2012, accessed November 2012.)[3]

Cancers of the oesophagus and stomach tend to present at a late stage[5]. Curative treatments, primarily surgery plus or minus neoadjuvant therapy are reserved for those with localised disease, representing only 20-25% of patients with oesophago-gastric cancer[6]. The incidence increases with age, most presenting after the age of 72 years[7]. In patients with advanced disease and those unfit for surgery due to age or comorbidity, palliation with chemotherapy/radiotherapy/stenting are often the only treatment options. Between 20% and 42% of these patients receive chemotherapy (with or without endoscopic and radiological palliative therapy)[5].

Cancer sub-types

Histological

The great majority of oesophageal cancers fall into two main subtypes, adenocarcinoma and squamous cell carcinoma. The majority of malignant gastric cancers are adenocarcinomas.

Anatomical location

Traditionally, upper gastrointestinal cancers have been divided into gastric and oesophageal according to their anatomical location. However, there are problems associated with this classification. The incidence of adenocarcinomas of the gastro-oesophageal junction is increasing and it is becoming clear that they behave as a distinct subtype of their own. This group have been classified separately as cancers of the gastro-oesophageal junction. These tumours can be divided into subtypes depending on the exact anatomical relationship to the cardia/gastro-oesophageal junction and were described by Siewert and Stein in 1998[8] (Figure 6).

Type I	Adenocarcinoma of the distal oesophagus that usually arises from an area of specialised intestinal metaplasia (Barrett's oesophagus) and which may infiltrate the oesophago-gastric junction from above
Type II	True carcinoma of the cardia arising from the cardiac epithelium or short segments with intestinal metaplasia at the oesophago-gastric junction; this entity is also referred to as 'junctional carcinoma'
Type III	Subcardial gastric carcinoma, which infiltrates the oesophago-gastric junction and distal oesophagus from below

Figure 6 Siewert Classification of gastro-oesophageal junction tumours[8]

The following section describes how recent changes in the staging classification of oesophageal and gastric have taken account of these important anatomical considerations.

Staging in oesophago-gastric cancer

Classification

Since 1986, a single staging classification has been agreed by the American Joint Committee on Cancer (AJCC), the Japanese Joint Committee (JCC) and the International Union Against Cancer (UICC). It is an anatomical classification also referred to as the TNM classification where T represents the extent of the primary tumour, N nodal disease and M metastatic disease. There are specific classifications for each cancer subtype which are updated periodically as more accurate methods of staging cancers become available[9]. Figure 7 to Figure 12 show the up to date classifications (7th edition) for oesophageal/gastro-oesophageal junction and gastric cancer.

One of the most important changes in this updated classification compared to the 6th edition reflects the anatomical considerations mentioned above. Tumours involving the oesophago-gastric junction but arising in the proximal 5cm of the stomach (Siewert Type III) are staged along with all other junctional cancers staged using the oesophageal cancer subtype classification. T categories in gastric cancer have also been harmonized with those of the oesophagus and small and large intestine[10, 11].

Accurate staging is critical in determining the optimal treatment for patients with oesophageal and gastric cancer. One of the most important aspects of treatment is avoiding unnecessary surgery which involves identifying those patients who will not benefit. The main goal of surgery in oesophageal and gastric cancer is improved survival. It is known that patients with metastatic disease at presentation and those with an unresectable tumour will not gain a survival benefit from resection of the primary tumour. The identification of unresectable nodal disease, resectability of the primary

tumour and identification of metastatic disease are therefore crucial requirements of the staging process.

Stage	Description
TX	Primary tumour cannot be assessed.
T0	No evidence of primary tumour.
T1	Tumour invades lamina propria, muscularis mucosae, or submucosa.
T1a	Tumour invades lamina propria or muscularis mucosae.
T1b	Tumour invades submucosa.
T2	Tumour invades muscularis propria.
T3	Tumour invades adventitia.
T4	Tumour invades adjacent structures.
T4a	Resectable tumour invading pleura, pericardium or diaphragm.
T4b	Unresectable tumour invading other adjacent structures, such as aorta, vertebral body, trachea etc.

Figure 7 Local tumour staging for cancer of the oesophagus/oesophago-gastric junction, from AJCC: Esophageal and esophagogastric junction. In Edge SB, Byrd DR, Compton CC et al., eds.:AJCC Cancer Staging Manual. 7th ed New York, NY: Springer, 2010, pp103-15.[9]

Stage	Description
NX	Regional lymph nodes cannot be assessed.
N0	No regional lymph node metastasis.
N1	Metastases in 1-2 regional lymph nodes.
N2	Metastases in 3-6 regional lymph nodes.
N3	Metastases in ≥7 regional lymph nodes.

Figure 8 Nodal staging for cancer of the oesophagus/oesophago-gastric junction, from AJCC: Esophageal and esophagogastric junction. In Edge SB, Byrd DR, Compton CC et al., eds.:AJCC Cancer Staging Manual. 7th ed New York, NY: Springer, 2010, pp103-15.[9]

Stage	Description
M0	No distant metastasis.
M1	Distant metastasis.

Figure 9 Metastasis staging for cancer of the oesophagus/oesophago-gastric junction, from AJCC: Esophageal and esophagogastric junction. In Edge SB, Byrd DR, Compton CC et al., eds.:AJCC Cancer Staging Manual. 7th ed New York, NY: Springer, 2010, pp103-15.[9]

Stage	Description
TX	Primary tumour cannot be assessed.
T0	No evidence of primary tumour.
Tis	Carcinoma in situ: intraepithelial tumor without invasion of the lamina propria.
T1	Tumour invades lamina propria, muscularis mucosae, or submucosa.
T1a	Tumour invades lamina propria or muscularis mucosae.
T1b	Tumour invades submucosa.
T2	Tumour invades muscularis propria.
T3	Tumor penetrates subserosal connective tissue without invasion of visceral peritoneum or adjacent structures
T4	Tumor invades serosa (visceral peritoneum) or adjacent structures.
T4a	Tumor invades serosa (visceral peritoneum).
T4b	Tumor invades adjacent structures.

Figure 10 Local tumour staging for gastric cancer, from AJCC: Esophageal and esophagogastric junction. In Edge SB, Byrd DR, Compton CC et al., eds.:AJCC Cancer Staging Manual. 7th ed New York, NY: Springer, 2010, pp103-15.8

Stage	Description
NX	Regional lymph nodes cannot be assessed.
N0	No regional lymph node metastasis.
N1	Metastases in 1-2 regional lymph nodes.
N2	Metastases in 3-6 regional lymph nodes.
N3	Metastases in ≥7 regional lymph nodes.
N3a	Metastases in 7–15 regional lymph nodes.
N3b	Metastases in ≥16 regional lymph nodes.

Figure 11 Nodal staging for gastric cancer, from AJCC: Esophageal and esophagogastric junction. In Edge SB, Byrd DR, Compton CC et al., eds.:AJCC Cancer Staging Manual. 7th ed New York, NY: Springer, 2010, pp103-15.8

Stage	Description
M0	No distant metastasis.
M1	Distant metastasis.

Figure 12 Metastasis staging for gastric cancer, from AJCC: Esophageal and esophagogastric junction. In Edge SB, Byrd DR, Compton CC et al., eds.:AJCC Cancer Staging Manual. 7th ed New York, NY: Springer, 2010, pp103-15.8

Staging Guidelines

Guidelines for the management of oesophageal and gastric cancer were published in 2011 by the Association of Upper Gastrointestinal Surgeons of Great Britain and Ireland (AUGIS), the British Society of Gastroenterology (BSG) and the British Association of Surgical Oncology (BASO)[12]. The recommendations on staging are summarised. Initial staging should be performed with a computed tomography (CT) scan including multiplanar reconstructions of the thorax, abdomen and pelvis. In T1 oesophageal tumours or nodularity in high grade dysplasia, staging by endoscopic resection (ER)

should be used to define depth of invasion. EUS should be used in oesophageal, oesophago-gastric junctional and selected gastric tumours. PET-CT scanning should be used in combination with EUS and CT for assessment of oesophageal and oesophago-gastric junctional cancer. Staging laparoscopy should be undertaken in all gastric cancers and in selected patients with lower oesophageal and oesophago-gastric junctional tumours (whenever tumour extends below the diaphragm, according to the previous set of guidelines by the same group in 2002[13]).

Staging modalities

In addition to those modalities mentioned above (endoscopy, endoscopic resection, CT, EUS, PET-CT and staging laparoscopy), additional techniques such as video-assisted thoracosopic surgery (VATS), endobronchial ultrasound (EBUS) and ultrasound guided biopsy of lymph nodes or suspicious peripheral lesions are sometimes employed by individual specialist centres on an individual patient basis. The typical staging pathway used in our specialist multidisciplinary team (MDT) is summarised in Figure 13.

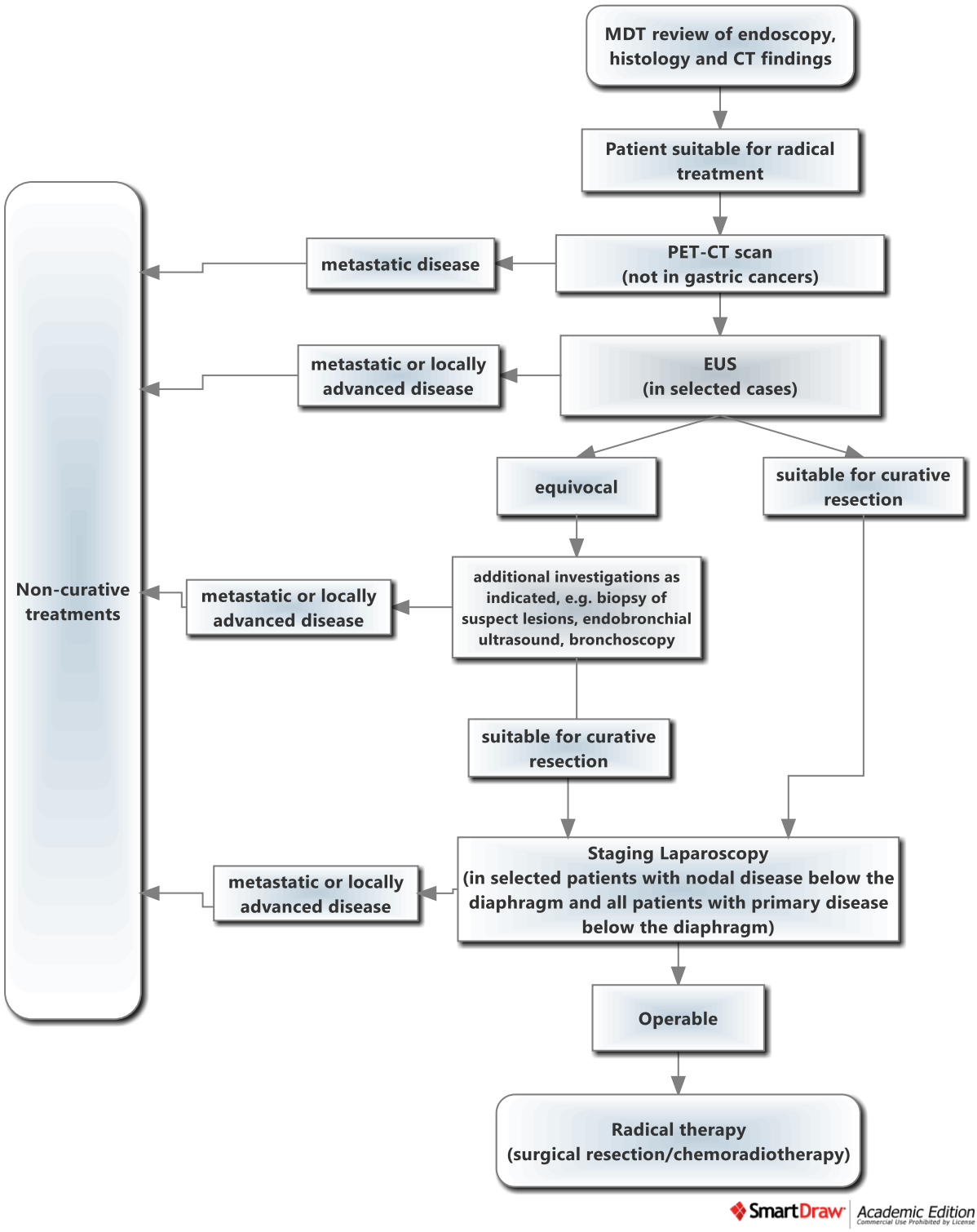

Figure 13 Flow chart showing typical staging pathway. MDT, multidisciplinary team; EUS, endoscopic ultrasound; PET, positron emission tomography; CT, computed tomography.

Each modality can contribute in some way; however, none of the modalities are able to stage the disease with complete accuracy. Published guidelines reflect this and tend to lead to over-investigation which is costly, time-consuming and can delay definitive treatment. The result is that many patients undergoing a staging investigation will not have their management altered as a result of undergoing the test.

In the same way that clinicians are trying to find ways of individualising treatment, it may be possible to tailor the staging investigations in each patient according to the results of initial tests rather than following a rigid protocol in all patients.

One particular problem in assessing the accuracy of various staging modalities is the rapid pace of improvements in technology which means that even relatively recent reports may rely on imaging solutions that lag behind what is currently available. Modern multidetector CT (MDCT) scanners are able to produce images with better resolution allowing multiplanar 2D and 3D reconstructions, improving the accuracy of staging. Endoscopic equipment has also advanced significantly in recent years with high definition now being the standard in many units.

This raises the question of whether there are features of endoscopy and CT examinations that are able to predict the tumour stage with sufficient accuracy that would obviate the need for further investigations such as PET scanning, endoscopic ultrasound, and staging laparoscopy in some patients.

Clinical assessment

Staging should always start with clinical assessment. This may direct investigations in order to identify metastatic disease at an early stage so that further unnecessary investigations can be avoided.

Endoscopy

Endoscopy is the most important initial investigation in suspected oesophageal and gastric cancer since it is required to establish the histological diagnosis and can provide useful staging information.

Endoscopic resection

ER is very useful in the staging of early cancer. It is used in Barrett's with dysplasia and nodularity where invasion is suspected. It has greater sensitivity than biopsy for detection of invasion and is superior to EUS in staging early T1 cancers[14].

CT scanning

The main role of CT scanning is in the detection metastatic disease. Up to 50% patients present with metastatic disease, many of whom will be identified on CT and can be spared further staging investigations. For example, the accuracy in detecting liver metastases is between 86% and 98%[15]. CT is also important in determining resectability of the primary tumour. After endoscopy, it is usually the next staging investigation to be performed. The accuracy and role of CT in staging is explored further in Chapter 4 - Staging.

Endoscopic ultrasound

Since the outcome in units performing EUS is related to operator experience, it has been recommended that only experienced sonographers at centres performing at least 100 examinations annually should be using this technique[12]. The main use of EUS is in assessment of T and N stage which it has been shown to do more accurately than CT. Accuracy is dependent on stage: for T-staging in gastric cancer, sensitivities ranges from 82.3%-99.2% and specificity ranges from 94.7%-100%[16]. For N-staging in gastric cancer, sensitivity is around 58.2%-64.9% and specificity 87.2%-92.4%[16]. In oesophageal cancer,

the sensitivity for T stage ranges between 81.6% and 92.4%. The specificity ranges from 97.4% to 99.4 %[16]. The main limitations of EUS are poor accuracy in staging early (intramucosal) neoplasia and in advanced (T3 and T4) lesions where the tumour cannot be traversed by the scope. Despite good overall accuracies, there has been limited evidence for improved outcomes associated with the additional information that EUS provides until results of the pragmatic randomised trial COGNATE (Cancer of Oesophagus or Gastricus - New Assessment of Technology of Endosonography) were published. These showed EUS improves survival and has the potential to reduce health-care resources[17]. Perhaps the best use of EUS is in the assessment of mediastinal lymphadenopathy when a linear probe is used to facilitate guided fine needle aspiration (FNA). This improves sensitivity for nodal (N) staging from 84.7% to 96.7%[18].

PET-CT

Metabolic imaging (PET) when combined with CT provides functional as well as anatomical information. Its main role is in the detection of distant nodal and metastatic disease. PET has demonstrated significantly better specificity than CT (even when combined with EUS) for the identification of both nodal and distant metastasis[19, 20]Whilst some studies have shown that PET leads to a change in the staging in up to 20% patients compared to CT and EUS alone, a meta-analysis concluded that PET offered little improvement in the overall accuracy of staging in oesophageal cancer[21]. The role of PET-CT in staging of oesophageal cancer is explored further in CHAPTER 4 - INDIVIDUALISED STAGING INVESTIGATIONS.

Staging laparoscopy

CT, EUS and PET are poor at identifying low volume peritoneal/hepatic metastases and determining local resectability. Laparoscopy (which is usually performed in conjunction

with a second endoscopy to aid tumour localisation) offers the ability to identify low volume metastases and can give useful information on resectability. Reports have shown that this provides additional treatment information in 17% patients with oesophageal/junctional tumours and 28% patients with gastric tumours[22]. The role of laparoscopy in the staging of gastric and gastro-oesophageal tumours is further explored in Chapter 4 - Staging.

Video-assisted thoracoscopic surgery (VATS)

VATS can be used to examine and take biopsies of thoracic lesions that may represent metastatic deposits outside the field of resection. The resectability of thoracic primary tumours can also be assessed.

Endobronchial Ultrasound (EBUS)

EBUS is used to examine possible direct tumour invasion into the respiratory tree and can be used to biopsy suspicious mediastinal nodes the lie outside the field of resection.

Chemotherapy and Chemoradiotherapy

Purpose of chemotherapy/chemoradiotherapy

The aim of chemotherapy in the management of cancer is to improve overall quality of life and survival. Preoperative (neoadjuvant) chemotherapy is thought to improve operability and reduce local recurrence by primary tumour shrinkage. It is also thought to treat occult micro-metastases early, thereby reducing mortality from postoperative metastatic recurrence[23]. Survival benefits are achieved through cure in some and by prolonged survival in those that do recur. Neoadjuvant therapy is considered as preferable to adjuvant therapy in the treatment of oesophageal cancer not only due to the actions above but because it is associated with other factors such as improved tumour oxygenation at the time of therapy[24]; better tolerance of therapy before

surgery[24, 25]; improvements in swallowing allowing improved preoperative nutrition and it allows the sparing of surgery to those patients who progress early with metastatic disease[24]. Similarly, chemoradiotherapy is intended to down-stage disease in an effort to increase complete resection rates and reduce recurrence/survival[24].

Evidence - Oesophageal cancer

Neoadjuvant chemotherapy

The American Intergroup Trial (INT0113) randomised 440 patients to having neoadjuvant chemotherapy (3 cycles of cisplatin and fluorouracil) with or without postoperative chemotherapy or no chemotherapy at all[26]. They showed no difference in treatment related mortality, median survival or pattern of disease recurrence between the two groups although there was a higher R0 resection rate in those having chemotherapy. Criticisms of this study include a low (80%) operation rate in the chemotherapy arm put down to high levels of therapy-associated toxicity.

The Medical Research Council Adjuvant Gastric Infusional Chemotherapy (MAGIC) trial, which included 131 patients with oesophageal/junctional cancer, demonstrated that a combined strategy of three preoperative chemotherapy cycles (epirubicin, cisplatin & fluorouracil, ECF) and three postoperative chemotherapy cycles (ECF) decreased tumour size and significantly improved progression-free and overall survival[25].

The Medical Research Council (MRC) OE02 study is arguable the most influential study in the UK to date on this topic[27]. 802 patients were randomised to neoadjuvant chemotherapy with surgery (CS) or surgery alone (S) arms. Complete (R0) resection rate was somewhat higher in the chemotherapy group (60% vs. 53% $P<0.0001$) and the

overall survival was significantly better in the chemotherapy group with 5-year figures of 23% and 17% for CS and S groups respectively[28].

An updated Cochrane review of 11 randomised trials published in 2006 concluded that there was some evidence to suggest preoperative chemotherapy improves survival but that this was inconclusive (HR 0.88; 95% confidence interval (CI) 0.75-1.04)[29].

In an attempt to establish whether more chemotherapy (4 cycles epirubicin, cisplatin, capecitabine (ECX)) was more effective than 'standard treatment' given in the OE02 study (2 cycles of Cisplatin, 5-FU), the OE05 study randomised 897 patients to either treatment[30]. Early results were presented at the 2015 American Society of Clinical Oncology (ASCO) meeting in June. There was no survival difference between the groups and chemotherapy-related toxicity was higher in the ECX group. It is not known whether a sub-group analysis according to observed therapy response would give different results, however, in any case these results suggest that overall, the therapeutic benefit of neoadjuvant therapy may have been reached with the standard regime and that potential benefits of more additional agents and cycles are negated by toxic effects.

The ST03 trial set out to investigate whether the addition of the vascular endothelial growth factor monoclonal antibody, bevacizumab, to ECX neoadjuvant chemotherapy would improve overall survival. However, this part of the trial closed due to toxicity (high surgical complication rate) in the bevacizumab arm. This is further evidence supporting the notion that it is not possible to improve response rates and overall survival simply by adding more therapeutic agents.

Neoadjuvant chemoradiotherapy

Walsh *et al* conducted a randomised trial comparing surgery alone with combined chemotherapy, radiotherapy and surgery[31]. 13 of 55 patients (25 per cent) treated with neoadjuvant therapy had complete pathological responses. Neoadjuvant chemoradiotherapy was associated with longer median survival, (16 months vs, 11 months, P=0.01) and 3-year survival of 32% vs. 6%, P=0.01. This study has been criticised for the very poor survival in the surgery alone group of 6% at 3 years which is much lower than that expected even at the time the study was conducted. Staging during this time was less accurate than it is now and it could be that patients were under-staged in the control arm[24].

A meta-analysis of both chemotherapy (1724 patients) and chemoradiotherapy (1209 patients) comparing each multimodality treatment with surgery alone demonstrated a significant survival benefit with the use of preoperative chemoradiotherapy compared to surgery alone amounting to an absolute benefit of difference in survival at 2 years of 13% with similar findings in both adenocarcinoma and squamous cell carcinoma[32]. The survival benefit in patients undergoing chemotherapy compared to surgery alone was 7% at 2 years. Outcomes in chemotherapy and chemoradiotherapy were not directly compared in this study.

An updated meta-analysis published in 2011 comparing neoadjuvant chemotherapy with chemoradiotherapy reviewed 19 studies and showed strong evidence for a survival benefit of neoadjuvant chemotherapy or chemoradiotherapy over surgery alone however a clear advantage of chemoradiotherapy over chemotherapy was not demonstrated[33].

In 2012, results from the Chemoradiotherapy for Oesophageal Cancer Followed by Surgery Study (CROSS) were published, demonstrating that chemoradiotherapy improved median survival (49.4 months vs. 24.0 months) among patients with potentially curable oesophageal or oesophago-gastric junctional cancer[34]. The regimes used in this study form the basis of current neoadjuvant chemoradiotherapy regimes in many specialist units in the UK.

Stahl et al compared preoperative chemotherapy with preoperative chemoradiotherapy in 119 patients with adenocarcinoma of the oesophagus or gastric cardia[35]. The 3-year survival was 27.7% in the chemotherapy group compared to 47.4% in the chemoradiotherapy group, however, this difference was insignificant, possibly because the study closed early due to low accrual.

Adjuvant therapy

Most studies investigating the use of postoperative chemotherapy have used it together with pre-operative chemotherapy in a 'peri-operative' regime which makes it impossible to measure its individual impact[25, 36]. Only 32%-40% patients in these studies completed the postoperative phases, which highlights one of the main disadvantages of adjuvant therapy.

Although there may be a role for postoperative radiotherapy in advanced squamous cell carcinoma, the evidence in support of its use in adenocarcinoma is not so clear.

Evidence - Gastric cancer

Chemotherapy

Rather than downsizing primary tumours to facilitate greater R0 resection rates (as with oesophageal cancer) the goal of chemotherapy in gastric cancer has tended to focus on

reducing local/regional recurrence. Treatment has therefore concentrated on adjuvant and 'peri-operative treatments'. The best evidence to date comes from the MAGIC trial mentioned in the oesophageal section above[25]. It included 372 patients randomised to have either peri-operative chemotherapy and surgery or surgery alone. The combination regime of epirubicin, cisplatin and fluorouracil (ECF) decreased tumour size and stage and significantly improved progression-free survival (hazard ratio for progression, 0.66; 95% confidence interval, 0.53-0.81; P<0.001) and overall survival (hazard ratio for death, 0.75; 05% confidence interval 0.60-0.93; P=0.009; five-year survival rate 36% vs 23%). This paper has radically changed the standard treatment of gastric cancer and pre-operative chemotherapy is now the standard of care for any tumours more advanced than T2N0 on initial staging. Post-operative chemotherapy tends to be considered after recovery from surgery depending on individual pathological and patient factors.

Intraperitoneal chemotherapy has been used in an effort to reduce peritoneal and hepatic recurrence, particularly in Japan although there are concerns over toxicity and overall or recurrence-free survival have not been proven.

Chemoradiotherapy

In an effort to reduce local recurrence and improve survival, postoperative chemoradiotherapy has been used. The most significant trial in the area is the American Intergroup 0116 study[37]. It randomised patients to receiving adjuvant chemoradiotherapy or not and showed improvements in disease-free (49% vs 32%) and overall (52% vs 41%) survival, however, it has been criticised for poor radiotherapy technique, chemotherapy toxicity and less than adequate surgery with a D0 resection

rate as high as 54%. Survival in the surgery alone arm was surprisingly poor at 41%. As a result, postoperative radiotherapy has not been widely adopted.

Guidelines for neoadjuvant chemotherapy/chemoradiotherapy

When appraising the evidence for chemotherapy/chemoradiotherapy treatment, it must be remembered that patients with oesophago-gastric cancer are a heterogeneous group. In particular the cancer subtypes (adenocarcinoma, squamous carcinoma etc.) behave differently in relation to chemotherapy. Likewise, the effectiveness of chemotherapy is dependent on anatomical tumour location[38]. Whilst most reports separate gastric and oesophageal cancer types, there is evidence to suggest that the three subtypes of gastro-oesophageal junction adenocarcinomas (types I, II and III) have different aetiologies, pathogeneses and natural histories and may therefore respond differently to chemotherapy[38]. Before the introduction of the latest version of the TNM staging system (7th edition in 2010) many junctional cancers now staged with the oesophageal classification would have been staged as gastric cancers. In studies using different staging systems it can be difficult to compare individual studies or combine results in a meta-analysis.

There have been a number of important guidelines produced covering the management of oesophageal and gastric cancers in recent years. The Scottish International Guidelines Network (SIGN) produced a national clinical guideline in 2006[39]. Following this, in 2011, guidelines produced on behalf of AUGIS, BSG and BASO were published in *Gut*[12].

Oesophageal cancer

The 2006 SIGN guidelines suggest that patients with operable oesophageal cancer who are treated surgically should be considered for two cycles of preoperative chemotherapy with cisplatin and 5-flourouracil or offered entry into a clinical trial[39]. Preoperative

chemoradiotherapy was not recommended outside clinical trials and preoperative radiotherapy was not recommended. There is a role for chemoradiotherapy when surgery is not being considered. Postoperatively, neither chemotherapy nor chemoradiotherapy was recommended. Radiotherapy could be considered in those with a high risk of local recurrence but there was not sufficient evidence to for a recommendation. The guidelines were designed to be applicable to both squamous cell and adenocarcinoma subtypes.

The AUGIS/BSG/BASO guidelines (2011) state that chemoradiation is the definitive treatment of choice in patients with localised squamous carcinomas of the proximal oesophagus[12]. Squamous tumours of the middle and lower oesophagus can be treated with chemoradiation alone or a combination of chemoradiation and surgery. In the treatment of adenocarcinoma, there is Grade IA evidence for preoperative chemoradiotherapy improving long-term survival over surgery alone. Similarly, chemotherapy using cisplatin and 5-flourouracil improves long-term survival over surgery alone[12]. Combined preoperative and postoperative (perioperative) chemotherapy is the preferred option for oesophago-gastric junctional types II and III adenocarcinoma[12].

Gastric cancer

The 2006 guidelines suggested there is no evidence for the use of chemotherapy or radiotherapy preoperatively or postoperatively for patients with gastric cancer outside a clinical trial[39].

The 2011 guidelines differ from the above significantly; suggesting that perioperative chemotherapy should be the standard of care in patients with gastric adenocarcinoma[12]. Postoperative chemotherapy should be considered in patients at a high risk of

recurrence who have not received preoperative chemotherapy. Chemoradiotherapy is an alternative in this group of patients.

There are no guidelines supporting the use of intraperitoneal chemotherapy which remains experimental.

Current chemotherapy practice

In practice, most centres in the UK including ours adopt a policy based on individual patients' comorbidity and disease staging. Patients with oesophageal cancer and disease equal to or more advanced than T2N0 are considered for neoadjuvant therapy. As a result, the majority of patients with oesophageal and gastric cancer are now being offered neoadjuvant therapy because the majority present with advanced disease. In the UK, neoadjuvant chemotherapy rather than chemoradiotherapy tends to be the standard of care. Chemoradiotherapy has been more popular in the United States but is being increasingly used in this country. Of those patients not undergoing neoadjuvant therapy, 7-9% patients receive chemotherapy/radiotherapy after surgery[5]. Moreover, the proportion of patients receiving chemotherapy is increasing both in those undergoing surgical resection and those not[5]. This trend is seen in oesophageal, gastric and junctional cancer types.

Limitations of current chemotherapy regimes

Resistance & Response rates

Resistance to chemotherapy is a common problem. A complete histological response is achieved in only 0-12.5% (typically less than 6%) in patients receiving neoadjuvant chemotherapy[40-43]. Partial response rates are typically reported at 11-12%[36, 40, 44]. Complete response rates with chemoradiotherapy are somewhat higher, typically reported between 17 and 29%[45-48].

Patients who do not respond to neoadjuvant therapy will have an unnecessary delay to surgery whist undergoing ineffective and potentially toxic chemotherapy which may be associated with poorer outcomes[26, 36]. In oesophageal squamous cell carcinoma for instance, it has been suggested that only complete pathological responders to neoadjuvant chemotherapy benefit from such additional treatment[41].

Current guidelines for multimodality therapy are based on studies reporting survival overall without considering whether outcomes differ according to how patients have responded to neoadjuvant therapy.

If it can be demonstrated that outcome depends on the degree of response to neoadjuvant therapy, this raises the important question of whether we can predict which patients will benefit from neoadjuvant chemotherapy/chemoradiotherapy at any stage during the treatment process and a number of authors have recognised that this should be the focus of more research[38, 44, 49-58] (see Figure 14). Recently this issue has received attention in the national press[59]. Predicting the response to therapy may enable clinicians to tailor treatment individually. This important issue is addressed in detail in the remainder of this thesis.

There are numerous methods of measuring response to chemotherapy/chemoradiotherapy but no universally agreed definition. It is essential to understand what defines a response when analysing outcomes in this way or when investigating prediction of response. This merits further discussion and is detailed in Chapter 3.

Figure 14 Authors declaring a need for further research into finding a means of predicting the response to neoadjuvant therapy.

Toxicity

The Cochrane review on preoperative chemotherapy for resectable thoracic oesophageal cancer reviewed the chemotherapy associated toxicity amongst the 12 studies containing 2097 patients[29]. Not all studies reported the toxicity and those that did varied in the range of toxicities defined. However, overall toxicity was reported between 11% and 90%. Preoperative deaths in the chemotherapy arm ranged between 0% and 9%. Preoperative deaths in the surgery only arm ranged from 0.5% to 2% (2 studies), however this cannot be compared to the chemotherapy deaths since the timeframe to surgery is much longer in the chemotherapy group. A number of gastrointestinal complications were reported in the chemotherapy group including nausea and vomiting but comparison with the surgery-alone group was not possible due to heterogeneity.

Therapeutic index

In addition to the specific problem of resistance, any chemotherapy treatment is limited by its maximal clinical effectiveness and its adverse effects, together determining the therapeutic index. The investigation of novel chemotherapy targets for chemotherapy is an important area of on-going research with the intention of developing newer agents that may have a wider therapeutic index. The UK MRC ST03 trial will evaluate whether the addition of the bevacizumab to peri-operative ECX is associated with improved survival in oesophago-gastric adenocarcinoma. The REAL 3 trial looked to investigate the addition of the anti-EGFR monoclonal antibody, panitumumab to EOX treatment but was stopped early due to inferior outcomes in the experimental arm which highlights the difficulties encountered in the development of new therapeutic agents.

CHAPTER 2 – AIMS & OBJECTIVES

The survival in oesophago-gastric cancer is poor but may be improved by the use of multimodality therapy. Neoadjuvant treatment agents are limited by a relatively narrow therapeutic index. The toxic effects of such treatment can be significant and are particularly important in patients who fail to respond to therapy. Reported rates of response to neoadjuvant therapy are poor and there is no agreed definition of response.

Aim 1

- To review the toxicity associated with neoadjuvant therapy in patients with potentially curable oesophago-gastric cancer treated with neoadjuvant therapy at the Peninsula Oesophago-gastric Surgery Unit. The efficacy of neoadjuvant therapy with respect to therapeutic response and disease stage will be analysed. Methods of measuring response to neoadjuvant therapy will be discussed and a revised definition of response will be defined from the regional population.

Multi-modality treatments are thought to have a beneficial role in more advanced stages of disease. Pre-treatment clinical staging is used to determine the need for multimodality therapies; however, staging modalities have several limitations. The staging thresholds beyond which treatments become effective are not known. Poor accuracy of radiological staging methods may mean that the wrong patients are being stratified for additional therapy. In the context of multiple imaging modalities, often individual investigations do not influence clinical management. Multiple staging investigations may introduce a delay in initiating potentially curative treatments.

Aim 2

- To investigate accuracy of imaging modalities used to determine pre-therapy disease stage and to seek whether the staging pathway could be streamlined by

limiting the use of certain modalities to those patients in whom management is likely to be altered.

The problems of poor response rates to therapy can be tackled in a number of ways. Newer additional agents may offer a solution but recent evidence suggests that we may have reached a therapeutic plateau with the risks of additional treatment outweighing any potential benefit.

Successful prediction of an individual patient's response to therapy would enable personalised treatment. The relatively recent discovery of multiple genetic markers in oesophago-gastric cancers has helped us to appreciate the importance of individual tumour biology. This provides an explanation for the observation that individual tumours can behave differently and supports the development of personalised treatment strategies.

Aim 3

- To review the published literature on methods of predicting response to neoadjuvant therapy.

Aim 4

- To investigate the potential of a number of circulating biomarkers in predicting response to neoadjuvant therapy.

CHAPTER 3 – NEOADJUVANT THERAPY: EFFICACY, TOXICITY AND DEFINING RESPONSE

Introduction

Background

Early studies investigating the potential benefits of neoadjuvant therapy showed conflicting results and it took many years before a survival advantage was demonstrated in randomised trials[25, 27]. This evidence is reflected in recent national guidelines supporting the use of neoadjuvant therapy (or perioperative therapy) in oesophago-gastric cancer which has now been adopted into routine practice[25, 28, 29, 32-34, 41].

Not only has an overall benefit taken some time to establish but the gains associated with neoadjuvant therapy are modest with many patients developing progressive disease despite treatment. These factors may be explained by low response rates and therapy-associated toxicity.

It can be difficult to know which of our patients are benefitting from this additional treatment. It has been suggested that only patients with a complete pathological response to therapy will benefit[41]. However, other factors such as tumour stage may also determine whether or not patients stand to gain any advantage. For instance, patients with early tumours without lymph node involvement are known to have good outcomes with surgery alone. Therefore, more advanced tumours may have more to gain from the addition of chemotherapy. Add to this the potential toxic effects of chemotherapy and it is easy to see why neoadjuvant therapy is often not given in early tumours.

The MAGIC trial included patients with adenocarcinomas of the lower oesophagus, oesophago-gastric junction and stomach. It demonstrated 5-year-survival of 36% in

patients undergoing perioperative chemotherapy compared to 23% in those undergoing surgery only[25]. Patients with stage II disease or higher were included, which amounts to any tumours staged equal to or more advanced than T3 or N1, except that gastric T1N1 cancer is considered stage I and oesophageal T2N0 tumours are considered stage II if poorly differentiated. In contrast, the Intergroup study did not show any survival advantage associated with preoperative chemotherapy and included some patients with stage I disease[36]. It may be that patients with more advanced disease gained some benefit but this was negated by a lack of benefit and/or toxic effects in patients with early stage disease.

It is not known exactly what constitutes an early tumour and how this should be best determined. The latter is a matter of pre-operative staging which is subject to debate regarding accuracy and usefulness of different modalities. Chapter 4 further investigates which patients have the potential to benefit from the additional staging information provided by staging laparoscopy and PET-CT. CT is the only staging investigation whose benefit has not recently been questioned and is used routinely. Although other modalities may provide information that can modify preoperative staging to a degree, accuracy is critical when this information is used to make decisions on neoadjuvant therapy use.

Questions raised

The issues above have raised important questions that need addressing. Firstly, there are numerous ways of measuring response to neoadjuvant therapy but no established definition of what constitutes an adequate or beneficial response.

Accurately defining response would give prognostic information, would inform decisions on adjuvant therapy, and if adopted widely, could help to standardise reporting in the

scientific literature. If a means to predict response is identified in order to provide personalised treatment then it will be essential to have a robust and standardised definition of response.

Whichever methods are used to define response, they must be associated with improved survival in order to be clinically useful and these methods must reflect a true measure of neoadjuvant therapy response rather than simply acting as prognostic indicators or they will not influence neoadjuvant therapy decision making.

The fate of patients who do not respond to neoadjuvant treatment is unknown although anecdotally, patients developing progressive disease on therapy seem to have poor outcomes.

Clinical staging is an important aspect of neoadjuvant treatment for two reasons. Firstly, the decision whether or not to treat with neoadjuvant therapy is partly based on preoperative staging. Secondly, radiological staging is used as a means of measuring and defining response to neoadjuvant therapy. Therefore, clinical staging accuracy is of critical importance in multimodality therapy.

Accuracy and usefulness of staging modalities aside, the relationship between stage of tumour and efficacy of neoadjuvant therapy is not known. Finally, any perceived benefit of therapy must be weighed against its toxic effects. Neoadjuvant therapy adverse events are therefore worthy of further discussion.

Chapter aims

This chapter aims to redefine response to neoadjuvant therapy. It aims to describe the incidence and significance of neoadjuvant therapy-associated toxicity. It also aims to examine the efficacy of neoadjuvant therapy with respect to disease stage.

Chapter overview

A clinical review of the available methods used to define response to neoadjuvant therapy will be reported with particular attention paid to those methods that have been validated by association with survival outcomes.

A hypothesis describing the most appropriate method for defining response to neoadjuvant therapy in patients with oesophago-gastric cancer will be proposed.

The proposed definition of response will be applied in a survival analysis of a retrospective patient cohort.

The accuracy of clinical staging will be investigated in a retrospective patient cohort to identify whether aspects of staging are valid for use in defining response. The hypothesis will be modified accordingly.

The incidence and significance of neoadjuvant therapy-associated adverse events will be investigated in our patient cohort.

The efficacy of neoadjuvant therapy with respect to disease stage and response to neoadjuvant therapy will be investigated using an analysis of survival.

Measuring response to neoadjuvant therapy: clinical review

Why do we measure response to chemotherapy/radiotherapy?

In the palliative setting, response to chemotherapy/radiotherapy is used to gauge efficacy of treatment and plan further treatment. Since there is usually no surgical resection, information on histopathological regression is lacking and response is therefore measured by clinical or radiological means. In the neoadjuvant setting, response after therapy is used to inform prognosis and is important in restaging, to rule out progressive disease that may deem a patient inoperable. It is also used after surgery with the benefit of histological measures of response when it can help in deciding whether or not to offer adjuvant chemotherapy and which agents to use.

How is response to chemotherapy/chemoradiotherapy measured?

In the clinical and radiological measures described here, accuracy in defining response to treatment is often compared to the postoperative histological response which although has its own limitations (discussed below), is considered the standard by which other tools should be compared.

Clinical measures

Forshaw et al investigated whether changes in dysphagia and weight correlated with radiological and pathological assessment of response and clinical decision making in patients with locally advanced oesophago-gastric cancer[60]. Although swallowing was improved in radiological responders, there was no association between pathological response and either swallowing or changes in body weight. A separate study showed that resolution of symptoms in patients undergoing neoadjuvant chemoradiotherapy for oesophageal cancer does not accurately correlate with pathologic response[46].

Endoscopic measures

Endoscopic assessment after neoadjuvant chemotherapy is not considered reliable for assessing the response largely because it cannot determine whether residual bulk is due to oedema, scarring or residual tumour[51]. Likewise, after chemoradiotherapy, endoscopic appearance was not able to accurately identify responders[46]. Biopsies can be taken but a negative result cannot be taken to indicate no residual tumour.

Radiological measures

Radiological measures are commonly used to assess the response to chemotherapy/chemoradiotherapy particularly in the palliative setting and after neoadjuvant therapy, prior to proceeding with resection. The classification proposed by the World Health Organisation (WHO) in 1981 for reporting results of cancer treatment is widely used to define clinical response[61]. A reduction in the tumour size of 50% when measured in two perpendicular diameters is considered a partial response. A working group set up by the National Cancer Institute of the United States, the National Cancer Institute of Canada and the European Organisation for Research and Treatment of Cancer revised this classification with a new system in 2000 known as the Response Evaluation Criteria in Solid Tumours (RECIST) guidelines. This proposed that a partial response should be defined by a decrease in the maximal tumour diameter of 30% rather than using bi-dimensional measurements[62]. Progressive disease is defined by a 20% increase in maximal diameter and a complete response is marked by the disappearance of all target lesions. When using RECIST criteria, CT is the only valid assessment modality in oesophageal cancer (see below).

Barium oesophagram

A barium oesophagram (barium swallow) can be performed before chemotherapy and after chemotherapy. Response can be measured according to set criteria for complete and partial radiographic response[36]. In the USA Intergroup 113 study 7% patients were considered as complete responders and 19% as partial responders. Survival was greater in responders with a hazard ratio of 2.83, 95% confidence interval 1.84-4.35, P<0.0001[36]. This result was seen as very significant because initial results of the trial had shown no overall survival benefit for patients undergoing neoadjuvant chemotherapy. However, this is not a widely used technique for measuring response and similar findings have not been demonstrated elsewhere.

Endoscopic ultrasound (EUS)

In a systematic review of CT, EUS and PET in the assessment of response to neoadjuvant therapy in oesophageal cancer, EUS was shown to have better accuracy than CT (85% vs 54%)[58]. However, EUS was not feasible in 6% patients and in many centres it is only used selectively. As with endoscopy, EUS is unable to distinguish between oedema, fibrosis and residual tumour[51].

CT Scanning

CT scanning has been used to assess response to therapy, usually alongside the WHO classification of clinical response and related updated guidelines (RECIST)[61, 62] . However, the decrease in tumour size is a late event and fibrotic or necrotic tissue does not accurately reflect viable tumour tissue[38, 58]. In the systematic review by Westerterp, sensitivity of CT was between 33% and 55% and specificity was between 50% and 71%[58]. Accuracy of CT was significantly lower than that of EUS (*P*<0.003) or PET (*P*<0.006).

Griffith et al used CT to assess response to therapy in patients with squamous cell cancer of the oesophagus and found no correlation between tumour volume reduction at serial CT scan and pathological response or survival[63].

A recent study investigating the accuracy of 64-slice MDCT in restaging of oesophageal cancer after neoadjuvant chemotherapy demonstrated that prediction of complete histopathological response was poor with 80% patients over-staged[64]. They concluded that assessing response using CT has not improved with the use of MDCT compared to older generation CT.

CT perfusion and the new area of radiomics may increase the potential for predictive information from CT, however, this is an area requiring further evaluation before any clinical utility can be established[65-67].

A combination of tumour size and density according to CT has been used to devise the Choi criteria described for use in gastrointestinal stromal tumours (GISTs)[68]. It can identify a subgroup of apparent non-responders according to the RECIST criteria that have decreased tumour density and improved survival. The system has not been validated in adenocarcinoma or squamous cell carcinoma.

MRI

Magnetic resonance imaging (MRI) is rarely used in the staging and assessment of oesophageal tumours although it is a modality of interest in current research. As yet there is no evidence available on its role in measuring response to therapy although as with CT, the new area of MRI radiomics is being investigated.

PET scanning

PET scanning with fluorodeoxyglucose (FDG) gives a measure of the metabolic activity of tissues. Tumour tissues are more metabolically active than non-tumour tissues and take up more radio-labelled glucose therefore give a higher metabolic signal. A successful response to chemotherapy may be associated with a decrease in the standardised uptake value (SUV) of the tumour or involved lymph nodes even when there is no appreciable decrease in bulk on CT. For this reason, the use of PET assessing response has been extensively investigated. However, when compared to histological examination, PET has limitations. Complete metabolic response is not uniformly predictive of a pathologic response[69]. In one study, 59% patients with a complete metabolic response on PET, had residual disease on histopathological examination of the resected specimen[69]. A systematic review showed that PET was more accurate than CT in evaluating response when using pathological regression as the reference standard ($P<0.006$)[58]. Sensitivities ranged from 71% to 100% and specificities ranged from 55% to 100%.

Brucher et al reviewed 13 studies investigating the FDG–PET in assessment of response to neoadjuvant therapy in patients with oesophageal cancer[51]. Most studies demonstrated some association between PET-avidity and histological response. 10 studies reported the relationship to survival and of these, 8 demonstrated a significant association. The authors of this review point out that studies differ with respect to the PET variables used to measure response (maximum SUV, change from baseline SUV, mean SUV) all of which can have different thresholds, which makes studies difficult to compare and consensus statements are difficult to generate. Whilst PET therefore demonstrates some association with histological response and survival, further studies

would benefit from standardisation of how accuracy is reported and which parameter is used to measure response.

Updated RECIST guidelines published in 2009 acknowledge the potential of moving from the anatomic uni-dimensional assessment offered by CT to functional assessment using FDG-PET, however, in part due to the standardisation problems outlined above, they felt further clinical validation studies are required[70]. Likewise, new approach of volumetric assessment has been recognised but requires further validation[70].

Histopathological response

Whilst clinical and radiological features have been used, the methods of measuring response to neoadjuvant therapy with the greatest prognostic value and most widely used are pathological[38, 40, 51]. There are a variety of histological measures used which involve examining the resected primary tumour or lymph nodes and measuring changes in size[71, 72], residual tumour cells[40, 73-75], fibrosis[40, 73, 74], stage (e.g. T,N)[44, 76-78], other morphological features[79, 80] or a combination of the above. Of these, measurement of histological regression based on the degree of residual tumour cells and fibrosis have shown the most promise and there a number of systems described in the literature that use this approach.

The Mandard scoring system was described in 1994 and was designed to classify the response to neoadjuvant chemoradiotherapy in patients with oesophageal cancer into one of five grades according to the relative proportions of residual tumour cells and fibrosis[74] (see Figure 15). Tumour response grade (TRG) 1 is considered a complete response and TRG2 is considered a near-complete response[40]. It is standard practice in prognostic scoring to consider all patients with no or minimal residual tumour cells as responders (TRG 1 and TRG 2)[40, 42, 44] . TRG 4-5 are considered as non-responders[40].

There is some debate regarding the fate of TRG 3 patients, some authors classing them as responders[76] and others as non-responders[44].

Grade	Description
TRG 1	Absence of residual cancer, fibrosis extending through different areas of oesophageal wall
TRG 2	Rare residual cancer cells scattered through fibrosis
TRG 3	Increase in number of residual cancer cells but fibrosis still predominant
TRG 4	Residual cancer outgrowing fibrosis
TRG 5	Absence of regressive changes

Figure 15 Mandard scoring system for response to neoadjuvant chemoradiotherapy in oesophageal cancers. Tumour Regression Grade (TRG).

In 1994, Ninomiya et al described a system for assessing response to chemotherapy in patients with gastric cancer based on the degree of tumour necrosis[81] (Figure 16).

Grade	Description
Grade 0	No change ± neither necrosis nor cellular or structural change can be seen throughout the lesion
Grade 1a	Necrosis or disappearance of the tumour is present in less than 1/3 of the whole lesion
Grade 1b	Necrosis or disappearance of the tumour is present in no more than 2/3 of the whole lesion
Grade 2	Moderate change ± necrosis or disappearance of the tumour is present in more than 2/3 of the whole lesion, but viable tumour cells remain
Grade 3	Marked change ± the whole lesion falls into necrosis and/or is replaced by fibrosis, with or without granulomatous changes. No viable tumour cells

Figure 16 Ninomiya scoring system for histological response to neoadjuvant chemotherapy in gastric cancers

In 2003, Becker et al described a system based on the proportion of viable tumour cells for assessing response in patients undergoing chemotherapy for gastric cancer (Figure 17). It classifies patients into four grades that can be roughly matched to Mandard

grades: Grade 1A equating to TRG1 (complete response), Grade 1B roughly equivalent to TRG2 (near complete response), Grade 2 encompasses both TRG grades 3 and 4 (partial/minimal response) and Grade 3 is equivalent to TRG5 (no response).

Grade	Description
1A	No residual tumour/tumour bed
1B	<10% tumour cells
2	10-50% residual tumour/tumour bed
3	>50% no signs of neoplastic regression

Figure 17 Becker scoring system for histological response to neoadjuvant chemotherapy in gastric cancers.

The Japanese Society for Esophageal Diseases devised a set of response evaluation criteria on a scale of 0 to 3 indicating increasing effectiveness based on the proportion of viable cancer cells[82].

Grade	Description
0	no recognizable cytologic or histologic therapeutic effect
1	slightly effective with apparently viable cancer cells accounting for one-third or more of the tumor tissue
2	moderately effective with viable cancer cells accounting for less than one-third of the tumor tissue
3	markedly effective, with no evident viable cancer cells (pathologic complete response, or pCR).

Figure 18 Japan Esophageal Society scoring system for response to chemoradiotherapy in oesophageal cancer.

When examining the literature on the relationship between histopathological response to neoadjuvant therapy and survival (below), for clarity, chemoradiotherapy and chemotherapy are considered separately as historically they have been used in different patient groups and have utilised different regression scoring systems.

Histopathological response to neoadjuvant chemoradiotherapy in oesophageal cancer

In the case of chemoradiotherapy, the goal is usually considered to be complete pathological response and as such the particular type of regression/scoring system used is often of minimal consequence since a complete response is easily defined and comparable across scoring systems. Pathological complete response rates to neoadjuvant radiotherapy in oesophageal cancer are reported between 15% and 40%[42, 43, 45-48, 78]. Rohatgi et al demonstrated that patients with a complete pathological response (29%) had longer median overall survival (133 months vs. 34 months, P=0.002) and disease-free survival (P=0.001) compared to those without a complete pathological response[48]. Hermann et al also showed that only patients with a complete pathological response defined as TRG 1 (17%) had longer overall survival (P=0.0008)[43]. A separate study investigating survival in 171 patients with oesophageal cancer demonstrated that complete response to preoperative chemoradiotherapy (35%) is associated with significantly improved survival[42]. Whether there is any benefit of neoadjuvant chemoradiotherapy to the majority of patients who do not achieve a complete response is not so clear. One study was able to demonstrate that a partial or complete response to chemoradiotherapy is associated with improved survival[83].

Histopathological response to neoadjuvant chemotherapy in oesophago-gastric cancer

Compared to chemoradiotherapy, even fewer patients respond to neoadjuvant chemotherapy with rates of complete response reported at 0-13%[40, 41, 44, 78] and rates of complete/near-complete response of 11-27%[36, 40, 44]. In the case of chemotherapy, lower rates of complete response mean that there is perhaps more attention paid to near-complete/partial response and arguably, therefore, the choice of regression score used

becomes more important than after chemoradiotherapy where the focus is on complete response, which is simpler to define.

In 2001, a randomised, controlled trial of preoperative chemotherapy (47 patients) versus surgery alone (47 patients) for resectable oesophageal squamous cell carcinoma showed that only pathological complete response to neoadjuvant chemotherapy improves significantly the long term survival[41]. 5-year survival was 60% in responders, which was significantly better than in non-responders (12%, P=0.0002) and in those undergoing surgery alone (26%, P=0.01).

In a study of 66 patients with gastric/gastro-oesophageal junction adenocarcinoma undergoing neoadjuvant chemotherapy according to the MAGIC protocol, Mirza et al investigated the usefulness of the three main histopathological scoring systems described above[40]. Both the Mandard[74] and Becker[84] systems yielded prognostic information with acceptable inter-observer agreement whereas the Ninomiya[81] system did not. In fact, although the Mandard system has 5 grades compared to the Becker system's 4 grades, when patients were classified as either responders or non-responders, outcomes were very similar. Of the 66 patients in total, The Mandard system identified 12 responders compared to the 11 using the Becker system. Using the Mandard system, 5-year overall survival was 100% for responders (TRG 1&2, 12% of total) and 35% for non-responders (TRG 3-5), P=0.035. There were similar findings using the Becker system with a 5-year survival of 100% for responders (Grades 1A and 1B, 11% of total) and 34% for non-responders. This study to some extent validates the Mandard score for measuring response to chemotherapy in gastric adenocarcinoma when considering the system was first described in relation to chemoradiotherapy for squamous cell carcinoma of the oesophagus[74].

A recent study from Southampton in patients with oesophageal and gastro-oesophageal junction adenocarcinoma receiving neoadjuvant chemotherapy showed patients with a complete or near-complete response to chemotherapy (TRG 1&2) had a significant survival advantage compared to non-responders (TRG 3-5). Mean disease-free survival (DFS) in the TRG 1-2 group was 5.1 years and in the TRG 3-5 group was 2.8 years, $P < 0.0001$[44]. The effect of lymph node down-staging was also investigated in this study and is discussed below.

The prognostic value and resulting widespread use of histological regression scoring systems mean that they are considered the gold standard, to which all other methods of measuring response are compared.

Limitations of histopathological regression

Unlike the radiological assessments above, histological regression depends on having a surgical resection for examination and can therefore only be used postoperatively.

Whilst the relative proportions of viable tumour cells and areas of regression/fibrosis used in the systems above are valid means to assess response, these systems have limitations. Firstly, microscopic regression often displays heterogeneity within a tumour with some areas appearing as predominantly fibrosis with other areas of tumour not displaying any signs of regression. This system does not take into account any change in stage according to tumour depth which is frequently observed by histopathologists and is not necessarily matched by a proportional degree of regression. These systems also do not take account of any regression or down-staging in lymph nodes which is also frequently observed by histopathologists[85]. Lymph node stage is one of the most important prognostic indicators so down-staging as a result of neoadjuvant chemotherapy could reflect a clinically significant response.

The limitations above together with poor neoadjuvant therapy histological regression rates have led authors to seek other histological parameters which may identify a partial response and confer a survival advantage in patients otherwise considered non-responders.

Tumour (T) and Lymph node (N) stage response

Staging according to tumour depth (T stage) is routinely recorded radiologically before starting anti-tumour therapy and after resection histologically. It is recognised that T stage can regress after neoadjuvant treatment, which together with the limitations of regression scoring above has prompted the suggestion that tumour (T) down-staging could be useful to define a response to therapy[40, 84, 86].

It has long been known that lymph node staging is an important and independent factor associated with poor prognosis. It has also been recognised that neoadjuvant therapy can downstage the nodal (N) status[44, 85, 86]. These observations have led to authors proposing that lymph node (N) down-staging may act as a measure of clinically significant response to chemotherapy[44, 85].

Korst et al evaluated the frequency of T and N down-staging after neoadjuvant chemotherapy in patients with oesophageal cancer and the relationship to survival[78]. Pre-treatment, clinical T and N stage were compared to post-resection, pathologic stage in the context of survival. Patients eliciting a down-staging of T or N (48%) had a 5-year survival of 63% compared with 23% for those who were not down-staged (p=0.002).

In a retrospective non-randomised study comparing survival in patients receiving neoadjuvant therapy and surgery with that in patients having just surgery, only

responders had improved survival when defined by a down-staging of tumour or lymph node stage, or complete pathological response[86].

Noble et al assessed survival in 218 patients with adenocarcinoma of the oesophagus, 136 of whom underwent neoadjuvant chemotherapy[44]. Histological non-responders (TRG3-5) were also subdivided according to whether they had evidence of lymph node down-staging (reduction in N stage between pre-chemotherapy radiological staging and postoperative N stage). Nodal down-staging was identified in 30% of non-responders and conferred a significant DFS advantage (mean DFS; TRG3-5 and nodal down-staging: 5.5 years *vs* TRG3-5 and no nodal down-staging: 1.1 years, $P < 0.0001$). Therefore, a sub-group of patients that would be classed as histological non-responders and therefore would be considered to have gained no benefit from chemotherapy demonstrate an improved prognosis if they have evidence of lymph node down-staging.

Limitations of T and N down staging

Both T and N down-staging rely on comparing pre-operative radiological staging with postoperative histological staging. A major criticism of this method is that apparent down-staging may simply reflect pre-operative over-staging. More specifically, there may be a tendency to over-stage earlier tumours. Over-staged tumours will appear to be down-staged and an observed survival benefit may be ascribed to the apparent down-staging when it is simply due to earlier disease stage.

Noble et al have attempted to address this by comparing survival in patients with pathological N0 staging after chemotherapy with survival in patients undergoing surgery alone who had pathological N0 staging. Reduced disease free survival in the chemotherapy patients was found and used to indicate that pre-operative N stage must have been adequate. However, T-stage was also higher in the chemotherapy group

which would give rise to poorer survival. Furthermore 37.5% patients undergoing surgery alone had 'nodal down-staging' or rather must have been over-staged pre-operatively.

In the paper by Allan et al where T or N down-staging is shown to correlate with survival in patients undergoing neoadjuvant therapy, pre-operative T/N stage is not given in the results table and the 'down-staging' rate in the control group having surgery only is not reported which raises concern over the preoperative staging accuracy[86].

What is the outcome in non-responders compared to those undergoing surgery alone?

The observation that non-responders have such a poor prognosis raises the question of whether there is any benefit at all in them receiving neoadjuvant therapy or even whether toxic effects and a delay to attempted resection outweigh any benefit and have an overall adverse effect on outcomes[12, 28, 36, 41, 50, 56].

The original results of the USA Intergroup 113 study failed to show any benefit from using neoadjuvant chemotherapy in patients with oesophageal cancer[26]. In the long-term results the authors tried to identify a subset of patients that did benefit[36]. They classified patients according to chemotherapy response measured by barium swallow appearances and demonstrated improved overall survival in responders compared to non-responders and compared to those undergoing surgery alone. Therefore, it may be that overall the benefit in responders was negated by reduced survival in the non-responders[12]. There was a trend towards poorer survival among non-responders compared to those randomised to surgery alone although this was not significant.

In a randomised controlled trial comparing surgery alone with preoperative chemotherapy, 5-year survival was 26% in those undergoing surgery alone, 60% in responders, and 12% in non-responders although there was no significant difference in the rates between the surgery only and non-responder groups[41].

A study in 84 patients undergoing neoadjuvant chemoradiotherapy for advanced oesophageal cancer demonstrated a trend towards poorer 2-year survival in non-responders compared to those undergoing surgery alone (32% vs 54.3% *P*=0.06)[87]. Patients undergoing chemoradiotherapy were younger and had higher anatomically located tumours. Prognosis is known to be better in younger patients and those with upper oesophageal cancers which would have tended to increase the survival in the chemotherapy group although breakdown of demographics between responders and non-responders is not reported.

In one study comparing survival in 63 neoadjuvant therapy non-responders with that in 81 patients treated with primary oesophagectomy, overall survival and disease free survival were significantly poorer in the non-responders (P=0.024 and P<0.001 respectively) within patients with stage 2 disease[88].

Summary of clinical review

Whilst it is clear that patients responding to neoadjuvant chemotherapy/chemoradiotherapy gain a survival benefit from such therapy, it is also evident that those not responding, do not benefit from improved survival as a result of the additional treatment.

Histopathological regression is still thought to be the most accurate method of assessing response to neoadjuvant therapy, it correlates well with survival and is likely to form the

basis of any method used to define response. The Mandard and Becker systems appear to be the most reliable. However, typical response rates are low and a proportion of non-responders defined by these methods may carry a survival benefit from therapy which could be considered a partial response. These patients may be identifiable from down-staging of the lymph node (N) or tumour (T) stage. If N of T down-staging is to be used in defining response, it is vital that we investigate pre-operative staging accuracy to ensure that there is no systematic over-staging which could lead to an apparent down-staging after therapy. In fact, the decision whether or not to offer neoadjuvant chemotherapy itself is determined largely by pre-operative staging, based on whichever staging modalities have been used and agreed by the MDT. Pre-operative staging accuracy is therefore fundamental to the rationale behind multimodality treatment. The next part of this chapter specifically addresses the accuracy of pre-operative staging by comparing pre-operative clinical/radiological staging with postoperative histological staging in a historic cohort of patients not undergoing neoadjuvant therapy.

Hypothesis

Based on the clinical review of methods used to measure and define response to neoadjuvant therapy, it can be hypothesised that responders could be defined as those with a histological regression according to either the Mandard or Becker systems. Tumour (T) and Lymph node (N) down-staging may also contribute to the definition if the pre-operative staging is reliable and they are associated with improved survival.

Cohort

A historical cohort of patients was used for the following sections of this chapter: 'Clinical staging accuracy', 'Redefining response to neoadjuvant therapy', 'Neoadjuvant therapy toxicity' and 'Neoadjuvant therapy efficacy'.

Patients

Since January 2010, a database has been kept of all patients discussed in the MDT meetings at the Peninsula Oesophago-gastric Surgery Unit, Derriford Hospital, Plymouth. From this database, all patients planned to undergo surgical resection for malignant oesophago-gastric cancer and starting treatment between January 2010 and January 2015 were identified (i.e. patients undergoing radical chemoradiotherapy were not included).

Data collection

Data were collected on patients' demographics, tumour characteristics, preoperative staging, neoadjuvant treatments, surgical treatment, adverse events, final outcomes and survival. Patients were divided according to prognostic stage groupings (e.g. 1, 2, 3, or 4) using the preoperative TNM staging data.

Figure 19 shows the 587 patients identified from the database. All patients were started on a curative treatment pathway for oesophago-gastric cancer that included intention for surgical resection. Mean age was 66.7 years and 73.9% were male. 64.1% underwent neoadjuvant therapy. Patient demographics, tumour characteristics, staging and neoadjuvant treatment details are shown in Table 1 below.

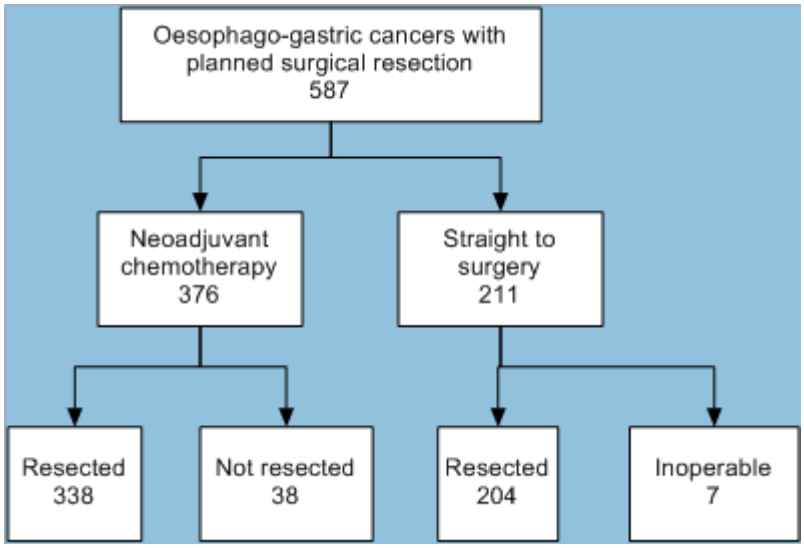

Figure 19 All patients identified from the database with broad treatment categories and outcomes shown.

Patient characteristics, n=587	
Demographics	
Age, in years; mean (range)	66.7 (25.4 to 87.9)
Male; n (%)	434 (73.9)
Tumour characteristics	
Histological type; n (%)	
Adenocarcinoma	518 (88.2)
Squamous cell carcinoma	63 (10.7)
Undifferentiated/other epithelial	6 (1.0)
Anatomical tumour location; n (%)	
Upper third oesophagus	8 (1.4)
Middle third oesophagus	21 (3.6)
Lower third oesophagus	186 (31.7)
Gastro-oesophageal junction	248 (42.2)
Gastric	124 (21.1)
Pre-operative staging	
Pre-operative T stage; n (%)	
≤T2	200 (34.1)
T3	355 (60.5)
T3/4 and T4	22 (3.7)
Tx	10 (1.7)
Pre-operative N stage; n (%)	
N0	316 (53.8)
N1	216 (36.8)
N2	54 (9.2)
Nx	1 (0.2)
Prognostic stage group (stage); n (%)	
0	15 (2.6)
1	125 (21.3)
2	230 (39.2)
3	217 (37.0)
Neoadjuvant therapy; n (%)	
Yes	376 (64.1)
No	211 (35.9)
Neoadjuvant regimen; n (%)	
CROSS	9 (2.4)
CF/CX	57 (15.2)
ECX/ECF/EOX/EOF	260 (69.1)
ECarbo	1 (0.3)
Carbo + paclitaxel/etoposide	3 (0.8)
ECX+B	2 (0.5)
OE05	3 (0.8)
STO3	11 (2.9)
missing	30 (8.0)

Table 1 Patient cohort characteristics.

Abbreviations: CROSS, as per the CROSS trial, carboplatin, paclitaxel and concurrent radiotherapy; CF, cisplatin and 5-flourouracil; CX, Cisplatin and capecitabine, ECX, epirubicin, cisplatin, capecitabine; ECF, epirubicin, cisplatin, capecitabine; EOX, epirubicin, oxaliplatin, capecitabine; EOF, epirubicin, oxaliplatin, 5-flourouracil; ECarbo, epirubicin and carboplatin; ECX+B, ECX and bevacizumab; OEO5, as per the OEO5 study (ECX or CF); ST03, as per the ST03 study (ECX +/- bevacizumab).

Clinical staging accuracy

Methods

Patients

To investigate staging accuracy, pre-operative staging according to CT, EUS, PET-CT and final pre-operative stage were compared to the postoperative histological staging in patients going straight to surgery (no neoadjuvant therapy).

Staging protocol

After histological confirmation of oesophago-gastric cancer via endoscopic biopsy, patients underwent staging with CT of thorax, abdomen and pelvis using 64-slice multi-detector scanners using the same oesophago-gastric staging protocol (0.625-1.25mm slices, oral water as negative contrast and intravenous contrast with portal venous phase imaging). Staging was reported according to the AJCC 7th edition manuals for oesophageal and gastric cancer respectively (TNM staging). All CT scans were reviewed by at least one of three specialist upper gastrointestinal CT radiologists at a specialist MDT meeting. Oesophageal tumours were staged according to criteria similar to that described by Ba-Ssalamah *et al*[89]. Specifically, T2 tumours were characterised as having thickening of the oesophageal wall of less than 15mm with slight/mild stenosis and outer borders which are smooth or show stranding for less than one third of the tumour extension. T3 lesions were represented by thickening of greater than 15mm with mild to severe stenosis and marked stranding for over one third of the tumour extension or extensive blurring of the outer border. T4 lesions required invasion into adjacent structures such as pericardium, diaphragm, pleura, tracheobronchial tree or aorta. Gastric cancers were staged according to criteria similar to those described by Makino et al[90]. Tumours appearing as minimal thickening were staged as T2, those with more

demonstrable thickening of the stomach wall and a smooth outer layer with preserved perigastric fat plane were staged as T3 and those with a nodular/irregular outer border of the gastric wall or infiltration of the perigastric fat or adjacent structures were staged as T4.

PET-CT was performed for N and M staging in oesophageal/gastro-oesophageal junction cancers with the potential for radical treatment and curative intent in accordance with current guidelines[12]. EUS was used in selected traversable oesophageal/gastro-oesophageal junction tumours to further assess T and N stage which helped to determine resectability and the need for neoadjuvant therapy.

Staging laparoscopy was performed in accordance with current guidelines to assess operability[12]. Specifically, laparoscopy was undertaken in all potentially resectable gastric cancers and lower oesophageal/gastro-oesophageal junction cancers with a component below the level of the diaphragm.

US, EUS or Endobronchial ultrasound (EBUS) with Fine needle aspiration (FNA)/biopsy were used in selected cases where positive nodal involvement would change management. On the basis of all available staging modalities, the final pre-operative stage was then decided by the MDT and recorded on the database.

Surgery

Patients with resectable tumours who were still fit for operation underwent resection. Ivor-Lewis gastro-oesophagectomy was performed for lower oesophageal and gastro-oesophageal junction tumours. Subtotal or total gastrectomy was performed for gastric tumours depending on site and extent of the tumour. Histopathological reporting followed the minimum dataset (minimum reporting detail) for oesophageal cancer in all

cases of oesophageal and gastro-oesophageal junction tumours and following the minimum dataset for gastric cancers in all gastric cancers. Routine histopathological reporting included recording the Mandard score (TRG) and pathological TNM staging according to the AJCC 6th or 7th Edition manual and was performed by a specialist gastrointestinal pathologist.

Analysis

Tables were used to present the data according to each staging modality. Over-staging was defined as recording a pre-operative stage (T or N) that was higher than the subsequent post-operative histological stage. Under-staging was defined similarly. For each staging modality, the percentage of patients over-staged or under-staged for each histopathological stage group was calculated.

Patients were also divided into two groups depending on whether the post-operative histology was above the threshold for offering treating with neoadjuvant chemotherapy. All >T3 or >N0 tumours (locally advanced) were considered for such treatment whereas those with ≤T2, N0 tumours were generally not offered it. The accuracy of pre-operative staging was then analysed with respect to identification of patients with a stage above this threshold.

Results

Patient characteristics

Of the 587 patients who were identified from the database, 211 patients were planned to have surgical resection without neoadjuvant therapy. 7 patients were inoperable leaving 204 who were resected and are included in the analysis (Figure 19). Patient characteristics are shown in Table 2. Mean age was 69.9 years and 67.2% were male. The majority of patients were staged as T2 or less (64.7%) and the majority of patients were staged as N0 (82.8%).

Patient characteristics, n = 204	
Demographics	
Age, in years; mean (range)	69.9 (25.4 to 87.9)
Male; n (%)	137 (67.2)
Tumour characteristics	
Histological type; n (%)	
Adenocarcinoma	175 (85.8)
Squamous cell carcinoma	25 (12.3)
Other	4 (2.0)
Anatomical tumour location; n (%)	
Upper third oesophagus	5 (2.5)
Middle third oesophagus	6 (2.9)
Lower third oesophagus	51 (25.0)
Gastro-oesophageal junction	73 (35.8)
Gastric	69 (33.8)
Final (multimodality) pre-operative staging	
Pre-operative T stage; n (%)	
≤T2	132 (64.7)
T3	58 (28.4)
T3/4 and T4	4 (2.0)
Tx	10 (4.9)
Pre-operative N stage; n (%)	
N0	169 (82.8)
N1	29 (14.2)
N2	6 (2.9)
Post-operative histology	
Post-operative T staging; n (%)	
≤T2	116 (56.9)
pT3	66 (32.4)
pT4	22 (10.8)
Post-operative N stage; n (%)	
N0	112 (54.9)
N1	50 (24.5)
N2	23 (11.3)
N3	19 (9.3)
R0 vs. R1 resection; n (%)	
R0	149 (73.0)
R1	39 (19.1)
missing	16 (7.8)

Table 2 Patient characteristics. Note: percentages may not sum to 100 due to rounding.

T staging

CT staging (refer to Table 3)

CT over-stages at least 10% of tumours pathologically staged as T1 or earlier. Patients staged as T1/2 on CT and ≤T1 pathologically were not considered as over-staged. CT over-stages 38.7% (12/31) T2 tumours. A high proportion of T3 and T4 tumours (60.7% (37/61) and 90.5% (19/21)) are under-staged.

EUS staging (refer to table 4)

65 patients underwent staging with EUS. Of 3 Tis (carcinoma in situ) lesions, 2 were over-staged as T1. 48% (12/25) T1 lesions were over-staged and 14.3% (1/7) T2 lesions were over-staged. 61.9% (13/21) and 100% of T3 and T4 tumours were under-staged respectively.

Best pre-operative staging (refer to table 5)

Final pre-operative staging over-staged at least 41.7% (30/72) T1 (or earlier) tumours and 25.7% (9/35) T2 tumours. Overall, 22.7% (39/172) patients (T1-T3) were over-staged and 44.3% (54/122) patients (T2-T4) were under-staged. 53.8% (35/65) and 81.8% (18/22) of T3 and T4 tumours were under-staged respectively.

	Pathological stage			
CT stage (n=204)	≤T1	T2	T3	T4
≤T2	53	16	31	2
T2	6	3	6	3
T3	1	12	23	14
T4	0	0	1	2
Tx	20	5	5	1
Totals	80	36	66	22
Over-staged	11.7%	38.7%	1.6%	0%
Correctly-staged	88.3%	61.3%	37.7%	9.5%
Under-staged	0%	0%	60.7%	90.5%

Table 3 Comparison of pre-operative T staging according to CT and post-operative pathological staging

	Pathological stage				
EUS stage (n=65)	Tis	T1	T2	T3	T4
Tis/T0	1	2	0	0	0
T1	2	11	0	1	0
T2	0	10	6	12	1
T3	0	2	1	8	1
T4	0	0	0	0	0
Tx	0	2	1	4	0
Totals	3	27	8	25	2
Over-staged	66.7%	48.0%	14.3%	0%	0%
Correctly-staged	33.3%	44.0%	85.7%	38.1%	0%
Under-staged	0%	8.0%	0%	61.9%	100%

Table 4 Comparison of pre-operative T staging according to EUS and post-operative pathological staging

	Pathological stage			
Pre-op staging (n=204)	≤T1	T2	T3	T4
Tis/T0/T1	34	1	2	0
T1/2	8	6	8	0
T2	26	19	25	3
T3	4	9	30	15
T4	0	0	0	4
Tx	8	1	1	0
Totals	80	36	66	22
Over-staged	41.7%	25.7%	0%	0%
Correctly-staged	58.3%	71.4	46.2%	18.2%
Under-staged	0%	2.9%	53.8%	81.8%

Table 5 Comparison of final pre-operative T staging and post-operative pathological staging

N staging

CT staging (refer to table 6)

CT over-staged 9.3% (10/107) N0 patients. 1 N1 patient was over-staged and no N2 patients were over-staged. Overall, a high proportion (87.8%; 79/90) of all node positive patients (N1, N2 or N3), were under-staged.

EUS staging (refer to table 7)

EUS over-staged 5.3% (2/38) N0 patients and 8.3% (1/12) N1 patients were over-staged. Overall 89.5% (17/19) patients with node positive disease were under-staged including all those with N2 or N3 disease.

PET-CT staging (refer to table 8)

PET-CT over-staged 7.7% (4/52) N0 patients. No N1 or N2 patients were over-staged. Overall, 90.0% (36/40) node positive patients were under-staged including all those with N2 or N3 disease.

Best pre-operative staging (refer to table 9)

Final pre-operative stage over-staged 8.0% (9/112) N0 patients and 4.0% (2/50) N1 patients. Overall, 88% (81/92) node positive patients were under-staged and 5.9% (11/185) N0-N2 patients were over-staged.

	Pathological stage			
CT stage (n=204)	N0	N1	N2	N3
N0	97	40	17	9
N1	10	8	6	7
N2	0	1	0	2
N3	0	0	0	0
Nx	5	1	0	1
Totals	112	50	23	19
Over-staged	9.3%	2.0%	0%	0%
Correctly-staged	90.7%	16.3%	0%	0%
Under-staged	0%	81.6%	100%	100%

Table 6 Comparison of pre-operative N staging according to CT and post-operative pathological stage

	Pathological stage			
EUS stage (n=65)	N0	N1	N2	N3
N0	36	10	5	2
N1	2	1	0	0
N2	0	1	0	0
Nx	4	1	1	2
Totals	42	13	6	4
Over-staged	5.3%	8.3%	0%	0%
Correctly-staged	94.7%	8.3%	0%	0%
Under-staged	0%	83.3%	100%	100%

Table 7 Comparison of pre-operative N staging according to EUS and post-operative pathological stage

	Pathological stage			
PET-CT stage (n=99)	N0	N1	N2	N3
N0	48	17	10	3
N1	4	4	3	3
N2	0	0	0	0
N3	0	0	0	0
Nx	3	2	1	1
Totals	55	23	14	7
Over-staged	7.7%	0%	0%	0%
Correctly-staged	92.3%	19.0%	0%	0%
Under-staged	0%	81.0%	76.9%	100%

Table 8 Comparison of pre-operative N staging according to PET-CT and post-operative pathological stage

	Pathological stage			
Pre-op stage (n=204)	N0	N1	N2	N3
N0	103	39	17	10
N1	8	9	6	6
N2	1	2	0	3
N3	0	0	0	0
Nx	0	0	0	0
Totals	112	50	23	19
Over-staged	8.0%	4.0%	0%	0%
Correctly-staged	92.0%	18.0%	0%	0%
Under-staged	0%	78.0%	100%	100%

Table 9 Comparison of final pre-operative N staging according to CT and post-operative pathological stage

Staging according to neoadjuvant therapy threshold

According to the pathological stage, 114/204 (55.9%) patients were above the threshold for consideration of neoadjuvant chemotherapy (locally advanced), see Table 10. The sensitivity for identifying this advanced stage was 51.8% (95% CI: 42.6% to 60.9%) with a specificity of 86.7% (95% CI: 79.6% to 93.7%). 48.2% patients with locally advanced tumours were under-staged and 13.3% patients with early tumours were over-staged.

	Pathological stage		
Pre-op stage (n=204)	≤T2 and N0	≥T3 or ≥N1	Totals
≤T2 and N0	78	55	133
≥T3 or ≥N1	12	59	71
Totals	90 (44.1%)	114 (55.9%)	204
Over-staged	13.3% (12/90)	-	
Correctly-staged	86.7%	51.8%	
Under-staged	-	48.2% (55/114)	
Specificity		86.7% (95% CI: 79.6% to 93.7%)	
Sensitivity		51.8% (95% CI: 42.6% to 60.9%)	

Table 10 Comparison of pre-operative and post-operative staging according to neoadjuvant therapy threshold

Discussion

Staging accuracy cannot directly be assessed in patients undergoing neoadjuvant therapy due to the therapy effect itself. Therefore, a cohort of patients going straight to surgery is used to estimate staging accuracy. This is necessary to ensure that any definition of response to neoadjuvant therapy that relies on staging would be valid. Specifically, evidence suggests that lymph node (N) down-staging and tumour depth (T) down-staging may be able to identify amongst patients considered non-responders according to histopathological regression, a sub-set who are partial responders and carry a survival benefit above the remaining non-responders.

Of particular concern is that lower stage tumours (e.g. T1 or N0) may be systematically over-staged more so than higher stage tumours. This would result in an apparent down-staging when examining the post-operative histology even in the absence of any therapy effect. Therefore, staging modalities that have a lower tendency to over-stage earlier tumours would be preferable. However, the tendency to under-stage tumours must also be taken into account since any under-staging will reduce the power to identifying true responders to therapy. I.e. if an N1 tumour is mistakenly staged as N0, then even if a true response to therapy resulted in pathological N0 staging, this would not be recognised as a response.

Whilst the final pre-operative staging is based on an MDT discussion of all available staging information, the accuracy of each individual modality was also investigated because these modalities may have different tendencies to over-stage disease.

T staging

It is generally considered that CT is unable to differentiate reliably between T1 and T2 tumours and therefore early tumours are often staged as Tx or T1/2 on CT. It is difficult to comment on the over-staging of ≤T1 tumours although the data shows that 10% are over-staged even when the benefit of doubt is given to tumours staged as T1/2. 38.7% and 48.0% T2 tumours were over-staged by CT and EUS respectively. These findings are reflected in the final pre-operative T staging which over-stages 36.4% of T1/T2 tumours when grouped together. A large proportion of T3 and T4 tumours are under-staged.

There are a number of reasons why early tumours may be over-staged. Firstly, muscle wall thickening caused by peri-tumour inflammation will over-stage a T1 tumour to T2 and any irregularity of the outer muscle wall may give rise to T3 staging even if not representative of true tumour spread. There may also be a tendency to overcall the stage of T2 tumours so as not to deny patients potentially useful therapy such as neoadjuvant chemotherapy by under-staging. Conversely, advanced tumours (T3/4) are less likely to be over-staged, not least because a T4 tumour cannot be over-staged. This phenomenon in statistical trends has been known as regression to the mean.

It must be remembered that this group of patients going straight to surgery differs from the group having neoadjuvant therapy not least because the reason for offering such therapy is often due to the stage of disease (≥T2 or ≥N1 disease). For this reason, staging accuracies will be different between the groups and the analysis must be used cautiously as an estimate for accuracy in the neoadjuvant group. This may explain why there are a

relatively low proportion of tumours staged at T3/4 pre-operatively and also why so many pathological T3/4 tumours are under-staged in this group. For this reason, the proportion of over-staging in early tumours may be an underestimate compared to the degree of over-staging in patients undergoing chemotherapy who have higher pre-operative stages. The accuracy of T staging when addressed from the point of view of over and under-staging is poor and individual modalities appear no better than the final pre-operative staging.

N staging

Regarding N staging, CT over-staged 9.3% of node-negative (N0) tumours. Results were similar with EUS and PET-CT. This is reflected in the final staging which shows 8.0% N0 tumours and 4.0% N1 tumours are over-staged. These figures compare favourably with a recent study in which 37.5% patients undergoing surgery alone for pN0 oesophageal cancer were staged as N1 pre-operatively[44]. A high percentage of our node-positive tumours were under-staged. CT staging of nodal disease is based on size criteria, which is known to have poor sensitivity and specificity because small nodes may contain metastases and large nodes may be inflammatory and benign. Although PET-CT has been shown in the literature to have a better specificity than CT in nodal metastasis detection (see Chapter 4), it performed no better than CT in this patient cohort. The small number of predicted node positive patients in this cohort of patients not undergoing neoadjuvant therapy may explain this and is also likely to be the reason for a high rate of under-staging in node-positive disease.

There is no magnitude of over-staging that is considered acceptable although nodal over-staging of 37.5% in patients going straight to surgery has been reported by others[44]. T over-staging was 22.7% overall (41.7% in early tumours) and N over-staging

was 5.9% overall (8.0% in N0 patients). The significance of such over-staging is further examined in the next section (redefining response to neoadjuvant therapy).

Neoadjuvant therapy treatment threshold

It is generally accepted that patients with early tumours have less to gain from neoadjuvant chemotherapy since surgical resection with clear resection margins in patients without lymph node metastases carries a very good prognosis. Pre-operative staging is used to determine whether tumours are likely to be of an early stage. Patients staged as T2N0 or earlier (early tumours) have generally not been offered chemotherapy and those staged as at least T3 or N1 disease (locally advanced) are offered chemotherapy. There is perhaps a trend towards offering neoadjuvant therapy to earlier stage (T2N0) patients, perhaps because of the concern regarding possible under-staging. The whole basis of multimodality treatment is therefore entirely dependent on accurate pre-operative staging. The importance of staging in discriminating, for example, T1bN0 tumours versus T2N0 tumours and T2N1 tumours versus T3N1 tumours is questionable since the management is likely to be the same with regards to neoadjuvant therapy and surgery. However, discriminating those early tumours where neoadjuvant chemotherapy would not be considered from more advanced tumours is critical. In the cohort of 204 patients not undergoing neoadjuvant therapy, 55.9% (114/204) were above this threshold on histopathological analysis. This suggests that the majority could have had the potential for benefit from neoadjuvant chemotherapy if given. However, just over half of these 114 patients with locally advanced disease appear to have been accurately staged with regards to this threshold and were presumably considered for chemotherapy but deemed not medically fit enough. Of the 204 patients, nearly 1 in 7 patients (13.3%) with early tumours were over-staged. The clinical effect of this in all-

comers would be an overuse of chemotherapy according to current protocol. Since this group of patients is likely to be of lower pre-operative stage than those over the same time period who underwent chemotherapy, this is likely to be an underestimate of the over-staging in all patients. Thus a proportion of patients are being exposed to chemotherapy which is associated with toxic effects and a low probability of responding when in fact they may have little to gain from neoadjuvant therapy.

Perhaps even more concerning is that 48.2% of locally advanced tumours were under-staged. Whilst this will be an over-estimate of the error in all comers for the same reasons as above and a proportion of these patients would not be fit for neoadjuvant chemotherapy, the clinical effect of this would be to deny the opportunity of neoadjuvant therapy for a significant proportion of those patients who have potential to benefit.

Summary

Assessment of staging accuracy is problematic now that neoadjuvant therapy forms part of the standard of care. Determining staging accuracy in neoadjuvant patients directly is not possible. The best estimation of this would be a measure of staging accuracy in a randomised control group going straight to surgery. Any comparison of non-randomised groups such as ours is a limitation. However, most ongoing randomised trials are currently comparing one neoadjuvant treatment with another rather than a straight to surgery control group, making assessment of staging accuracy impossible in these trials[30, 91].

With the proviso of the limitation above, this study of staging accuracy in our cohort uncovered a significant problem with the MDT decision making process with regards to neoadjuvant therapy. Treatment is considered on the basis of pre-operative staging

when the T stage is ≥T3 and the N stage is ≥N1. However, staging accuracy according to this cut-off is poor, leading to over or under-treatment. This raises the question of whether there is a better way to determine which patients should receive neoadjuvant chemotherapy. Another consideration relating to the threshold for neoadjuvant therapy treatment aside from the staging accuracy is exactly what constitutes the optimal cut-off level. This will be addressed in the section on

Neoadjuvant therapy efficacy.

Redefining response to neoadjuvant therapy: Survival analysis

Methods

Chemotherapy regime

Patients with tumours staged above T2N0 were considered for neoadjuvant chemotherapy. Chemotherapy consisted of 2 cycles of cisplatin and fluorouracil (OE02 trial protocol); 3 cycles of ECF (MAGIC trial protocol); ECX with or without bevacizumab (ST03 trial protocol); or EOX. In a small number of patients, other combinations of these agents were used and a small number of patients underwent adjuvant therapy. After the completion of the final cycle of neoadjuvant chemotherapy, patients were restaged with a CT scan of the thorax, abdomen and pelvis for estimation of radiological response and operability.

Surgery

Patients with resectable tumours who were still fit for operation underwent surgery. Ivor-Lewis gastro-oesophagectomy was performed for lower oesophageal and gastro-oesophageal junction tumours and distal, subtotal, total or extended gastrectomy were performed for gastric tumours depending on site and extent of the tumour. A small number of patients underwent left thoraco-abdominal oesophagectomy or 3 phase (McKeown) oesophagectomy.

Pathological reporting

Histopathological reporting followed the minimum dataset (recommended reporting guidelines) for oesophageal cancer in all cases of oesophageal and gastro-oesophageal junction tumours and following the minimum dataset for gastric cancers in all gastric cancers. Routine histopathological reporting included recording the Mandard score (TRG) and pathological TNM staging and was performed by a specialist gastrointestinal pathologist.

Data collection

Data were recorded on patient demographics, histological tumour type, anatomical tumour location, pre-operative staging, chemotherapy regime, operative details, postoperative histopathology, length of stay and survival.

Data analysis

The Mandard scoring system was chosen over the broadly similar Becker system primarily because it is already used routinely in our unit and is therefore likely to benefit from greater reliability compared to introducing a new system. The distribution of tumour TRG scores was established. Kaplan-Meier estimates were used to show survival probabilities according to TRG scores and therefore indicate which scores represented histological responders.

Tumour/Nodal down-staging analysis

To further explore the validity of nodal down-staging, patients that were pre-operatively staged with disease at least as advanced as the threshold for considering neoadjuvant therapy (>T2 and/or >N0) were selected and patients not proceeding to resection were excluded. Patients were grouped according to whether they were TRG responders (Group Ai), non-responders (Group Aii) or had surgery only (Group B). Groups were then compared with respect to the apparent tumour/nodal down-staging in order to assess the validity of down-staging for use in defining response.

N down-staging was defined as a lowering of the stage from pre-operative staging of N1/N2 to postoperative (pathological) staging of N0 which has been described elsewhere[44]. T down-staging was defined as any reduction in T stage, i.e. T3 to T2, or T2 to T1 etc. also described previously[78].

Results

Survival according to TRG

Of 587 patients identified in the database with a planned surgical resection, 376 underwent neoadjuvant chemotherapy. 45 patients did not have pathological data available; 38 patients did not proceed to resection and 7 patients had missing/awaited TRG data. This left 331 patients for the analysis, see Figure 20. Patient characteristics are shown in Table 11.

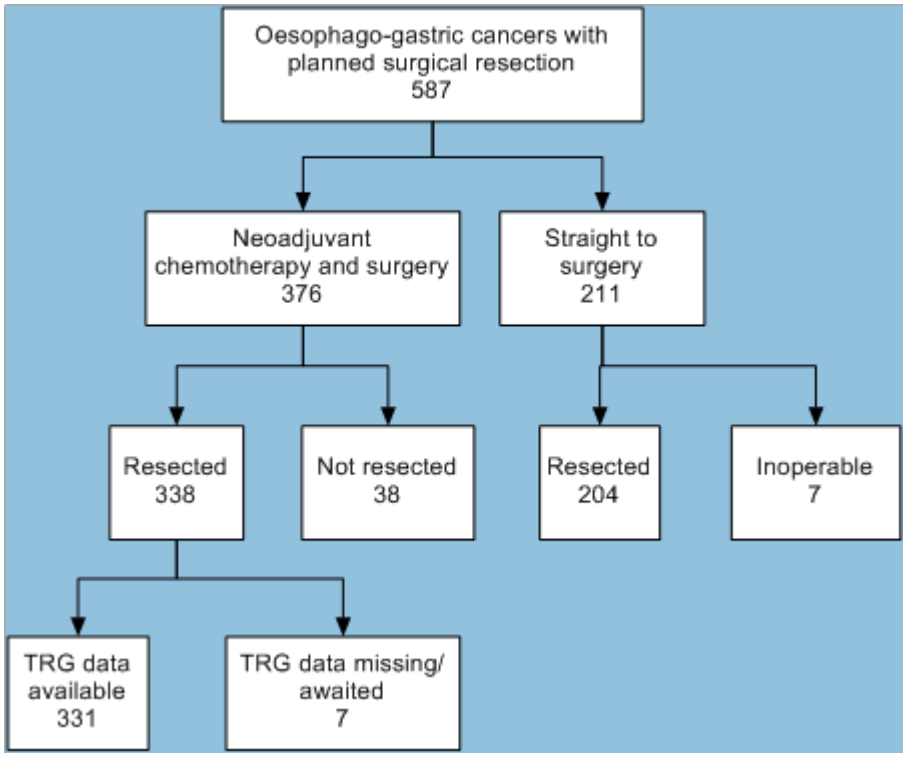

Figure 20 Patients undergoing neoadjuvant therapy and proceeding to resection with TRG data available.

Patient characteristics, n = 331	
Demographics	
Age, in years; mean (range)	64.5 (29.3 to 82.7)
Male; n (%)	255 (77.0)
Tumour characteristics	
Histological type; n (%)	
Adenocarcinoma	293 (88.5)
Squamous cell carcinoma	35 (10.6)
Other carcinoma	3 (0.9)
Anatomical tumour location; n (%)	
Upper third oesophagus	2 (0.6)
Middle third oesophagus	12 (3.6)
Lower third oesophagus	57 (17.2)
Gastro-oesophageal junction	216 (65.3)
Gastric	44 (13.3)
Pre-operative staging	
Pre-operative T stage; n (%)	
≤T2	60 (18.1)
T3	256 (77.3)
T3/4 and T4	15 (4.5)
Pre-operative N stage; n (%)	
N0	133 (40.2)
N1	167 (50.5)
N2	31 (9.4)
Neoadjuvant regimen; n (%)	
Cisplatin/5-FU	50 (15.1)
ECX/ECF/EOX	245 (74.0)
CROSS	8 (2.4)
Carbotaxol +/- epirubicin	4 (1.2)
Missing	24 (7.3)
Post-operative histology	
Post-operative T staging; n (%)	
≤T2	99 (29.9)
pT3	199 (60.1)
pT4	32 (9.7)
missing	1 (0.3)
Post-operative N stage; n (%)	
N0	118 (35.6)
N1	102 (30.8)
N2	58 (17.5)
N3	52 (15.7)
Nx	1 (0.3)
R0 resection	
R0	193 (58.3)
R1	122 (36.8)
R2	1 (0.003)
missing	15 (4.5)

Table 11 Patient characteristics.

Abbreviations: CF; cisplatin, 5-flourouracil; ECX, epirubicin, cisplatin, capecitabine; ECF, epirubicin, cisplatin, capecitabine; EOX, epirubicin, oxaliplatin, capecitabine; EOF, epirubicin, oxaliplatin, capecitabine; CROSS, as per the CROSS trial, carboplatin, paclitaxel and concurrent radiotherapy; OEO5, as per the OEO5 study (ECX, CF); ST03, ECX +/- bevacizumab. Note: percentages may not sum to 100 due to rounding.

The distribution of TRG scores is shown in Figure 21. If patients with a TRG score of 1 or 2 are considered as responders (as has been described elsewhere) then they account for 13.9% of patients.

TRG distribution

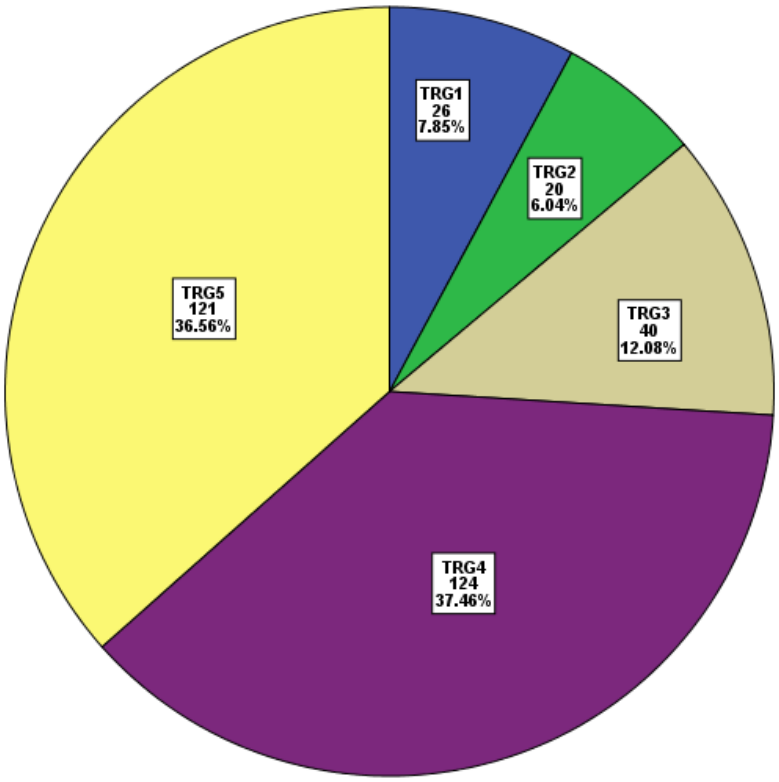

Figure 21. TRG distribution in 331 patients undergoing neoadjuvant chemotherapy followed by surgical resection for oesophageal or gastric cancer.
Note: percentages may not sum to 100 due to rounding.

Survival according to TRG score is shown in Figure 22. There appears to be a difference in survival according to whether patients have a TRG score of 1-3 where median values are not reached or a TRG score or 4 or 5 where they are reached (all mean and median survival times by TRG group are shown in Table 12). TRG3 patients have better survival than TRG 4/5 patients considered together, Log Rank (Mantel-Cox) P=0.008, Table 13 and Figure 23. There is no difference in survival between TRG3 patients and TRG1/2 patients considered together Log Rank (Mantel-Cox) P=0.69 (Table 14 and Figure 24). When response is defined by a TRG score of 1-3 (with TRG 4-5 considered non-responders) rather than TRG 1-2, the proportion of responders increases from 13.9% (46/331) to 26.0% (86/331) (Figure 21). Mean survival in responders is 49.5 months compared to 35.7 months in non-responders, Log Rank (Mantel-Cox) P=0.0001, see Table 15 and Figure 25.

Survival according to TRG score

TRG	n	Mean survival (months)	95% confidence interval (months)	Median survival
1	26	48.0	38.6-57.4	>20.7
2	20	53.9	45.9-61.9	>19.4
3	40	46.9	40.3-53.6	>53.8
4	124	37.0	32.5-41.6	37.8
5	121	33.7	28.8-38.5	25.3
Overall	331	39.3	36.3-42.2	51.3

Table 12 Mean survival according to TRG

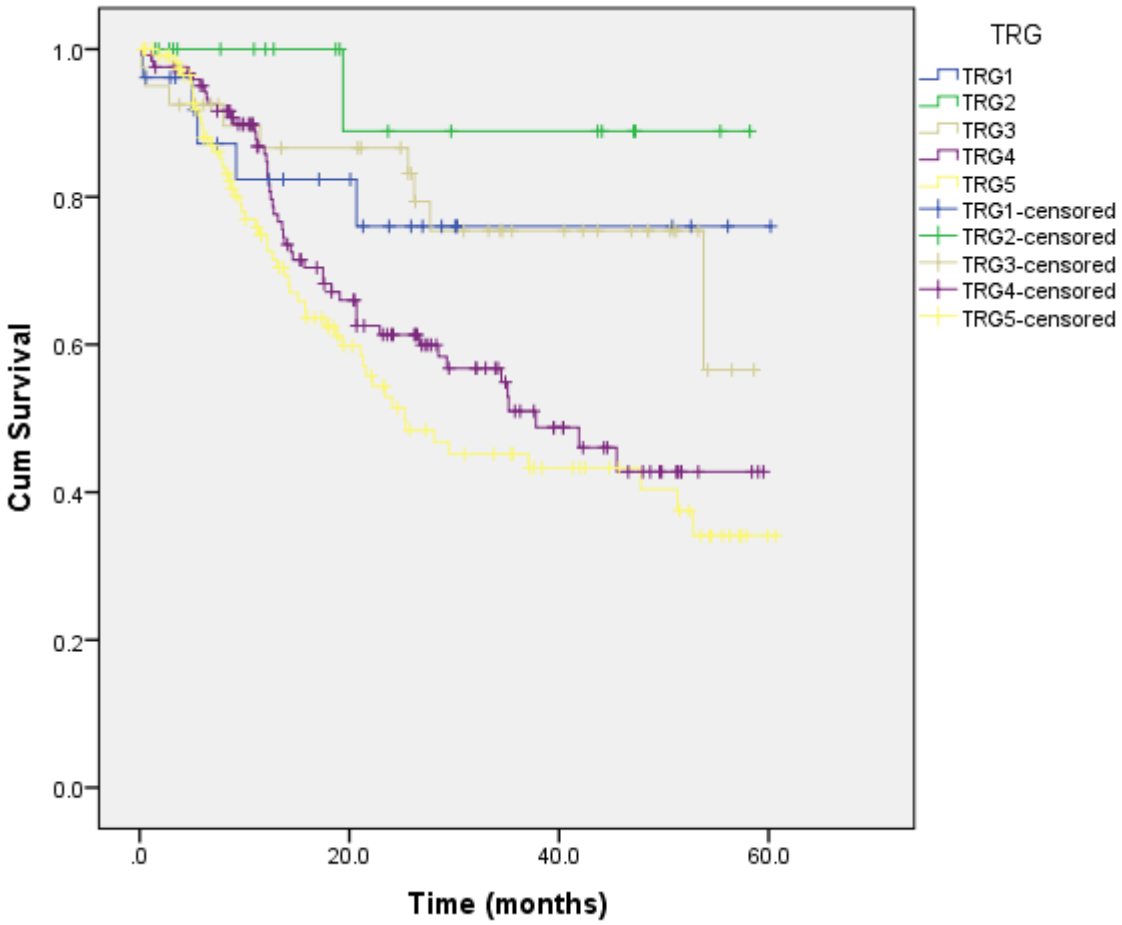

Figure 22 Kaplan-Meier estimates of survival according to TRG score.
Log Rank (Mantel-Cox) P=0.002.

Survival TRG3 compared to TRG4-5

TRG	n	Mean survival (months)	95% confidence interval (months)	Median survival
3	40	46.9	40.3-53.6	>53.8
4-5	245	35.7	32.3-39.1	35.1

Table 13 Survival in TRG3 patients compared to TRG 4-5

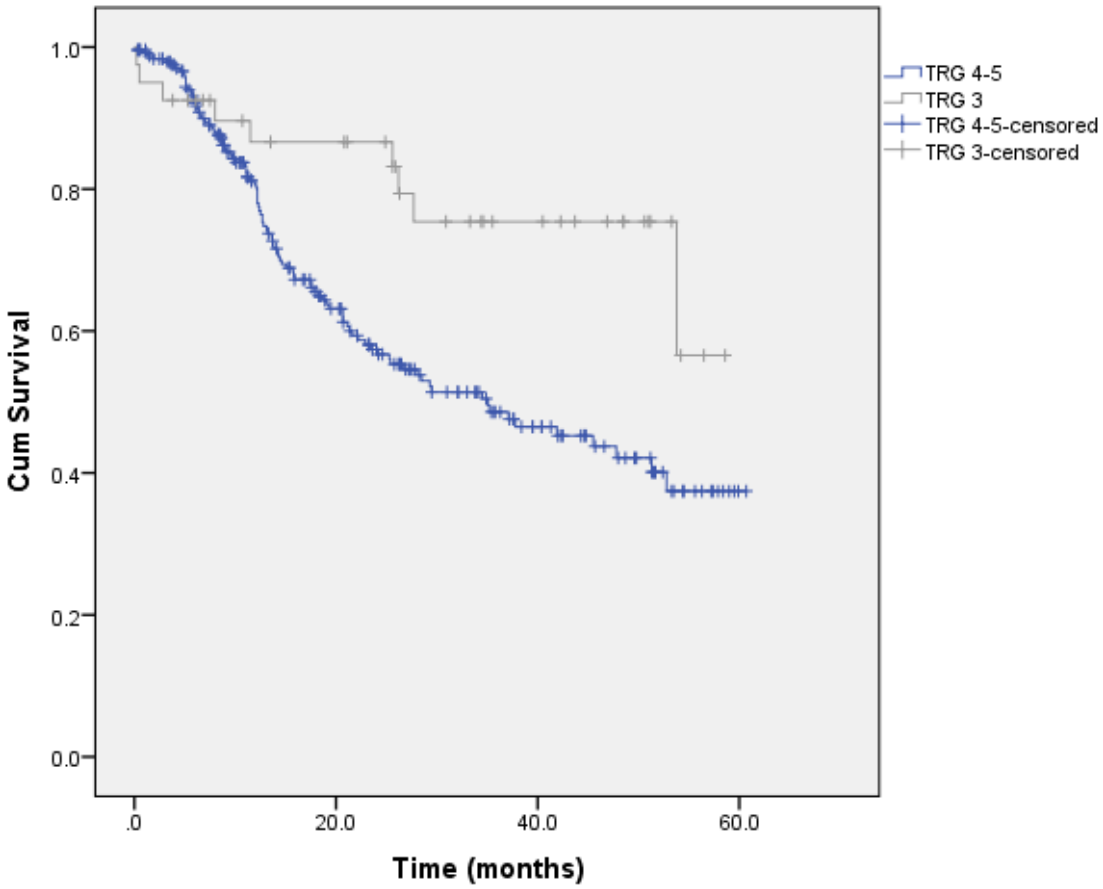

Figure 23 Kaplan-Meier estimates of survival in patients with TRG3 compared to TRG 4-5.
Log Rank (Mantel-Cox) P=0.008.

Survival TRG3 compared to TRG1/2

TRG	n	Mean survival (months)	95% confidence interval (months)	Median survival
1-2	46	51.3	44.7-57.8	
3	40	46.9	40.3-53.6	>53.8

Table 14 Survival in TRG3 patients compared to TRG 1-2 patients

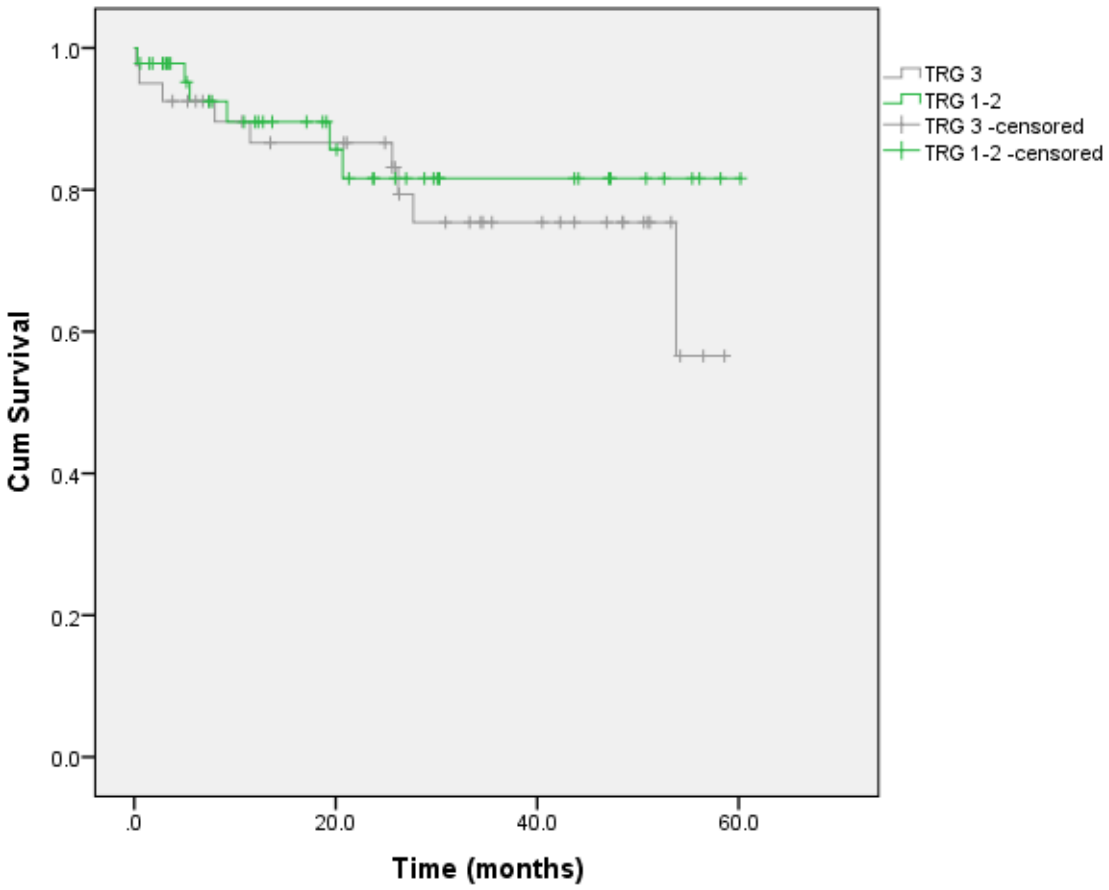

Figure 24 Kaplan-Meier estimates of survival in patients with TRG3 compared to TRG 1/2. Log Rank (Mantel-Cox) P=0.69.

Survival according to TRG (TRG 1-3 responder vs. TRG 4/5 non-responder)

TRG responder	n	Mean survival (months)	95% confidence interval (months)	Median survival
Yes (TRG 1-3)	86	49.5	44.7-54.3	>53.8
No (TRG 4-5)	245	35.7	32.3-39.1	35.1

Table 15 Mean survival according to TRG response

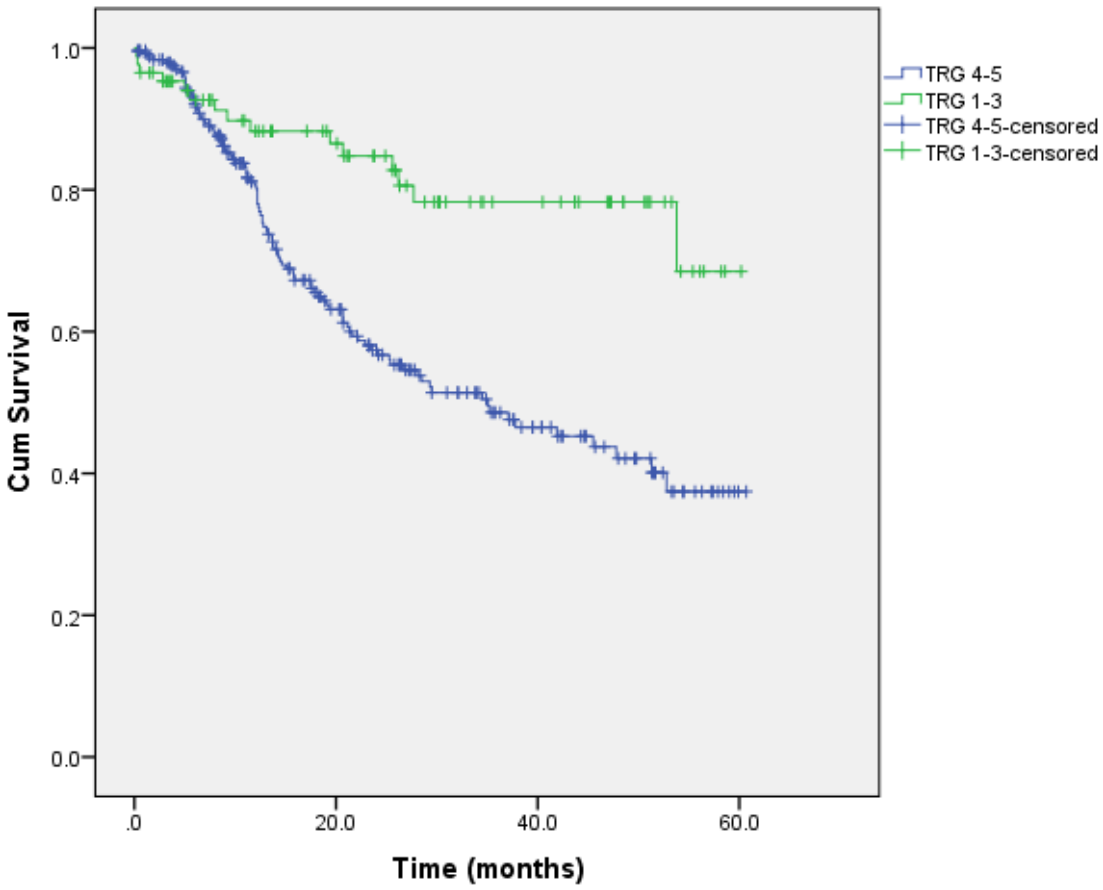

Figure 25 Kaplan-Meier estimates of survival according to TRG response (TRG 1-3 = responder; TRG 4-5 = non-responder). Log Rank (Mantel-Cox) P=0.000.

Tumour/Nodal down-staging analysis

Figure 26 shows the cohort of patients used in the analysis of T/N down-staging. Groups A, B, Ai and Aii and referred to in the tables and text presented below.

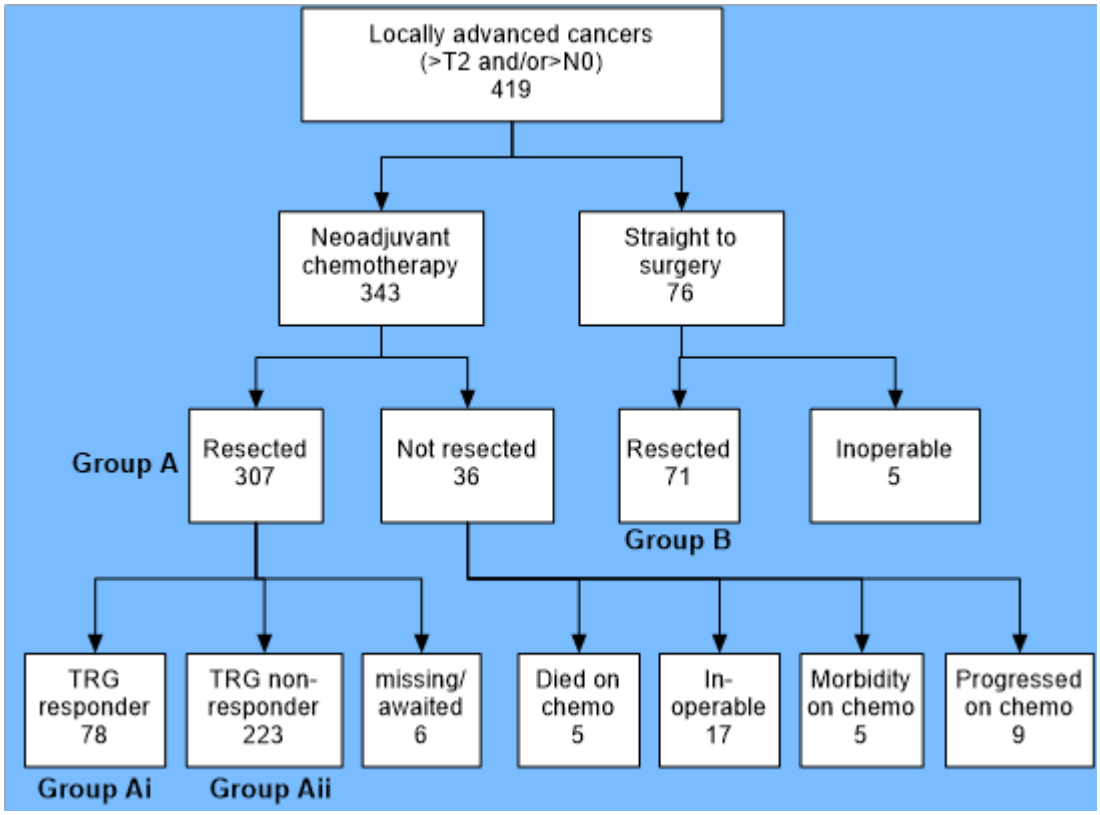

Figure 26 Treatment groups and outcomes in patients meeting threshold for neoadjuvant therapy.

Amongst patients who proceeded to resection and had full pathological results available, a comparison of lymph node down-staging in those having neoadjuvant therapy and those undergoing surgery alone was performed (shown in Table 16).

Group	Nodal Down-staging, number (% of group)	
	Yes	No
Neoadjuvant therapy (Group A) n=307	58 (18.9)	249 (81.1)
Surgery only (Group B) n=71	9 (12.7)	62 (87.3)

Table 16 Nodal down-staging according to treatment group
(see Figure 26 for explanation of groups), P=0.29 (chi-square)

When the neoadjuvant therapy group is subdivided according to TRG response, responders (Group Aii) have a significantly higher proportion of lymph node down-staging compared to both non-responders (Group Aii), P<0.0001 and to the surgery only

group (Group B), P=0.002, but there is no difference in nodal down-staging between non-responders (Group Aii) and the surgery only group (Bi), 13.0% and 12.7% respectively.

Group	Nodal Down-staging, number (% of group)	
	Yes	No
TRG responder (Group Ai) n=78	28 (35.9)	50 (64.1)
TRG non-responder (Group Aii)	29 (13.0)	194 (87.0)
Surgery only (Group B) n=71	9 (12.7)	62 (87.3)

Table 17 Nodal down-staging according to treatment group

(see also Figure 26 shows the cohort of patients used in the analysis of T/N down-staging. Groups A, B, Ai and Aii and referred to in the tables and text presented below.

), P<0.0001 (Pearson Chi square)

The rate of T down-staging in Group A was no different from Group B, *P*=0.24 (Table 18).

When patients undergoing neoadjuvant therapy were sub-divided according to therapy response, there was a difference in the rates of tumour down-staging between Groups Ai, Aii and Bi, P<0.001 (Table 19).

In a 2x2 contingency analysis, there was no difference in down-staging rates between Groups Aii and B. The rate of tumour down-staging was higher in Group Ai compared to both Group Aii (*P*<0.0001) and Group B (*P*<0.0001).

Group	Tumour Down-staging, number (% of group)	
	Yes	No
Neoadjuvant therapy (Group A) n=307	87 (28.3)	220 (71.7)
Surgery only (Group B) n=71	15 (21.1)	56 (78.9)

Table 18 Tumour down-staging according to treatment group
(see Figure 26 for explanation of groups), P=0.28, Chi-square.

Group	Tumour Down-staging, number (% of group)	
	Yes	No
TRG responder (Group Ai) n=78	49 (62.8)	29 (37.2)
TRG non-responder (Group Aii) n=223	35 (15.7)	188 (84.3)
Surgery only (Group B) n=71	15 (21.1)	56 (78.9)

Table 19 Tumour down-staging according to treatment group

(see Figure 26 shows the cohort of patients used in the analysis of T/N down-staging.

Groups A, B, Ai and Aii and referred to in the tables and text presented below.

for explanation of groups), P<0.0001, Pearson Chi-square.

Discussion

It has become clear that responders to neoadjuvant therapy have improved survival compared to non-responders. Authors suggest that if non-responders can be predicted or identified early, then they should proceed directly to surgery to avoid excessive chemotherapy-associated toxicity, a delay to surgery and an unnecessary cost of ineffective neoadjuvant therapy[86].

The quoted proportion of patients defined as responders varies widely. This could be explained by differences in patients, tumour characteristics, treatments and not least by the existence of many methods for measuring response to neoadjuvant therapy. However, regardless of the method used to evaluate response, rates of response are low, typically 11-27%[40, 44]. Therefore, either 73%-89% of patients are receiving neoadjuvant therapy without any benefit, or the system is failing to identify all patients with a meaningful response.

There is a need to re-define what constitutes an adequate response to neoadjuvant therapy although there have been few attempts to do this and standardise the definition. This section therefore has set out to achieve this.

Firstly, a literature review was performed to identify possible methods of defining response. The most promising methods would then be applied to a historical patient cohort and survival analysed to see which method or combination provides the most accurate measure of response. Any proposed definition must reflect a true neoadjuvant therapy response and must be associated with survival. Any use of pre-operative staging information would depend on its accuracy.

The literature review provided a short-list of variables that show promise as measures of response to therapy. Whilst clinical and radiological features have been used, the methods best associated with survival are pathological. Histological regression based on the degree of residual tumour cells and fibrosis has the most evidence to support its use. The Mandard TRG score and Becker systems provide the best prognostic information and are broadly similar. The Mandard score is arguably the most widely used and it has been shown to be associated with survival in oesophageal cancer[43, 44, 92] and gastric cancer[92, 93]. It has also been used routinely by our specialist GI pathologists for a number of years and so was chosen as the basis for defining response to therapy.

There is evidence that combining histopathological regression and down-staging of primary tumour (T) or lymph node (N) stage may be associated with survival[44, 78, 86]. Specifically, it has been suggested that such down-staging may be a more sensitive or an additional independent measure of response that could help to identify more responders. It is not known if this represents a valid measure of response to neoadjuvant therapy, whether it should be included as part of the definition and if so, how it would be used alongside histopathological regression. Furthermore, there has been little consideration of pre-operative staging accuracy on which the validity of these definitions critically depends.

The study of pre-operative staging accuracy in our cohort of patients having surgery only showed that pre-operative T and N staging were associated with a degree of inaccuracy, leaving concern over the validity of such measures.

The issue of nodal-down-staging has been recently investigated by others. In their study, Noble et al acknowledged that the notion of down-staging is controversial due to difficulties in evaluating pre-therapy staging and specifically that apparent down-staging

may simply represent over-diagnosis of lymph node metastases on clinical imaging[44]. To address this, they compared the survival of patients undergoing neoadjuvant chemotherapy with those undergoing surgery alone, only amongst patients that were staged N0 in the resection specimen. Those undergoing chemotherapy had marginally poorer survival compared to those undergoing surgery alone. It is suggested that this poorer survival can be explained by and proves the existence of preoperative lymph node metastases. However, the neoadjuvant patients also had more advanced T stage ($P<0.001$) which could also explain the poorer survival in this group. Also of concern is the fact that 37.5% pN0 patients in their surgery only group had apparent nodal down-staging, i.e. were clinically over-staged, suggesting that their neoadjuvant patients may also be prone to similar over-staging.

Down-staging or T and/or N has been shown to be associated with improved survival after neoadjuvant chemotherapy treatment in 77 patients with oesophageal cancer[78]. Histological regression was not assessed so it is not known if these effects are independent of regression. Also there was no consideration of pre-operative staging accuracy bringing into question the validity of down-staging.

A separate study in patients with oesophageal adenocarcinoma undergoing neoadjuvant chemotherapy/chemoradiotherapy, a combination of the two approaches above was used[86]. Whilst complete responders were identified by histopathological regression, partial responders were identified by down-staging of T and or N status. Response was associated with improved survival. Again, there is no consideration given to the validity of measuring response in this way with regards to accuracy of pre-operative staging.

It is clear that histological regression (using a validated score) is likely to represent a true beneficial response to therapy with responders having improved survival. Our analysis showed that TRG scores 1, 2 and 3 (representing 26.0% patients) were associated with improved outcomes and could therefore be considered as responders. The issue of debate is amongst the histological non-responders and whether a subset of these patients could be considered partial responders with some survival benefit. If such a group exists and is represented by lymph node or tumour down-staging, then the proportion of down-staged patients in the neoadjuvant therapy group (and specifically in histological non-responders) should be greater than the proportion of apparently down-staged patients in the surgery only group. However, patients are selected to these groups on the basis of staging so an overall comparison of stage change is unreliable. There is a group of patients within the surgery only group in whom it can be argued disease stage was not used to select treatment group. These are the patients that meet the threshold for neoadjuvant therapy (>T2 and/or >N0) but who are unfit for or decline such therapy (n=76). They can be compared to a group of patients with similar staging undergoing neoadjuvant therapy (n=343). Figure 26 shows the broad outcomes in patients meeting the threshold for neoadjuvant therapy according to whether or not they did indeed undergo neoadjuvant therapy or had surgery only. Any patients not proceeding to surgery or without pathological data available were excluded, leaving patients in Groups Ai, Aii and B from Figure 26 in the analysis. This showed the tumour and nodal down-staging rates were no different between non-responders and the surgery only group, suggesting that the down-staging observed in non-responders is simply a result of clinical over-staging rather than representing a true treatment effect. Any true down-staging in neoadjuvant patients appears to be limited to the group of TRG responders (responders do express higher rates or T and N down-staging compared

to surgery only patients). Therefore, nodal down-staging should not be used as a method of identifying responders to neoadjuvant therapy in the TRG non-responder group.

Based on the clinical review of methods used to measure response to neoadjuvant therapy and based on the investigation on staging accuracy above, it is suggested that responders are defined as those with histological regression defined by a Mandard score of 1 to 3. Neither T down-staging nor N down-staging are reliable enough to be included in the definition.

Neoadjuvant therapy toxicity

From the same cohort of patients planned to undergo surgical resection, all patients undergoing neoadjuvant therapy were selected and therapy-associated adverse effects were recorded. These were graded according to the Common Terminology Criteria for Adverse Events v3.0 (CTCAE)[94], see Table 20.

Grade	Severity
0	No adverse event
1	Mild adverse event
2	Moderate adverse event
3	Severe adverse event
4	Life-threatening or disabling adverse event
5	Death related to adverse event

Table 20 CTCAE (Common Terminology Criteria for Adverse Events) grading system for chemotherapy/chemoradiotherapy related adverse events.

Statistical analysis

Survival curves were constructed using the Kaplan-Meier technique in SPSS v.21. The Log Rank (Mantel-Cox) method was used to assess differences in survival between defined groups of patients.

Results

Figure 27 shows the broad outcomes in patients undergoing neoadjuvant therapy divided according to whether they experienced therapy-associated adverse effects.

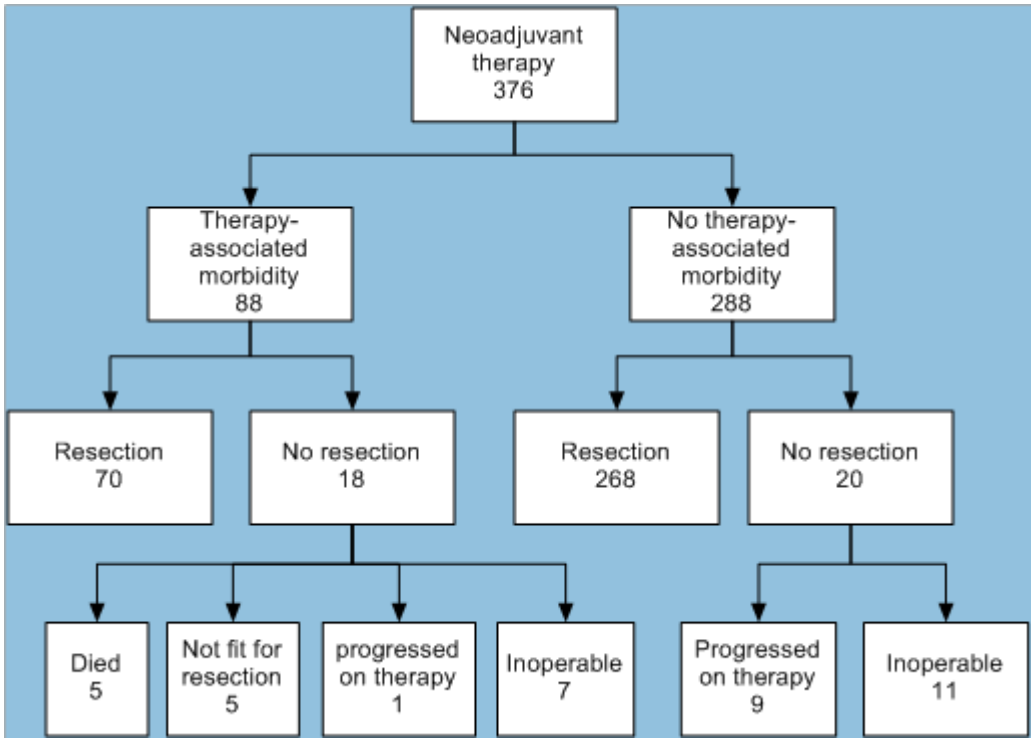

Figure 27 Outcomes in patients undergoing neoadjuvant therapy according to whether adverse events were experienced.

Neoadjuvant therapy associated adverse effects were identified in 88 patients, 23.4% of those 376 undergoing therapy. The grades of severity recorded are shown below in Table 21.

CTCAE Grade	Severity	Number (% of all patients undergoing therapy)
0	No AE	288 (76.6)
1	Mild AE	1 (0.3)
2	Moderate AE	26 (6.9)
3	Severe AE	25 (6.6)
4	Life-threatening or disabling AE	31 (8.2)
5	Death related to AE	5 (1.3)

Table 21 Grade of adverse events, AE, (CTCAE, Common Terminology Criteria for Adverse Events).

Of the five therapy-associated deaths, two were due to pulmonary embolism, two were due to myocardial infarction and one was due to gastrointestinal bleeding.

Figure 28 shows survival curves according to the presence or absence of adverse effects. Survival is significantly better in patients without adverse effects (P=0.002). Median

survival in those with and without adverse effects was 21.1 and 52.8 months, respectively (Table 22).

Chemotherapy morbidity	n	Mean survival (months)	95% confidence interval (months)	Median survival
No	288	43.0	39.2-46.8	52.8
Yes	88	28.2	23.6-32.8	21.1

Table 22 Survival according to the presence or absence of chemotherapy morbidity

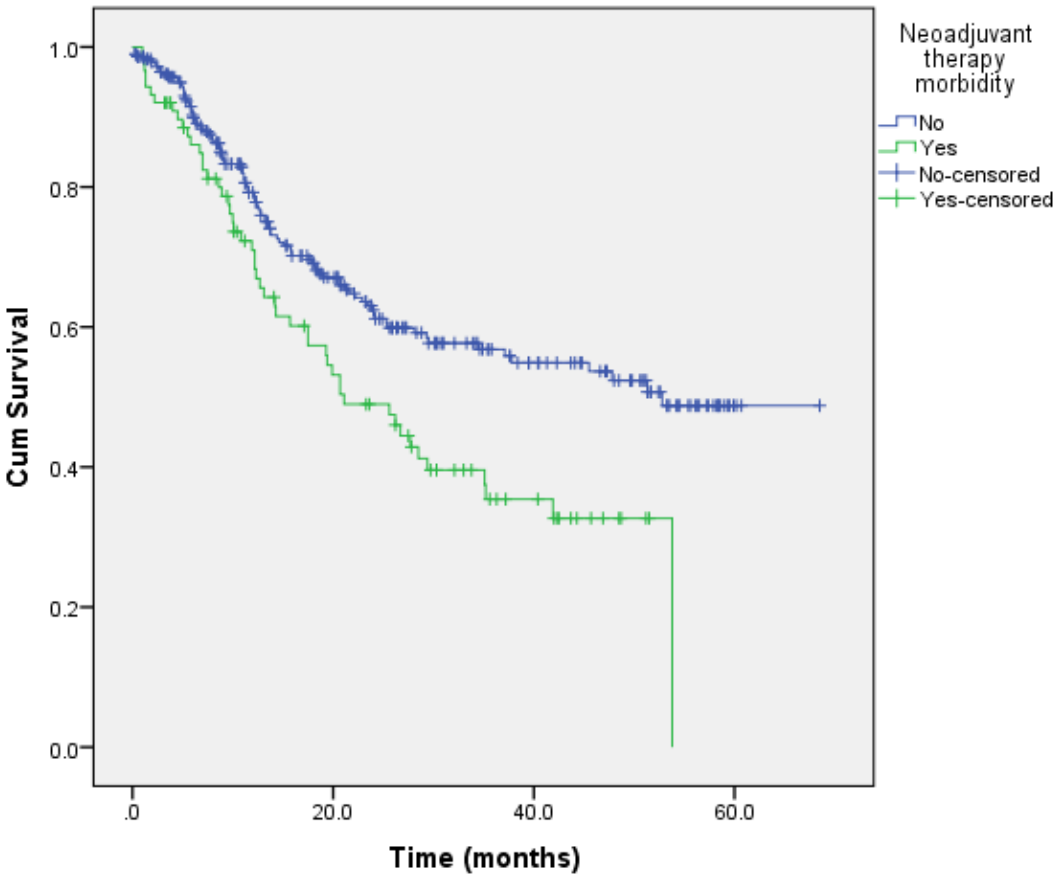

Figure 28 Kaplan-Meier estimates of survival according to presence or absence of neoadjuvant therapy morbidity. Log Rank (Mantel-Cox) P=0.002.

Number of chemotherapy cycles completed

Of the 376 patients undergoing neoadjuvant therapy, 226 (60.1%) patients had data available on the number of cycles of chemotherapy administered, see Table 23. 26 of 226 patients (11.5%) did not receive the intended number of chemotherapy cycles. 15 of these were due to documented adverse effects. In the remaining 11 patients, the reasons for not completing the neoadjuvant course were often not recorded but included poor tolerance of side-effects and signs of clinical progression, such as worsening dysphagia.

In patients with adverse effects, the proportion with reduced cycles was 22.4% compared to 6.9% in those without adverse effects (P=0.002, chi-square).

	Number of cycles completed			
	reduced	**complete**	**missing**	**Total**
No AE, n (%)	11 (6.9)	148 (93.1)	129	288
AE, n (%)	15 (22.4)	52 (77.6)	21	88
Total, n (%)	26 (11.5)	200 (88.5)	150	376

Table 23 Relationship between presence of adverse events, AE and number of cycles completed

There was no survival difference between those patients completing the full course of neoadjuvant therapy and those with reduced cycles, Table 24 and Figure 29 (P=0.6, log-rank).

Cycles completed	n	Mean survival (months)	95% confidence interval (months)	Median survival
Yes	200	36.3	32.7-40.0	35.2
No	26	37.7	28.5-46.9	53.8

Table 24 Survival in patients according to neoadjuvant course completion

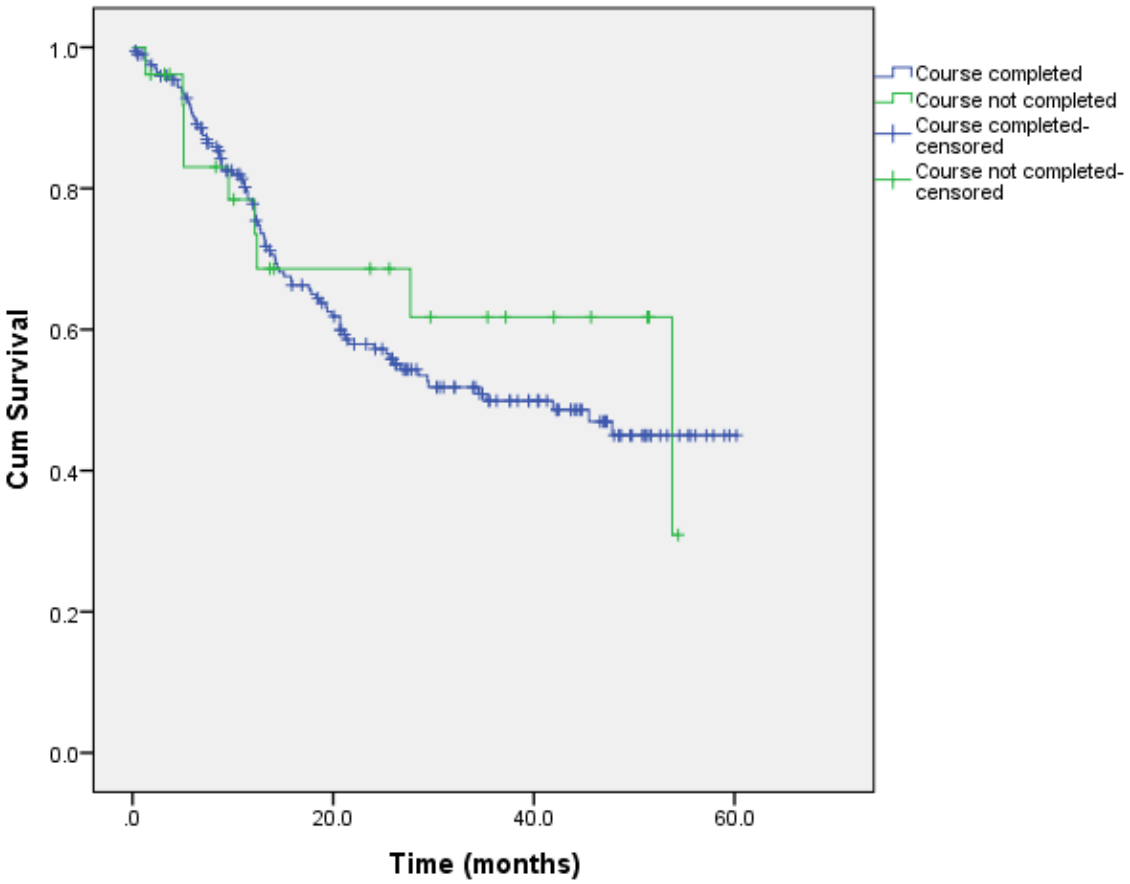

Figure 29 Kaplan-Meier estimates of survival according to whether chemotherapy cycles were completed. Log Rank (Mantel-Cox) P=0.64.

Proportion of patients proceeding to resection

The proportion of patients not proceeding to resection after therapy associated adverse events was 18/88 (20.5%); compared to 20/288 (6.9%) in those without adverse events, P=0.0002, chi-square (Table 25).

	Resection status		
	No resection	**resection**	**Total**
No AE	20	268	288
AE	18	70	88
Total	38	338	376

Table 25 Relationship between resection status and AE

Regardless of the presence or absence of adverse effects, it can be seen from Figure 27 that overall, 38/376, 10% patients who undergo neoadjuvant therapy do not proceed to resection.

Survival in patients proceeding to resection is greater than those failing to have a resection, Table 26 and Figure 30, (P<0.0001, log-rank). Median survival in those having a resection was 51.3 months, compared to 8.8 months in those failing to have a resection.

When considering only patients having a resection, there is some evidence of improved survival among those who did not suffer an adverse effect on chemotherapy, compared to those who did, although this difference did not reach statistical significance (P=0.06, log-rank; Figure 31).

Resection	n	Mean survival (months)	95% confidence interval (months)	Median survival
Yes	338	42.8	39.4-46.2	37.8
No	38	10.5	7.4-13.6	8.8

Table 26 survival according to whether patients proceeded to resection

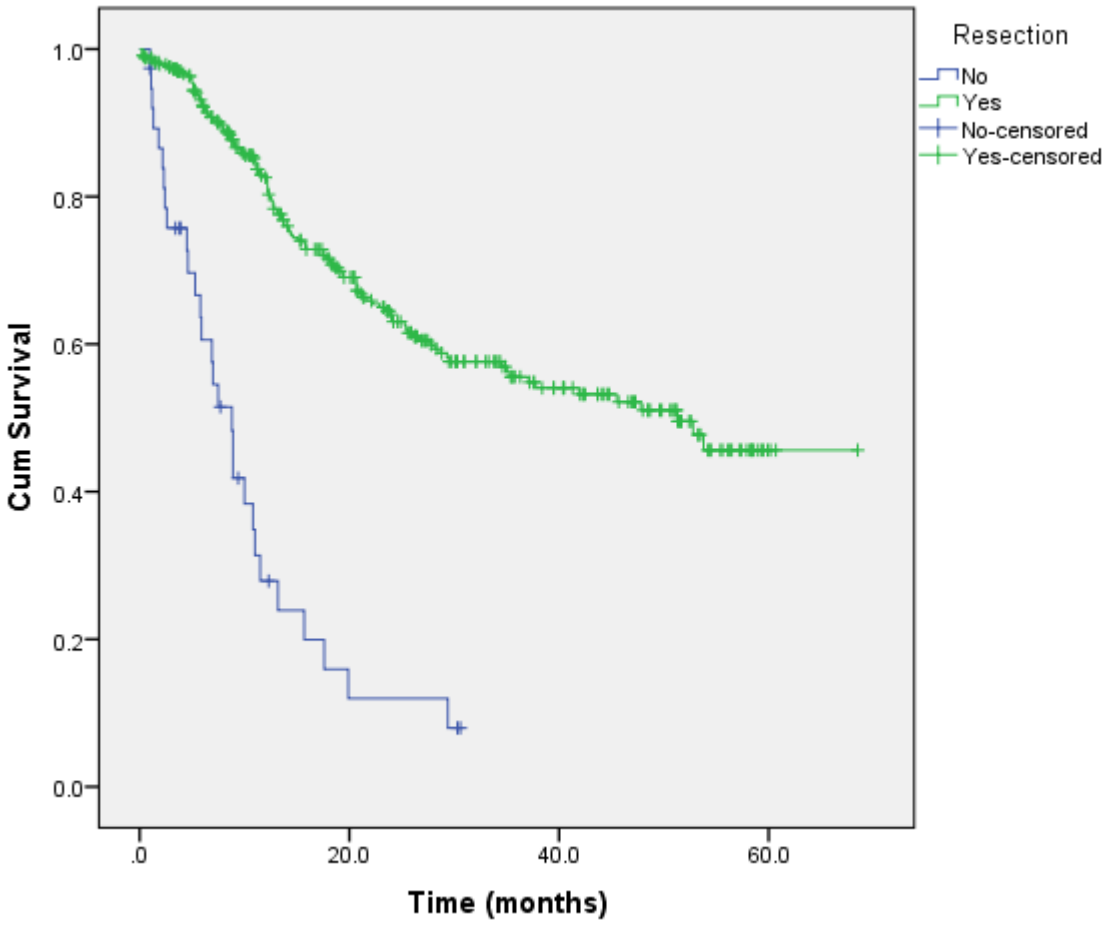

Figure 30 Kaplan-Meier estimates of survival according to whether patients proceeded to resection. Log Rank (Mantel-Cox) P≤0.001.

Neoadjuvant therapy morbidity	n	Mean survival (months)	95% confidence interval (months)	Median survival
No	268	44.8	41.0-48.7	Not reached
Yes	70	32.6	27.6-37.6	28.5

Table 27 Survival according to neoadjuvant therapy toxicity in patients undergoing surgical resection

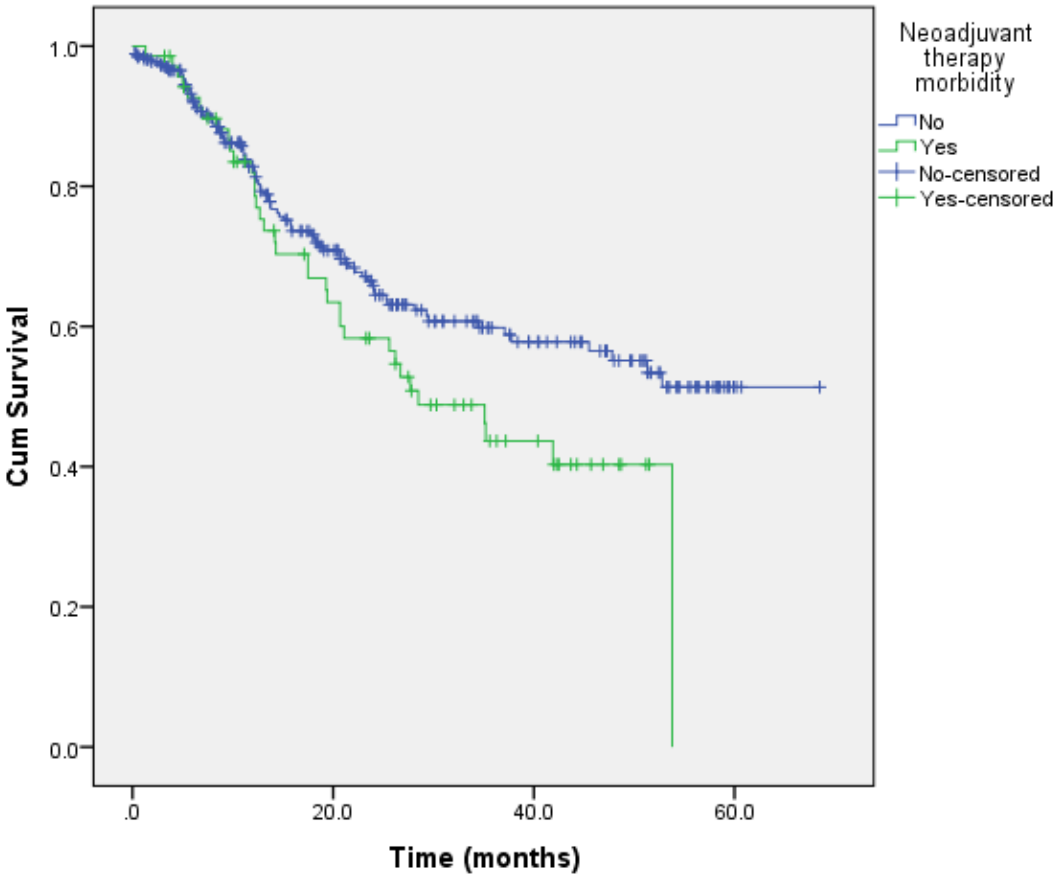

Figure 31 Kaplan-Meier estimates of survival according to whether there was neoadjuvant therapy associated morbidity in resected patients only.
Log Rank (Mantel-Cox) P=0.063.

TRG response

The proportion of TRG responders did not differ between patients suffering therapy-associated adverse events and those not, 16/70 (22.9%) and 70/261 (26.8%) respectively, P=0.6, chi-square (Table 28).

	TRG response		Total
	Responder	Non-responder	
No AE	70	191	261
AE	16	54	70
Total	86	245	331

Table 28 Comparison of TRG response and presence of adverse effects

Likewise, the proportion of TRG responders did not differ between patients not receiving all 3 pre-operative cycles of chemotherapy (15/61; 24.6%) and those completing the course (35/139; 25.2%), P=1, chi-square (Table 29).

	TRG Response		Total
	Responder	Non-responder	
< 3 cycles	15	46	61
3 or more cycles	35	104	139
Total	50	150	200

Table 29 Comparison of TRG response and whether all 3 cycles of chemotherapy were given

Discussion

Of patients undergoing neoadjuvant therapy, nearly 1 in 4 (23.4%) suffered adverse effects and 69% of these were graded as severe, life-threatening or fatal. There are perhaps a surprisingly low number of mild adverse events. The types of events that would be included in this category include mild, transient symptoms, such as skin rashes and asymptomatic anaemia, leukopenia or thrombocytopenia. It is likely that we do not have the pathways in place to record all such mild/moderate events. As a result, we probably underestimate the total number of events but overestimate the proportion of events that are classed a severe. The proportion of all patients undergoing neoadjuvant therapy that experience severe toxicity would be unchanged by the under-reporting of minor events.

Only 72% patients received the full number of preoperative chemotherapy cycles and those with adverse effects were less likely to complete the course. Course completion was not associated with better survival.

At least 1 in 5 patients with adverse effects did not proceed to resection in comparison to less than 1 in 15 patients without adverse effects. Not surprisingly, whether patients are resected or not is strongly associated with survival.

Adverse events are associated with a failure to complete the planned neoadjuvant cycles, resection rate and survival. Although reduced resection is associated with survival, reduced number of cycles is not, suggesting that the reduced survival in those with adverse events is due to failure to proceed to resection rather than failure to complete the full course of neoadjuvant therapy. In patients undergoing resection, the apparent trend towards poorer survival in those with adverse events suggests that there

may be additional factors beyond being well enough to have a resection that have an ongoing negative effect on survival.

Neoadjuvant therapy efficacy

Methods

Survival according to stage and within stage groups

Amongst the 587 patients identified in the cohort, survival across all prognostic stage groups was plotted and compared. To investigate whether any beneficial effects of neoadjuvant therapy are dependent on disease stage, each stage grouping was considered separately and survival compared in patients with a plan for neoadjuvant therapy and surgery (Group A, Figure 32) with survival in those going straight to surgery (Group B Figure 32). In an attempt to further characterise the patients that benefit from additional therapy, those undergoing neoadjuvant therapy were further sub-divided according to whether they were TRG responders (Group Aii) or not (Group Aiii) and survival curves compared to a similar group of patients having resection only (Group Bi). Patients not proceeding to surgical resection were excluded from both neoadjuvant therapy and surgery only groups in the latter analyses.

Overall effect of neoadjuvant therapy

To determine the overall effect of neoadjuvant therapy on survival, Kaplan-Meier estimates were plotted and compared across responder, non-responder or surgery only groups. In an attempt to reduce the natural differences in stage, the cohort was limited to those patients who on staging reached the threshold for neoadjuvant therapy (>T2 and/or >N0), see Figure 32. Patient characteristics of the Groups are presented in table and differences between groups analysed using T-test or Chi-Square tests as appropriate without adjusting for multiple comparisons. The relationships between any factors differing between groups and survival were analysed in a Cox Regression model.

Results

Survival according to stage and within stage groups

Figure 32 shows the broad outcomes with patients divided accordingly to whether they underwent neoadjuvant therapy and then whether they underwent surgical resection. It may be helpful to refer to this diagram when interpreting the survival curves that follow in this results section.

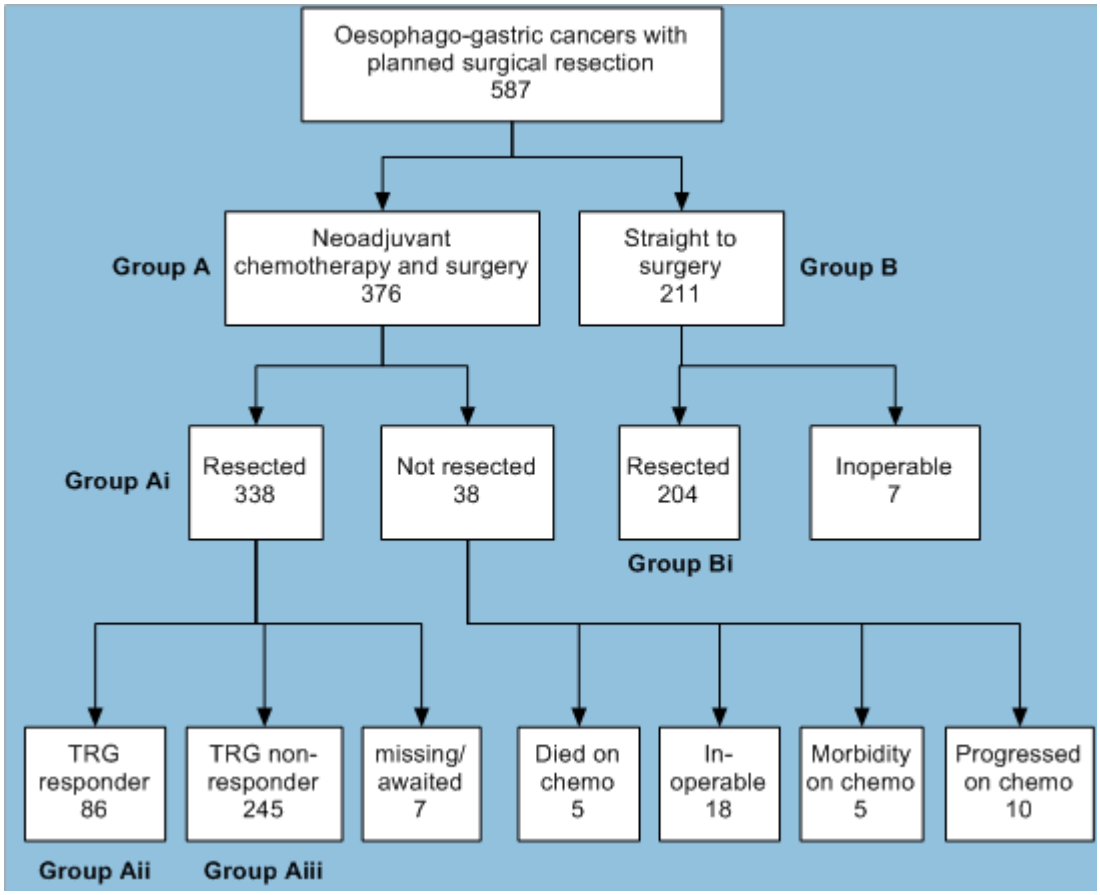

Figure 32 Outcomes in all according to whether or not neoadjuvant therapy was given in addition to surgery Named groups are referred to in the text.

Figure 33 and Table 30 show the survival according to the prognostic stage grouping (stage) based on pre-operative stage data. As stage increases, survival decreases significantly, P<0.0001 (Log Rank).

Prognostic stage group	n	Mean survival (months)	95% confidence interval (months)	Median survival
0	15	-	-	-
1	125	47.7	43.5-51.9	-
2	231	40.0	35.9-44.1	21.1
3	216	33.6	29.9-37.3	19.4

Table 30 Survival according to prognostic stage group.

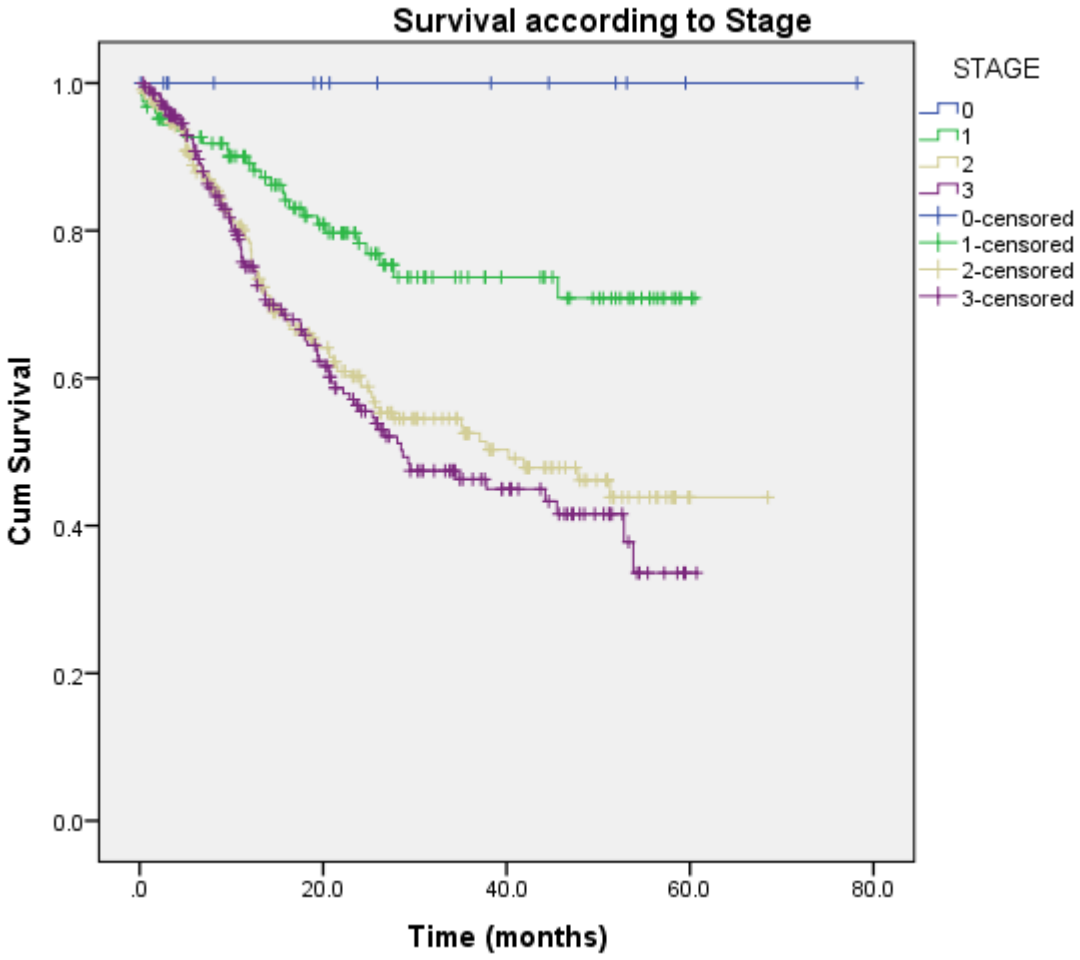

Figure 33 Kaplan-Meier estimates of survival in all patients according to pre-operative prognostic stage group. Log Rank (Mantel-Cox) P=0.000.

Figure 34 and Table 31 include only patients in stage 1 and compare survival in patients undergoing neoadjuvant therapy (Group A from Figure 32, green line) and those

undergoing surgery alone (Group B from Figure 32, blue line). There is no difference in survival between the groups (P=0.81, Log-rank).

	n	Mean survival (months)	95% confidence interval (months)	Median survival
Neoadjuvant therapy and surgery (Group A)	21	49.1	39.4-58.8	Not reached
Surgery Only (Group B)	104	47.4	42.7-52.0	Not reached

Table 31 Survival according to treatment plan within prognostic stage group 1 patients only

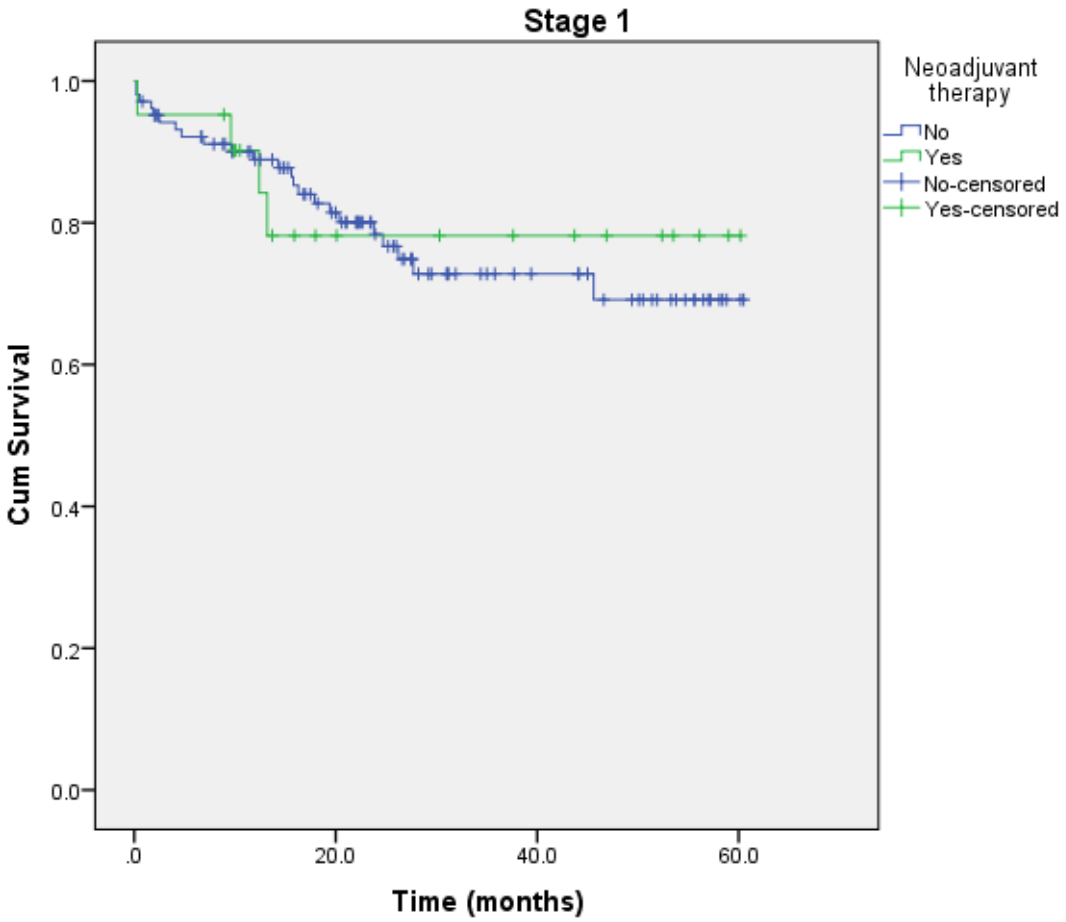

Figure 34 Kaplan-Meier estimates of survival according to treatment type in prognostic stage 1 patients only (neoadjuvant therapy, green line or surgery only, blue line). Log Rank (Mantel-Cox) P=0.81.

Figure 35 and Table 32 include only patients in stage 2 and compares survival curves in patients undergoing neoadjuvant therapy (Group A from Figure 32, green line) and those undergoing surgery alone (Group B from Figure 32, blue line). There is no difference in survival between the groups (P=0.89, Log-rank).

	n	Mean survival (months)	95% confidence interval (months)	Median survival
Neoadjuvant therapy and surgery (Group A)	165	39.9	35.0-44.8	37.1
Surgery Only (Group B)	66	36.4	30.1-42.8	37.8

Table 32 Survival according to treatment plan within prognostic stage group 2 patients only

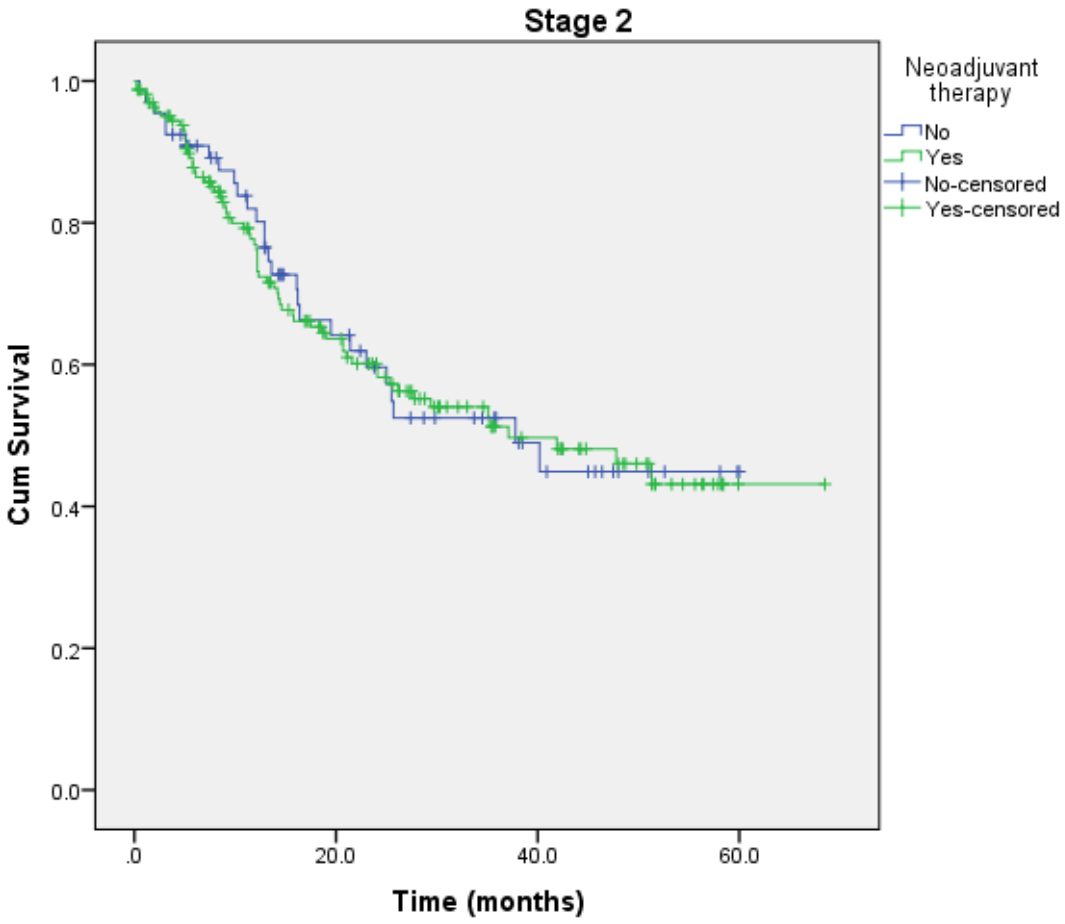

Figure 35 Kaplan-Meier estimates of survival according to treatment type in prognostic stage 2 patients only (neoadjuvant therapy, green line or surgery only, blue line). Log Rank (Mantel-Cox) P=0.91.

Figure 36 and Table 33 include only patients in stage 3 and compares survival curves in patients undergoing neoadjuvant therapy (Group A from Figure 32, green line) and those undergoing surgery alone (Group B from Figure 32, blue line). Survival is better in patients undergoing neoadjuvant therapy, P=0.015 (Log-rank).

	n	Mean survival (months)	95% confidence interval (months)	Median survival
Neoadjuvant therapy and surgery (Group A)	190	35.1	31.1-39.0	29.3
Surgery Only (Group B)	26	21.1	13.9-28.3	19.5

Table 33 Survival according to treatment plan within prognostic stage group 3 patients only

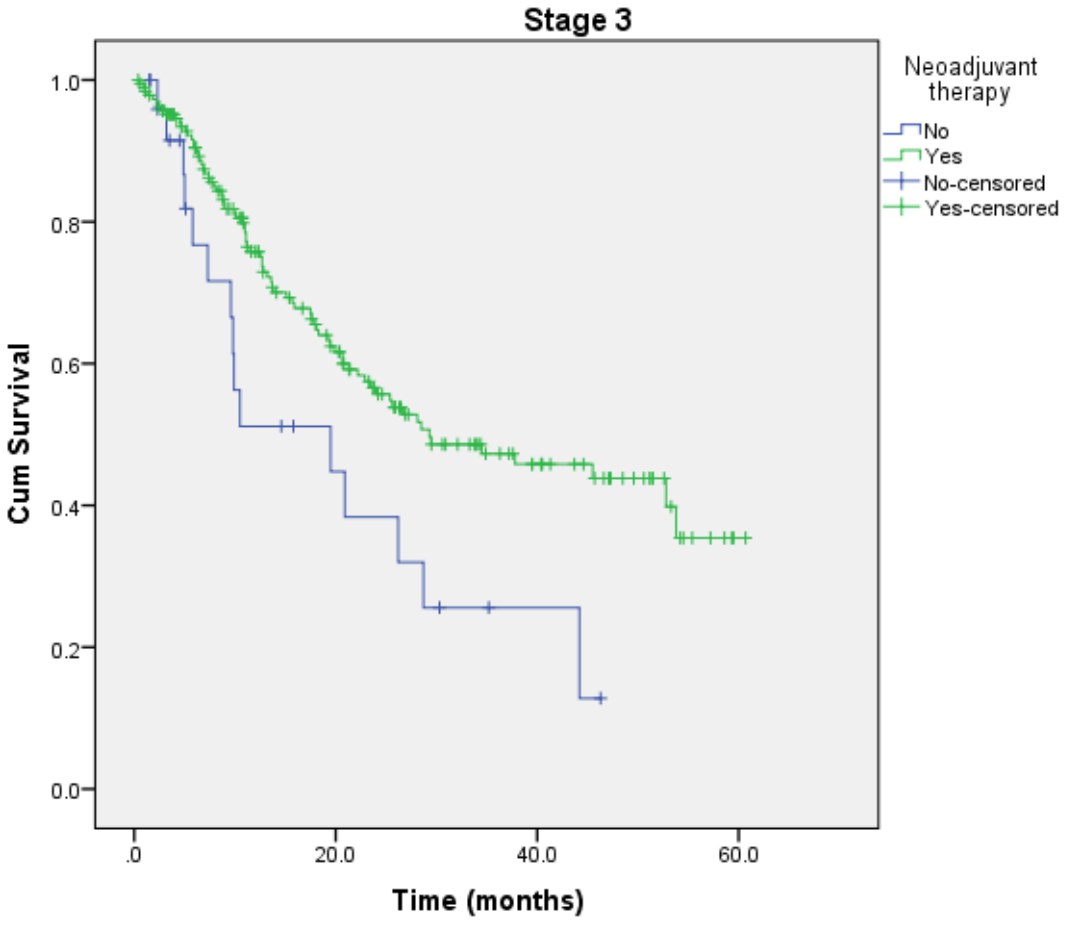

Figure 36 Kaplan-Meier estimates of survival according to treatment type (neoadjuvant therapy or surgery only) in prognostic stage 3 patients only.
(Green line - neoadjuvant therapy; Blue line - surgery only). Log Rank (Mantel-Cox) P=0.015.

Figure 37 and Table 34 include only stage 1 patients who underwent surgical resection and compares survival in TRG responders to neoadjuvant therapy (Group Aii from Figure 32, blue line), TRG non-responders (Group Aiii, green line) and patients undergoing surgery alone (Group Bi, tan line). There is no difference in survival between groups (P=0.92, Log-rank).

	n	Mean survival (months)	95% confidence interval (months)	Median survival
TRG Responders (Group Aii)	6	50.2	32.4-68.1	-
TRG Non-responders (Group Aiii)	13	49.5	37.6-61.5	-
Surgery only (Group Bi)	102	48.2	43.7-52.8	-

Table 34 Survival according to whether resected, stage 1 patients were neoadjuvant therapy responders, non-responders or underwent surgery alone.

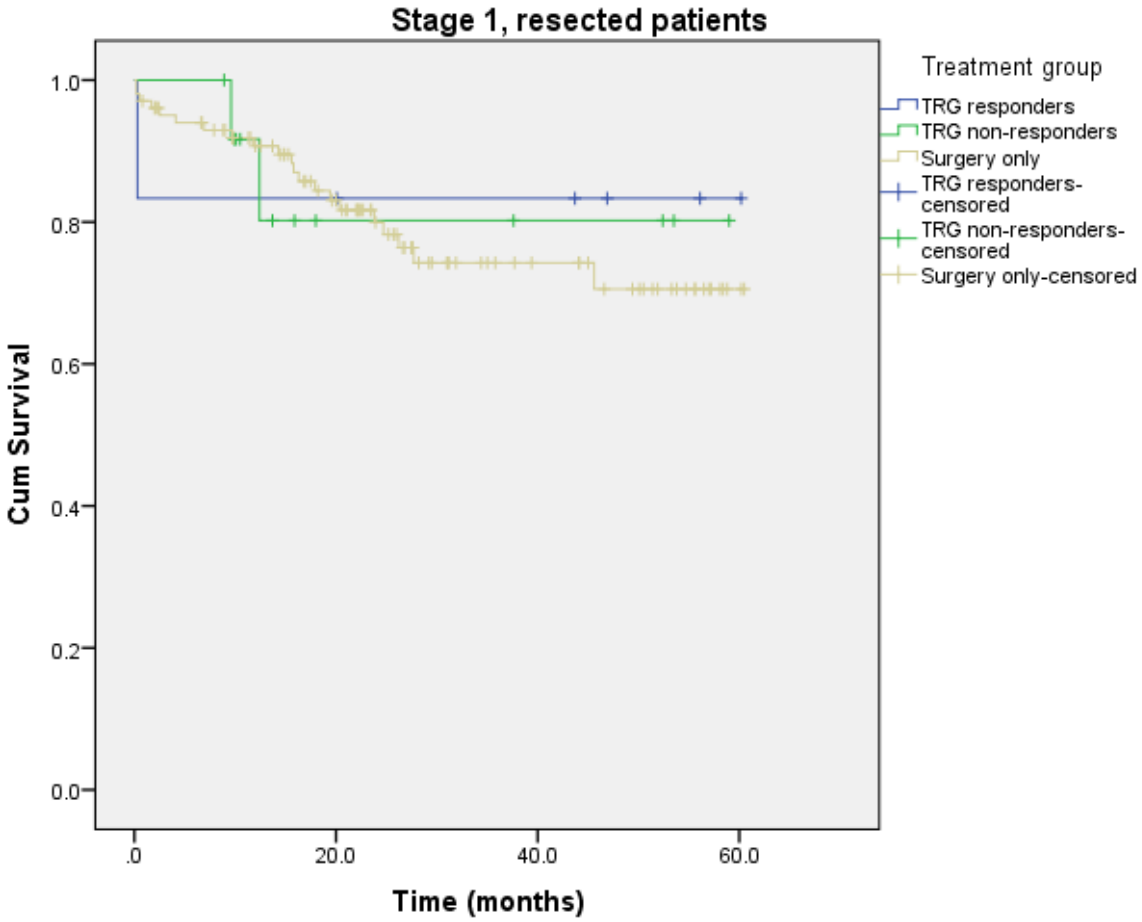

Figure 37 Kaplan-Meier estimates of survival according to treatment type and histological response to therapy in prognostic stage I patients only.
(Blue line – neoadjuvant therapy responder; green line – neoadjuvant therapy non-responder; Brown line – surgery only). Log Rank (Mantel-Cox) P=0.92.

Figure 38 includes only resected patients in stage 2 and compares survival curves in TRG responders to neoadjuvant therapy (Group Aii from Figure 32, blue line), TRG non-responders (Group Aii from Figure 32, green line) and patients undergoing surgery alone (Group Bi from Figure 32, tan line). There is some indication of a difference in survival between the groups but this does not reach significance. (P=0.101, log-rank).

	n	Mean survival (months)	95% confidence interval (months)	Median survival
TRG Responders (Group Aii)	40	45.2	37.9-52.5	-
TRG Non-responders (Group Aiii)	111	35.0	30.1-39.9	35.2
Surgery only (Group Bi)	66	36.4	30.1-42.8	37.8

Table 35 Survival according to whether resected, stage 2 patients were neoadjuvant therapy responders, non-responders or underwent surgery alone.

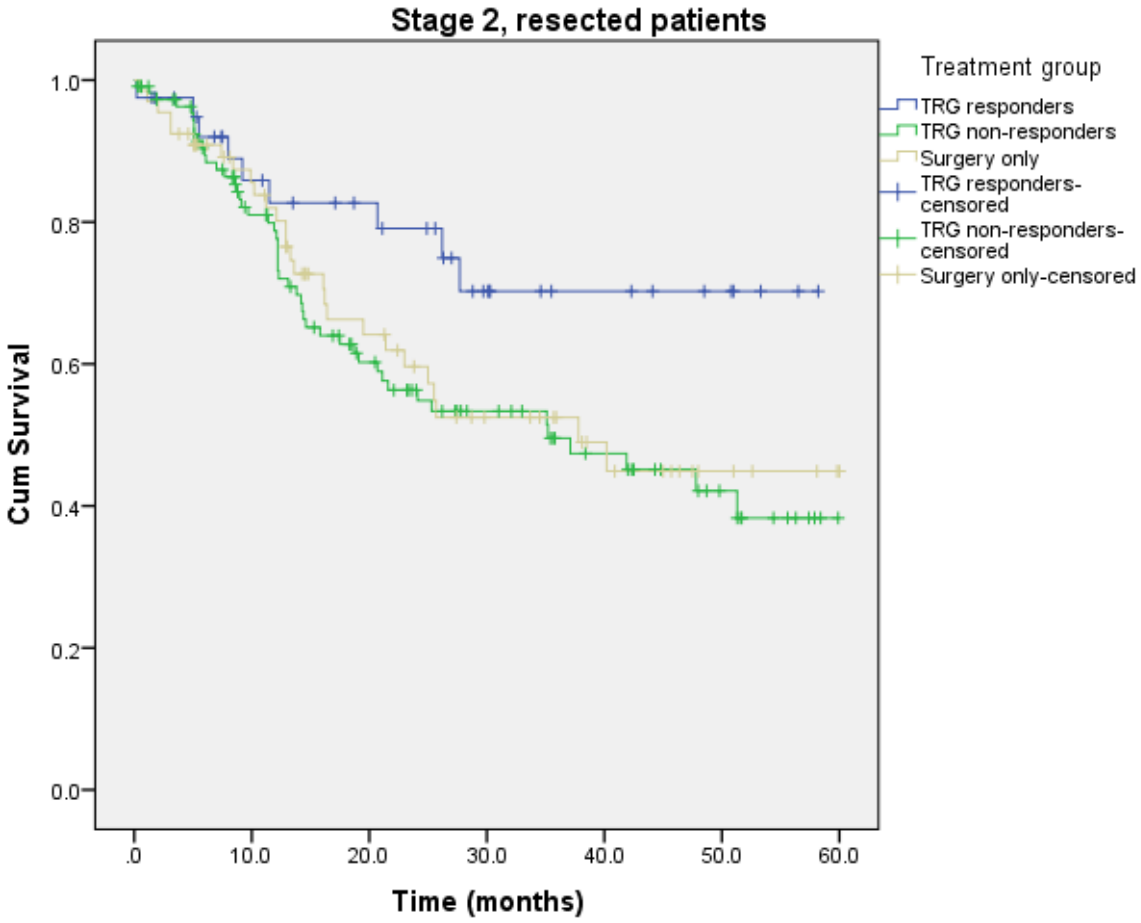

Figure 38 Kaplan-Meier estimates of survival according to treatment type and histological response to therapy in prognostic stage II patients only.
(Blue line – neoadjuvant therapy responder; green line – neoadjuvant therapy non-responder; Tan line – surgery only). Log Rank (Mantel-Cox) P=0.101.

Table 36 and Figure 39 includes only patients in stage 2 and compares survival curves in TRG responders to neoadjuvant therapy (Group Aii from Figure 32, blue line) and TRG non-responders (Group Aiii from Figure 32, green line). Survival is better in responders, Mean survival 45.2 months vs. 35.0 months, P=0.036 (Log-rank).

	n	Mean survival (months)	95% confidence interval (months)	Median survival
TRG Responders (Group Aii)	40	45.2	37.9-52.5	-
TRG Non-responders (Group Aiii)	111	35.0	30.1-39.9	35.2

Table 36 Survival according to whether resected, stage 2 patients were neoadjuvant therapy responders or non-responders.

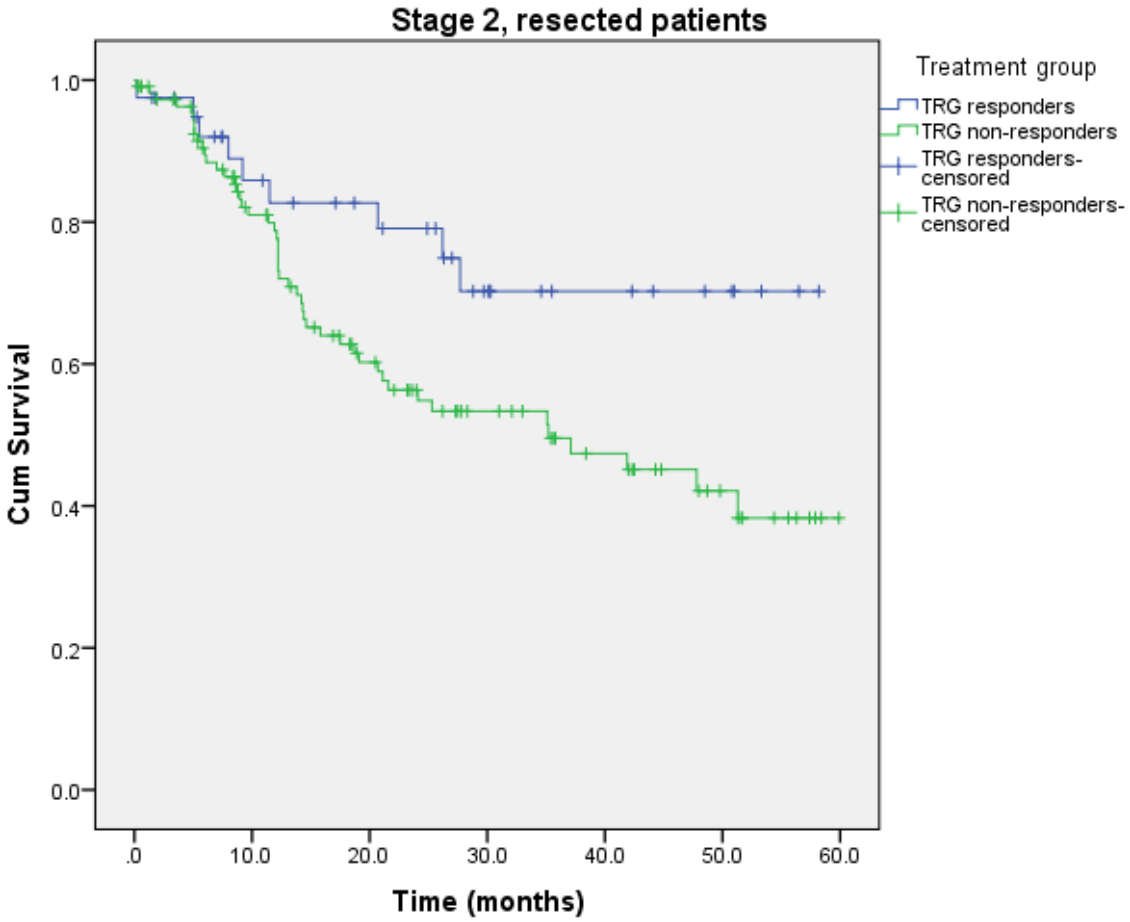

Figure 39 Kaplan-Meier estimates of survival according to histological response to therapy in prognostic stage 2 patients only.
Blue line – TRG responders (TRG1-3); green line – TRG non-responders (TRG4/5). Log Rank (Mantel-Cox) P=0.036.

Table 37 and Figure 40 include only patients in stage 2 and compares survival curves in TRG responders to neoadjuvant therapy (Group Aii from Figure 32, blue line) and patients undergoing surgery only (Group Bi, tan line). There is some evidence of better survival in responders, mean survival 45.2 months vs. 35.9 months, P=0.074 (Log-rank).

	n	Mean survival (months)	95% confidence interval (months)	Median survival
TRG Responders (Group Aii)	40	45.2	37.9-52.5	-
Surgery only (Group Bi)	66	36.4	30.1-42.8	37.8

Table 37 Survival according to whether resected, stage 2 patients were neoadjuvant therapy responders or underwent surgery alone.

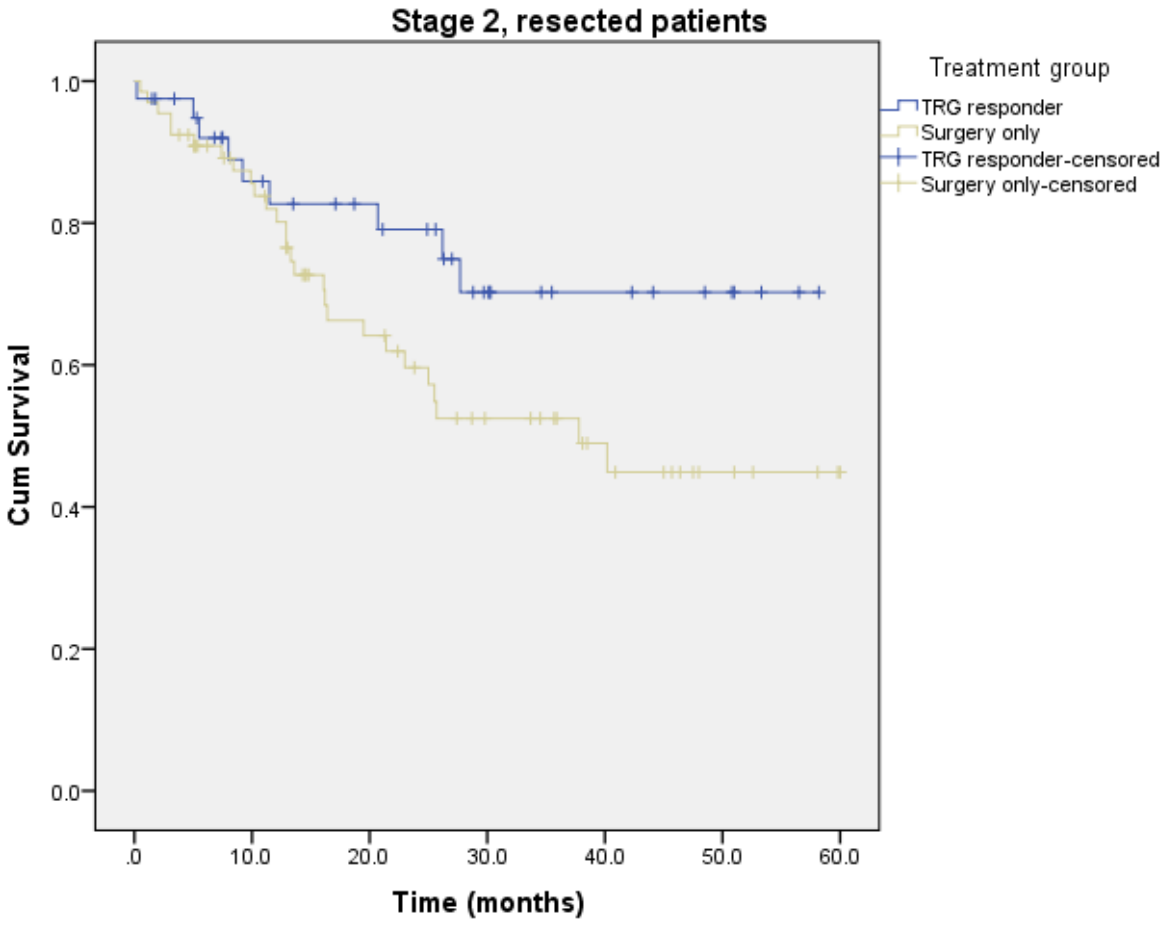

Figure 40 Kaplan-Meier estimates of survival in TRG responders and surgery only patients within prognostic stage II.
Blue line – neoadjuvant therapy responders; Tan line – surgery only. Log Rank (Mantel-Cox) P=0.074.

Table 38 and Figure 41 include patients in stage 3 and compare survival in TRG responders to neoadjuvant therapy (Group Aii from Figure 32, blue line), TRG non-responders (Group Aiii, green line) and patients undergoing surgery alone (Group Bi, tan line). Responders have a better survival than non-responders or patients having surgery only, P=0.001 (Log-rank).

	n	Mean survival (months)	95% confidence interval (months)	Median survival
TRG Responders (Group Aii)	40	51.4	45.6-57.1	-
TRG Non-responders (Group Aiii)	121	34.7	29.9-39.5	29.3
Surgery only (Group Bi)	21	25.3	16.9-33.7	26.2

Table 38 Survival according to whether resected, stage 3 patients were neoadjuvant therapy responders, non-responders or underwent surgery alone.

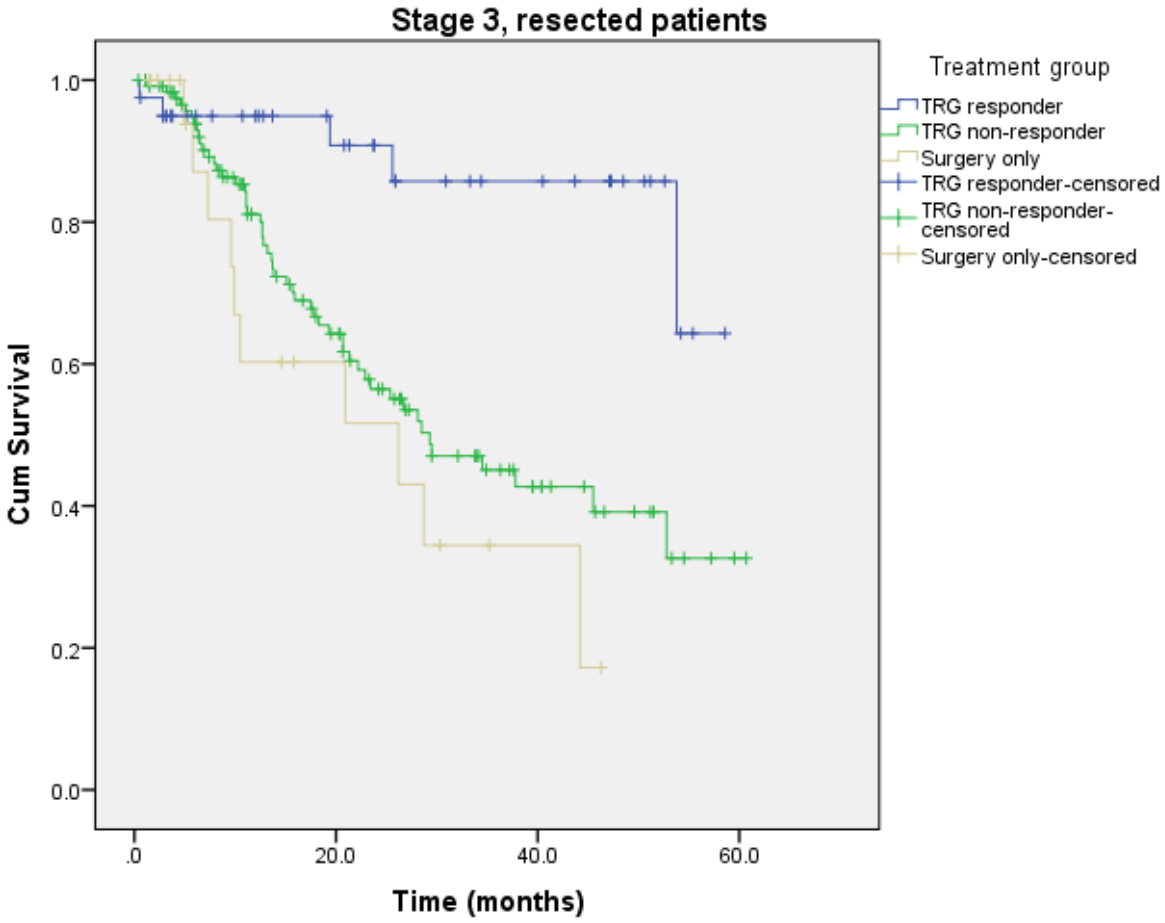

Figure 41 Kaplan-Meier estimates of survival according to treatment type and histological response to therapy in resected prognostic stage 3 patients only.
Log Rank (Mantel-Cox) P=0.001.

Overall effect of neoadjuvant therapy

Broad treatment groups and outcomes in all patients staged at or above the threshold for neoadjuvant therapy are shown in Figure 42, n=419. Patients not proceeding to resection or without post-operative pathological data available were excluded and the remaining 372 patients are detailed in Table 39 along with patient characteristics, staging information and neoadjuvant treatment details where applicable.

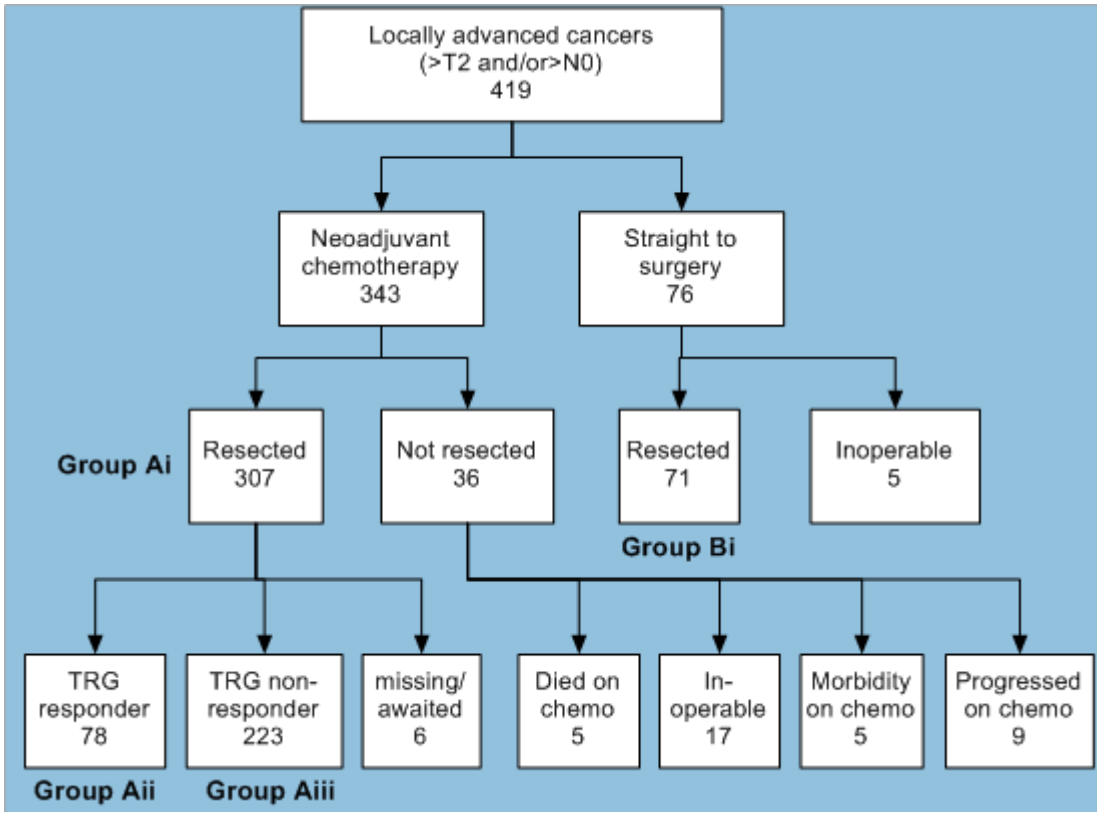

Figure 42. Treatment groups and outcomes in patients meeting threshold for neoadjuvant therapy.

Patient characteristics n= 372	Neoadjuvant therapy		Surgery only	Sig.
	Responders Group Aii, n=78	Non-responders Group Aiii, n=223	Group Bi, n=71	Group Aiii vs. Bi
Demographics				
Age in years; mean (range)	64.0 (31.5 to 82.7)	64.7 (29.3 to 80.7)	72.8 (51.3 to 85.7)	P=0.000
Male, number (%)	57 (73.1)	173 (77.6)	45 (63.4)	P=0.059
Performance score				
0	61 (78.2)	158 (70.9)	24 (33.8)	P=0.000
1	16 (20.5)	62 (27.8)	41 (57.7)	
2	1 (1.3)	3 (1.3)	4 (5.6)	
3	0	0	2 (2.8)	
Tumour characteristics				
Histological type, n (%)				
Adenocarcinoma	69 (88.5)	198 (88.8)	59 (83.1)	P=0.14
Squamous cell carcinoma	9 (11.5)	23 (10.3)	9 (12.7)	
Other carcinoma	0	2 (0.9)	3 (4.2)	
Tumour location, n (%)				
Upper third oesophagus	1 (1.3)	2 (0.9)	2 (2.8)	P=0.000
Middle third oesophagus	5 (6.4)	7 (3.1)	1 (1.4)	
Lower third oesophagus	17 (21.8)	63 (28.3)	9 (12.7)	
GO junction	47 (60.3)	124 (55.6)	29 (40.8)	
Gastric	9 (11.5)	27 (12.1)	30 (42.3)	
Pre-operative staging				
T stage, number (%)				
≤T2	5 (6.4)	25 (11.2)	8 (11.3)	P=0.36
T3	69 (88.5)	187 (83.9)	58 (81.7)	
T3/4 and T4	4 (5.1)	11 (4.9)	4 (5.6)	
Tx	0	0	1 (1.4)	
N stage, number (%)				
N0	31 (39.7)	72 (32.3)	36 (50.7)	P=0.018
N1	38 (48.7)	129 (57.8)	29 (40.8)	
N2	9 (11.5)	22 (9.9)	6 (8.5)	
Neoadjuvant regimen n				
Cisplatin/5-FU	7 (9.0)	37 (16.6)	n/a	n/a
ECX/ECF/EOX	62 (79.5)	162 (72.6)	n/a	
CROSS style	5 (6.4)	3 (1.3)	n/a	
Carbotaxol +/- epirubicin	1 (1.3)	2 (0.9)	n/a	
Missing	3 (3.8)	19 (8.5)	n/a	
Post-operative histology				
T stage, number (%)				
≤T2	48 (61.5)	34 (15.2)	18 (25.4)	P=0.003
pT3	30 (38.5)	156 (70.0)	33 (46.5)	
pT4	0	32 (14.3)	20 (28.2)	
pTx	0	1 (0.4)	0	
N stage, n (%)				
N0	47 (60.3)	54 (24.2)	25 (35.2)	P=0.32
N1	20 (25.6)	74 (33.2)	21 (29.6)	
N2	9 (11.5)	46 (20.6)	11 (15.5)	
N3	2 (2.6)	49 (22.0)	14 (19.7)	
Nx	0	0	1 (1.4)	
R0 status n (%)				
R0	61 (78.2)	108 (48.4)	45 (63.4)	P=0.07
R1	12 (15.4)	107 (48.0)	22 (31.0)	
R2	0	1 (0.4)	0	
missing	5 (6.4)	7 (3.1)	4 (5.6)	

Table 39 Characteristics and staging data in patients above the threshold for neoadjuvant therapy in those progressing to resection with pathological data available. Abbreviations: CF; cisplatin, 5-flourouracil; ECX, epirubicin, cisplatin, capecitabine; ECF, epirubicin, cisplatin, capecitabine; EOX, epirubicin, oxaliplatin, capecitabine; EOF, epirubicin, oxaliplatin, capecitabine; CROSS, as per the CROSS trial, carboplatin, paclitaxel and concurrent radiotherapy; OEO5, as per the OEO5 study (ECX, CF); ST03, ECX +/- bevacizumab; Sig, significance.

Table 40 and Figure 43 include patients above the neoadjuvant threshold, comparing survival in TRG responders (Group Aii from Figure 42, blue line), TRG non-responders (Group Aiii, green line) and patients undergoing surgery alone (Group Bi, tan line). Survival is better in responders compared to the other two groups, P=0.001, Log-rank.

	n	Mean survival (months)	95% confidence interval (months)	Median survival
TRG Responders (Group Aii)	78	48.6	43.7-53.4	-
TRG Non-responders (Group Aiii)	223	35.3	31.7-38.9	34.5
Surgery only (Group Bi)	71	31.7	25.5-37.9	25.0

Table 40 Survival according to whether resected patients above the threshold for neoadjuvant therapy were responders, non-responders or underwent surgery alone.

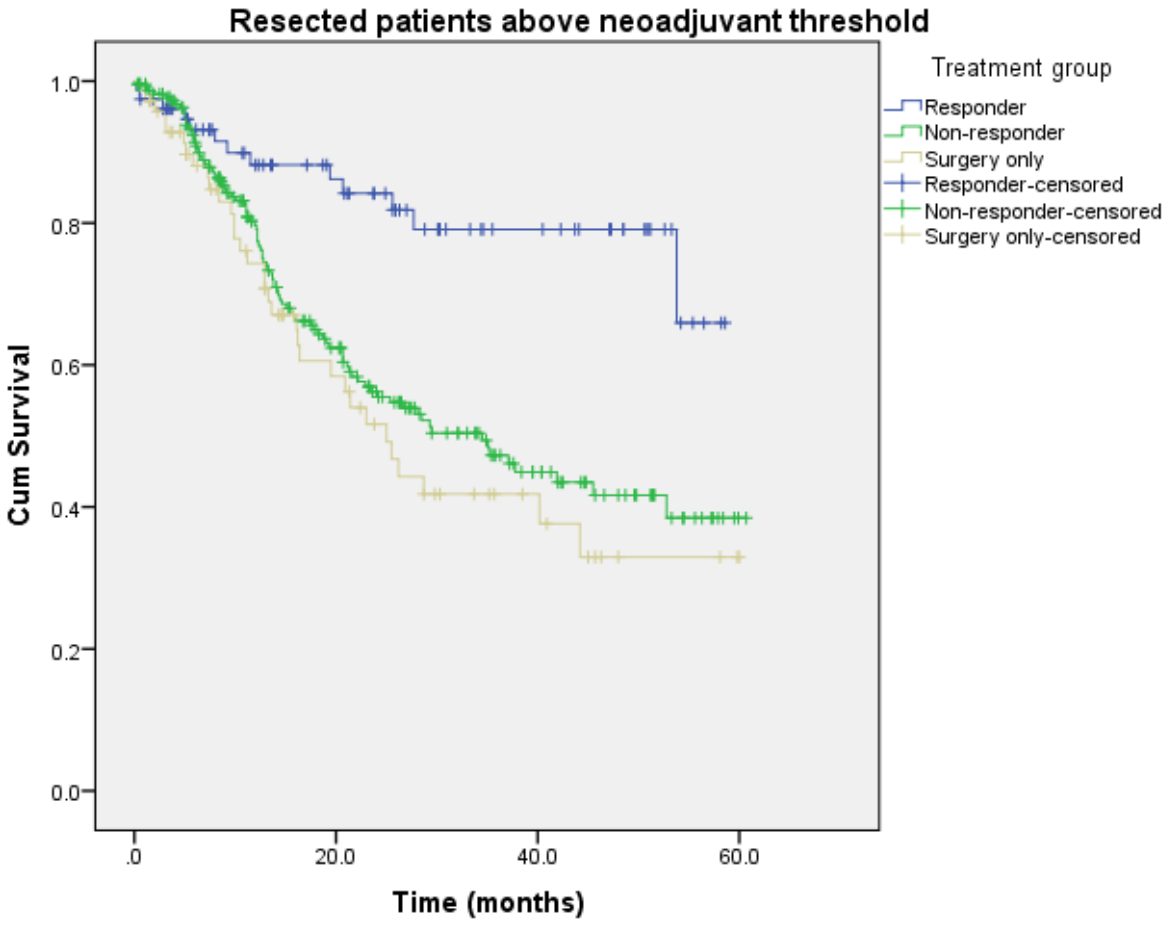

Figure 43 Kaplan-Meier estimates of survival in TRG responders, TRG non-responders and patients undergoing surgery, limited to patients above the threshold for considering neoadjuvant therapy.
Log Rank (Mantel-Cox) P=0.000.

The overall survival in patients staged above the neoadjuvant threshold was compared in TRG non-responders (Group Aiii from Figure 42, blue line) and patients undergoing surgery alone (Group Bi, green line). Results are shown in Figure 44 and Table 41. There is no difference in survival between the two groups (P=0.35, Log-rank).

	n	Mean survival (months)	95% confidence interval (months)	Median survival
TRG Non-responders (Group Aiii)	223	35.3	31.7-38.9	34.5
Surgery only (Group Bi)	71	31.7	25.5-37.9	25.0

Table 41 Survival according to whether resected patients above the threshold for neoadjuvant therapy were non-responders, or underwent surgery alone

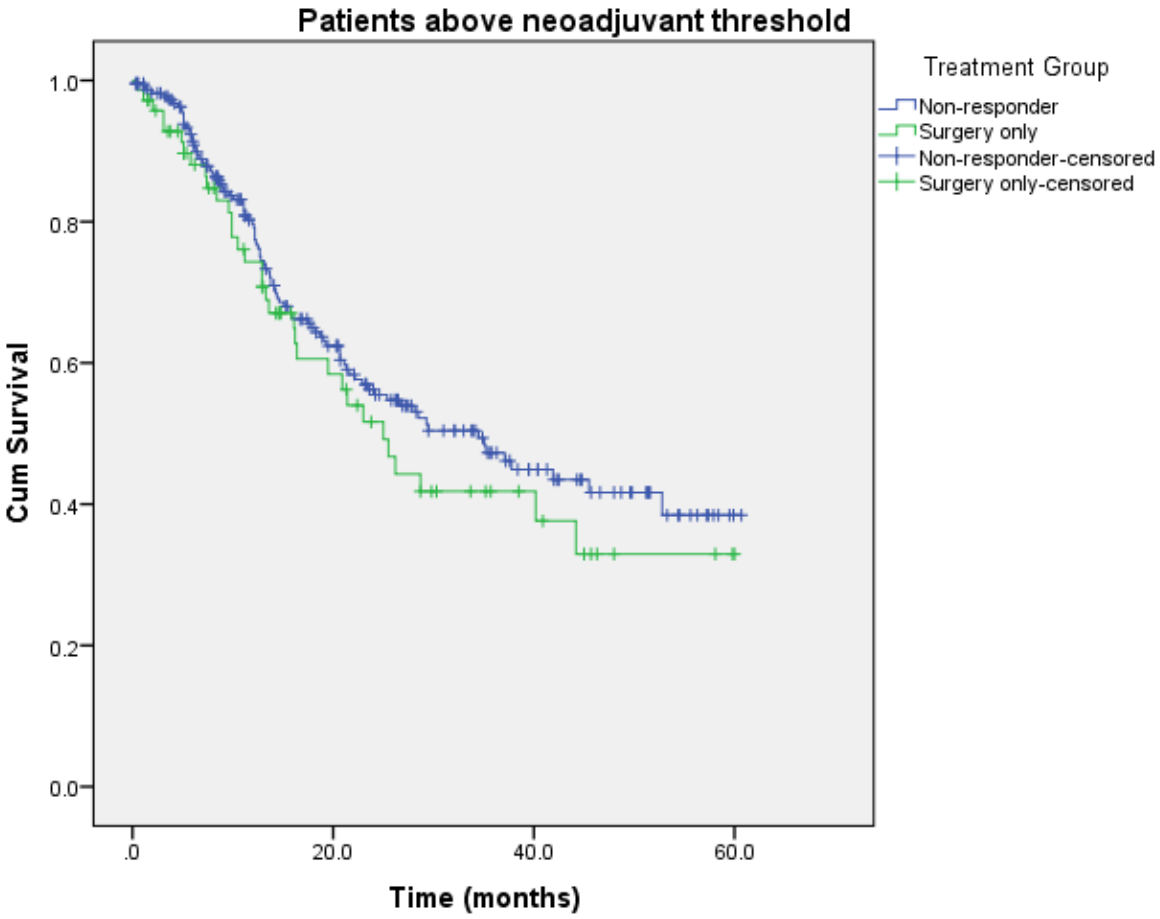

Figure 44 Kaplan-Meier estimates of survival in TRG non-responders (blue line) and patients undergoing surgery only (green line), limited to patients above the threshold for considering neoadjuvant therapy.
Log Rank (Mantel-Cox) P=0.35.

From Table 39 above, it can be seen that compared to non-responders (Group Aiii), patients undergoing surgery only (Group Bi) are older and have a higher performance score. They have a greater proportion of gastric tumours and have a lower proportion of tumours staged as N1 pre-operatively.

To identify whether any of the above factors differing between groups are associated with survival, hazard ratios (HR) were estimated using a cox regression model for the various demographic variables. Groups Aiii and Bi are presented separately (Table 42) and also combined (Table 43). The only significant factor identified was age above median in the surgery only group (Bi) associated with improved survival.

Variable		Group Aiii Est. HR (95% CI)	P value	Group Bi Est. HR (95% CI)	P value
Performance score 1, 2 or 3 vs. 0		0.85 (0.53 to 1.35)	0.49	1.31 (0.56 to 3.10)	0.52
Age (above median vs. below)		1.34 (0.88 to 2.06)	0.18	0.42 (0.19 to 0.93)	0.03
Female vs. Male		1.22 (0.70 to 2.10)	0.49	0.77 (0.37 to 1.60)	0.48
Pre-op N stage	**N0** **N1** **N2**	1.40 (0.57 to 3.41) 1.81 (0.78 to 4.21)	0.46 0.17 0.27	0.64 (0.14 to 2.89) 0.38 (0.08 to 1.93)	0.56 0.25 0.33
Tumour location	**Oesophagus** **Gastric** **Junctional**	0.94 (0.40 to 2.22) 1.48 (0.81 to 2.70)	0.26 0.89 0.21	1.45 (0.51 to 4.03) 0.86 (0.30 to 2.43)	0.42 0.48 0.78

Table 42 Estimated hazard ratios (HR) from cox regression model, non-responder and surgery only groups considered separately.

Variable		Est. HR	95% CI	P value
Performance score 1, 2 or 3 vs. 0		0.98	0.68 to 1.46	0.75
Age (above median vs. below)		1.08	0.74 to 1.58	0.98
Female vs. Male		1.10	0.72 to 1.68	0.75
Pre-op N stage	**N0**	1.38	0.65 to 2.96	0.40
	N1	1.51	0.72 to 3.14	0.27
	N2			0.53
Tumour location	**Oesophagus**			0.73
	Gastric	1.18	0.65 to 2.16	0.58
	Junctional	1.23	0.74 to 2.03	0.43

Table 43 Estimated hazard ratios (HR) from cox regression model, non-responder and surgery only groups combined.

Discussion

The AJCC Cancer Staging Manuals for cancers of the stomach and the oesophagus/oesophago-gastric junction not only describe the TNM staging system but specify ordered stage groupings into which any patient can be classified according to their individual TNM stages. These stages correspond to prognosis. There are different stage groupings in oesophageal/oesophago-gastric junction cancer according to whether histology is of adenocarcinoma or squamous cell carcinoma type and gastric cancer also has a separate stage grouping.

In our cohort, prognostic stage grouping based on preoperative clinical staging is associated with survival which is expected and suggests some degree of accuracy in clinical staging.

When patients undergoing neoadjuvant therapy and those having surgery alone are compared within each stage group, only stage 3 patients show a clear benefit from the addition of neoadjuvant therapy. However, when patients undergoing neoadjuvant therapy were further divided according to TRG response, not only do stage 3 patients benefit from neoadjuvant therapy but amongst stage 2 patients, survival was better in responders compared to non-responders (P=0.036). There was no significant difference in survival in responders compared to those undergoing surgery alone although there may be some association (P=0.062). In our cohort, there are few patients in stage 1 undergoing neoadjuvant therapy, and mortality is generally very low in this group; therefore, a meaningful comparison of survival between responders, non-responders and surgery only patients in stage 1 was not possible.

In considering the relationship between neoadjuvant therapy efficacy and stage, the inaccuracy of clinical pre-operative staging must be kept in mind. As shown earlier in

this chapter – there is a high rate of under-staging of T3N1 disease, whereby a T2/T3N0 (stage 2) patient may actually have T3N1, and therefore stage 3 disease.

These results together suggest that only patients with stage 3 disease stand to gain overall benefit from neoadjuvant therapy, however, stage 2 patients may also benefit on the basis that they may be under-staged and they could achieve a pathological response. This would support the use of neoadjuvant therapies in patients with at least stage 2 disease.

The inaccuracy of clinical staging raises another issue here. The results of this study show quite different results according to whether a patient is clinically staged as prognostic group 2 or 3. However, since staging is known to be inaccurate with lower stage patients tending to be over-staged and more advanced stage patients tending to be under-staged, we could be underestimating the magnitude of differences between stage 2 and stage 3 groups. In other words, actual stage 3 patients may have even more to gain than we think; however, actual stage 2 patients may have even less to gain from neoadjuvant therapy. If pre-operative staging accuracy was to improve and this phenomenon was observed, then this would provide a counter argument for using neoadjuvant therapy in stage 2 disease.

These arguments for and against the use of neoadjuvant treatment apply in the current situation where response to therapy cannot be predicted. If it were possible to predict response, there is a strong argument for using neoadjuvant therapy in predicted responders with stage 2 and 3 disease, with insufficient data in stage 1 patients to comment. In predicted non-responders, any potential benefit of neoadjuvant therapy has to be balanced against the potential harm caused by such additional therapy in the event of not responding. The section above on neoadjuvant therapy toxicity highlighted

the incidence and significance of therapy-associated morbidity. It is therefore important to consider whether there is any survival difference between non-responders and patients undergoing surgery only.

Survival was also analysed in patients with a pre-operative stage above the threshold for considering neoadjuvant therapy treatment in order to minimise stage differences between groups. Although responders had improved survival over non-responders and patients having surgery only, there was no difference in survival between non-responders and surgery only groups (Figure 43 and Figure 44).

This could be interpreted that that no overall harm is caused by undergoing neoadjuvant therapy in non-responders, implying that even if a means to predict therapy were available, such therapy could be given safely to predicted non-responders. Neoadjuvant therapy bears an additional cost, necessitates further investigations and introduces treatment delay. Since there is also no demonstrable benefit in non-responders; if response could be predicted, perhaps a stronger argument would be for predicted non-responders to proceed directly to surgery or alternative therapies.

The Intergroup trial reported no difference survival between non-responders and surgery only patients with median survival times of 1.1 years and 1.3 years respectively[36]. Although response was determined clinically using barium oesophagram, the findings agree with ours. In a randomised trial of neoadjuvant chemotherapy in patients with oesophageal squamous cell carcinoma, Ancona et al failed to show any overall benefit from neoadjuvant treatment. Although responders had improved survival compared to non-responders and surgery only groups, no difference in survival was shown between patients undergoing surgery only and non-responders[41].

The major limitation of comparing neoadjuvant therapy and surgery only groups in non-randomised groups has been touched on above. Even when doing so within stage groups in an attempt to reduce confounding by stage, patients may differ with respect to a number of variables. Factors of concern are likely to be those used to influence treatment decisions initially.

A comparison of demographics and tumour details between the latter two groups showed that patients having surgery only tended to have older age, poorer performance status, a higher proportion of gastric tumours and a lower pre-operative N stage although there was no adjustment for multiple comparisons. Whilst it might be expected that these factors are associated with survival, on Cox regression modelling, the only significant factor was age in the surgery only group. Of 71 patients in this group, survival was poorer in the 21 patients of the lower age group. The reasons for this are not clear but may be related to different patient characteristics between the groups that are difficult to interpret due to small group sizes or a type I statistical error, identifying an effect in the sample that is not present in the population.

The issue of neoadjuvant therapy efficacy is difficult to answer outside the context of a randomised trial. However, in addition to concerns which have been present for some time over the questionable efficacy especially in lower stage patients, there is ongoing concern that non-responders to therapy may suffer from poorer survival compared to patients having surgery alone. Given that non-responders typically make up 73-88% patients undergoing chemotherapy and 60-85% patients undergoing chemoradiotherapy, the fact that these patients may be disadvantaged by this treatment is of huge concern.

Chapter summary

There are many ways to measure response to neoadjuvant therapy but those that correlate best with survival and therefore thought to be the most valid representation of true therapeutic response involve measurement of histological regression. The Mandard and Becker systems have been validated for use in oesophageal and gastric cancer. Whilst primary tumour and lymph node down-staging have been put forward as additional methods of identifying responders, these techniques are associated with significant limitations that have been further investigated in this chapter. Such down-staging relies on pre-operative clinical staging accuracy which is poor, particularly in primary tumour (T) staging. The rate of lymph node (N) clinical over-staging was shown to be the same in non-responders and patients undergoing surgery only, indicating that this phenomenon does not represent true down-staging and is unable to identify a subgroup of partial responders amongst the TRG non-responders. We therefore defined response to neoadjuvant therapy as achieving a TRG score of 1-3 using the Mandard system which is in routine use in out unit.

23.1% patients undergoing neoadjuvant therapy suffered a moderate or severe adverse event. Such toxicity was associated with reduced survival which is thought to be largely related to failure to proceed to surgical resection. Overall 10% having chemotherapy do not proceed to surgical resection which compares to 3.3% patients undergoing surgery alone.

Whilst patients responding to therapy have the potential to benefit from it, it is not so clear whether the risks of chemotherapy and delay to surgery associated with its use will outweigh the benefits and have a negative influence on survival in patients who do not gain a histological response to therapy. It is also thought that the potential to benefit

from neoadjuvant therapy may be related to disease stage, with the risk: benefit ratio only in favour of neoadjuvant therapy in more advanced stages of disease. In our cohort, there was only a clear benefit for patients with disease stage III although it is thought reasonable to offer such therapy to those with stage II disease on the basis that they may be under-staged; they may benefit if they respond to therapy and even if they don't respond, there is as yet no conclusive evidence that they will do worse than if not having the additional therapy.

However, regardless of whether patients are stage 2 or 3, the most important factor determining outcome after neoadjuvant therapy is whether there is a histopathological response. It is conceivable that stage 1 patients may also stand to benefit if they respond to neoadjuvant therapy, particularly when considering the chance of under-estimating stage although small number of patients in this treatment group limits the analysis.

The overwhelming survival advantage of histological responders together with the poor accuracy of clinical staging on which neoadjuvant treatment decisions are made indicates that if response can be predicted then this should be used in addition to or perhaps even instead of clinical staging in the decision whether or not to offer neoadjuvant therapy.

CHAPTER 4 - INDIVIDUALISED STAGING INVESTIGATIONS

Introduction

The range of treatment modalities available means that accurate staging is necessary in order to determine whether patients should be selected for curative treatments and if so, which treatments. Accurate staging also allows comparison of different treatments and outcomes in different units on a stage by stage basis.

There is a now a wide range of techniques available for the staging of oesophago-gastric cancers including endoscopy, ultrasound, CT, PET, PET-CT, MRI, bronchoscopy, thoracoscopy, laparoscopy, endoscopic ultrasound, EBUS, endoscopic resection and many of these techniques allow the opportunity for sampling or biopsy for cytological/histological diagnosis.

Clearly it is not practical or useful for all patients to have all investigations. Each carries a healthcare cost and has the potential to introduce a delay in starting treatment whether curative or palliative. Figure 45 shows the typical staging pathway used in our unit. Some investigations are considered mandatory in all cases such as endoscopy and CT. Others are useful only in specific incidences at the discretion of a specialist Upper Gastrointestinal MDT such as EBUS with lymph node biopsy. Other techniques have been widely adopted despite a lack of evidence of their importance.

In order to standardise staging pathways, the available evidence has been reviewed and guidelines produced. However, these often carry only Grade C recommendations which are based on level IV evidence, i.e. committee reports and opinions of respected authorities or at best Grade B recommendations, based on non-randomised clinical studies[12]. Therefore, the evidence is weak and this type of clinical research tends to

consider all patients the same rather than allowing for individual factors that may be relevant.

It is widely accepted that endoscopy and CT are mandatory and that specialist investigations such as endobronchial ultrasound, MRI, thoracoscopy and endoscopic mucosal resection are used for specific indications as directed by the MDT. However, staging laparoscopy, PET scanning and EUS are used routinely (according to tumour location) in many units according to the Grade B/C recommendations without any randomised evidence of any benefit and without consideration of individual patient factors.

In the same way that there is a growing trend towards providing individualised treatment for patients, staging investigations should also be tailored to meet patients' specific needs.

This chapter explores the evidence for the most controversial of the routinely used staging investigations, staging laparoscopy and PET-CT scanning.

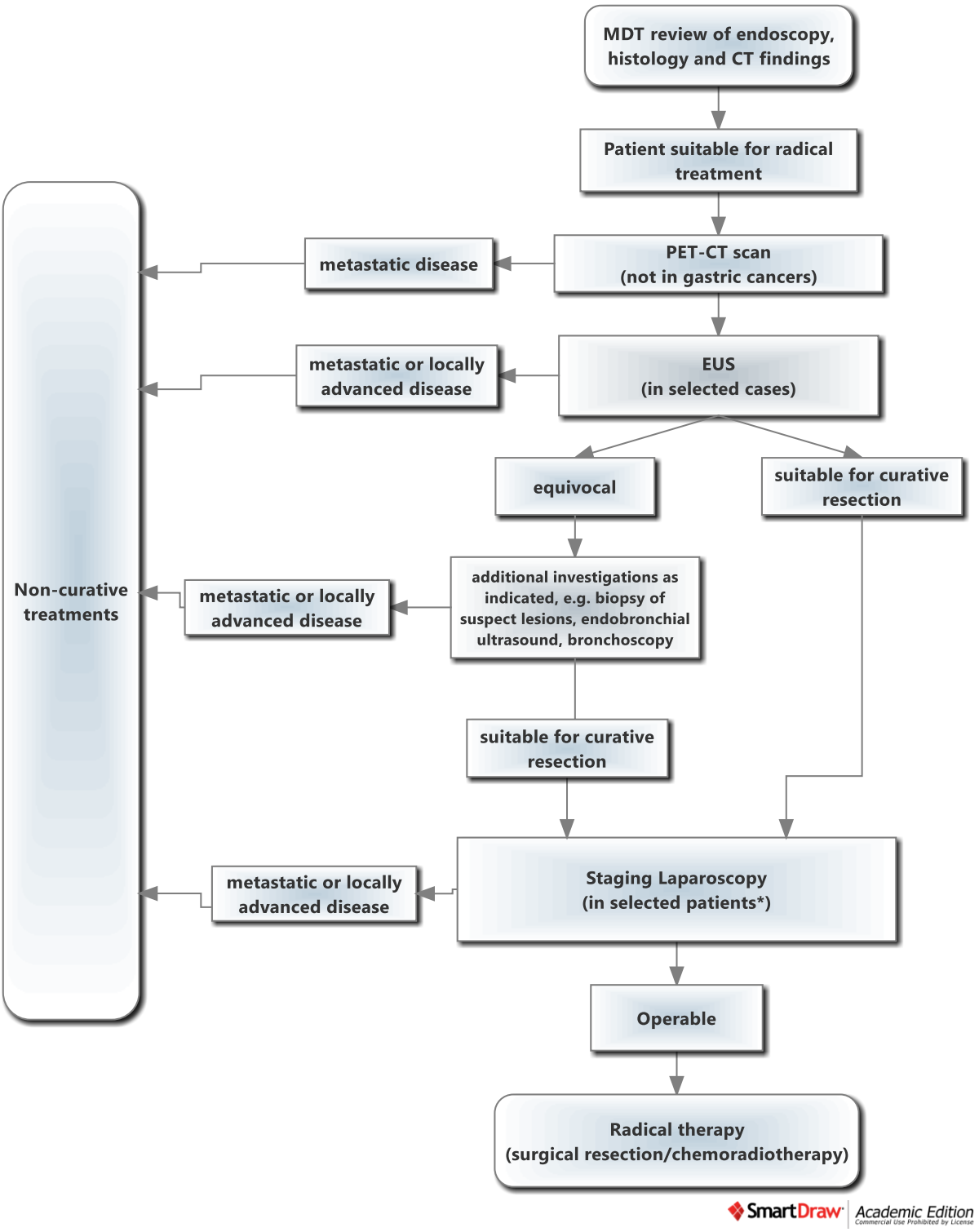

Figure 45 Flow chart showing typical staging pathway, MDT, Multidisciplinary Team.

*all patients with primary disease below the diaphragm and selected patients with nodal disease below the diaphragm.

Staging laparoscopy

Introduction

Staging laparoscopy is used in patients with potentially resectable oesophago-gastric cancer to assess operability before committing to surgical resection with or without neoadjuvant therapies. Laparoscopy can identify advanced local spread (by tumour or nodal disease) and low volume liver/peritoneal metastases not detectable on computed tomography (CT) or positron emission tomography (PET) scanning[12, 95-99]. Laparoscopy can avoid the morbidity associated with unnecessary laparotomy or radical therapies in patients with unresectable disease. The 2002 guidelines from the Association of Upper Gastrointestinal Surgeons of Great Britain and Ireland (AUGIS), the British Society of Gastroenterology (BSG) and the British Association of Surgical Oncology (BASO) suggested the routine use of laparoscopy following CT and Endoscopic Ultrasound (EUS) prior to consideration of radical resection in patients with gastric cancer and in gastro-oesophageal junctional tumours where there appears to be a gastric component[13]. Updated guidelines in 2011 suggest laparoscopy should be undertaken in all gastric cancers and selected patients with lower oesophageal/oesophago-gastric junctional cancer[12]. The guidelines do not define how to select patients for laparoscopy and this is therefore open to interpretation.

Historically, staging laparoscopy has been shown to change management in over ten per cent of patients with oesophago-gastric junctional cancer and over twenty per cent of patients with gastric cancer[98]. Whilst considered generally safe, laparoscopy is associated with complications and may delay the start of treatment in patients who go on to have neoadjuvant therapy or surgery[98, 100]. The procedure also brings an additional cost and is an inconvenience to the patient.

Advances in CT scanning technology produce higher resolution and multiplanar reconstructions that are able to stage oesophageal and gastric cancers more accurately. Consequently, studies published 10 years ago may be out of date in the context of staging with modern multi-detector scanners.

The aim of the present study was to re-evaluate the role of staging laparoscopy in the management of oesophago-gastric cancer in the context of a modern MDT environment. The authors seek to validate a classification for determining the risk of resectability in patients with oesophago-gastric cancer.

CT accuracy in T and N staging

Oesophageal Cancer

T staging

The accuracy of CT in T staging for oesophageal cancer compared to histopathological stage has been reported between 43 and 92%[101-105]. In 2010 Umeoka et al demonstrated that the accuracy of CT for T staging of oesophageal carcinoma was improved by using dual phase imaging, particularly in early cancers which are difficult to identify on CT. Overall accuracy was 68% with the arterial phase compared to 51% with traditional venous phase imaging[106].

Ba-Ssalamah et al recently evaluated the accuracy of multi-detector CT using water as negative contrast in T-staging of patients with oesophageal cancer. Accurate local staging was achieved in 76.3% and 68.7% for the two reporters[89]. Sensitivity was 95% and positive predictive value 96%.

N staging

A meta-analysis of studies published prior to January 2006 demonstrated pooled sensitivity and specificity of CT for detection of regional lymph nodes metastases of 50%

and 83% respectively[107]. More recent papers have demonstrated accuracies in N staging between 27% and 86%[101-103, 108].

CT staging of lymph nodes relies on size criteria. The threshold for consideration of malignant involvement ranges from 5-15mm with 10mm historically being the most widely used. However, lymph nodes less than 10mm can harbour metastatic disease and indeed lymph nodes of greater than 10mm may not be metastatic. Nevertheless, the possibility of involved nodal disease indicates a higher risk of having inoperable disease which is why our high risk criteria in table 1 include any patients with lymph nodes larger than 10mm or with multiple (≥3) 5-10mm nodes.

Gastric Cancer

T staging

The accuracy of CT T staging in gastric cancer has been reported as between 77% and 89%[109-114]. Makino et al compared the T-staging by multi-detector CT with operative and pathologic findings in 276 patients with gastric cancer visible on CT. Overall accuracy was 90.9% and only 3% were under-staged[90]. All patients with positive cytology or peritoneal metastases diagnosed at laparotomy had been diagnosed as T4a or deeper, so would have been stratified as at increased risk using the proposed criteria.

N staging

The accuracy of CT staging for nodal disease in gastric cancer has been reported at between 63% and 80%[110, 113, 114]. A recent prospective validation study in 315 patients with gastric cancer using MDCT demonstrated overall diagnostic accuracy for N staging of 75.9%. It is recognised that most studies in the literature have used previous versions of the AJCC staging manual. CT scanning technology continues to improve along with greater staging accuracy.

Methods

Consecutive patients diagnosed with localised oesophageal or gastric cancer over a 48-month period between 2010 and 2013 were identified retrospectively from a database of all patients discussed at a regional MDT meeting. All patients undergoing staging laparoscopy during the time period were included in the study. The Health Research Authority National Research Ethics Service deemed that ethics approval was not required for this study.

Predictive algorithm

A proposed algorithm for stratifying patients according to the likely risk of having inoperable disease was devised. In a pragmatic approach including a literature review, anecdotal experience and a pilot study in 24 patients at this institution; criteria based on endoscopy and CT findings were identified that are thought to increase the risk of finding inoperable disease. These criteria are shown in Table 44. Specifically, tumour length was included because it provides information in addition to the T stage and has been shown to be an independent predictor of long-term survival[115]. We included multiple lymph nodes (5-10mm) or any lymph node with a diameter of greater than 10mm as signs of more advanced disease that imply an increased risk of peritoneal disease. PET-CT criteria were not used in the algorithm because the investigation is not performed in most gastric cancers and the value of PET over CT is largely confined to more sensitive detection of distant metastases. EUS criteria were not used because in many centres including ours, it is being used selectively and staging is limited in obstructing lesions.

Risk of unresectable disease	Description
Increased	• Junctional tumour >3cm length on endoscopy or not traversable with scope *or* • T3/4 on CT *or* • ≥3 regional lymph nodes (5-10mm) or any lymph node ≥ 1cm *or* • Bulky gastric tumour/>4cm ulcer
Low	• all other tumours

Table 44 Risk of unresectable disease based on upper GI endoscopy and CT findings.

Patient staging

Patients underwent staging with CT of thorax, abdomen and pelvis using 64-slice multi-detector (MD) scanners on 5 different hospital sites using the same oesophago-gastric staging protocol (0.625-1.25mm slices, oral water as negative contrast and intravenous contrast with portal venous phase imaging). Staging was reported according to the AJCC 7th edition manuals for oesophageal and gastric cancer respectively. All CT scans were reviewed by at least one of four specialist upper gastrointestinal CT radiologists at a SMDT meeting. Tumours were staged using assessment criteria similar to that described by Ba-Ssalamah *et al*[89]. Specifically, T2 tumours were characterised as having thickening of the oesophageal wall of less than 15mm with slight/mild stenosis and outer borders which are smooth or show stranding for less than one third of the tumour extension. T3 lesions were represented by thickening of greater than 15mm with mild to severe stenosis and marked stranding for over one third of the tumour extension or extensive

blurring of the outer border. T4 lesions required invasion into one of the adjacent structures such as pericardium, diaphragm, pleura, tracheobronchial tree or aorta. Gastric cancers were staged according to criteria similar to those described by Makino et al[90]. Tumours appearing as minimal thickening were staged as T2, those with more demonstrable thickening of the stomach wall and a smooth outer layer with preserved perigastric fat plane were staged as T3 and those with a nodular/irregular outer border of the gastric wall or infiltration of the perigastric fat or adjacent structures were staged as T4.

Figure 45 shows the MDT pathway and the order of staging investigations used in the region. Patients underwent staging with PET-CT and EUS according to national guidelines[12]. Specifically, PET-CT was performed in all patients with potentially resectable oesophageal and gastro-oesophageal junction tumours but not gastric cancers. Operability was assessed by EUS in selective patients.

Staging laparoscopy

Staging laparoscopy was performed in accordance with current guidelines[12]. Specifically, laparoscopy was undertaken in all potentially resectable gastric cancers and lower oesophageal/GOJ cancers with a component at the level of the diaphragm. The technique included selective exploration of the lesser sac where unresectable nodal disease was suspected. Peritoneal washings for cytology were not taken due to the lack of consensus on how to interpret the result.

Patient stratification

Patients were stratified according to the risk of having unresectable disease based on the criteria shown in Table 44 without knowledge of the EUS findings, PET-CT result or staging laparoscopy outcome. Laparotomy results and the final outcome were recorded.

Staging laparoscopy results and patient outcomes were recorded and represented on a flowchart according to whether they were predicted as having a low risk or increased risk of finding inoperable disease at laparoscopy. Outcomes were also reported according to anatomical tumour location. Patients were divided into those with tumours of the lower oesophagus (tumour centre >5cm from the GOJ), gastro-oesophageal junction (component at the junction with tumour centre within 5 cm above or below the GOJ, i.e. Siewert I, II and III lesions) and stomach (tumour confined to stomach or centre >5cm below GOJ).

Results

227 patients were identified during the 48-month recruitment period. The mean age at diagnosis was 67.0 years and 74.0% were men (see Table 45). 3.1%, 59.5% and 37.4% tumours were located in the lower oesophagus, gastro-oesophageal junction and stomach respectively. Tumours staged clinically as T3 tumours made up the great majority of cancers.

Patient characteristics	N=227
Demographics	
Mean age, years (range)	67.0 (28.9-89.7)
Male proportion, n (%)	168 (74.0)
Histological type, number (%)	
Adenocarcinoma	218 (96.0)
Squamous cell carcinoma	8 (3.5)
Other	1 (0.5)
Anatomical tumour location, number (%)	
Lower Oesophagus	7 (3.1%)
Gastro-oesophageal junction	135 (59.5%)
Stomach	85 (37.4%)
Neoadjuvant chemotherapy, number (%)	
Yes	131 (57.7%)
No	96 (42.3%)
Staging laparoscopy result, number (%)	
Inoperable disease	33 (14.5%)
Operable	194 (85.5%)
T stage (clinical)	
T1/2	10 (4.4)
T2	34 (15.0)
T3	163 (71.8)
T4a	19 (8.4)
Tx	1 (0.4)
N stage (clinical)	
N0	98 (43.2)
N1	98 (43.2)
N2	30 (13.2)
N3	1 (0.4)

Table 45 Characteristics of patients undergoing staging laparoscopy. Note: percentages may not sum to 100 due to rounding.

Overall, staging laparoscopy identified inoperable disease in 33 (14.5%) patients, Figure 46.

Table 46 and Figure 46 show the outcomes of patients divided into two groups according to the predicted risk of having inoperable disease. Of the 48 patients predicted to be at low risk, none had inoperable disease found on laparoscopy and none were subsequently found to be unresectable at operation. 45 patients underwent resection,

two declined resection and one patient was eventually deemed not fit for radical therapy.

Within the 179 patients predicted to be at increased risk of having inoperable disease, laparoscopy identified inoperable disease in 33 (18.4%) patients (see figure 2 and table 3). The breakdown by anatomical tumour location is shown in table 3. Of the 33 patients with inoperable disease, according to pre-operative CT, 30 were staged as T3 or T4 and were therefore deemed before staging laparoscopy to be at increased risk of inoperable disease. 20 of these patients also had lymph node burden on CT indicating higher risk of inoperability and 8 had advanced endoscopic findings (>3cm long or not traversable) suggesting increased risk. Of the 3 patients staged as T1/2, 2 had N1 disease on CT and the other had a fundic ulcerating lesion over 4cm in length indicating increased risk of inoperable disease. Therefore, all 33 patients with inoperable disease found at laparoscopy were identified beforehand as at increased risk of inoperability according the criteria in table 1. The reasons for inoperability included liver metastases; unresectable lymph node disease; peritoneal metastases and unresectable primary tumour, Table 47. These patients were referred on for palliative therapies.

Tumour location	**Predicted risk of inoperability**			
	Low risk		**High risk**	
	Number	**Inoperable at laparoscopy**	**Number**	**Inoperable at laparoscopy**
Lower Oesophagus	2	0 (0%)	5	1 (20%)
Gastro-oesophageal Junction	15	0 (0%)	120	17 (14%)
Gastric	31	0 (0%)	54	15 (28%)
Total	48	0 (0%)	179	33 (18%)

Table 46 Predicted risk of inoperability and staging laparoscopy outcome according to anatomical tumour location.

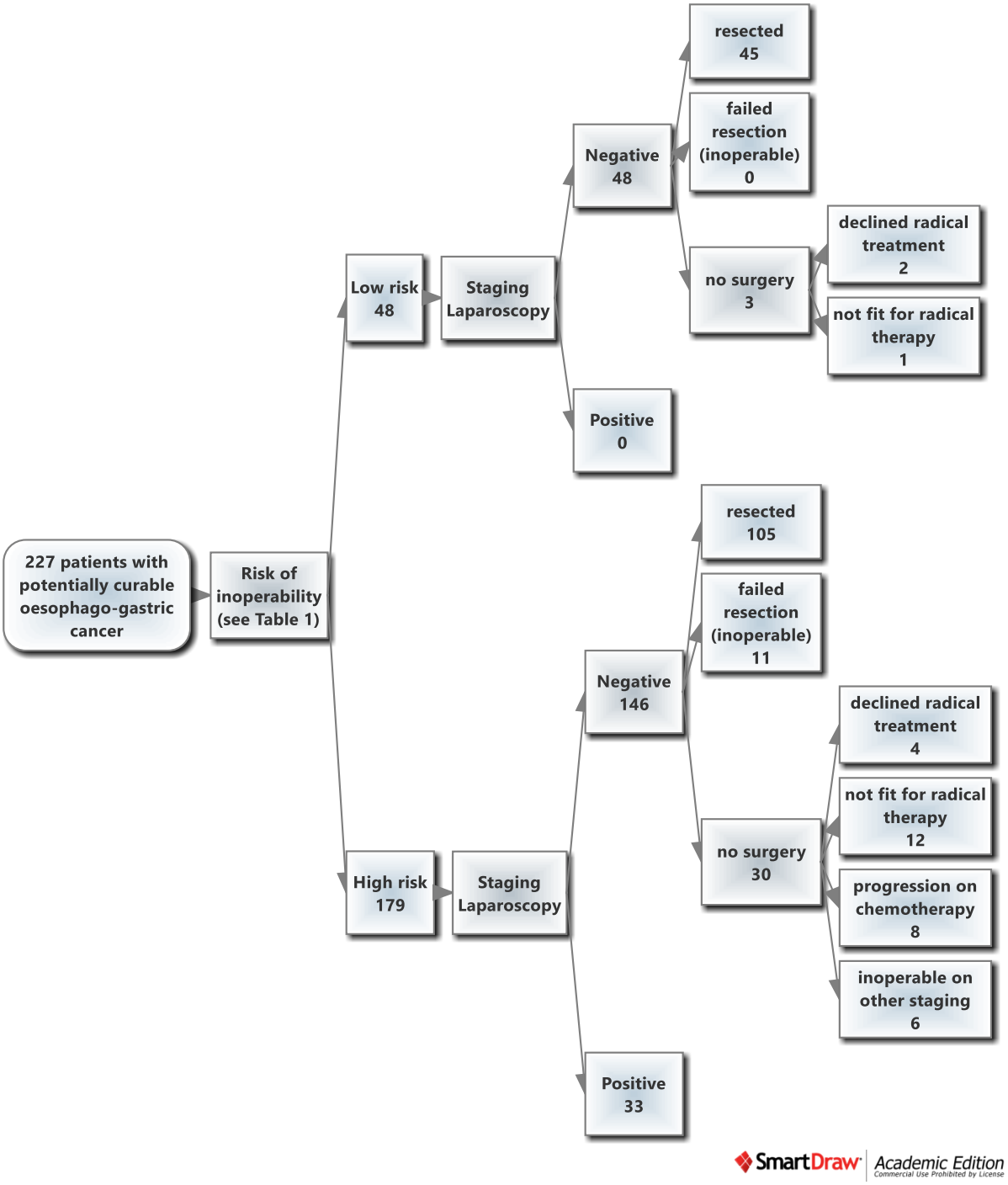

Figure 46 Flow chart showing outcomes in patients after classifying according the risk of having unresectable disease.

***Risk of unresectable disease is based on criteria from Table 44.**

Reason for inoperability at staging laparoscopy	n (%)
Liver metastases	4 (11.8)
Unresectable lymph node disease	3 (8.8)
Peritoneal metastases	17 (50.0)
Unresectable primary tumour	10 (29.4)
Totals	34

Table 47 Reason for inoperability at staging laparoscopy.

Table 48 shows the pathological TNM stage of patients according to high and low risk groups. The large proportion of patients in the high risk group without staging data represents those patients not undergoing resection.

Pathological staging		Predicted risk of inoperability	
		Low risk n=48	High risk n=179
T stage	**T0**	0	6 (3%)
	T1	17 (35%)	9 (5%)
	T2	4 (8%)	16 (9%)
	T3	19 (40%)	52 (29%)
	T4a	5 (10%)	19 (11%)
	T4b	0	8 (4%)
	Unknown	3 (6%)	68 (38%)
N stage	**N0**	18 (38%)	29 (16%)
	N1	16 (33%)	27 (15%)
	N2	9 (19%)	19 (11%)
	N3	2 (4%)	29 (16%)
	unknown	3 (6%)	75 (42%)
M stage	**M0**	45 (94%)	100 (56%)
	M1	0	52 (29%)
	unknown	3 (6%)	27 (15%)

Table 48 Pathological TNM stage according to predicted risk of inoperability.

Of 146 patients deemed to be at increased risk but having no sign of inoperable disease at laparoscopy, 116 underwent attempted resection. Of these, 105 underwent successful resection and 11 were found to be unresectable at laparotomy. This was due to direct posterior invasion into the pancreas in 5 patients, deposits on the visceral

pleura in two undergoing Ivor Lewis oesophagectomy, extranodal peritoneal spread in the lesser sac in one, direct invasion of posterior mediastinum in one, extensive direct invasion of the right diaphragmatic crus/overlying peritoneum in one and direct tumour extension into the lesser omentum in one. Outcomes in the remaining 30 patients deemed at increased risk with no sign of inoperable disease at laparoscopy and not undergoing resection are shown in Figure 46.

The algorithm can predict inoperable disease with a sensitivity of 100% and specificity of 25%; i.e. of all patients deemed resectable on laparoscopy (194), 25% (48) were correctly predicted to be resectable and could have been spared the procedure. The proposed staging pathway taking into account this algorithm is shown in Figure 47.

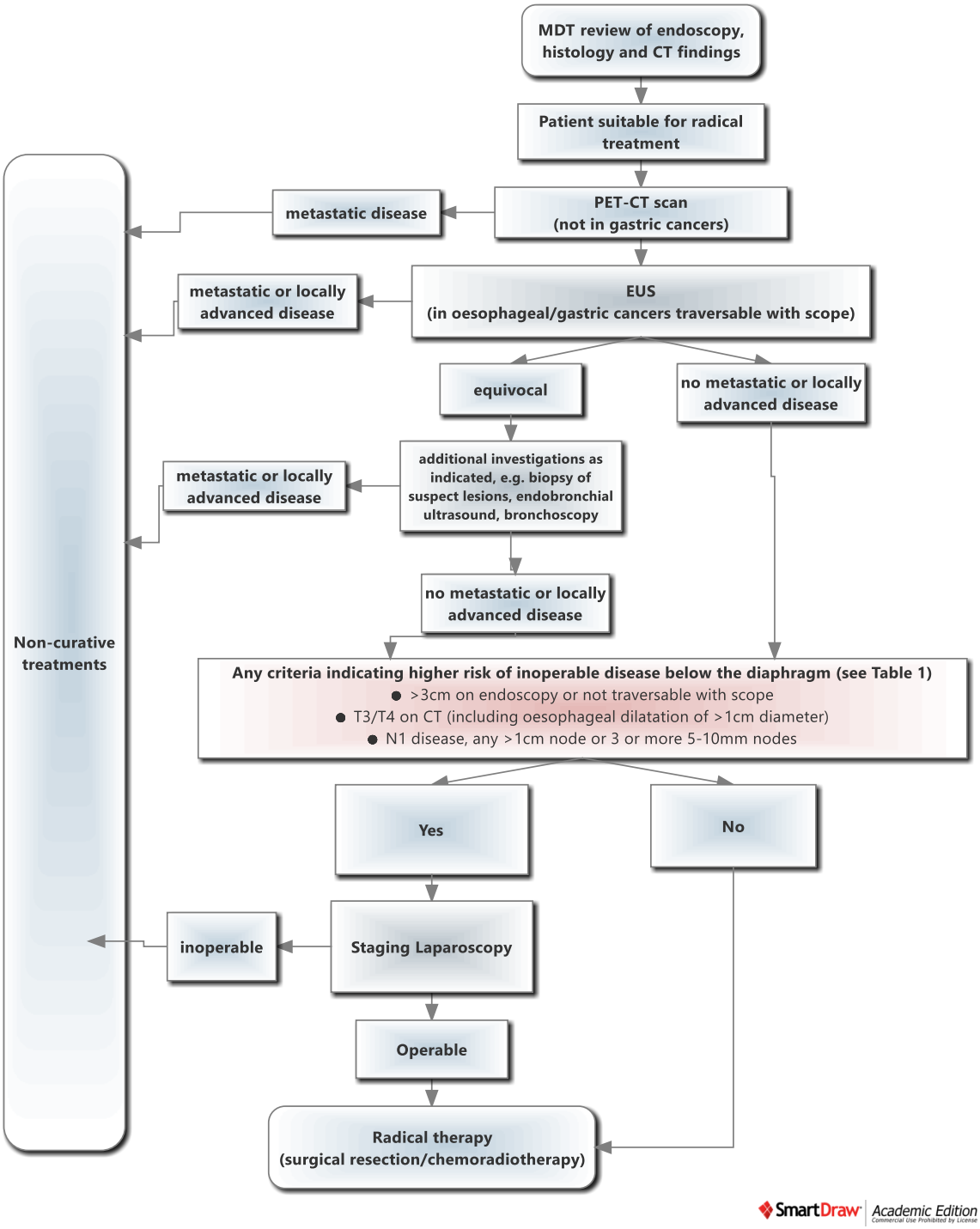

Figure 47 Flow chart showing proposed staging pathway including use of staging laparoscopy depending on specified criteria.

MDT, multidisciplinary team; EUS, endoscopic ultrasound; PET, positron emission tomography; CT, computed tomography.

Discussion

Staging laparoscopy has a role in the work-up of patients with gastric and gastro-oesophageal junction tumours, although the management of most patients will not be altered. A pilot study identified a number of pre-operative factors associated with increased risk of finding inoperable disease laparoscopy. Based on these factors, we proposed a classification to stratify risk of inoperability. This was investigated in a validation cohort and was shown to accurately predict a group of patients with low risk of having inoperable disease. 21.1% patients were predicted as low risk, all of whom underwent a staging laparoscopy showing operable disease.

We have described a heterogeneous group of patients including those with lower oesophageal, junctional and gastric cancer types that behave differently and are staged according to two different AJCC systems. None of the gastric cancers and 86% of the other groups underwent PET-CT as part of staging whereas CT was performed in all patients. For this reason, PET-CT findings were not used in the criteria for predicting the risk of unresectable disease shown in Table 44. In addition, the value of PET over CT is largely confined to more sensitive detection of patients with distant metastases who will be excluded from staging laparoscopy. PET adds no additional benefit to tumour (T) or regional lymph node (N) staging. In a pragmatic approach, the proposed criteria have been kept as simple as possible and the study aims to demonstrate its validity in all anatomical groups so that it can be applied to all patients who would normally undergo staging laparoscopy.

Richardson and Khan used a best evidence technique to answer the question of whether patients with radiologically-staged resectable oesophago-gastric junctional tumours undergoing an oesophagectomy benefit from additional useful staging information

provided by diagnostic laparoscopy[98]. They reviewed five studies, finding that laparoscopy appears to detect previously occult peritoneal metastases as well as liver metastases and lymph nodes leading to changes in management in over 10% of patients. They note that the procedure is associated with morbidity; complications included small bowel perforation, pulmonary oedema and moderate hepatic bleeding[100, 116]. They also surmise that routine PET-scanning may reduce the efficacy of routine staging laparoscopy. Only one of the five studies was published within the last 12 years so their conclusions are based on outdated CT technology. CT scanners have been replaced with newer multi-detector models in all of our regional units since 2007.

In the largest study to investigate the role of staging laparoscopy, De Graaf *et al* reported on 416 patients undergoing the procedure in two UK hospitals between 1997 and 2003[22]. A change in treatment decision was made in 17.1%, 17.2% and 28% of patients with distal oesophageal, GOJ and gastric tumours respectively. Staging was performed with CT and/or ultrasound, the latter being very poor at predicting resectability therefore many of their patients may have been under-staged. With modern staging investigations applied, more patients with unresectable disease may have been identified before laparoscopy which may explain the higher proportion of patients with a change in management in this study compared to ours. In those that did undergo CT, there is no data regarding the predicted T and N staging and it is therefore impossible to apply the proposed classification. The other 4 studies reviewed are smaller with less than 80 patients in each[100, 116-118]. Only three directly commented on changes in management which were reported in 8%, 10% and 17% of patients [100, 116, 117]. Changes in management were reported as avoiding resection due to peritoneal disease or unresectable lymph node disease in the majority and down-staging or altered choice of

operation in others. Again, no information is given on the T staging, lymph node status or tumour length.

With specific regards to the use of CT in determining the presence of peritoneal disease, in a recent UK-based study of 46 patients with gastric cancer and no direct evidence of peritoneal disease according to CT, 6 (13%) were found to have peritoneal disease following laparoscopy[96]. Of these 6 patients, according to CT, one had T3 disease, one N1 disease, one T3N1 disease and one was a perforated tumour (T4). Therefore, at least 4 of the 6 'false-negative' cases would have been categorised as moderate/high risk in this study and would have gone on to have staging laparoscopy as part of our protocol. There is no information on the other two cases. Of 67 patients in the study without CT signs of peritoneal disease and no evidence of such at laparoscopy/laparotomy, it is not known how many would have been stratified as low risk according to the proposed criteria. The authors conclude that CT is not sensitive enough to directly detect early peritoneal disease in gastric cancer, however, they indicate that staging laparoscopy is likely to be of most use in tumours staged as T3 on CT which in accordance with our results. It should be acknowledged that transcoelomic dissemination requires breach of the visceral peritoneum by the tumour itself or extracapsular lymph node spread and therefore is dependent on T and N staging. It follows therefore that T and N staging may be more useful in determining the risk of peritoneal disease or unresectable lymph node spread than direct identification of peritoneal metastases on CT scanning.

Studies indicate that staging laparoscopy can change management in more patients with gastric cancer compared to oesophageal/oesophago-gastric junctional tumours[22]. Our findings are in accord with this view (Table 50); gastric cancer has a greater propensity for transcoelomic spread.

Conclusions

Guidelines recommend the use of staging laparoscopy in patients with potentially resectable gastric cancer and in oesophageal cancers with a component at the diaphragm level in an attempt to identify inoperable disease not identified on radiological staging. We have introduced a proposed classification to stratify risk of inoperability which can accurately predict a group of patients at a low risk of having inoperable disease identified at staging laparoscopy, thereby potentially avoiding laparoscopy in 21.1% patients. Validity has been demonstrated in our patient cohort of cancers affecting the lower oesophagus, gastro-oesophageal junction and stomach.

PET-CT

Introduction

Positron emission tomography (PET) and PET-CT have been used for a number of years in the staging of oesophageal cancer primarily to rule out metastatic disease in patients suitable for radical treatment. The goal of any staging modality is to differentiate between those patients with potentially curable disease and those without, which should ultimately lead to an improvement in survival and/or quality of life.

In 2009, the Scottish National PET Advisory Group recommended the routine use of PET-CT in staging patients with potentially operable oesophageal cancer, as it can be beneficial in selecting patients for curative treatment[16]. Recent guidelines from AUGIS, BSG and BASO suggest that PET-CT scanning should be used in combination with EUS and CT for the assessment of oesophageal and oesophago-gastric junctional cancer[12]. In 2012 the Royal College of Radiologists and Royal College of Physicians published joint guidelines suggesting that oesophago-gastric cancer staging is a suitable indication for PET-CT in the UK[119].

PET-CT scanning is significantly more accurate than PET in loco-regional lymph node staging[120] and is useful in local staging when EUS is incomplete or not tolerated[121]. Studies now tend to agree that PET-CT is superior to both PET and CT in the detection of distant metastases and is therefore the current modality of choice for this purpose[122-124].

Routine PET-CT scanning has therefore become commonplace and it may have uses beyond staging. PET-CT is useful in identifying other incidental pathology such as colorectal or prostatic neoplasia. Despite guidelines and the successful uptake of PET scanning, controversy still exists over its impact on the staging of upper GI cancers since

there is a lack of data on quality of life or survival[125]. There is some debate over which isotope has the best performance, although F18-fluorodeoxyglucose (FDG) is widely available and has the most data supporting its use.

Advances in multi-detector CT (MDCT) scanning technology in recent years, with increasingly higher resolution and multiplanar reconstructions allow for more accurate staging of oesophageal cancers. The role of PET therefore needs re-evaluation in the context of staging with modern technology. There is a rising trend to provide a tailored approach to the treatment of cancer patients with regards to the requirement and timing of neoadjuvant/adjuvant therapies[44]. A similar approach should be applied to staging investigations rather than adopting a 'one-size fits all' strategy.

Compared to CT alone, PET-CT has the potential to alter the staging and change management in typically 12-18% patients[125-128]. Management in most patients is unaffected by the investigation. PET-CT incurs additional exposure to ionizing radiation, may delay definitive treatment and bears a financial cost which is significant when delivering a resource-limited healthcare service.

The aim of this study was to re-evaluate the role of PET-CT in the management of oesophageal cancer in the context of a modern MDT environment. We have investigated whether a classification based on CT and endoscopy criteria can accurately predict the PET-CT result.

Methods

CT and endoscopic criteria that may be able to stratify patients' risk of having metastatic disease on PET-CT were identified. The criteria encompass T and N staging, with additional features and are detailed in Table 49. Tumour length was included because it

provides information in addition to the T stage and has been shown to be an independent predictor of long-term survival[115]. The presence of multiple lymph nodes of 5-10mm was included because CT is increasingly able to identify sub-centimetre nodes that may be involved with tumour and increase the risk of metastatic disease. Whilst EUS can add to the accuracy of nodal (N) staging when used in addition to CT, EUS results were not used in the criteria to stratify risk because as an invasive investigation, it is usually performed after PET-CT and it is unable to fully assess patients with stricturing, non-traversable tumours.

Risk of metastatic disease	Description
Low	• ≤3cm on endoscopy, traversable with scope *and* • ≤T2 on CT *and* • N0
Increased	• >3cm on endoscopy or not traversable with scope *or* • ≥T3 on CT (including oesophageal dilatation of >1cm diameter) *or* • N1 disease, any ≥ 1cm node or ≥3 5-10mm nodes *or* • Suspicion of M1 disease

Table 49 Risk of metastatic disease based on computed tomography (CT) and endoscopy findings. 'T', 'N' and 'M' refer to the tumour, node, metastasis (TNM) staging according to the American Joint Committee on Cancer (AJCC) 7th edition manual for oesophageal cancer.

Patients

Consecutive patients with localised oesophageal or gastro-oesophageal junction malignant tumours diagnosed over a 39-month period between 2010 and 2013 were identified retrospectively from a prospectively maintained database of all patients discussed at a specialist MDT meeting. Patients with all histological cancer types were included.

Staging protocol

After histological confirmation of the diagnosis of an oesophageal or junctional cancer via endoscopic biopsy, patients underwent staging with CT of the thorax, abdomen and pelvis using multi-detector scanners on 5 different hospital sites according to the same oesophago-gastric staging protocol. This included 0.625-1.25mm slices, oral water as negative contrast and intravenous contrast with portal venous phase imaging. Staging was reported according to the AJCC 7th edition manual for oesophageal cancer. All CT scans were reviewed by at least one of three specialist upper gastrointestinal CT

radiologists at a specialist MDT meeting. Tumours were staged according to criteria similar to that described by Ba-Ssalamah *et al*[89]. Specifically, T2 tumours were characterised as having thickening of the oesophageal wall of less than 15mm with slight/mild stenosis and outer borders which are smooth or show stranding for less than one third of the tumour extension. T3 lesions were represented by thickening of greater then 15mm with mild to severe stenosis and marked stranding for over one third of the tumour extension or extensive blurring of the outer border. T4 lesions required invasion into one of the adjacent structures such as pericardium, diaphragm, pleura, tracheobronchial tree or aorta.

Figure 47 shows the MDT pathway and the order of staging investigations used in the region. EUS was used in traversable tumours to further assess T and N stage which helps determine resectability and the need for neoadjuvant therapy. PET-CT was performed in all patients with the potential for radical treatment and curative intent in accordance with current guidelines[12]. All patients undergoing PET-CT were included in the study.

Data recording and analysis

Data were recorded on dates and results of all staging investigations. Time intervals from diagnosis to CT scan and from diagnosis to PET-CT were calculated. Data were captured on any incidental pathology identified by PET-CT.

Patients were stratified according to the risk of finding inoperable disease on PET-CT based on the criteria shown in without knowledge of the EUS or PET-CT results. The results of the PET-CT scan results and patient outcomes were recorded.

It is not possible to calculate sensitivity or specificity of PET-CT for detection of distant metastases since the true positive and true negative numbers are impossible to define for any study such as this.

Results

Patients and demographics

383 patients undergoing PET-CT were identified. Mean age at diagnosis was 66 years and 74% were male. Patient characteristics are shown in Table 50.

Patient characteristics	n=383
Demographics	
Age, in years; mean (range)	66.4 (31.2 to 85.4)
Male; n (%)	285 (74.4)
Histological type; n (%)	
Adenocarcinoma	305 (79.6)
Squamous cell carcinoma	75 (19.6)
Adenosquamous carcinoma	3 (0.8)
Anatomical tumour location; n (%)	
Upper third oesophagus	7 (1.8)
Middle third oesophagus	43 (11.2)
Lower third oesophagus	163 (42.6)
Gastro-oesophageal junction	170 (44.4)
Treatments	
Curative - Surgery only; n (%)	74 (19.3)
• Resected	69
• Inoperable	5
Curative - Neoadjuvant therapy and surgery; n (%)	187 (48.8)
• Progressed/inoperable after chemotherapy	20
• Chemotherapy morbidity preventing surgery	7
• Resected	158
• Unknown	2
Curative – Chemoradiotherapy; n (%)	26 (6.8)
Not fit for radical therapy; n (%)	14 (3.7)
Declined radical treatment; n (%)	8 (2.1)
Palliative; n (%)	68 (17.8)
Local therapies; n (%)	6 (1.6)
T Stage (on staging CT)*; n (%)	
≤T2	140 (36.6)
T3	220 (57.4)
T3/4 and T4	19 (5.0)
Tx	4 (1.0)
PET-CT result; n (%)	
Metastatic disease	52 (13.6)
No metastatic disease	331 (86.4)

Table 50 Characteristics of all patients undergoing positron emission tomography-computed tomography (PET-CT) scan.
***'T' refers to tumour staging according to the American Joint Committee on Cancer (AJCC) staging manual 7th edition for oesophageal cancer.**

Outcomes according to PET-CT result

Overall, PET-CT identified possible metastatic disease in 71 (18.5%) patients. Table 51 shows the anatomical distribution of metastases and Table 52 shows the outcomes according to PET-CT result. 52 patients had metastases that were unequivocal or confirmed on biopsy. They were not offered radical treatment (surgery or chemoradiotherapy) and were referred for palliative options. Of these patients, according to pre-operative CT, 51 of these patients were staged with at least T3 or N1 disease. The remaining patient had a long stricturing lesion on endoscopy. Therefore, all 52 patients with metastatic disease on PET-CT were identified as being at increased risk according to the criteria in Table 49.

Whilst metastases were unequivocal in some, others underwent further investigation to confirm or refute the presence of metastases. M1 disease was disproven in 19 patients. Of these, 3 progressed on neoadjuvant therapy; 4 had unresectable disease diagnosed on other investigations (staging laparoscopy/EUS); 7 were not fit for radical therapy; 4 had radical treatment (2 chemoradiotherapy and 2 surgery); and 1 patient declined radical treatment.

Of the 312 patients without suspicion of metastatic disease on PET-CT, 22 underwent radical chemoradiotherapy; 8 declined radical treatment; 6 underwent local therapies; 9 had unresectable disease diagnosed by other means (staging laparoscopy (7), EUS (1), CT (1)); 20 were not fit for radical therapy; 225 underwent resection; 13 had inoperable disease at the time of surgery and 9 patients had progression of disease on neoadjuvant therapy (Table 52).

Distribution of Metastases	M1 disease suspected	
	M1 confirmed	M1 disproven
Lymph nodes	31	9
Bone	11	3
Liver	6	1
Adrenal	2	2
Other	2	4
Totals	**52**	**19**

Table 51 Distribution of metastases and PET-CT results.

Main outcome	PET-CT result			Totals
	M0	M1		
		M1 confirmed	M1 disproven	
Surgical resection	225	0	2	225
Planned resection - inoperable	13	0	0	13
Declined radical therapy	8	0	1	9
Radical chemoradiotherapy	22	0	2	23
Local therapies	6	0	0	6
Not fit for radical therapy	20	0	7	26
Progression of disease on neoadjuvant therapy	9	0	3	12
Unresectable disease diagnosed by other means (CT, laparoscopy or EUS)	9	0	4	13
Palliative options	0	52	0	52
Totals	**312**	**52**	**19**	**383**

Table 52 Patient outcomes according to initial PET-CT results.

Outcomes according to predicted risk of metastatic disease

83 (21.7%) patients were predicted as low risk and 300 (78.3%) as increased risk (Figure 48). Table 53 shows the breakdown according to anatomical location. Within the low risk group, none had metastatic disease on PET-CT. Within the high risk group, 52 (17%) patients had metastatic disease on PET-CT. A further 46 (15%) patients developed progression of disease during neoadjuvant treatment or went on to have a failed resection due to inoperable disease (see Figure 48).

Lymph node staging and neoadjuvant therapy decision making

Nodal involvement was identified by PET-CT in 127 of 383 patients. 34 of these patients did not have enlarged lymph nodes on CT. Management in these patients were analysed further to identify whether PET-CT contributed to decision making regarding chemotherapy. Of the 34 patients (N0 on CT and N1 on PET-CT), 25 were categorised as at increased risk of metastatic disease on the basis of the primary tumour size or endoscopy criteria and would therefore have had PET-CT under the criteria anyway. Of the remaining 9 patients, 5 did not undergo chemotherapy despite PET-CT suggesting N1 disease, one underwent radical chemoradiotherapy and the remaining 3 had chemotherapy based on EUS findings.

Time to PET-CT

Median time from diagnosis to CT scan was 11.5 days and from diagnosis to PET-CT was 23.6 days.

Patients not proceeding to radical therapy

Overall, in patients not undergoing radical therapy after PET-CT (130), this was due to inoperable disease seen on the PET-CT in 40% (n=52). 20% (n=26) were unfit for surgery or declined surgery; 35% (n=46) had inoperable disease or progressed on neoadjuvant therapy and 5% (n=6) underwent local therapies.

PET-CT identification of incidental pathology

PET-CT identified possible incidental pathology in 43 (11.2%) patients (data not shown in tables or figures). These patients underwent further examination or investigation leading to benign or normal findings in 39 and to neoplastic diagnoses in 4 (1.0%). In two of these patients, a colonic adenomatous polyp was removed. In one patient, a sigmoid colon cancer was identified and simultaneous Ivor-Lewis oesophago-gastrectomy and

sigmoid colectomy were performed. One patient had a PET-avid pelvic lymph node which was excised and proved to contain metastatic prostate adenocarcinoma.

Tumour location	Predicted risk of metastatic disease			
	Low risk		Increased risk	
	Number	Metastatic disease on PET-CT	Number	Metastatic disease on PET-CT, number (%)
Upper third oesophagus	0	0	7	3 (43)
Middle third oesophagus	8	0	35	9 (26)
Lower third oesophagus	45	0	118	19 (16)
Junctional tumours	30	0	140	21 (15)
Total	**83**	**0**	**300**	**52 (17)**

Table 53 Predicted risk of metastatic disease and positron emission tomography-computed tomography (PET-CT) outcome according to anatomical tumour location.

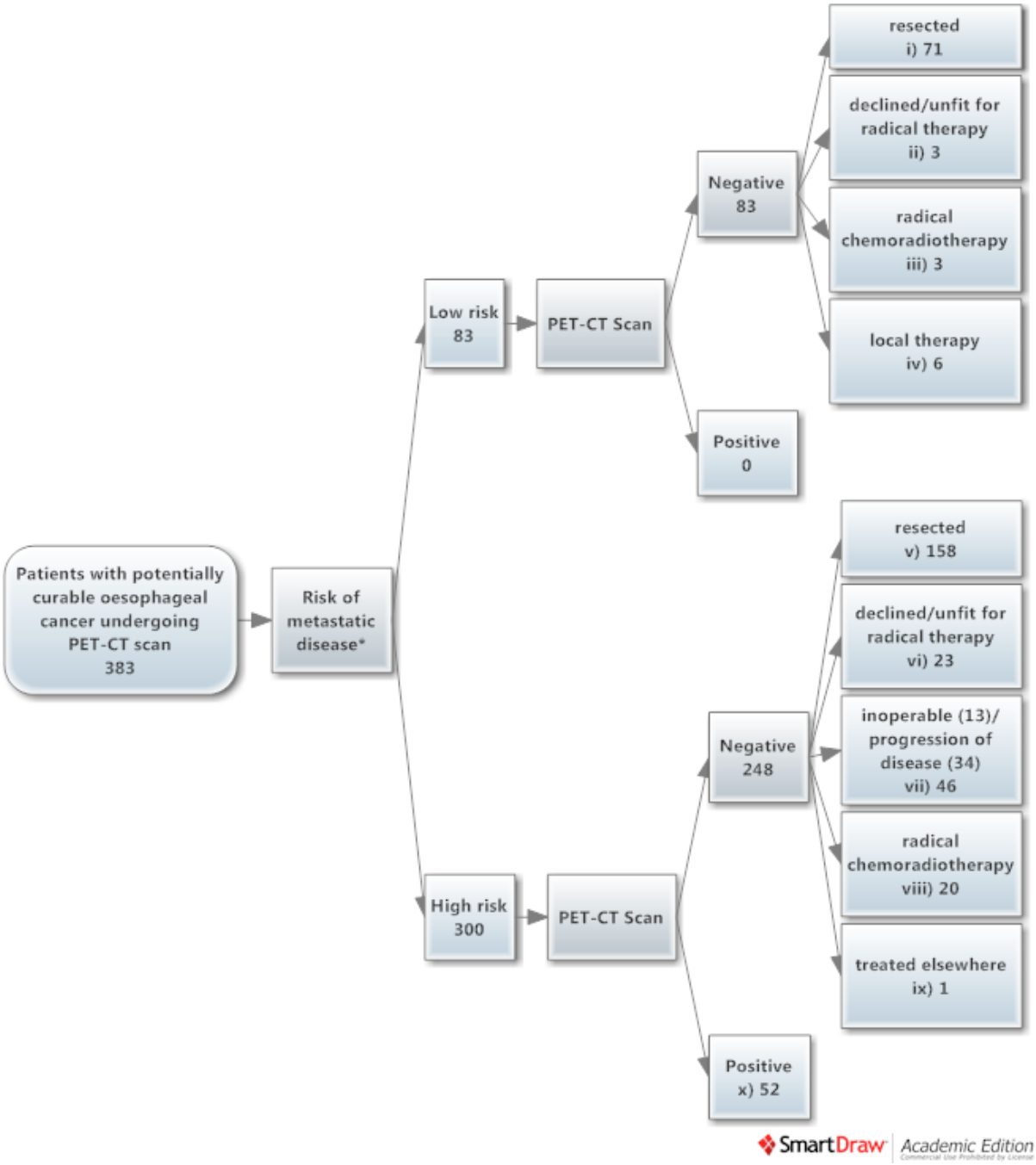

Figure 48 Flow chart showing outcomes in patients after classifying according to the risk of having metastatic disease.

PET, positron emission tomography; CT, computed tomography. *Risk of metastatic disease predicted according to criteria in Table 49. Outcomes are numbered i to x for reference in the text.

Discussion

PET-CT has an important role in the staging of patients with oesophageal cancer, however, only a minority will benefit. We review the evidence for use of PET-CT in the management of oesophageal cancer within a modern MDT environment and seek to validate a proposed classification for stratifying patients according to the risk of having metastatic disease.

A multicentre, prospective, UK study examined the role of PET-CT in the staging of 191 patients with oesophageal cancer[126]. Metastatic disease was identified by PET-CT and confirmed in 9.4% patients overall which included 13% patients undergoing selective PET-CT. The criteria for selective PET-CT were not reported. In the 85% patients undergoing PET-CT routinely, metastatic disease was confirmed in 5%. When subdivided by CT/EUS staging, metastatic disease was found in 13%, 0%, 6% and 0% of patients with stage 3/4, stage 2b, stage 2a and stage 1 disease respectively. Results concur with ours demonstrating that early tumours infrequently show evidence of metastasis on PET-CT. The authors suggest further data are required to determine in which patients PET-CT has no additional value.

Berrisford *et al* assessed the role of PET-CT in staging 50 patients with oesophageal/oesophago-gastric junctional tumours[127]. Patients were assigned to one of two groups according to CT and EUS staging (group A CT N0M0, group B CT N1 and/or borderline M1). PET-CT re-categorised 6 (12%) patients as inoperable based on the presence of distant metastases. Four of these patients were from the group without confirmed nodal or suspected metastatic disease. It is not known whether these patients had any characteristics that could have predicted the likelihood of metastatic disease.

In a study of 199 patients with oesophageal cancer from the Netherlands, the addition of PET to staging investigation led to surgery being avoided in only 6 (3%) patients, all of whom had clinical stage III-IV disease before PET. No patients with stage I-II disease went on to have distant metastases on PET scanning[129]. These results support the notion that early tumours rarely demonstrate metastatic disease on PET scan and casts doubt over the suggestion that all patients should undergo PET scan as part of staging.

A recent study from Bristol by Blencowe *et al*, [128] investigated the influence of PET-CT on decision making in MDTs. M1 disease was identified in 43 of 238 (18%) patients. Tumour stage was not reported so it is not known whether patients upstaged on PET-CT would have been identified as at high risk according to our criteria. Whilst MDT recommendations were said to be changed in 91 patients, 23 of these were due to incidental findings which were of minimal consequence and 25 were due to refuting CT suspicion of M1 disease which arguably may not have altered the key management decisions. This study was not designed to investigate which patients stood to gain the most from PET-CT scanning. It did raise the important issue of how to evaluate the influence of PET-CT on decision making. The authors correctly recognise that many studies do not define what a change in management constitutes. The majority of studies do report the frequency of finding previously undetected metastases which is the most important factor in determining suitability for radical therapy. The authors also suggest calculating survival in patients assigned to groups according to the influence of PET-CT but this will not answer the question of whether PET-CT improves outcomes.

None of the above studies recognise that the ultimate aim of improving survival/quality of life can only be demonstrated by comparing patients that undergo PET-CT as part of staging with control patients that don't and few have attempted to address this. The

increase in PET/PET-CT scanning has been supported with guidelines now recommending routine use so there may be a reluctance to perform controlled trials. There have been no randomised studies to date. A good example of such study is the pragmatic randomised EUS trial, COGNATE (Cancer of Oesophagus or Gastricus - New Assessment of Technology of Endosonography) which showed that EUS improves survival and has the potential to reduce health-care resources[17]. This demonstrates that randomised study designs can be performed in a modern MDT environment.

An important study from the Three-Counties Cancer Network used a control group to investigate whether integrated PET-CT improved staging, reduced early recurrence or increased survival in oesophageal cancer[130]. Patients were retrospectively divided into two groups according to whether they had undergone PET-CT or not. Early recurrence and survival rates were the same despite more patients in the PET-CT group undergoing neoadjuvant chemotherapy (as a result of change in routine practice over time). PET-CT was responsible for upstaging only 4% patients and down-staging 5.5%. In the 5.5% patients down-staged, it is not clear whether the suggestion of metastatic disease on CT was clear enough to deny patients radical treatment if PET-CT were not performed but regardless this seems a good indication for the use of PET scanning. Inaccurate identification of occult metastatic disease prior to the introduction of PET-CT did not appear to be the primary cause of early recurrence in their patients. The study compared two patient groups treated over different time periods. Although multiple potential confounding factors make comparison unreliable, if the findings are realistic then doubt is cast over the true influence of PET-CT on early recurrence and survival.

When comparing the pre-PET-CT MDT recommendation with the post-PET-CT recommendation, (a recognised method of evaluating the influence of PET-CT[128], our

MDT recognised a problem common to all studies above but not addressed by any. The recommendation before PET-CT may be influenced by the knowledge that a PET-CT is planned. Specifically, if it is known that a PET-CT will be performed, the MDT may be more likely to suggest a patient is suitable for radical treatment. In one study, three quarters of the advanced disease found on PET-CT had already been identified by other staging investigations even though PET-CT was said to be restricted to those without metastatic disease[130].

Furthermore, patients who are not fit for surgery or who have signs of advanced disease on other staging modalities may be undergoing PET unnecessarily. This is implied by the high percentage of patients that have a negative PET-CT but do not proceed to surgical resection or radical therapy, 24% (78 of 331 patients) in our study (represented by outcome groups ii, iv, vi and vii from Figure 48. This compares to 26-28% in the Bristol study[128] and 35% in the study by Noble *et al*[126]. When considering all patients not undergoing curative therapy (surgery/CRT) after PET-CT scan (groups ii, iv, vi, vii and x from Figure 48), the reason for this was due to advanced disease seen on PET-CT in only 39.4% in our study (represented by group x from Figure 48) and 23% to 35% in others[126, 130]. The remainder of patients not undergoing surgery in our study were unfit for surgery, declined surgery, underwent local therapies, had inoperable disease identified through other investigations or progressed on neoadjuvant therapy. The main intended use of PET-CT is to rule out incurable disease in patients suitable for radical therapy, however, many patients deemed curable do not end up undergoing radical therapy. A positive PET-CT can make decision making easier for patients with borderline fitness for surgery and if all patients were to undergo formal cardiopulmonary

investigations/exercise testing before PET-CT scanning, staging and treatment could be delayed, however, a negative PET-CT at this time is frequent (86.4%) and unhelpful.

In our study, the median time from diagnosis to CT was 11.5 days and from diagnosis to PET-CT was 24 days. This compares to median times of 11 days and 35 days in patients from the Three-Counties Cancer Network study[130]. A concern is that having a PET-CT scan will risk a delay in definitive treatment.

False positive rates for PET-CT have been reported at between 1.5% and 7.5%[126, 129-131]. In these cases, metastatic disease suggested by PET-CT has been subsequently disproved after further investigations or MDT discussion. This limitation of PET-CT is sometimes poorly quantified and the true false positive rate often unknown since positive PET-CT results are not always challenged[128]. This may lead to over-staging and withholding potentially curative treatments. Biopsy-confirmation of metastases identified by PET-CT in selected/all cases has been discussed without consensus[128, 131, 132]. A balance may need to be struck between avoiding unnecessary confirmatory investigations and ensuring that patients are not over-staged.

PET-CT offers the potential to identify incidental pathology that may change the management with regards to the oesophageal cancer or the patient in general. In our cohort, 11.2% (43/383) had uptake in other organs and 1.0% (4/383) of patients had incidental neoplastic disease confirmed. This compares to 6.6% and 1.6% respectively in the study by Noble *et al*[126]. In the three-counties study, 8% patients had uptake in other organs all of whom were shown to have benign conditions not requiring treatment[130]. Therefore, although incidental neoplastic disease can be identified in a small percentage of patients and other treatments are occasionally undertaken, the poor prognosis of

oesophageal cancer is such that this dictates outcome (survival) rather than any incidental disease.

PET usage is commonplace; however, as part of routine staging, its influence on management may be minimal and restricted to those patients with advanced stages of disease. Whilst it is becoming clear that patients with less advanced cancers may not benefit from undergoing a PET scan, no method for accurately identifying such patients is currently available. The authors have developed criteria based on endoscopy and CT findings that can accurately identify a proportion of patients (22%) in whom PET scan will be negative for metastatic disease and could be spared this investigation. Of the 52 patients with metastatic disease demonstrated on PET, none were categorised as having a low probability of metastatic disease.

It may be argued that PET can identify nodal disease not reaching CT criteria for lymph node metastases. This could impact on the decision to give neoadjuvant therapy. However, in our series none of the patients that were upstaged on PET and would not have had a PET under the new criteria had a decision to give chemotherapy based on the PET result. Therefore, limiting the use of PET-CT in this series would not have changed the decision making process regarding neoadjuvant chemotherapy.

Financial implications must be considered with the routine use of PET-CT in the staging of patients with oesophageal cancer. Whilst a reduction in unnecessary operations as a result of identifying occult metastatic disease may lead to cost savings, the investigation itself bears a cost which could be reduced by restricting its use to those with a realistic chance of benefit.

This study is limited by its retrospective design. A digital, centralised radiology package gave us reliable CT reports, but endoscopy results were only recorded on local systems leading to some missing data. It can be difficult to determine the true treatment intent and MDT recommendation before PET-CT scan in an era when the investigation is used routinely. In our study, as in others, the false positive rate/specificity of PET-CT has not been quantified since biopsy confirmation of positive lesions is not performed in all cases. Likewise, it is not possible to calculate sensitivity of PET-CT in detecting metastatic disease but the treatment outcomes in patients are shown in order to give an idea of the influence of PET-CT results on patient management.

In order to demonstrate its external validity, this classification needs to be applied in other institutions to be sure that similar results can be achieved in the presence of locally variable factors such as radiologist reporting and CT technology.

Conclusions

PET-CT undoubtedly makes a contribution to the staging of patients with oesophageal cancer and guidelines recommend its routine use in patients with potentially curable disease. The investigation is useful to confirm suspected sites of metastases and in localising the most suitable sites for biopsy. Unfortunately, only a small proportion of patients benefit from the information provided by PET-CT. In the largest study of this kind to date, the authors re-evaluate the use of PET-CT in the staging of oesophageal cancer and introduce a classification based on endoscopy and CT findings that can predict a group of patients who will not benefit from PET-CT scanning and could be spared this investigation.

Summary

The risk-stratification criteria and results above were presented to the MDT business meeting on 24th July 2014. The criteria will now be applied to patients discussed at the forthcoming MDT meetings and decisions regarding whether to perform staging laparoscopy or PET scan will be made accordingly. Results will be monitored over the next 12 months to audit the adoption of this service improvement tool and to ensure its safety.

CHAPTER 5 - PREDICTION OF NEOADJUVANT THERAPY RESPONSE: CLINICAL REVIEW

Introduction

In Chapter 1 it was shown that outcomes in oesophago-gastric cancer are poor. Neoadjuvant therapy has been used in an attempt to improve survival. The widespread adoption of neoadjuvant treatments was initially hampered by conflicting results of randomised trials[26, 27]. A large US trial[36] and an updated Cochrane review[29] failed to demonstrate any significant benefit from the use of pre-operative chemotherapy. However, two important trials, the MRC OEO2 study[28] and the MAGIC trial[25], have demonstrated a benefit associated with the use of pre-operative chemotherapy. The lack of benefit in non-responders may explain why initial trials failed to demonstrate any overall benefit[26, 36]. Trials in chemoradiotherapy have suffered from similar limitations but again recent reports indicate that pre-operative chemotherapy is likely to be associated with a benefit in patients with oesophageal cancer[31-33]. As a result of the more recent studies above, guidelines now suggest using neoadjuvant chemotherapy/chemoradiotherapy in patients with oesophageal cancer and chemotherapy in patients with gastric cancer[12]. As a result, these multimodality treatments have become the standard of care in the UK.

Whilst neoadjuvant treatment has been shown to improve outcomes, typical 5-year survival rates of 23%-47% are still poor[12, 25, 34]. The findings shown in Chapter 3 that only responders to neoadjuvant therapy gain any benefit may be partly responsible for this[36, 40, 41]. A wide range of response rates is quoted in the literature with complete response rates of 0-13% [40-44, 78] and complete/near-complete responses of 11-40%[36, 40, 44]. Poor overall survival in patients despite the use of neoadjuvant therapy may be due to poor survival in non-responders that make up the majority. The wide range in reported

response rates may be due to patient factors or differences in treatment but could also result from the varying methods of defining response. The latter was addressed in the previous chapter and response has been re-defined in our population. Though there are many ways to measure response to therapy, it must be remembered during this review that those methods relying on histological analysis have been shown to be the most reliable and correlate best with survival.

Despite not gaining any benefit from neoadjuvant therapy, non-responders are still exposed to the risks of therapy and face a delay to potentially curative surgery. These factors together with the observations above have led to a number of authors calling for research into how the response to neoadjuvant chemotherapy/chemoradiotherapy can be predicted in patients with oesophageal and gastric cancer[24, 38, 41, 49, 51, 54, 56, 133, 134].

Predicting response

The earlier in a patient's treatment pathway that response can be predicted, the greater the potential to influence changes in management. Any factors identifiable before starting neoadjuvant therapy are truly predictive. These could be used to change management in predicted non-responders by directing clinicians towards second-line chemotherapy regimens or by avoiding potentially harmful neoadjuvant treatment altogether and proceeding directly to surgery. Patients predicted to respond may even be considered for having all 'peri-operative' cycles of chemotherapy up front, before surgery although there is no evidence to support this as yet.

Early assessment of response

Early assessment of the response to neoadjuvant treatment (after treatment has been started) can be thought of as an in vivo way of assessing chemo-sensitivity and could

also influence a change in treatment at this stage although it would not have all the benefits of pre-therapy prediction[135].

Measuring response after treatment

Finally, response can be assessed after completion of neoadjuvant treatment. Although too late to influence chemotherapy, it may give valuable information on prognosis and influence whether or not to proceed with attempted curative resection.

Biomarker research

What is a biomarker?

The National Institute of Health Biomarkers Definition Working Group defined a biomarker as a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention"[136]. Matuszcak *et al* go further to suggest this definition implies it should be easily detectable and measurable in the relevant patient samples; be consistently expressed and refractory to degradation in these samples in order to allow proper analysis on clinical samples[137].

What are the ideal properties of a biomarker?

Fareed *et al* state that the ideal predictive marker should be reliable, readily available and detectable by reasonably acceptable laboratory techniques[57]. In a commentary for the Journal of the National Cancer Institute, Pepe *et al* suggest that the ideal marker should be sensitive, specific and cost-effective[138].

Biomarker development

In order to define the process of biomarker development; to set standards for development and to standardise development, organisations have described the

process using a number of phases. In 2001, The Early Detection Research Network (EDRN) backed by the National Cancer Institute established a development sequence of five phases of biomarker development for cancer diagnosis/screening[138], see Table 54.

Phase	Description
I	Preclinical exploratory studies
II	Clinical assay development for clinical disease
III	Retrospective longitudinal repository studies
IV	Prospective screening studies
V	Cancer control studies

Table 54 Phases of biomarker development according to the Early Detection Research Network (EDRN).

In a further news article published in the Journal of the National Cancer Institute in 2004, it was acknowledged that development of biomarkers used as prognostic indicators is much more complicated than for markers used strictly for detection because there is an additional step in the validation where the relationship between biomarker and outcome has to be investigated[139]. Not only is the association important, but the issue of causation and a true biological effect has to addressed. A separate process was described by the American Society of Clinical Oncology's tumour marker guideline committee which includes three phases and is more relevant to prognostic/predictive markers[140], see Table 55:

Phase	Description
I	Identification of potential association between marker and outcome
II	Measurement of association between marker and outcome
III	Randomized marker validation trial

Table 55. Phases of biomarker development according to the American Society of Clinical Oncology[140].

Whilst the process of biomarker development has been described in phases as above, put simply, it is clear that there are two main aspects of development, namely biomarker discovery and biomarker validation. Discovery aims to identify potential candidate markers and validation aims to independently establish accuracy and clinical usefulness.

Biomarker discovery

There are multiple approaches being used to identify potentially useful biomarkers. These approaches can be classified into two groups:

Hypothesis-driven approaches

Firstly, knowledge-based methodologies focus on specific markers or pathways and can include seeking novel applications of existing biomarkers; investigating new biomarkers and hypothesis-driven immunohistochemical techniques[134].

Examples of existing markers include routine haematological/biochemical parameters measured in peripheral blood such as white cell count or alkaline phosphatase, which have recently been examined for their predictive and prognostic potential.

Biochemical research frequently identifies new factors such as cell surface antigens, receptors or intracellular enzymes. If these are differentially expressed in cancer patients or tissues compared to controls this will stimulate interest in them as potential screening or prognostic markers. Any molecular factors identified from pathways involved in carcinogenesis will be subject to interest as potential diagnostic/prognostic

markers or targets for therapy. Similarly, with specific regards to potential markers of chemosensitivity/chemoresistance, molecular cancer research has identified a large number of markers that function in pathways related to the mechanism of action of anti-cancer therapies. Such markers are often seen as having predictive potential and are investigated accordingly.

Therapeutic trials are often used as a means of retrospectively investigating the value of such potential markers since issues of ethical approval, consent, sample acquisition and data collection are already covered.

Mass discovery approaches

Secondly, high-throughput or 'fishing' approaches such as gene expression RNA profiling, single nucleotide polymorphism (SNP) array analysis, microRNA expression analysis and proteomics have been used to screen for potentially predictive biomarkers[80, 134, 141-145].

Biomarker validation

Biomarker development can be lengthy, costly and complex. The key to successful biomarker development is validation and although the term is used frequently and there are much written on the subject, it is poorly understood[139]. It is beyond the scope of this chapter to enter a lengthy discussion on the concept of validation, however, it is important to understand that once the relationship between a biomarker and outcome has been investigated and normal marker ranges/thresholds set as necessary, the marker needs to be tested in a further prospective validation cohort. Whether a predictive marker can achieve a difference in outcome by changes in management can then only be identified as part of a randomised trial. The process of biomarker

development requires collaboration between clinicians, biologists and statisticians which to date has been limited[138]

Aims

This chapter aims to provide an up to date summary of the published literature on predicting response to neoadjuvant therapy in oesophago-gastric cancer. This is separated into two distinct parts. Firstly, in Part 1, a review of clinicopathological and radiological predictors is presented. In Part 2, molecular biomarkers derived from blood serum/plasma (known as circulating biomarkers) and those derived from tumour tissue and are summarised.

Part 1: Clinical, pathological and radiological markers

Methods

A MEDLINE search using the PubMed service was performed using the terms esophageal cancer, oesophageal cancer, gastric cancer and predicting response to neoadjuvant therapy, chemotherapy or chemoradiotherapy. Papers including clinical, pathological and radiological criteria were reviewed. Summaries of potential clinicopathological and radiological predictors are presented, the latter presented according to radiological modality.

Results

Clinicopathological markers

Ajani *et al* devised a model based on clinicopathological parameters to predict pathological response in 322 patients undergoing preoperative chemoradiation for oesophageal cancer[49]. The following parameters were identified as contributing to the model: the post-chemoradiation biopsy, post-chemoradiation PET, sex, histologic

tumour grade and baseline T stage (Endoscopic Ultrasound). The area under the receiver-operating characteristic curve was 0.70; however, they admit that other parameters (biomarkers) would be required to accurately predict response. 3 of the 5 parameters were measured after chemoradiation treatment which limits its predictive nature and clinical usefulness.

A further study identified nutritional status, T stage, M stage and alkaline phosphatase (also considered as a circulating molecular marker, see below) as significant factors that contributed independently to the response of oesophageal cancer to chemoradiotherapy[146]. Other factors were associated with response on univariate analysis but lost significance on multivariate analysis including body mass index, N stage, and tumour length. Odds ratios of the four independently predictive factors were approximated and scored. The overall response score was able to differentiate between responders and non-responders and a high score was associated with a greater chance of complete response (72.7% vs. 14.8%, $P<0.001$). In this study chemoradiotherapy was with palliative/curative intent rather than neoadjuvant and no patients went on to resection. Response was measured radiologically using the WHO criteria.

MacGuill *et al* investigated a wide range of pre-treatment clinicopathologic factors in 176 patients undergoing neoadjuvant chemoradiotherapy for oesophageal cancer in Dublin and showed that only tumour length differed significantly between responders and non-responders to chemotherapy[147]. A smaller tumour length was predictive of a greater response to chemotherapy ($p<0.05$). They concede that results may indicate existing dose and treatment schedule are inadequate in larger tumours rather than representing different tumour biology and a true increased responsiveness to therapy in smaller tumours.

Improvement in dysphagia has been investigated by two studies regarding its potential to predict the pathological response to chemotherapy/chemoradiotherapy. Neither study demonstrated significant predictive ability[60, 148].

The National Centre of Tumor Diseases in Heidelberg evaluated the prognostic significance of various clinicopathological parameters in 410 gastric cancer patients treated with neoadjuvant chemotherapy[149]. Multivariate analysis identified three parameters associated with improved response to therapy and prognosis. These were tumour localization in the middle third of the stomach (P=0.001), well-differentiated tumours (P=0.001) and intestinal tumour type (P=0.001). A scoring system was developed from these parameters separating patients into low, medium and high risk groups. Suggestions of how this information should be used to alter management are not given only to suggest the system needs to be prospectively validated in another cohort. Even if patients deemed at high risk were considered for alternative strategies, this group only accounts for 15.4% patients. Even in the intermediate and low risk groups, 75% and 67% patients respectively did not respond to chemotherapy indicating that the system is not sensitive in identifying non-responders.

Other authors have suggested that tumour subtypes based on anatomical location may behave differently in many ways including response to therapy, however, it is suggested that these subtypes may have less relevance to systemic chemotherapy treatment compared to for example surgical approach and pattern of recurrence[38]. A study in 1775 patients with cancers of gastric, junctional and oesophageal subtypes demonstrated no significant difference in response rate to palliative chemotherapy between the three types[150].

Brown *et al* investigated the value of endoscopic assessment of tumour regression after neoadjuvant chemoradiotherapy in patients with oesophageal cancer. Endoscopy can predict some complete responders but it is not sufficiently accurate to be used for excluding patients from further treatment on the basis of inadequate response[151].

Radiological criteria

CT

Westerterp *et al* published a systematic review on the use of CT (and EUS/PET, see below) to assess response to neoadjuvant therapy using histopathological response as the reference standard[58]. CT has limited sensitivity (33%-55%) and specificity (50%-71%), the authors concluding that CT has poor accuracy for assessment of response when compared to EUS or PET.

More recently, Motoori *et al* assessed the use of CT in the early evaluation of tumour response to neoadjuvant chemotherapy (after completion of the first cycle of neoadjuvant chemotherapy)[152]. Patients with a >20% decrease in the size of the primary tumour were defined as early responders and the remainder as non-responders. 20% patients were classified as responders. The progression-free survival and response to 2nd chemotherapy cycle were poorer in the non-responder group. In addition to non-responder status, clinical T3 stage was an independent predictor or poor survival. In non-responders with T3 disease survival was poor and no difference was observed between those undergoing one or two cycles of neoadjuvant chemotherapy therefore authors suggest discontinuation of neoadjuvant chemotherapy in early non-responders with T3 disease according to CT.

To summarise the use of CT in assessment of chemotherapy response, it is becoming clear that progression of disease on CT after completion of neoadjuvant chemotherapy is usually associated with a very poor histopathological response and overall prognosis which presents a strong argument in such cases for considering avoidance of surgery[38]. However, the value of CT in early assessment of response and determining whether patients should continue neoadjuvant chemotherapy is not proven.

Endoscopic ultrasound

The main role of EUS before commencing neoadjuvant chemotherapy is in accurate determination of the T-stage and this may be used in part to determine whether a patient is offered neoadjuvant chemotherapy although not on the basis of whether or not a patient is likely to respond but rather whether it is required at all.

In Westerterp's systematic review, EUS was show to be more accurate than CT in the assessment of response to chemotherapy, with sensitivities of 50%-100% and specificities of 36%-100%[58].

EUS is an invasive procedure, its use is not as widespread at CT and it is not possible to pass the tumour in a proportion of cases, limiting its usefulness. As with endoscopy and CT, at best EUS could be used in the early assessment of response to therapy which has already started rather than acting as a predictive test before initiation of treatment.

Metabolic criteria (FDG-PET)

Metabolic imaging (FDG-PET) currently provides the most promising data in the prediction of histopathological response to neoadjuvant therapy using radiological means.

Single scan

The ability of absolute pre-treatment PET to predict response is unclear. Studies have shown that initial maximum standardized uptake value (SUVmax) is unable to predict response to chemotherapy[153-155]. In a study of patients with oesophago-gastric junctional adenocarcinoma undergoing neoadjuvant chemotherapy absolute SUVmax levels from scans performed 2 weeks after initiation of therapy and preoperatively did not demonstrate a significant correlation with histopathological response or survival.[155] One study suggests that the number of PET abnormalities (reflecting the regional nodal metastases) correlates with overall survival but not pathologic response[156].

Serial scans

A number a reports have investigated the use of serial FDG-PET measurements in predicting the pathological response to chemotherapy. These have been reviewed by Bain and Petty in 2010[38].

The strongest evidence supporting serial PET in the predicting histological response comes from the Munich group. A reduction in metabolic activity (SUV decrease ≥35%) 14 days after initiation of chemotherapy was shown to correlate with histopathological tumour regression, a higher rate of curative resections and longer survival[157, 158].

The Metabolic response evalUatioN for Individualisation of neoadjuvant Chemotherapy in esOphageal and esophagogastric adeNocarcinoma trial (MUNICON) assessed the feasibility of an algorithm based on PET response[159]. Metabolic responders underwent further chemotherapy and non-responders went straight to surgery after the initial 2 weeks' chemotherapy. The metabolic responders did have a higher histopathological response rate, however 50% of those predicted to respond did not and therefore did not receive any clinical benefit from neoadjuvant therapy. The hazard ratio for survival between those with a PET response and histopathological response and those with a

PET response but no histopathological response was 4.55 (95% CI, 1.37-15.04; *p*=0.004). This demonstrates clearly that the histopathological response is still a stronger indicator of clinical outcome and that PET is only partly able to predict this.

In the protocols above, chemotherapy has to be given for two weeks before any predictive value is gained. One study performed PET on day 7 after initiating chemotherapy and found no correlation between the change in SUV from baseline and the TRG[160]. Delaying the PET until 3 months after starting chemotherapy or after completion does not result in a better correlation with histological response suggesting that 14 days is the optimal period[155]. The optimal timing may be dependent on the type of chemotherapy regime used.

It has been hoped that similar results could be achieved with the use of neoadjuvant chemoradiotherapy rather than chemotherapy. The Munich group showed similar results with chemoradiotherapy using a threshold of 30% reduction in SUVmax for patients with oesophageal squamous cell cancer[161]. In a study from the Netherlands, SUV decrease at 14 days was associated with histopathological tumour response but the accuracy in detecting non-responders was too low to justify the use of PET for early discontinuity of chemoradiotherapy[162]. Other centres have failed to demonstrate any positive results[163, 164]. In the study by Malik *et al* from Dublin, the negative results may have been due to differences in the chemotherapy part of the regime which ceased at day 6, allowing time for an inflammatory radiation response to develop in the oesophagus and obliterating any potential reduction in SUV values by the time of the PET scans at day 9-14[163]. This study included only adenocarcinoma whereas the Munich study included only squamous cell cancer which could also explain the differing results. However, a further study including both histological types demonstrated a higher

metabolic response to chemoradiotherapy in adenocarcinoma compared to squamous cell carcinoma and different optimal threshold values[165]. This adds further complexity to interpretation of studies that include differing cancer subtypes and typifies the difficulties in appraising such literature.

A systematic review and meta-analysis of prediction of tumour response to neoadjuvant chemotherapy/chemoradiotherapy in patients with oesophageal cancer using PET was published in 2010[166]. It reviewed 20 studies totalling 849 patients including both adenocarcinoma and squamous carcinoma types. Pooled sensitivity and specificity were 67% and 68% respectively. The area under the ROC curve was 0.7815. They concluded that PET should not be used to guide neoadjuvant therapy decision in patients with oesophageal cancer.

PET Parameters

Rather, than simply the maximal tumour SUV, other parameters have been considered as potentially more useful in prognostic scoring and may be more powerful predictors of chemotherapy response. The number of baseline PET abnormalities (reflecting the regional nodal metastases) was significantly associated with overall survival[156]. A separate study showed that post-chemoradiotherapy uptake in a focal distribution compared to diffuse uptake predicted residual disease when maximal tumour SUV and length of uptake did not suggesting that this qualitative measure may be a more accurate predictor of response to therapy[167]. A recent paper found the post-chemoradiotherapy metabolic tumour volume correlated with the TRG score but that the maximum tumour SUV did not[153].

Although PET shows some promise in predicting the response to neoadjuvant therapy, there are clearly outstanding issues that need to be overcome before it could be used

reliably in the clinical setting to make treatment decisions. Many issues relate to heterogeneity between studies and these would need to be addressed with standardisation of various factors including PET acquisition, image analysis, optimal PET parameter, chemotherapy/radiotherapy regime and tumour subtype. These could be overcome with a well-designed multicentre study and followed by randomised trials in order to prove that outcomes could be improved by using PET as a predictor of response[166, 168].

Part 2: Molecular biomarkers

Methods

A systematic search of the MEDLINE, Embase, and Cinharl databases was performed using the NICE Healthcare Databases Advanced Search facility by combining the search terms “oesophageal cancer” or “esophageal cancer” or “gastric cancer” and “therapy”, “chemotherapy” or “chemoradiotherapy” and “predict” and “response”. Publications up to and including December 2014 were included. Titles were reviewed for suitability and after excluding irrelevant papers, the remaining abstracts/full-texts were reviewed as necessary. Additional studies were identified through reference lists, by a PubMed search and using the PubMed related articles feature. Only clinical studies were included rather than those using in-vitro methods. Studies using adjuvant therapy (after surgery) only were excluded. Only markers demonstrating a statistically significant relationship to therapy response were included.

Search criteria - tissue markers

Biomarker discovery is far more advanced in the case of tumour tissue markers compared to serum/plasma markers and the great number of publications relating to tissue biomarkers reflects this. It was therefore possible to be much more selective in the review of tissue biomarkers. Only studies in patients undergoing neoadjuvant chemotherapy/chemoradiotherapy and those measuring response using histological means were included. Studies using palliative treatments and those only reporting clinical response were excluded. Negative studies, studies in palliative/adjuvant therapy and those measuring only clinical response although not included in the main results, are referred to in the discussion where the additional information is helpful.

Search criteria - serum/plasma markers

In contrast, for serum/plasma biomarkers, there are far fewer studies published and limiting the search to neoadjuvant therapies and those studies measuring histological response would risk excluding potentially useful studies with biomarkers that are yet to be investigated in the neoadjuvant role. Therefore, studies in palliative patients and those measuring only clinical responses were included. Once again, negative studies whilst not reported in the results section are referred to in the discussion where necessary.

Presentation of results

It was helpful to divide the large number of candidate markers identified into groups according to the function of the marker and the predominant cellular pathway in which it acts.

A table including all studies on tissue markers was compiled and included data on anatomical tumour location, histological type, type of therapy (chemoradiotherapy/chemotherapy), number of subjects, the type of histological response assessment used, the biomarker of interest and the direction of correlation between biomarker level and response. Any systematic reviews/meta-analyses of specific markers are discussed separately in the text.

A separate table was compiled for studies on plasma/serum marker which also included whether therapy was neoadjuvant or palliative. Again, systematic reviews/meta-analyses are discussed separately in the text.

Results

Search results

The initial search identified 542 articles (see Figure 49). 479 were excluded on the basis of title alone. Abstracts and full texts were reviewed with further exclusion of 35 papers. 28 studies were therefore included. A further 53 studies were identified through reference lists, a PubMed search and by using the PubMed related articles feature. Of the 81 studies identified in total, 51 investigated tissue markers and 30 investigated plasma/serum markers.

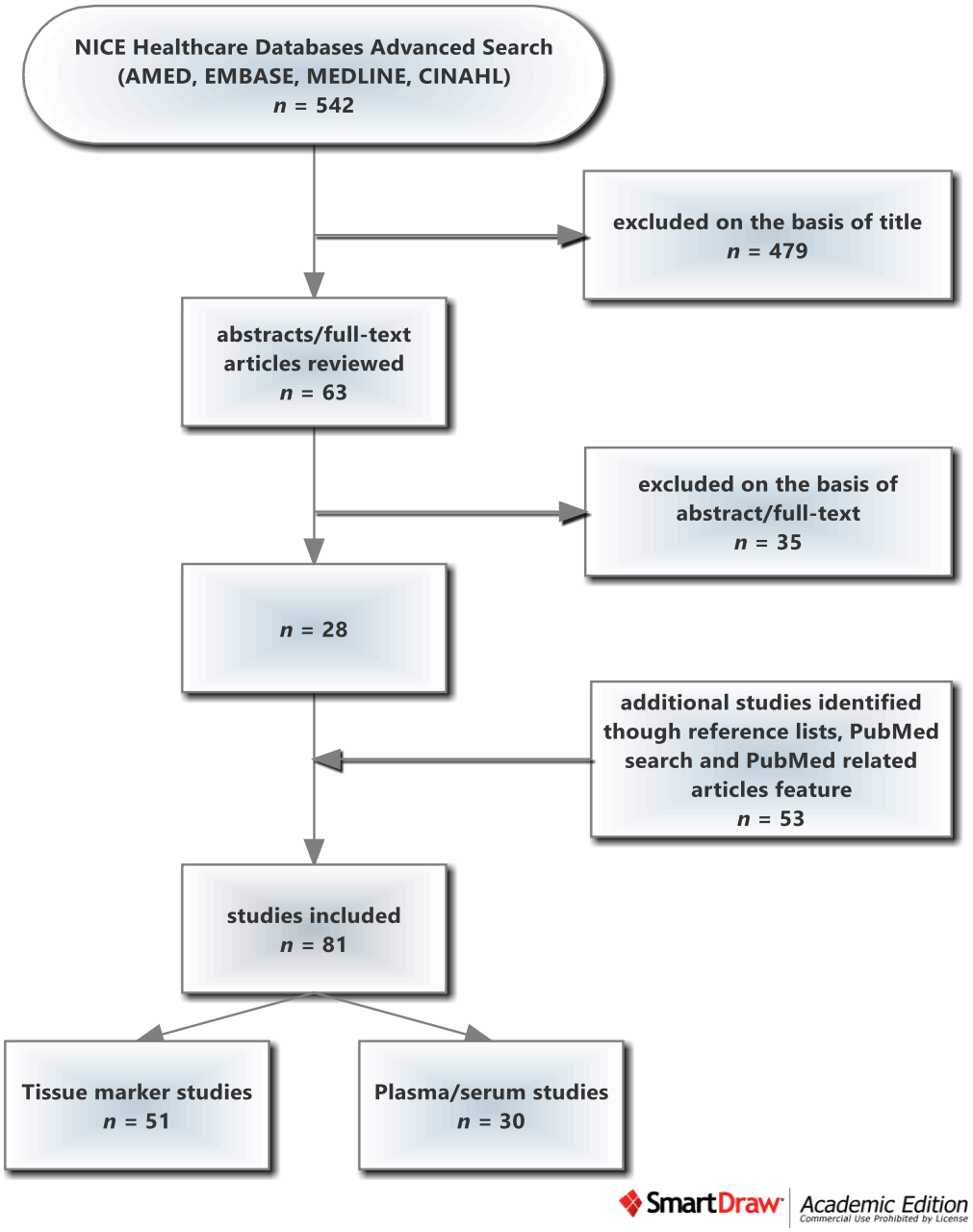

Figure 49 Flow chart of biomarker search

Tissue Biomarkers

Growth factor receptors

Epidermal growth factor receptor (EGFR), is also known as ErB-1 or HER-1 and is a member of the ErbB family of receptors. EGFR mutations are known to be associated with cancers and may form a heterodimer by pairing with another ErbB family receptor such as human epidermal growth factor receptor 2 (HER-2). HER-2 has a direct activating ligand and its activity may be dependent on this EGFR pairing. Proliferating cell nuclear antigen (PCNA) is essential for DNA replication and repair. In a small study, Hickey *et al* showed that EGFR and or PNCA activity were associated with increased pathological response ($P < 0.05$).

The search identified 4 studies demonstrating a relationship between HER-2 expression and histopathological response to chemotherapy/chemoradiotherapy. One study measured HER-2 mRNA using polymerase chain reaction (PCR) and the other 3 measured protein using IHC. In 3 of the 4 studies, increased mRNA/protein levels were associated with reduced response[53, 54, 169]. In the other study, increased HER-2 levels were associated with increased response[170]. The latter study included only AC, 2 of the other 3 included only SCC and the final paper (Miyazono *et al*) included both AC and SCC types. Interestingly when Miyazono performed a subgroup analysis according to tumour type, HER-2 was associated with response in SCC but no significant relationship was found in patients with AC. This suggests that whilst the majority of positive studies looking at HER-2 show a relationship to response in SCC patients, the situation in AC is less clear with one study showing the opposite relationship and the other showing none.

Angiogenic factors

Vascular endothelial growth factor (VEGF) is a signal protein that stimulates vasculogenesis/angiogenesis necessary for tumour growth. Imdahl *et al* showed lower levels of VEGF expression in patients was associated with a complete pathological response (*P*=0.035) and better long term survival (*P*=0.0205).

Tumour suppressor genes

p53 is a protein encoded by the *TP53* gene, known as a tumour suppressor gene. *TP53* is frequently mutated in human cancers with mutations occurring in at least 40% oesophageal cancers[171]. Mutated p53 is more resistant to degradation and p53 levels are considered as a marker of mutation. It is a critical transcription factor and involved in cell functions that are key to cancer growth such as cell cycle regulation, apoptosis and DNA repair. It is not surprising then that its status is related to prognosis and it has been considered as an important potential predictive marker of response to therapy.

In all 9 studies identified in this review, the wild-type *TP53I* or low p53 expression was associated with better pathological response.

p21 is a cyclin-dependent kinase inhibitor that operates downstream of p53-dependent cell cycle regulation. One study has shown that increased p21 expression is associated with greater pathological response to hyperthermochemoradiotherapy in SCC oesophageal cancer(P=0.0213)[172].

DNA repair system/DNA synthesis

Oesophageal and gastric cancers are commonly treated with 5-FU and platinum-based agent as part of multimodality therapy. Recently other agents such as epirubicin have been added to these regimes. 5-FU drugs are known as antimetabolites and act by inhibiting DNA and RNA synthesis. There are a number of proteins involved in the

metabolism of 5-FU products that affect chemotherapy sensitivity and protein levels, mRNA levels and polymorphisms of such enzymes have all been investigated as potential markers of chemosensitivity. Thymidylate synthase (TS) catalyzes the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) necessary for the production of thymine which DNA synthesis is dependent upon. 5-FU metabolites compete with endogenous 5-dUMP for TS binding reducing its endogenous activity and therefore inhibiting DNA synthesis. This review identified 6 studies all showing that increased levels of TS protein or mRNA are associated with poorer response to chemotherapy presumably by overcoming the actions of 5-FU-based chemotherapy.

Platinum-based drugs such as cisplatin and oxaliplatin act by binding DNA, producing cross-links and by free radical production. Therefore, the DNA repair pathway is important in resistance to platinum-based chemotherapy. Several genes and gene products acting in DNA repair pathways have been identified as potential markers for response to therapy. ERCC1 is involved in nucleotide excision repair of damaged DNA. This review identified 5 studies showing a significant association with response to platinum-based neoadjuvant therapy. Reduced ERCC1 levels and reduced mRNA levels were linked to increased response in 2 studies and 1 study respectively and two studies from the same research group, polymorphisms of the ERCC1 gene was associated with response.

High levels of the DNA double-strand break repair enzyme DNA-PKcs have been shown to correlate with increased response to chemoradiotherapy (P=0.0149).

The XRCC1 gene is involved in base excision repair and a polymorphism of this gene has been shown to correlate with response to chemoradiotherapy in oesophageal cancer (P=0.002)[173].

p53R2 is a ribonucleotide reductase regulated by p53 and supplies nucleotides to repair damaged DNA. Lower p53R2 expression was associated with better pathological response (P=0.0018)[174].

A study in 79 patients undergoing neoadjuvant chemoradiotherapy for oesophageal cancer created tissue micro arrays from tumour tissue and evaluated expression of a number of proteins involved DNA repair pathways[175]. Higher scores for MLH1 (P=0.018) and lower scores for FANCD2 (P=0.037) were associated with pathological response to chemoradiotherapy on multivariate analysis.

Apoptotic factors

Regardless of the precise upstream mechanism of toxicity, chemotherapy and radiation induce apoptosis through the intrinsic (mitochondrial) pathway. Therefore, inhibitors of apoptosis (e.g. survivin) and pro-apoptotic factors (e.g. Bax) have been investigated as potential markers for response to chemotherapy and radiotherapy. Reduced survivin expression is associated with increased pathological response (P=0.043) and survival (P=0.0023)[176].

Cox-2 may also be associated with resistance to apoptosis as well as mediating angiogenesis, tumour growth and tumour invasion[57, 177]. Both Cox-2 expression (P=0.01) and Cox-2 mRNA (P=<0.05) levels are associated with reduced pathological response[178, 179].

DNA transcription factors

Nuclear factor kappa B (NF-κB) controls transcription of DNA and high levels have been associated with reduced pathological response to neoadjuvant chemoradiotherapy in three studies (two from the same institution) that include patients with oesophageal cancer of both AC and SCC types.

Cell cycle regulators

Normal cell cycle function is a requirement for dividing cancer cells and cell cycle regulatory factors have therefore been suggested as targets for response to therapy.

Three studies have demonstrated an inverse relationship between cyclin D1 levels and pathological response with 2 of those demonstrating an associated survival advantage[180, 181]. High CDC25B levels have been shown to correlate with increased response in two studies from the same group [182, 183]. 14-3-3sigma is a conserved regulatory protein of the p53 family and is associated with increased response to chemoradiotherapy[184].

Chemotherapy/pyrimidine degradation

Dihydropyrimidine dehydrogenase (DPD) inactivates 5-FU and not surprisingly, increased levels of the protein or mRNA are associated with reduced response to 5-FU based chemotherapy[185-187].

Proliferation index

Ki-67 is a protein that is associated with and may be necessary for cellular proliferation. To date, 3 separate studies have shown an association between higher Ki-67 expression (one using the MIB-1 antibody) and increased pathological response to chemoradiotherapy[188-190].

Other markers associated with chemotherapy resistance

There are a number of other individual markers that that are involved in various pathways thought to influence sensitivity to chemotherapy/radiotherapy. Some of these have been shown to correlate with pathological response to therapy and are included in Table 56 below. Results from all the relevant studies are summarised in Table 56 below.

Mechanism/ pathway	Marker and direction of change	Site	n	T type	Therapy	Analytical method	Outcome measures	*P* value	Author
Growth factor receptors	EGFR and/or PCNA -ve	OC	14	SC	CRT	IHC	PR↑ OS↑	<0.05 0.0003	Hickey *et al*. 1994[191]
	HER-2 ↑	OC	36	AC/SC	CRT	PCR	PR↓(Junker)	0.015	Miyazono *et al*. 2004[53]
	HER-2 ↑	OC	34	SC	CRT	IHC	PR↓	0.02	Akamatsu *et al*. 2003[169]
	HER-2 ↑	OC	51	SC	CRT	IHC	PR↓	unknown	Predescu *et al*.2012[54]
	HER-2 ↑	OC/ OGJ	42	AC	CRT	IHC	PR↑ OS↑	<0.05 0.010	Duhaylongsod *et al*. 1995[170]
Angiogenic factors	VEGF ↓	OC	56	AC/SC	CRT	IHC	PR↑ S↑	0.035 0.021	Imdahl *et al*. 2002[192]
Tumour suppressor genes	p53 ↑	OC	59	SC	CT	IHC	PR↓	0.032	Shimada *et al*. 2000[193]
	p53 ↓	OC	47	SC	RT	IHC	PR↑	<0.0001	Miyata *et al*. 2000[182]
	p53 ↓	OC	62	SC	CRT	IHC	PR↑ Clinical↑ survival↑	0.0001 0.016 0.0011	Okumura *et al*. 2005[174]
	p53 ↓	OC	77	SC	CRT	IHC	PR↑	0.005	Kishi *et al* 2002[183]
	p53 ↑	OC	48	AC/SC	CRT/CT	IHC	PR↓	0.024	Beardsmore *et al*. 2003[194]
	p53 ↑	OC	28	SC	CRT	IHC	PR↓	0.08	Sobajima *et al*. 2012[195]
	p53 mutation p53 ↑	OC	64	SC	CRT	PCR IHC	PR↓ PR↓	0.004 0.042	Makino *et al*. 2010[196]
	p53 ↑	OC OGJ	42	AC	CRT	IHC	PR↓ OS	0.01 n/s	Duhaylongsod *et al*. 1995[170]
	p53 ↑ p21 ↑	OC	30	SC	NACT	IHC	PR↓ PR↑	<0.01 <0.01	Nakashima *et al* 2000[197]
	p21↑	OC	32	SC	NACRT	IHC	PR↑	0.0213	Ishida *et al*2007[172]
DNA repair	ERCC1 ↓	OC	129	SC	CRT	IHC	PR↑ OS ↔	<0.001 ns	Kim *et al*. 2008[198]
	ERCC1mRNA↓	GC	38	AC	CT	PCR	PR↑ OS↑	0.003 0.034	Metzger *et al* 1998[199]
	ERCC1 ↓	OC	36	AC/SC	CRT	PCR	PR, Junker ↑	<0.001	Warnecke-Eberz *et al*. 2004[200]
	ERCC1 (rs11615) CT	OC	153	AC	CRT	PCR	PR↑	<0.001	Metzger *et al*.2012[201]
	ERCC1 C118T	OC	52	AC/SC	CRT	PCR	PR↑	<0.003	Warnecke-Eberz *et al*. 2009[202]
	DNA-PKcs ↑ (protein)	OC	67	SC/AC	CRT	IHC	PR↑	0.0149	Noguchi *et al* 2002[203]
	XRCC1 Arg399Gln GG type	OC	210	SC	CRT	PCR	PR↑ MST↑	OR 2.75 (CI 1.14-6.12) 0.0002	Wu *et al* 2006[173]

	p53R2-	OC	62	SC	CRT	IHC	PR↑ CR↑ OS↑	0.0018 0.041 0.0057	Okumura *et al*. 2005[174]
	MLH1↑ FANCD2↓	OC	79	AC/SC	CRT	TMA	PR↑ PR↑	0.018 0.037	Alexander *et al*2012[175]
DNA synthesis/5-FU metabolism	TS protein↑	OC	129	SC	CRT	IHC	PR↓	0.04	Kim *et al* 2008[198]
	TS protein↑	GC/ GOJ	22	AC	CT	Western Blot	PR↓	0.03	Alexander *et al* 1995[204]
	TS↑	GC	62	AC	CT	IHC	PR↓	<0.05	Fukuda *et al* 2006[185]
	TS ↑	OC	99	AC/SC C	CRT	PCR	PR↓ Survival ↓	<0.001 0.003	Joshi *et al*. 2005[205]
	TS mRNA↑	GC	38	AC	CT	PCR	PR↓	0.024	Metzger *et al* 1998[199]
	TS mRNA↑	GC	65	AC	CT	PCR	PR↓ OS↓	<0.001 0.003	Lenz *et al*1996[206]
	TP ↓	OC	21	AC	CT	PCR	PR↑(Becker)	0.013	Langer *et al*. 2007[186]
Apoptotic factors	Survivin ↓	OC	51	AC/SC	CT	PCR	PR↑ survival↑	0.043 0.0023	Kato et a.l 2001[176]
	COX-2 ↓	OC	18	SC/AC	CRT	IHC	PR↑	0.01	Kulke *et al* 2004[178]
	COX-2↓ mRNA	OC	29	SC	CRT	PCR	PR↑	<0.05	Takatori *et al* 2005[179]
DNA Transcription factors	NF-κB -ve	OC	58	AC	CRT	IHC, Western blot	PR↑ survival ↑	0.0001 <0.05	Abdel-Latif *et al*. 2004[207]
	NF-κB +ve	OC	43	AC/SC	CRT	IHC	PR↓ OS↓	0.05 0.06 (ns)	Izzo *et al*. 2006[208]
	NF-κB +ve	OC	80	AC/SC	CRT	IHC	PR↓ OS↓	0.006 0.009	Izzo *et al*. 2006[209]
Cell cycle regulators	Cyclin D1 ↑	OC	38	SC	CRT	IHC	PR↓ Survival ns	0.026	Sarbia *et al*.1999[210]
	Cyclin D1 ↓	OC	34	SC	CRT	IHC	PR/CR↑ OS	0.0025 0.038	Samejima *et al*.1999[180]
	Cyclin D1↓	OC	26	SC	CRT	PCR	PR↑ survival↑	0.047 0.02	Brucher *et al* 2009[181]
	CDC25B ↑	OC	47	SC	CRT	IHC	PR↑	0.0168	Miyata *et al* [182]
	CDC25B ↑	OC	77	SC	CRT	IHC	PR↑ Survival ns	0.038 ns	Kishi *et al*.2002[183]
	14-3-3sigma	OC	36	SC	CRT	IHC	PR↑	0.01	Okumura *et al* 2005[184]
Chemo-therapy/ pyrimidine degradation	DPD↓	GC	62	AC	CT	IHC	PR↑	<0.01	Fukuda *et al* 2006[185]
	DPD↓	OC	21	AC	CT	PCR	PR Becker ↑	0.032	Langer *et al*. 2007[186]
	DPD mRNA ↓	GC	61	AC	CT	PCR	PR↑ OS	0.006 ns	Napieralski *et al* 2005[187]
Proliferation index	MIB-1 ↑	OC	42	AC/SC	CRT	IHC	PR↑ S↑	0.018 0.0149	Imdahl *et al*. 2000[188]

	Ki-67 ↑ & p53-	OC	95	AC/SC	CRT	IHC	PR↑	0.0013	Kitamura *et al*. 2000[189]
	Ki-67↑	OC	41	SC	CRT	IHC	PR↑	0.033	Takeuchi *et al*2003[190]
Other genes/proteins associated with chemotherapy resistance	ABCB1 C3435T (rs1045642)	OC	262	AC/SC	CRT	Real-time PCR	Lymph node formation↑	0.012	Narumiya *et al*. 2011[211]
	CHK2 ↑	OC	94	SC	CRT	IHC	PR↑Becker	0.0011	Sarbia *et al* 2007[212]
	Caldesmon ↑	OC	38	AC	CT	PCR	PR, ↑Becker	0.016	Langer *et al*. 2005[213]
	MTHFR ↑	OC	38	AC	CT	PCR	PR, ↑Becker Survival ↑	0.012 0.015	Langer *et al*. 2005[213]
	MRP1↑	OC	38	AC	CT	PCR	PR, ↑Becker survival↑	0.007 0.017	Langer *et al*. 2005[213]
	MT↓	OC	30	SC	CRT	IHC	PR↑ Survival ns	0.0024	Yamamoto *et al* 1999[214]
	MT↓	OC	77	SCC	CRT	IHC	PR ↑	0.033	Kishi *et al* 2002[183]
	ALDH-1↓	OC/GOJ	167	SC/AC	CRT	IHC	PR↑	<0.001	Ajani *et al* 2014[215]
	hsa-miR-296↓ HS-240↓ hsa-miR-141↑ hsa-miR-31↑ HS-217↑	OC	25	SC/AC	NACRT	PCR	PR↑	0.007 0.040 0.019 0.018 0.048	Ko *et al*.2012[144]
	let-7b let-7c	OC	74	SC	NACT	PCR	PR↑ OS ns PR↑ OS↑	0.014 ns 0.032 0.032	Sugimura *et al* 2012[216]
	Lin28↓	GC	47	AC	NACT	IHC	PR↑	0.006	Teng *et al*2013[217]
	c-MYC↑ PSEN1↑	GC	69	AC	NACT	PCR	PR↓ PR↑	0.013 0.033	Munzig *et al* 2014[218]

Table 56 Tumour tissue biomarkers demonstrating potential in prediction of the response to chemotherapy/chemoradiotherapy.

EGFR, epidermal growth factor receptor; HER-2, Human epidermal growth factor receptor 2; VEGF, vascular endothelial growth factor; NF-κB nuclear factor kappa-light-chain-enhancer of activated B cells; MRP1, multidrug resistance protein 1; GST, glutathione-S-transferase; GC, gastric cancer; OC, oesophageal cancer; AC, adenocarcinoma; SC, squamous carcinoma; CRT, chemoradiotherapy; CT, Chemotherapy; IHC, immunohistochemistry; PCR, polymerase chain reaction; OS, overall survival; PFS, progression-free survival; PR, pathological response; PCNA, Proliferating cell nuclear antigen; TP, Thymidine phosphorylase; DPD, Dihydropyrimidine dehydrogenase; MT, Metallothionein; TS Thymidylate synthetase; MTHFR Methylene-tetrahydrofolate reductase; CHK2, Checkpoint kinase 2. MST, Median survival time; ALDH-1, aldehyde dehydrogenase-1; TMA, tissue microarray

Plasma/Serum Biomarkers

Liver function tests

In one analysis, a higher level of pre-therapy serum alkaline phosphatase was shown to be associated with poorer clinical response to chemoradiotherapy in patients with oesophageal cancer (univariate, P=0.009, multivariate P=0.014)[146]. Alkaline phosphatase is found in all human tissues can be seen as a general, non-specific marker of malignancy and the authors found that higher pre-therapy T and M stage were associated with poorer response to chemoradiotherapy. It is therefore expected that alkaline phosphatase may simply be acting as a marker of more advanced disease but the multivariate analysis suggests there may be an independent association.

Serum albumin can be seen as a marker of nutrition which is thought to affect sensitivity to and tolerance of neoadjuvant therapy. In the study by Kogo *et al,* low albumin was found to be a predictor of poor clinical response on univariate analysis but not multivariate analysis[146]. In a retrospective analysis of 105 patients undergoing definitive chemoradiotherapy, a serum albumin level of >35g/l was shown in a multivariate analysis to be an independent predictive factor of complete pathological response[219]. A more recent and larger retrospective study on 246 patients showed that amongst a panel of baseline nutritional biomarkers, only serum albumin levels predicted pathological response to neoadjuvant chemotherapy on multivariate analysis (P=0.029)[220].

A lower alanine transaminase (ALT) was associated with a greater chance of clinical response to neoadjuvant chemotherapy in a study of 38 patients with locally advanced oesophageal squamous cell cancer[221].

Haematological markers

In a retrospective study of 123 patients undergoing neoadjuvant chemoradiotherapy for oesophageal cancer, pre-treatment haemoglobin (Hb) level was shown to be the sole independent predictive factor of a good pathological response[222]. Yi *et al* also found that Hb levels correlated with response to chemoradiotherapy(P=0.005)[223]. Hb levels of 12.0 to 14.0 g/dl were associated with the best response and patients with levels of >14.0 or <12.0 had poorer response rates.

In a 38 patients with oesophageal squamous cell cancer undergoing neoadjuvant chemotherapy, those with higher white blood cell counts, lymphocyte percentages, mononuclear cell counts, neutrophil counts, and eosinophil counts had a significantly greater chance of having an effective clinical response[221]. Elevated pre-treatment neutrophil to lymphocyte ratio predicts poor clinical response to palliative chemoradiotherapy in patients with metastatic gastric cancer(P=0.034)[224].

Pre-therapeutic d-dimer levels have been shown to be significantly lower in responders to neoadjuvant chemotherapy when response was measured clinically and pathologically[225].

Tumour cell antigens

In a study of 73 patients with advanced upper gastrointestinal adenocarcinoma undergoing palliative chemotherapy, baseline normal levels of CEA, TPA, CA19-9 and CA242 were associated with more clinical responses compared to elevated levels[226]. A model combining baseline levels with the change in levels after initiation of therapy provided a marginally but not significantly better prediction of outcome.

Yi *et al* demonstrated that CEA and CYFRA 21-1 may be helpful in predicting the responsiveness of oesophageal squamous cell carcinoma to chemoradiotherapy[227].

In 96 patients with SCC of the oesophagus, pre-treatment levels of Cyfra 21-1 correlated with histological response[228].

Complement

A study in 31 patients with oesophageal cancer, serum samples were analysed using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry and ELISA[143]. Samples were analysed before chemoradiotherapy treatment and at 24-hour and 48-hour time points. Peaks relating to complement C4a and C3a had different intensity in pathological responders compared to non-responders. These were then quantified using an ELISA technique. Pre-treatment serum levels of C4a (P=0.002) and C3a (P=0.035) were significantly higher in poor responders compared to non-responders. A leave-one-out analysis was used to determine that these proteins could predict response with a specificity and specificity of 78.6% and 83.3% respectively. In this proteomic profiling approach to biomarker discovery, these complement markers were not selected based on known mechanism relating to therapeutic sensitivity, however, as part of the complement family, they are linked to the inflammatory responses. Inflammation is known to drive the development of oesophageal cancer and a separate gene expression analysis by the same author has identified genes mediating inflammatory pathways that are differentially expressed in responders and non-responders[142, 143].

DNA repair/platinum dug action

Polymorphisms of the XRCC3 gene have been shown to predict clinical response to chemotherapy in gastric cancer but not histopathological response[229].

Gusella *et al* identified a polymorphism of the XRCC1 gene that is associated with pathological response in patients with oesophageal cancer[230].

Two separate ERCC1 polymorphisms have been associated with clinical response to 5-FU/oxaliplatin-based chemotherapy in a large study on 447 patients with advanced gastric cancer[231].

ERCC1 RNA levels can be measured in peripheral blood and have been shown to predict minor histopathological response to neoadjuvant chemoradiotherapy in patients with locally advanced cancer of the oesophagus[232].

A study in 89 patients with advanced gastric cancer showed that polymorphisms of the glutathione S-transferase P1 (GSTP1) gene was associated with clinical response to oxaliplatin/5-FU-based chemotherapy (P=0.026) and median overall survival (P=0.002)[233].

DNA synthesis/5-FU metabolism

In a multivariate analysis, a 6-bp deletion in TS-3'UTR was associated with a significantly higher clinical response rate to 5-FU/oxaliplatin-based palliative chemotherapy in 73 patients with advanced gastric cancer[234].

Increased levels of TS RNA measured in peripheral blood of 29 patients undergoing neoadjuvant chemoradiotherapy for oesophageal cancer. A high expression level of TS was associated with minor histopathological response[235].

DPD expression and mRNA levels measured tumour tissue were identified as promising markers of response to 5-FU therapy. One study investigated the predictive potential of 4 *DPYD* gene polymorphisms in 362 patients with gastric cancer undergoing 5-FU-based chemotherapy as neoadjuvant or palliative therapy with response measured

pathologically and clinically respectively[236]. The rs1801159A/A polymorphism was over-represented in responders (P=0.012).

Angiogenic factors

VEGF expression in tumour tissue was shown to act as a predictive biomarker of response to neoadjuvant chemotherapy in oesophageal cancer[192]. Polymorphisms of the gene can be identified from peripheral blood DNA. Oh *et al* demonstrated that the G/G genotype of VEGF-634G/C polymorphism is related to higher serum levels of VEGF and is predictive of the response to 5-FU/oxaliplatin-based chemotherapy in patients with advanced gastric cancer[237].

The predictive value of serum VEGF-A levels was investigated in 103 patients with oesophageal SCC undergoing neoadjuvant chemoradiotherapy[238]. Higher pre-therapy VEGF levels correlated with lower pathological response to treatment (P=0.042), poorer disease-free survival (0.009) and poorer overall survival (P=0.07).

Micro RNAs

There is increasing evidence that microRNA (miRNA) expression in cancer tissue can help to predict the prognosis in cancer patients. The relationship between a number of circulating miRNAs and response to neoadjuvant chemotherapy in oesophageal cancer has been investigated[239]. High levels of miR-200c levels were shown to correlate with poor response to chemotherapy (P=0.0211) and poor progression-free survival (P=0.0076).

Other markers

Germline polymorphisms have been identified in genes that are less well known and single studies have shown associations with response to therapy. These genes include the LRP5 and STK15 genes[240, 241].

Chen *et al* used genotyping arrays and mass spectrometry sequentially to determine germline polymorphisms that were associated with response to concurrent neoadjuvant chemoradiotherapy in patients with oesophageal cancer[145]. 2 SNPs were identified with a high accuracy for predicting response.

Huang *et al* used a mass spectrometry technique to identify potential predictive markers of response to palliative paclitaxel/capecitabine chemotherapy in patients with advanced gastric cancer[242]. 17 proteins were identified that were differentially expressed in responders and non-responders. These were investigated in a validation cohort of 24 patients and (Alpha-1-Microglobulin/Bikunin Precursor (AMBP) was identified as being higher in patients with progressive disease compared to those with partial response when measured using ELISA(P=0.06) and Western blotting (P=0.03).

Table 57 below includes all the serum/plasma biomarkers shown to be associated with response to chemotherapy/chemoradiotherapy in oesophago-gastric cancer.

Type of biomarker	Marker	n	Site	Tumour type	Therapy	Outcome measure	*P* value	Author
Liver function tests	Albumin ↑	105	OC	AC/SC	DefCRT	CR↑	0.009	Di Fiore *et al*. 2007[219]
	Albumin ↑	246	OC/ GOJ	AC/SC	NACT	PR (Mandard)↑	0.037	Noble *et al*. 2013[220]
	ALT ↓	38	OC	SC	NACT	CR (WHO)↑	0.003	Liu *et al*. 2014[221]
	Alk phos↑	108	OC	AC/SC	CRT	CR (WHO)↓	<0.05	Kogo *et al*. 2008[146]
Haematological markers	Hb ↑	123	OC	SC	CRT	PR↑ (JSED[243])	0.02	Hamai *et al*. 2014[222]
	Hb↑ (12-14g/dl)	181	OC	SC	DefCRT	CR ↑	0.005	Yi *et al*. 2010[223]
	WBC↓ Lymp%↑ Mon↓ Eos↓ Neut↓	38	OC	SC	NACT	CR (WHO)↑	0.003 0.047 0.027 0.038 0.005	Liu *et al*. 2014[221]
	D-dimer ↓	71	OC	SC	NACT	CR↑ PR↑	0.0491 0.0107	Tomimaru *et al* 2008[225]
	NLR↓	269	GC	AC	Pall CT	CR↑ PFS↑ OS↑	0.034 0.001 0.001	Cho *et al* 2012[224]
Tumour cell antigens	TPS↓ CA19-9↓ TPA ↓ CA242↓	73	UGI *	AC	Pall CT	CR↑ CR↑ CR↑ CR↑	0.007 0.001 0.036 0.002	Bystrom *et al*. 2010[226]
	CEA ↓ CYFRA21-1 ↓	181	OC	SC	DefCRT	CR↑	0.000 0.000	Yi *et al*. 2010[223]
	CYFRA21-1 ↓	96	OC	SC	NACRT	PR↑	0.03	Quillien *et al*. 1998[228]
Complement	C3a ↓	31	OC	AC/SC	NACRT	PR (Mandard)↑	0.035	Maher *et al*. 2011[143]
	C4a ↓	31	OC	AC/SC	NACRT	PR (Mandard)↑	0.002	Maher *et al*. 2011[143]

DNA repair/ platinum drug action	GSTP1-105VL or VV	89	GC	AC	PallCT	CR↑	0.026	Li *et al* 2010[233]
	XRCC1 XPA	105	OC	AC/SC	NACRT	PR↑		Gusella *et al* 2012[230]
	XRCC3 rs861539 XRCC3 rs861530	144	GC	AC	NACT	CR↑ PR	0.02 0.05 ns	Ott *et al* 2011[229]
	ERCC1 rs11615 TT/T ERCC1 rs2298881CC /C	447	GC	AC	PallCT	CR↑(WHO) OS↑ CR↑(WHO) OS↑	0.015 0.018 0.03 0.02	Lu *et al* 2014[231]
	ERCC1 RNA	29	OC	AC/SC	NACRT	PR ↓	0.004	Brabender *et al*.2008 [232]
DNA synthesis/ 5-FU Metabolism	TS RNA↑	29	OC	AC/SC	NACRT	PR↓	0.046	Grimminger 2009 *et al*[235]
	TS-3'UTR-6bp/-6bp XPD156 CA/AA	73	GC	AC	PallCT	CR↑	0.034 0.038	Keam *et al* 2008[234]
	DPYD rs1801159A/A	362	GC	AC	PallCT/NACT	CR(WHO)/PR(Becker)↑	0.012	Zhang *et al*2012[236]
microRNA	miR-200c ↑ (micro RNA)	64	OC	SC	NACT	PR↓ PFS↓	0.0211 0.0076	Tanaka *et al*. 2013[239]
Angiogenic factors	VEGF-A↓	103	OC	SC	NACRT	PR↑	0.042	Chiang *et al*[238]
	VEGF -634 GC or CC	190	GC	AC	PallCT	CR↑ PFS↑	0.034 0.043	Oh *et al*2013[237]
Other markers	AMBP↑	17	GC	AC	PallCT	CR↓	0.06	Huang *et al*[242]
	LRP5 rs3736228 CC	107	GC	AC	PallCT	CR↑(RECIST) PFS OS	<0.001 <0.001 <0.001	Liu *et al* 2014[240]
	rs16863886 (SGPP2) rs4954256 (ZRANB3)	116	OC	SC	NACRT	PR↑	0.0006 0.002	Chen *et al*. 2012[145]
	STK15-T91A (Phe31Ile)	134	OC	AC/SC	NACRT	PR ↓	0.048	Pan *et al*. 2012[241]

Table 57 Serum/plasma markers demonstrating potential in predicting the response to chemotherapy/chemoradiotherapy.

Abbreviations: ALT, Alanine transaminase; CEA, Carcinoembryonic antigen; TPA, tissue plasminogen activator; SNPs, single nucleotide polymorphisms; AC, adenocarcinoma; SC, Squamous cell carcinoma; NACRT neoadjuvant chemoradiotherapy; PallCT, palliative chemoradiotherapy; CRT, chemoradiotherapy; CT, chemotherapy; TPS, Tissue polypeptide-specific antigen; PR Pathological response; CR, Clinical response; UGI*, upper GI cancers including gastric, pancreatic and biliary. GST, glutathione S-transferase; VEGF-A, vascular endothelial growth factor A; NLR, neutrophil lymphocyte ratio; TS, thymidylate synthase; AMBP, Alpha-1-Microglobulin/Bikunin Precursor.

Discussion

A number of authors have recognised the need to predict the response to neoadjuvant therapy in the treatment of oesophago-gastric cancer and recognise the need for research in this area[38, 44, 49-58].

This chapter has reviewed the published literature on prediction of response to neoadjuvant therapy in patients with oesophago-gastric cancer. A summary of the literature relating to clinical, pathological and radiological markers has been presented and a detailed review of molecular biomarkers has been undertaken.

Clinical, pathological and radiological markers

Clinical, pathological and radiological markers are not the focus of the thesis but a summary of the published literature was presented in order to provide the background and historic context within which the field of molecular biomarkers has emerged. The most accurate model of prediction would take into account any factors that are shown to contribute to prediction in a multivariate analysis whether they are clinical, pathological, radiological or molecular and therefore any strong candidate variables should be further investigated.

Of the clinicopathological markers identified in the reviewed literature, the following were shown to be significantly associated with response to therapy: gender, T stage, nutritional status, M stage, tumour length, tumour localization in the middle third of the stomach, well differentiated tumours and intestinal tumour type. Of these factors, only T stage appeared in more than study.

The literature is limited by heterogeneity of subjects with regards to many variables including treatments, anatomical tumour locations, tumour types, tumour stage and the

means by which response to therapy was measured. The timescale over which studies are published adds further complexity as not only do patient selection and treatments change with time but staging criteria have also changed so the relevance of these studies to current practice remains uncertain.

Many of the clinicopathological factors identified are amongst those routinely measured during assessment and staging and could therefore be investigated in a retrospective patient group.

Molecular biomarkers

The field of molecular biomarkers in oesophageal and gastric cancer is relatively new and rapidly expanding. A simple plot of the publication trends when searching for "gastric cancer biomarker" and "oesophageal cancer biomarker" shows the rate of growth in publications, see Figure 50. The combined number of publications per year has doubled in less than 4 years. An up to date search is vital in order to keep up with the pace of research. A number of authors have suggested that molecular biomarkers are likely to provide to provide the solution to the problem of predicting response to neoadjuvant therapy, see [24, 38, 52-55, 57].

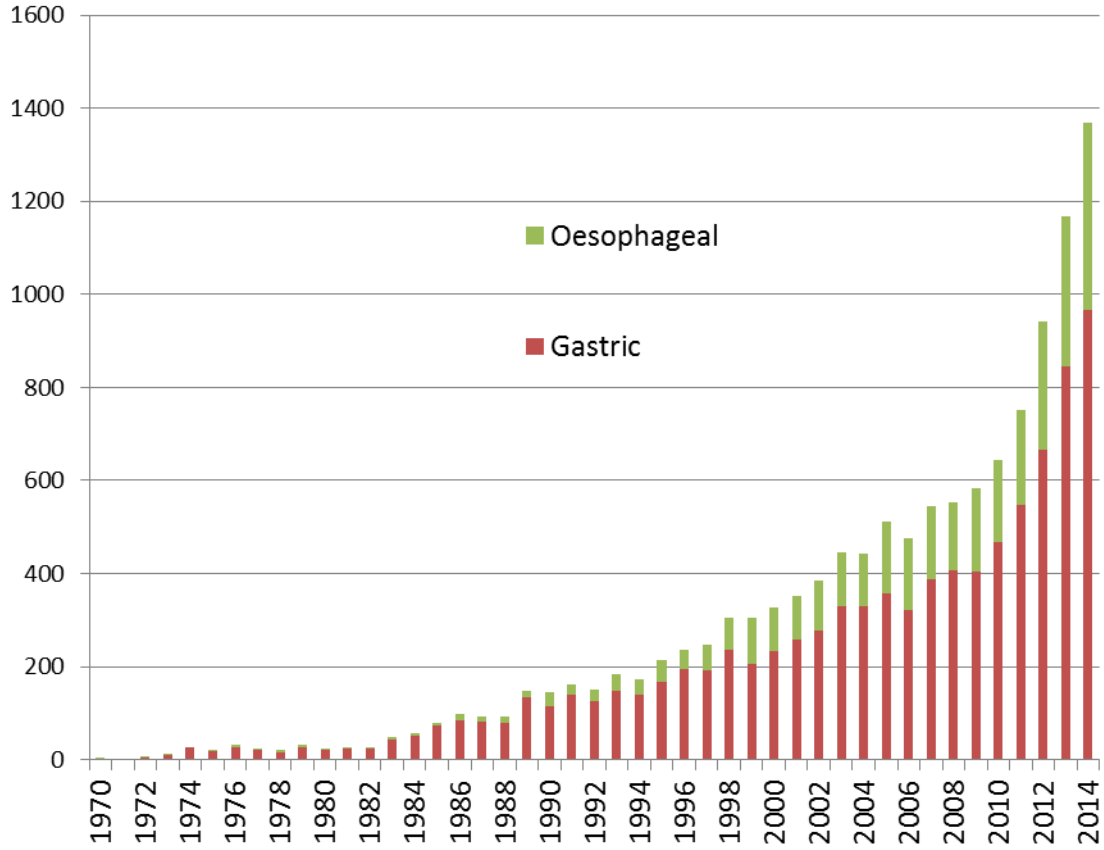

Figure 50. Publication trends for "oesophageal cancer biomarker" and "gastric cancer biomarker" from PubMed.gov, US National Library of Medicine National Institutes of Health.

Figure 51 Authors suggesting that the solution to the problem of finding a means to predict response to neoadjuvant therapy is likely to be provided by molecular biomarkers.

Tissue markers

A review of the literature on the role of molecular biomarkers in predicting the response to chemotherapy/chemoradiotherapy was performed. For this part of the review only those markers measured in tumour tissue were included. There are a huge number of potential biomarkers measurable in tissue and most will have no role in the prediction of response so only markers demonstrating statistically significant associations with response to therapy were included. Whilst there are a number of published biomarker discovery studies evaluating the predictive value of tissue markers for response to adjuvant or palliative therapy and these may be useful in identifying potential candidate markers for predicting response to neoadjuvant therapy, these were not included for two reasons. Firstly, the patients undergoing palliative therapies often have metastatic disease and therefore tumour biology may not reflect that in the less advanced tumours undergoing neoadjuvant chemotherapy. Secondly, in adjuvant and palliative therapy response can only be measured clinically which is known to be a poor surrogate marker of histopathological response. For the same reason, neoadjuvant studies where response was only measured clinically were also excluded.

Growth factor receptors

HER-2 is one of the first biomarkers to have been used successfully in the clinical management of solid tumours. It is used to determine sensitivity to trastuzumab in breast cancers over-expressing the receptor protein. There is in vitro evidence already reviewed by Miyazono *et al*[53] suggesting that sensitivity to several chemotherapeutic agents and radiotherapy may be dependent on ErbB receptor status. The evidence suggests that HER-2 is able to predict sensitivity to cisplatin/5FU-based chemoradiotherapy in SCC of the oesophagus, however evidence in AC suggest either

no association or perhaps even an opposite relationship. Whilst in vitro evidence has suggested c-erB-1 may also determine chemotherapy/radiotherapy sensitivity, the evidence is mixed. One study demonstrated an association between marker levels and response for HER-2 but not c-erbB-1[53] and another demonstrated increase in response when either c-erbB-1 or PCNA were positive[191].

Angiogenic factors

One study was identified supporting VEGF as a predictive biomarker and other authors have demonstrated associations with clinical response in patients undergoing NACRT or definitive CRT[244], however, other studies have failed to identify a significant relationship[178, 185].

Tumour suppressor genes

p53 is the most research biomarker for use in predicting response to therapy in oesophago-gastric cancer. All 9 studies identified in this review demonstrated an inverse relationship between p53 level/mutant form and histological response to therapy. However, other studies have not shown any such significant relationship[212, 244, 245].

A meta-analysis published in 2013 investigated the value of p53 status for predicting the response to chemotherapy/chemoradiotherapy in patients with oesophageal cancer[171]. A total of 28 studies with 1497 cases were included. Wild-type p53 (wild-type p53 gene and/or low p53 protein expression) was associated with a high rate of major pathological response to chemotherapy, risk ratio 1.15, CI 1.06-1.25, P=0.001. The authors concluded that p53 status might be a predictive biomarker for response to chemotherapy-based treatments in oesophageal cancer. The conflicting results in the literature were due to a lack of large-scale studies, no standardised evaluation of response, heterogeneity of

chemotherapy-based treatment and different methods of measuring p53 status which lack high sensitivity and specificity.

Regarding p21, in addition to the one study showing an association between increased expression and greater pathological response, a further study showed that the combination of HIF-1α levels, p21 levels and p53 type (mutant vs wild) is a powerful indicator of clinical response to CRT[246]. Patients with high p21 expression had high sensitivity to CRT but only compared to those with low p21 and mutant p53. The detailed underlying mechanisms are beyond the scope of this report suffice to say that p21 operates downstream of p53 and HIF-1α so it is perhaps not surprising that if p21 is looked at in isolation, results can be unpredictable and misleading.

DNA repair system & DNA synthesis

5-FU-based drugs predominantly act by inhibiting DNA synthesis and platinum-based drugs act by damaging DNA. Therefore, DNA synthesis and repair pathways encompass many markers that have the potential to influence sensitivity to these two drug types which form the basis of chemotherapy directed towards oesophago-gastric cancer.

High TS protein and mRNA levels were identified as being associated with fewer pathological responses. ERCC1 protein and mRNA levels also correlated with lower pathological response rates.

Hu *et al* published a systematic review in 2012 on the predictive value of TS in gastric cancer[247]. They analysed 24 studies including a total of 2,079 patients. 15 of the studies were in advanced gastric cancer undergoing palliative therapy and 9 were in localised cancers undergoing adjuvant therapy. They showed that high TS levels predicted poor response rates. They concluded that additional studies adhering to consistent

methodology would be needed to define the precise value of TS in these patients. Extrapolation to neoadjuvant therapy represents one further leap and therefore more studies in this defined population are needed.

The promising results here have led to a search for polymorphisms in the genes involved in DNA synthesis/repair in the hope that they may be predictive of response. Although individual studies have identified polymorphisms that appear to achieve this, a systematic review analysing 5 studies for TS and 4 studies for ERCC1 failed to demonstrate any overall significant predictive ability[248]. However, in an analysis of 6 studies investigating glutathione -S transferases (GSTs), they did find higher response rates in patients with the GSTT+ phenotype compared with the GSTT- phenotype including in a neo-adjuvant subgroup[248].

Whilst DNA-PKcs, XRCC1 polymorphism, p53R2, MLH1 and FANCD2 have been linked with response to therapy in isolated biomarker discovery projects, further studies will be required to substantiate their results.

Polymorphisms of other genes relating to chemotherapeutic sensitivity and DNA synthesis/repair pathways have been identified from other studies in patients with advanced cancers undergoing palliative chemotherapy. Although results cannot be directly extrapolated to neoadjuvant treatment, they are useful in identifying potentially predictive markers for further investigation. On such study by Goekkurt *et al* identified polymorphisms of the TS gene and GSTP1 gene that were associated with clinical response to 5-FU and cisplatin-based palliative chemotherapy[249].

Apoptotic factors

Although survivin has been shown in one study to predict pathological response to chemotherapy and it may act as an independent indicator of overall prognosis, another study did not demonstrate a relationship with response to therapy[250].

Cox-2 has been shown to inhibit chemotherapy and radiotherapy induced apoptosis in vitro and therefore has been considered as a potential clinical predictive marker[251]. It has not been extensively investigated as yet but two studies have demonstrated a relationship between increased levels and reduced response to neoadjuvant chemoradiotherapy. A further study failed to show a relationship between pre-therapy COX-2 mRNA or protein levels and response to neoadjuvant chemoradiotherapy in a cohort containing 38.5% AC [251]. The discrepancy in results cannot easily be explained by differences in tumour type or therapy regime, however, as is often the case, there were differences in techniques used to quantify of Cox-2 protein including the primary antibody. This illustrates a common and frustrating feature of biomarker discovery and development.

DNA transcription factors

NF-κB control the transcriptional regulation of target genes involved in cell survival. NF-κB activation is a highly regulated process which can be initiated by a range of stimuli and has been associated with cancer[209]. It suppresses apoptosis when cancer cells are exposed to radiotherapy or chemotherapy and therefore has been investigated as a predictor of response to neoadjuvant therapy. Two studies from one centre and a further study have all shown that NF-κB levels can predict pathological response to chemoradiotherapy.

Cell cycle regulators

Amongst isolated reports of individually promising markers, Cyclin D1 is emerging as the strongest candidate biomarker acting through cell cycle regulation 3 studies have shown a significant relationship with pathological response to therapy however, there are also two published studies that fail to show any relationship[182, 252]. All studies were in patients with SCC undergoing chemoradiotherapy. One of the negative studies assesses clinical rather than pathological response[252].

Chemotherapy degradation

Three studies have shown a correlation between DPD levels and the response to 5FU-based chemotherapy. In one study using chemoradiotherapy, DPD above the median correlated with pathological response (P=0.014) however on ROC analysis, the result did not reach significance[181]. DPD is also often considered in the DNA synthesis group due to the mechanism of action of 5-FU which is inactivated by DPD.

Proliferation index

Ki-67 is a marker of cell proliferation, may be a necessary for cells to continue proliferating and has been shown to correlate with increased pathological response rates. Three further studies have failed to report and significant association [180, 182, 252]. All these studies were in SCC whereas the positive studies all included AC except for one in which the P value was highest (0.033). This suggests Ki-67 may be better at predicting response in patients with AC compared to SCC.

Micro RNAs

The other group of markers worthy of discussion at this stage is microRNAs (miRNAs). These are short, non-coding RNAs that have a key role in post-transcriptional regulation of gene expression[144]. They are involved in cancer initiation and progression and there

is evidence that they impact on resistance towards various chemotherapeutics[137]. Since miRNAs are small, they are not easily degraded and can be extracted from formalin-fixed, paraffin-embedded (FFPE) tissue blocks, a process which is considered unsuitable for mRNA profiling[144]. Identification of example miRNAs that differ between pathological responders and non-responders to neoadjuvant chemoradiotherapy for oesophageal cancer has been attempted through expression profiling in a study by Ko *et al*[144]. This study identified 5 miRNAs from tumour tissue that had greater than 2-fold differences between responder and non-responder groups and are worthy of further investigation in external cohorts. A further study in 90 gastric cancer patients was not including in the main results table because therapy was palliative and time to progression was used as a measure of benefit from chemotherapy[253]. A 58-miRNA gene expression signature was able to differentiate between those with delayed time to progression and those with rapid time to progression. A review paper reported on 9 miRNAs identified from 7 *in vitro* studies that were associated with chemoresistance in gastric cancer and are worthy of further clinical evaluation.

Other markers associated with resistance

Of the other markers that have been liked to chemotherapy/chemoradiotherapy resistance, Metallothionein (MT) has the best evidence supporting is predictive properties. MTs are intracellular metal-binding proteins involved in zinc homeostasis and detoxification of heavy metals. They are thought to affect cisplatin-induced apoptosis. 3 studies have shown an inverse relationship between levels of the marker and response to chemoradiotherapy. There are no published reports of negative studies as yet, however, this may represent publication bias and reflect the small number of studies published on this marker (type I error).

Limitations

Interpretation of research into tissue biomarkers is made difficult by a number of factors. There is heterogeneity in many aspects making comparison of studies difficult. Patient factors include disease stage, staging classification used, anatomical tumour location and of course histological tumour type. Whilst mechanisms behind sensitivity to chemotherapeutic agents in AC and SCC are similar, it remains unclear whether there is a variation in clinical and histological responses between tumour types[143]. Not only do similar uncertainties exist in the potential differences between gastric tumours, oesophageal tumour and gastro-oesophageal junctional tumours with regards to sensitivity/resistance to therapeutic agents, but the treatments offered to such tumours are often different. Multimodal therapies now include a wide range of possible treatments that are all likely to have different molecular resistance mechanisms, some of which have been discussed above.

Most of the studies published to date are small including typically less than 100 subjects with sample sizes being determined by availability of tumour samples rather than being statistically powered. The studies are retrospective and often are of a discovery nature with no validation cohort. Whilst some of the markers above have been investigated with meta-analyses, these reviews are subject to the limitations above and none of the markers above have reached randomised trial phase.

As with any biomarker studies, publication bias is likely to be significant. The more factors are investigated, the more type I statistical errors will be made, generating associations which occur by chance rather than representing true biological effects. Overcoming this requires validation in other cohorts. It would be naïve to expect the publication of many negative biomarker studies but a step towards more openness

could involve compulsory registration of all biomarker studies on a public database. All studies being granted ethical approval in the UK are now required to be registered on such a database.

As we have seen in the previous chapter, there are a number of different methods used to define response to chemotherapy/chemoradiotherapy. An accurate measure of response is critical when making associations with biomarker levels since it is important that biomarkers are truly predicting what we think they are rather than representing a surrogate for other factors such as disease stage. If the measure is poor, then the prediction will be poor.

The major drawback of tissue markers is the reliance upon biopsy specimens which require invasive procedures and in general are not suitable for serial measurements. Many of the laboratory analytical techniques are not widely available yet although techniques such as PCR will become more commonplace.

Analytical techniques such as immunohistochemistry have a subjective, observer-dependent aspect to them and only give semi-quantitative results. There is often heterogeneity of expression within tumour tissue which may represent different clonal groups. These factors introduce problems with accuracy, repeatability and inter-observer/inter-unit agreement. Whilst different studies may use broadly similar techniques, each biomarker can often be targeted by different antibodies that exhibit different specificities for the marker in question.

Circulating markers

A systematic review of the published literature on biomarkers measured in blood plasma/serum was performed. The review on tissue markers was restricted to reports

in patients undergoing neoadjuvant chemotherapy and with response measured by histopathological regression. Due to the limited number of publications on circulating markers, this would have resulted in a very low yield and would have excluded reports on emerging biomarkers that may prove useful in the prediction of response in future studies, therefore in this section, reports in palliative therapy and those measuring clinical response to therapy were included.

Liver function tests

Whilst there have been a small number of studies published suggesting a relationship between liver function tests (alkaline phosphatase, albumin and ALT), these markers have been routinely measured for many years and if the results were reliable, one would expect numerous publications confirming similar results. However, since these tests are routinely performed, they can be readily investigated in retrospective studies.

In the case of alkaline phosphatase, it is found in all human tissues can be seen as a general, non-specific marker of malignancy. In the study by Kogo *et al*, in addition to alkaline phosphatase, pre-therapy T and M stage were associated with poorer response to CRT. Alkaline phosphatase may therefore simply be acting as a marker of more advanced disease although the multivariate analysis suggested there may be an independent association.

Although albumin has also been shown to correlate with response to therapy it may also be acting as a surrogate for poor nutritional health and more advanced tumour biology thereby acting as a prognostic marker rather than predictor of response. Prognosis is likely to be poor in patients predicted to respond poorly to therapy regardless of whether they undergo this or an alternative treatment plan.

Haematological markers

Although there were two reports suggesting a link between serum Hb level and response, there was a discrepancy in what levels are beneficial with one paper suggesting benefit over a cut-off at 13.0g/dl and the other suggesting a benefit within a range of 12.0-14.0g/dl.

Differential white cell counts, white cell differential proportions/ratios, total white cell counts and d-dimer levels have all been shown in single papers to be associated with response to therapy. Differential white cell counts and ratios were investigated in a recent study by Noble *et al* and not found to correlate with response to neoadjuvant chemotherapy in patients with oesophago-gastric cancer[220].

As with liver function tests, these haematological markers may simply represent poor nutritional status or advanced tumour biology which may explain why no link to histological response was shown in the above, latter study.

Tumour cell antigens

The markers CEA, TPA, CA19-9 and CA242 were significantly associated with clinical responses in patients undergoing palliative chemotherapy[226]. However, a separate study in patients with gastric cancer receiving palliative chemotherapy found no correlation between initial CEA, CA19-9 or CA-50 levels and radiological response to chemotherapy although there was a trend towards lower levels having a more favourable outcome[254].

Shimada *et al* reviewed the literature on the value of CEA, CA19-9 and CA72-4 in staging, evaluation of response to therapy and detection of recurrence in patients with gastric cancer although they did not specifically review the roles in predicting therapy response[255]. They conclude that a prospective trial is required to evaluate the clinical

significance of these markers and that a model combining these markers is likely to be the most effective way of staging before chemotherapy and that this could be used in the early evaluation of response to chemotherapy.

Complement

The findings of one study identifying through serum protein profiling C3a and C4a as predictive of response to chemoradiotherapy in oesophageal cancer are promising. Although these proteins were identified through a mass discovery approach, they are involved in inflammatory pathways and it has been hypothesised that these complement factors could alter the immune microenvironment of oesophageal tumours promoting differential responses to chemoradiotherapy[143]. C3a and C4a are therefore worthy of further study in other centres to externally validate results.

DNA repair/platinum-drug action

As outlined above platinum-based drugs work by damaging DNA, therefore DNA repair pathways are important in drug resistance mechanisms. The ERCC genes are involved in nucleotide excision repair and the XRCC genes are involved in base excision repair. Polymorphisms of both have been associated with clinical responses to chemotherapy in gastric cancer.

GSTP1 also directly participates in the detoxification of platinum compounds and polymorphisms of this gene are associated with clinical response to oxaliplatin-based chemotherapy.

A small study in 28 patients, Font *et al* investigated the relationship between polymorphisms of genes featuring in DNA repair pathways (XPD and XRCC3)[256]. The XRCC3 241M/T polymorphism showed a trend towards association with response to

CPT-11/docetaxel/cisplatin chemotherapy in patients with oesophago-gastric cancer (P=0.06). This finding merits further investigation in a larger patient cohort.

DNA synthesis/5-FU metabolism

As outlined above, 5-FU-based drugs can inhibit DNA synthesis and there are a number of pathways involved that could determine the sensitivity to this chemotherapy. In the case of tissue markers, thymidylate synthase was one of the most investigated factors from these pathways and not surprisingly authors have been interested in whether any markers relating to this enzyme can be identified in peripheral blood. Cellular tumour RNA has been extracted from peripheral blood and TS RNA levels were shown to be associated with a minor histopathological response to neoadjuvant chemoradiotherapy[235]. Although a review DNA synthesis/repair pathway polymorphisms identified from tumour DNA revealed limited association with response, there is interest in investigating the predictive value of polymorphisms in germline DNA which can be sampled peripherally. Of ten polymorphisms within 5 genes examined in genomic DNA from peripheral blood, a single TS polymorphism was found to be associated with a higher response rate to 5-FU/platinum-based palliative chemotherapy[234].

DPD expression measured in tumour tissue was another marker showing promise and one study has identified a genomic DPD polymorphism in DNA extracted from peripheral blood that was associated with better response to 5-FU-based chemotherapy.

Angiogenic factors

Not only does VEGF promote the neovascularisation require for tumour growth, but it increases the vascular permeability and capillary leakage. It has been suggested that this leads to elevated interstitial fluid pressure preventing effective transport of therapeutic

drugs into tumours and thereby reducing the efficacy of treatment[237]. Therefore, VEGF-A expression levels or polymorphisms that are known to alter the expression level could determine chemosensitivity. Lower serum VEGF-A levels have been associated with a greater histopathological response to neoadjuvant chemoradiotherapy and a polymorphism of the VEGF gene isolated from peripheral blood genomic DNA which is known to reduce expression of VEGF has been linked with clinical response to palliative chemotherapy[237, 238].

Micro RNAs

The role of micro RNAs in tumour growth and their potential association with chemosensitivity has been introduced above. They represent an area of rapidly developing research and there is particular interest in their ability to predict response to chemotherapy partly because of the discovery that they can either promote tumour development and growth or inhibit tumour progression through key processes such as cell proliferation, differentiation and apoptosis[144]. This is achieved by controlling translation and stability of mRNAs in a process downstream of transcription[137, 144]. MiRNAs are known to regulate the same genes that are targeted by chemotherapy agents[257]. MiRNA action is discreet from genetic polymorphism, DNA transcription or measurement of discreet protein levels but in a similar way to DNA/RNA analysis, miRNA expression patterns can be compared in different clinical subject groups – e.g. cancer patients vs. controls or responders vs. non-responders[137].

Not only can miRNAs be extracted from fixed tumour tissue but they can be identified in body fluids where they are known as circulating miRNAs[137, 239]. High levels of miR-200c in blood serum were shown to be associated with poor response to chemotherapy and shorter progression-free survival in patients with oesophageal cancer undergoing

neoadjuvant chemotherapy[239]. Whilst this is the only in vivo report describing a relationship between circulating miRNAs and response to therapy, there is background evidence that suggests this area warrants further study. A review of 22 studies has identified 35 circulating miRNAs that are differentially expressed in patients with gastric cancer and controls. Of these candidate markers, six (including miR-200c investigated above) have been shown to be deregulated in chemotherapy resistant gastric cancer cell lines adding further suggestion that they are implicated in drug resistance[137].

Other markers

Germline polymorphisms remain stable throughout disease progression unlike somatic mutations from tumour tissues and represent variations in genotype that may determine individual sensitivity to therapeutic agents. It is a relatively new field of exploration; however, it is rapidly expanding especially in the area of serum/plasma analysis because genomic DNA is so readily obtained from sampling peripheral blood.

Studies aimed at investigating germline polymorphisms using a range of methods including genotyping arrays and more direct SNP genotyping have identified a number of polymorphisms that show potential in predicting response to chemotherapy. Like miRNAs, germline DNA is readily extractable from peripheral blood and this research in area is likely to expand.

Limitations

Circulating biomarkers are subject to many of the same limitations as tissue biomarkers with respect to the heterogeneity between studies and therefore the difficulty comparing them. Whilst they don't require invasive testing and histological analysis, plasma and serum acquisition and analysis have their own limitations. Biomarker levels will depend on whether they are tested in plasma or serum which also affects marker

stability. The same applies to the different anti-coagulants used in plasma samples (e.g. EDTA, citrate, lithium-heparin). Biomarker levels in blood can depend on circadian timing, patient fasting status. Stability of markers in samples tubes also depends on handling issues such as ambient temperature, storage temperature, time to centrifuge/separation and time to freezing.

Research into circulating biomarkers is a rapidly expanding field highlighted by the fact that most of the key studies in the results section were published within the past few years. Reviews therefore need to take advantage of the most up to date literature and may need to be repeated a frequent intervals as new information becomes available.

Many serum/plasma assays like immunohistochemistry rely on immune techniques such as ELISA that are prone to the same issues of antibody specificity. Due to differences in sample handling and laboratory techniques, threshold values from one unit may not be valid in others even when using the same antibodies/kits. Cut-offs may need to be re-defined in separate validation studies

Interest in DNA/RNA extraction from peripheral blood has increased recently and techniques for achieving this are likely to become more commonplace. There are variations in these techniques with respect to probes, primers and cycling conditions.

When measuring a biomarker in peripheral blood, researchers need to be clear what exactly they are measuring. Is it a protein marker expressed and secreted by tumour cells or even host cells such as neutrophils or lymphocytes? Do circulating levels of the marker reflect expression levels? If it is a tumour marker, is it specific to the primary tumour in question and what is the relationship to secondary lesions? Secondary lesions may express clonal differences compared to primary lesions and will this affect the

circulating biomarker level. In DNA analysis, is it genomic DNA that is the desired target or circulating tumour DNA?

Summary

Some of the biomarkers identified in this review are promising but further studies are needed to validate and confirm the results in separate patient cohorts.

It is becoming clear that the most predictive pre-therapy markers are likely to be biomarkers[38, 54, 55]. Functional imaging (FDG-PET) is likely to be most useful when used after initiation of chemotherapy (early assessment of response)[166, 168]. There have been a number of promising tissue biomarkers identified but these rely on an invasive test and laboratory methods that may not be widely available[38, 55].

The potential role of serum/plasma biomarkers is under-investigated but offers exciting potential for many reasons[143, 146]. The technique relies only on a minimally invasive test, regularly undertaken in the routine management of cancer patients and universally available. Most laboratory analyses for circulating biomarkers rely on relatively low-cost techniques. Biomarker levels can be measured before neoadjuvant treatment when they offer the potential to be truly predictive. Serial measurements can also be recorded throughout treatment to monitor response to therapy in vivo.

No single biomarker or technique appears to be able to predict with sufficient accuracy the response to neoadjuvant therapy. The complexity of molecular carcinogenesis, the uniquity of individual cancers and the multiple mechanisms of chemoresistance mean that the ultimate model for accurately predicting response to chemotherapy is likely to require a combination of several predictive biomarkers[38, 51, 54, 133]. This will depend on having a panel of biomarkers validated in prospective trials showing in multivariate

analysis that they are independently associated with response. The goal should be to predict sensitivity/resistance to each and every potential therapeutic agent available. If in-vivo monitoring with the use of serial measurements alongside therapy could be shown to reflect changes in tumour biology during treatment, then this would be desirable.

There are a number of candidate biomarkers expressing potential to predict response to neoadjuvant therapy in oesophago-gastric cancer that are not included in this review. Whilst they may be involved in key cancer-cell pathways or have been studied in the in-vitro environment, they have not been subject to clinical investigation as yet. One such biomarker is the glycolytic enzyme M2-pyruvate kinase (M2-PK). M2-PK is known to be expressed by tumour cells and can be detected in peripheral blood. Although levels vary amongst patients with cancer, the significance of this is not known. There is in vitro evidence and a very limited amount of clinical evidence suggesting that M2-PK and in particular, the dimeric, tumour-specific form may be linked to chemotherapy resistance. Chapter 6 describes this in further detail.

CHAPTER 6 – M2-PYRUVATE KINASE

Introduction

In the search for a means to predict the response to neoadjuvant therapy in oesophago-gastric cancer, Chapter 5 demonstrated that molecular biomarkers have so far shown the most promise.

Pyruvate kinase (PK) catalyses the final step in the glycolysis pathway and is therefore common to cells of almost all living organisms. This action involves the dephosphorylation of phosphoenolpyruvate, yielding pyruvate and the production of ATP from ADP. Importantly, it is in independent of oxygen supply.

The Warburg effect

It has long been known that tumour cells have a different metabolism to that of normal cells particularly with regard to glycolysis[258]. In cancers, glucose is readily converted into lactate even in the presence of oxygen. This phenomenon is known as aerobic glycolysis or the Warburg effect and was first described in 1924. However, for many years the molecular basis behind this was poorly understood. It is now known that PK is a key regulator of this mechanism and it is this realisation that has prompted a wave of research into the molecular functions and interactions of PK[259]. In particular, the role of PK as potential target for chemotherapy is an area rapidly building momentum[260-265].

Pyruvate kinase isoforms

PK occurs in different isoforms according to tissue type[266]. The characteristics of each isoform depend on the needs of the tissues expressing them. Type L and type R isoforms are expressed in liver and red blood cells respectively and the M1 isoform is expressed in tissues requiring production of large amounts of energy such as brain and muscle[266, 267]. The type M2 isoform (M2-PK) is a splice variant of the M1 form[266]. It is an embryonic

form present during development and in most tissues is eventually replaced by other isoforms. M2-PK is found in some differentiated tissues and is characteristic of cells with a high rate of nucleic acid synthesis including many proliferating cell types and significantly, tumour cells[5, 268-272]. The PK isoenzymes are tetramers containing four identical subunits, however, M2-PK is unique in that it can exist both as a tetramer or dimer giving it exclusive properties[266, 273-277].

Pyruvate kinase and cancer cell metabolism

Other than its mere presence in cancer tissues which indicate that it may at least stand as a marker for cancer, several observations outlined below have led to the hypothesis that M2-PK and particularly its ability to exist in the dimeric form have a key role in the metabolic and genetic aspects of cancer cell survival and tumour growth.

Dimeric M2-PK has been demonstrated in metastatic cancer cells but not in adjacent lung tissue suggesting it is a tumour-specific form[273, 278, 279]. However, since this discovery, the dimeric form has been also been identified in non-cancerous proliferating tissues[270, 271]. Nevertheless, it is thought to be the predominant form in cancer cells and has therefore been termed tumour M2 pyruvate kinase[270, 280]. This terminology causes confusion in the literature whereby when total M2-PK (dimeric + tetrameric) is measured, it is sometimes referred to as tumour M2 pyruvate kinase due do it having the potential to exist in dimeric form rather than it actually occurring in the dimeric form.

The dimeric form has significantly lower affinity for the substrate phosphoenolpyruvate and therefore has much lower enzyme activity compared to the tetrameric form[273, 274, 281, 282]. Importantly, this allows glucose to be channelled into the synthesis of nucleic

acids, amino acids and phospholipids via the build-up of glycolytic intermediates which is thought to be essential for the proliferation of tumour cells[274, 282].

Tissues which do not normally express M2-PK start expressing it during tumorigenesis[283-285]. Cristofk *et al's* work showed that M2PK expression was associated with increased glucose uptake and lactate production but decreased oxygen consumption in cancer cells, supporting its role in the aerobic glycolysis and was published in *Nature*[285]. Genetic manipulation used to switch M2-PK to M1-PK reverses the Warburg effect in cancer cells and M2-PK but not M1-PK induces tumour xenograft growth in mice[285].

In addition to its role in glycolysis, M2-PK is thought to have other cytosolic and nuclear actions which may encourage the survival and proliferation of cancer cells. These are diverse, complex and may be equally if not more important to tumour cells than simply the role of PK in glycolysis[286-291]. The observation that cancer cells typically fail to respond to apoptotic stimuli and their ability to adapt to hypoxia by increasing the glycolytic rate via M2-PK has led to investigation of the relationship between M2-PK and apoptosis[291]. Agents that induce apoptosis interact with M2-PK which is then translocated to the nucleus. This is sufficient to induce cell death in a way that is independent of its enzyme activity and isoform specific[291].

Tissue pyruvate kinase expression in oesophago-gastric cancer

The majority of published research on M2-PK in humans has focussed on its role in disease screening, particularly for cancers, using blood plasma and faecal enzyme assays. Despite this, although there has been some quantification of M2-PK expression in colon cancer[270], pancreatic cell lines[272, 292], lung cancer[293, 294] and breast cancer[5, 294], there has been relatively little in oesophago-gastric cancers. The work that has been

done suggests that compared to established tumour markers, plasma M2-PK has similar or better sensitivity and specificity for oesophageal and gastric cancer[295-297].

Two recently published studies have compared M2-PK levels in gastric cancers to adjacent normal tissue[288, 298]. Kwon *et al* demonstrated an increased expression in tissue microarrays (semi-quantitative) of cancer tissues using an antibody recognising total M2-PK protein[288]. M2-PK expression correlated with reduced survival and tumour size suggesting it may have a prognostic value. Lim *et al* showed increased levels of M2-PK mRNA in gastric cancer compared to normal gastric epithelium[298]. Their results suggest higher total M2-PK levels are associated with poorer survival in signet cell cancers. Neither study quantified dimeric M2-PK protein.

Two published reports have measured total M2-PK in squamous oesophageal cancer using a range of techniques[269, 294]. These reports did not measure dimeric M2-PK and they did not examine adenocarcinoma, which is the predominant subtype in the UK.

Koss *et al* used immunohistochemical staining to identify dimeric M2-PK expression in oesophageal adenocarcinoma and Barrett's oesophagus[271]. They demonstrated an increase in expression through the metaplasia-dysplasia-carcinoma sequence and they also identified M2-PK expression in reflux oesophagitis. They did not measure M2-PK in blood sera/plasma and did not analyse normal oesophageal mucosa for the presence of M2-PK.

Pyruvate kinase and pharmacological manipulation

Small molecule M2-PK activators have been identified that promote the formation of the tetramer thereby suppressing tumorigenesis in xenograft tumours[265, 299]. Another agent (TLN-232/CAP-232) has been used in phase II trials for patients with metastatic

renal cell carcinoma with encouraging results[264]. Shikonin is an extract from the root of Lithospermum erythrorhizon used in traditional Chinese medicine for its various anti-inflammatory properties. It is now understood to act via M2-PK and has previously been used in effectively in patients with late-stage lung cancer[300, 301]. However, despite the obvious potential M2-PK has as a target for chemotherapy, further work is needed to develop new agents and further evaluate established compounds.

Pyruvate kinase and disease monitoring

In a cohort of lung cancer patients undergoing chemotherapy, tumour remission and progression correlated appropriately with M2-PK expression, demonstrating that M2-PK could be valuable as a diagnostic aid for therapy control and may be able to detect tumour relapse after treatment[278].

M2-PK and chemotherapy response

The majority of research measuring M2-PK in the blood plasma of cancer patients has been to investigate its role in screening[295]. Therefore, whilst preoperative/pre-chemotherapy re-therapy plasma M2-PK levels in cancer patients and controls have been established, much less is known about the relationship between disease activity or response to neoadjuvant therapy and M2-PK.

Platinum-based chemotherapy

Yoo *et al* undertook in vitro studies showing that cisplatin-resistant gastric cell lines have decreased total M2-PK protein levels and lower PK activity. In addition, suppression of M2-PK activity results in acquired cisplatin resistance[262].

Likewise, in colorectal cancer cell lines, using a proteomic approach, decreased M2-PK mRNA was associated with oxaliplatin resistance in human colorectal cancer cell lines and patients with colorectal cancer[261].

A further proteomic approach was used in an effort to understand the molecular mechanisms of multidrug resistance in ovarian cancer[302]. M2-PK was differentially expressed in cisplatin-resistant cell lines compared to parent cell lines and further analysis showed that M2-PK along with another marker (HSPD1) could contribute to the cisplatin resistance in the ovarian cell line.

The mechanism(s) behind platinum chemotherapy-resistance caused by low M2-PK activity is not clearly understood, however, Yoo *et al* offer a possible explanation[262]. M2-PK in its dimeric form has low enzyme activity leading to accumulation of upstream metabolites necessary for cell proliferation. As a result, glycolytic carbons are directed to the pentose phosphate pathway where they can be used in nucleic acid synthesis. Tumour cells with low M2-PK activity rely on glutaminolysis for energy production but may also have increased NADPH production from the oxidative pentose phosphate pathway. NADPH is a cofactor of GSH reductase, necessary for reduction of oxidised GSH (GSSH) back to GSH (2 molecules). It is known that cisplatin is inactivated by GSH-linking which could explain the mechanism of resistance in conditions with reduced M2-PK activity, such as the presence of the dimeric, tumour-form[262]. Activation of the Thioredoxin (Trx) system by NADPH may also be responsible for cellular resistance to cisplatin by scavenging intracellular toxic oxidants generated by cisplatin[303-305]. It is possible, therefore, that low M2-PK activity as a result of increased proportion of the dimeric form may lead to increased NADPH production via the pentose phosphate pathway and glutaminolysis[262]. Higher NADPH levels may lead to platinum-compound resistance via GSH reductase and the Trx system.

5-FU-based chemotherapy

In one study, Shin *et al* attempted to identify markers of 5-FU resistance in human colon cancer cell lines[260]. They identified secreted proteins that were up- or down-regulated in resistant cell lines compared to non-resistant parent cell lines. M2-PK was one of a number of glycolytic enzymes shown to be upregulated in the resistant cells. A separate study has also shown that inhibition of glycolysis can overcome drug resistance in colon cancer cells and lymphoma cells under hypoxic conditions in which cells exhibit high glycolytic activity[306]. Since the rate of glycolysis is known to be dependent on M2-PK effects, Shin *et al* investigated the effects of M2-PK substrate and product on proliferation of 5-FU resistant cells. They found a differential effect of addition of these intermediaries depending on the presence or absence of 5-FU. In the absence of 5-FU, intermediaries allowed increased proliferation but in its presence, intermediaries had no such effect. This suggests that M2-PK activity is perhaps sensitive to 5-FU and it is suggested that this biomarker could be a potential target for therapy in 5-FU resistant cancer. It would have interesting to known whether this effect is mirrored in 5-FU sensitive cells. The same authors demonstrated a trend towards increasing levels of M2-PK in sera ($p=0.23$) and tissues ($p=0.34$) from colorectal cancer patients responding poorly to 5-FU-based neoadjuvant chemotherapy compared to partial and complete responders[260]. Serum M2-PK was measured using a semi-quantitative western blot technique recognising total (rather than dimeric) protein which is thought to be less specific to cancer cell metabolism. Response to therapy was measured by CT scanning which is known to be an inaccurate predictor of histopathological response and clinical outcome.

Other agents

One study in 4 patients with lung cancer measured blood M2-PK, CEA and CYFRA-21 levels throughout the course of poly-chemotherapy treatment[278]. In two patients, radiological remission due to successful chemotherapy was associated with a fall in M2-PK levels. In one patient, surgical resection was associated with a fall in levels and in the final patient, progression of disease despite chemotherapy was associated with a rise in levels. M2-PK appeared to follow disease remission/progression more accurately than either of the other two biomarkers. No statistical analysis was possible in such a small number of patients.

DNA damaging agents such as H_2O_2 and UV radiation are associated with isoform-specific M2-PK translocation to the nucleus which is sufficient and necessary for programmed cell death[291]. Importantly, overexpression of an inactive form of the enzyme decreases the overall metabolic rate of cells but without triggering apoptosis[291]. However, it would be interesting to know specifically whether M2-PK dimers are as effective as M2-PK tetramers in stimulating apoptosis.

Whilst the studies above provide some evidence that M2-PK may be inked with sensitivity to chemotherapy, there are obvious limitations and further work has been called for [260-263, 266, 302]. Whilst it is known that the dimeric form of M2-PK carries its tumour-specific metabolic properties, most studies have examined total M2-PK m-RNA or protein levels. Much of the work has been in vitro with very little in human subjects. The association between chemotherapy resistance and M2-PK has been explored to an extent in colorectal, ovarian and lung cancer but there has been no work in oesophago-gastric cancers. Specifically, there is a need to investigate the relationship between pre-treatment dimeric M2-PK levels and response to neoadjuvant chemotherapy in patients

with oesophago-gastric cancer. Similarly, to establish the use of M2-PK in early assessment of response to neoadjuvant therapy, the relationship between M2-PK levels after initiation of treatment and response to therapy would be useful.

Summary

M2-PK is an isomer of the glycolytic enzyme pyruvate kinase. It can exist as a dimeric form which is over-expressed in tumour cells and is detectable in peripheral blood. There is evidence that M2-PK and PK activity may be linked to chemotherapy resistance although there have been few clinical studies in this area and none have been conducted in oesophago-gastric cancer patients.

CHAPTER 7 - PREDICTING RESPONSE TO NEOADJUVANT THERAPY

Background

The high proportion of patients with oesophago-gastric cancer who do not respond to neoadjuvant chemotherapy/chemoradiotherapy is concerning and leads to adverse outcomes. Currently there are no reliable methods of predicting the response to neoadjuvant therapy in clinical use. Early assessment of the response, or better still pre-therapy prediction would enable non-responders to proceed directly to surgery or be considered for alternative therapies, avoiding unnecessary toxicity and a delay to surgery.

The use of biomarkers in predicting response is promising, but there is a need to evaluate new markers and investigate the potential of established markers. Such biomarkers need to be safe, readily available, cost effective and acceptable to patients.

M2-PK is a novel biomarker involved in cancer cell metabolism. It has been investigated from a screening point of view and has shown good accuracy in detection of oesophago-gastric cancer compared to other makers. It can be quantified in tumour tissue, faeces and peripheral blood. A small number of studies have suggested links between M2-PK and sensitivity to chemotherapy, through the pentose phosphate pathway, the Trx pathway and via nuclear translocation/apoptosis. This is an under-researched area and to date there have been no clinical studies investigating the potential of M2-PK in predicting response to therapy in patients with oesophago-gastric cancer.

Biomarkers with established uses that have recently been shown to demonstrate potential to predict response to chemotherapy are attractive subjects for further

investigation because analysis is usually low-cost and widely available. Existing markers showing promise in oesophageal and gastric cancers are CEA, CA 19-9 and CA 72-4.

A number of clinicopathological radiological markers have been identified as having potential to predict response to neoadjuvant therapy and a model combining these with molecular biomarkers may offer the best prediction of response to therapy.

There are many methods available for defining the response to neoadjuvant therapy, which include measures of histopathological regression and tumour/lymph node down-staging. Such methods must be validated in the relevant population, must be associated with survival benefit and must reflect a true therapy effect, or they are of no clinical use in this setting. In Chapter 3 it was established that in our population, response is defined by a Mandard TRG score of 1-3.

Chapter outline

To investigate whether plasma M2-PK levels can be used as a biomarker alone or in combination with other biomarkers, imaging or clinicopathological parameters to predict the response to neoadjuvant chemotherapy/chemoradiotherapy, a number of project designs were considered.

The basic methodology involves biomarker analysis from blood tests taken before starting neoadjuvant treatment and investigating the relationship between these levels and the histological response. The background literature review also suggested that biomarkers may be useful in monitoring/early assessment of the response to therapy. Measurement of biomarker levels at a time point after initiation of therapy would be necessary to investigate this further.

Study designs can be classified as retrospective or prospective and are detailed below. Following this, preliminary studies were undertaken and are described in their own section. Finally, the main study is presented making the greatest contribution to this final thesis Chapter.

Retrospective study designs

Retrospective studies have the advantage of being able to immediately access readily available data without needing to recruit new patients and await sample collection. The number of available subjects could be identified at the start of the study. It was noted that blood tests are routinely taken during the management of potentially curable oesophago-gastric cancer before induction of chemotherapy and after completion of each cycle.

This type of study has the disadvantages of relying upon saved samples, not knowing how samples have been stored and being unable to control the timing of samples. Tissue biopsy and resection samples are routinely stored for clinical use but serum/plasma samples may not have been placed in long term storage.

Peninsula oesophago-gastric cancer database

The Peninsula Oesophago-gastric Surgery Unit is the tertiary referral centre for the Peninsula region. It has been responsible for undertaking all oesophageal and gastric cancer resections since 2010 and has kept an electronic database of all cancer patients since this time. This contains data on basic patient demographics, clinical history, tumour characteristics, investigations, regional hospital location, treatments, operative details and histopathology results.

Some of the haematological and biochemical markers identified in Chapter 5 as having potential to predict response to therapy would have been routinely measured in patients registered in the database before they commenced neoadjuvant therapy. This is done for reasons other than response prediction such as ensuring adequate hepatic/renal function, immune-nutritional assessment and to act as a baseline for comparing with subsequent tests. Likewise, clinicopathological and radiological factors identified as potential predictors of response are held in the database. An analysis of this historical data with relation to the subsequent histological response is possible and may be able to confirm or deny whether these markers have any useful predictive value.

Tissue specimens from cancer resections are routinely stored in the Derriford Laboratory. These may be useful in identifying additional novel biomarkers, however, it was found that plasma/serum samples taken from patients are not currently saved beyond 3-5 days and there is no biobank facility currently in place for blood samples. A retrospective study design utilising the general cohort of historic oesophago-gastric cancer patients would therefore not be feasible for investigation of novel blood markers.

Screening project data

The Peninsula oesophago-gastric centre recently undertook a feasibility study into the potential of faecal and blood biomarkers as a means of screening for oesophageal and gastric cancer. Data on demographics, history, tumour characteristics, treatment, and faecal/peripheral blood biomarker levels were available as a pilot study.

Prospective study design

A prospective study would ensure that the correct samples could be taken at the optimal time points and processed in the correct way. Consent could be taken to include storage of samples which could be used for future research pending further ethics approval. A

prospective study would also ensure that accurate and detailed demographic and clinical data could be taken at the time of recruitment. A prospective study, however, can be a lengthy process, slow to recruit participants and acquire data. This also involves an inbuilt delay in obtaining the necessary data (response to neoadjuvant therapy). When the anticipated low proportion of responders is taken into account, there is concern that it may take a long time to recruit the required sample size.

Preliminary studies

Screening project database

An examination of the screening project data provided pilot data on pre-therapy biomarker levels and the TRG in patients with oesophageal and gastric cancer. This was done as a pilot study in an attempt to demonstrate basic proof of concept that a formal biomarker discovery project was worthy of undertaking.

A group of 53 cancer patients had pre-therapy biomarker data available (plasma M2-PK, serum CA19-9, serum CA72-4 and faecal M2-PK). It was made up of a heterogeneous group of patients. 22 of these were not suitable for surgery either due to advanced stage or medical co-morbidity. Of the 31 patients undergoing surgery, nine did not receive neoadjuvant chemotherapy. Four patients had missing data on the TRG, leaving a total of 18 patients with data available on biomarker levels and TRG. It should be noted that at the time of this analysis, before starting the 2-year project, a clinically significant histopathological response was thought to be defined by a TRG score of 1-2. Two of the 18 patients were TRG 1-2. When the patients were plotted on a scatter diagram, it appeared that the responders had relatively low plasma M2-PK, CA19-9 and CA72-4 levels compared with non-responders (see Figure 52, Figure 53 and Figure 54). Serum CA19-9 levels were plotted on a logarithmic scale due to left-skewed data.

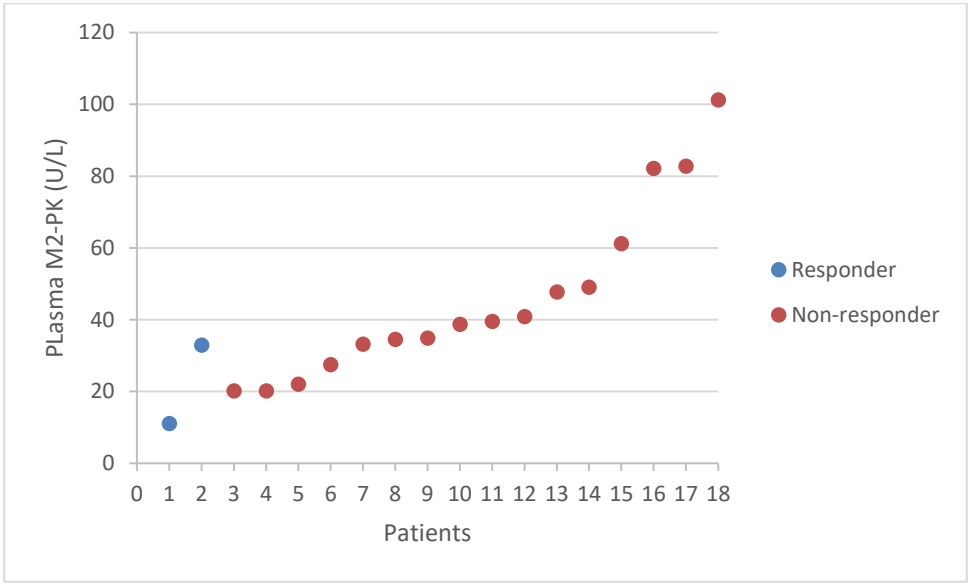

Figure 52 Plasma M2-PK levels in responders and non-responders to neoadjuvant therapy

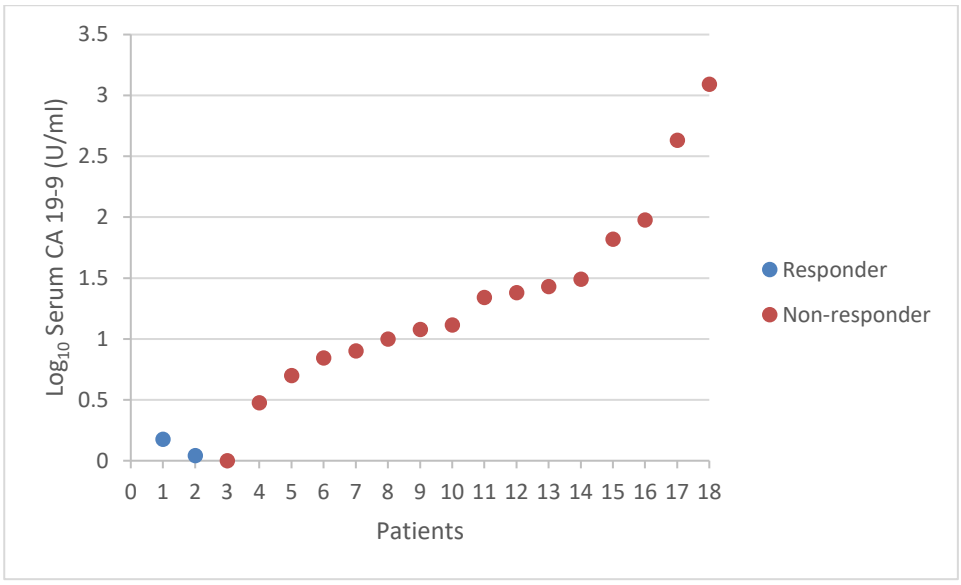

Figure 53 Log_{10} Serum CA 19-9 levels in responders and non-responders

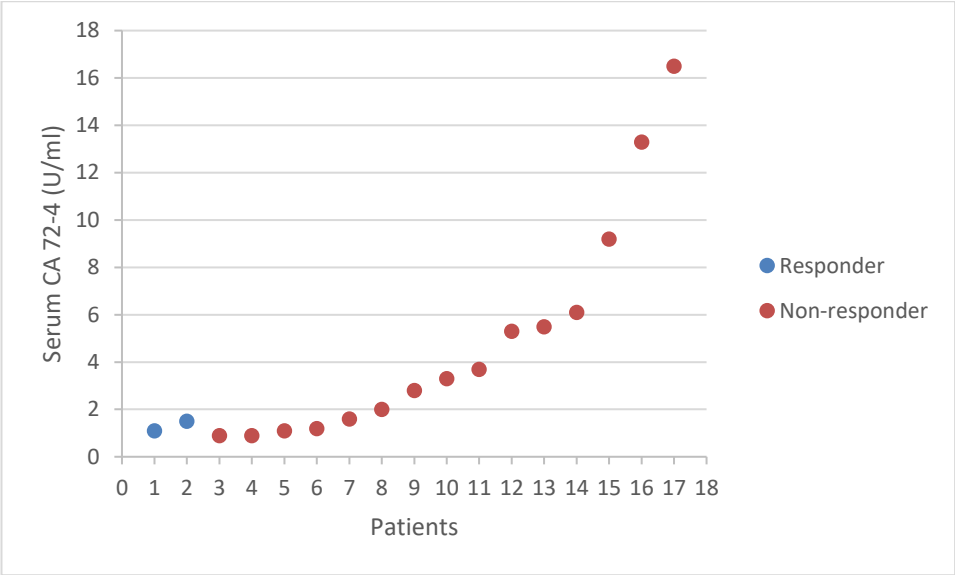

Figure 54 Serum CA 72-4 in responders and non-responders

Mean, median and range of biomarker levels are shown according to whether patients were neoadjuvant therapy responders or not (see Table 58). Average biomarkers levels appeared to be higher in responders compared to non-responders for all biomarkers. With only two patients in the responder group, this was felt too small for statistical analysis.

Marker	n		Mean		Median (range)	
	Resp.	Non-resp	Resp.	Non-resp.	Resp.	Non-resp.
M2-PK	2	16	22.0	46.0	22.0 (11.1-33.0)	39.2 (27.6-101.29)
CA 19-9	2	16	4.0	124.5	4.0 (2.0-6.0)	17.5 (1-1240)
CA 72-4	2	15	1.3	4.9	1.3 (1.1-1.5)	3.3 (0.9-16.5)

Table 58 Mean, median and range of biomarker levels are shown according to whether patients were neoadjuvant therapy responders or not.
Abbreviations: Resp, TRG responder; Non-resp, TRG non-responder

This limited amount of information adds support to the theory that it would be worth investigating the role of plasma and serum biomarkers in predicting the response to chemotherapy.

Historical data

Pre-treatment clinicopathological factors identified in the literature review (Chapter 5) as having potential to predict response to therapy in patients suitable for curative treatments included sex, T-stage, tumour grade, tumour length and tumour type (intestinal/diffuse).

Pre-treatment biochemical markers showing promise included alkaline phosphatase, albumin and alanine transaminase. Haematological markers identified included haemoglobin and white blood cell parameters including the neutrophil/lymphocyte ratio.

Methods

From the Peninsula Oesophago-gastric database, patients undergoing neoadjuvant therapy for oesophageal or gastric cancer and proceeding to resection over a five-year period from 2010 to 2014 inclusive were identified.

Clinicopathological markers

Demographics, tumour details, clinical staging information and treatments for all patients were recorded. The relationships between various pre-therapy clinical variables and TRG response were investigated to identify any factors displaying potential to predict response.

Blood biomarkers

From this 5-year cohort, pre-treatment blood biomarker data were available only in patients on the Plymouth site (PHNT). Levels of haematological and biochemical markers in these patients were identified from the electronic laboratory results system and the relationship between marker levels and TRG response investigated.

Results

376 patients had planned to undergo neoadjuvant therapy. 38 patients did not proceed to resection and 338 were resected. 7 patients did not have TRG data available leaving 331 patients for the analysis, see Figure 55. Demographic, tumour, staging and treatment details of these patients are shown in Table 59. 86 of the 331 (26.0%) patients responded to neoadjuvant therapy using the TRG 1-3 definition.

Clinicopathological markers

None of the clinicopathological variables were associated with TRG response (Table 60).

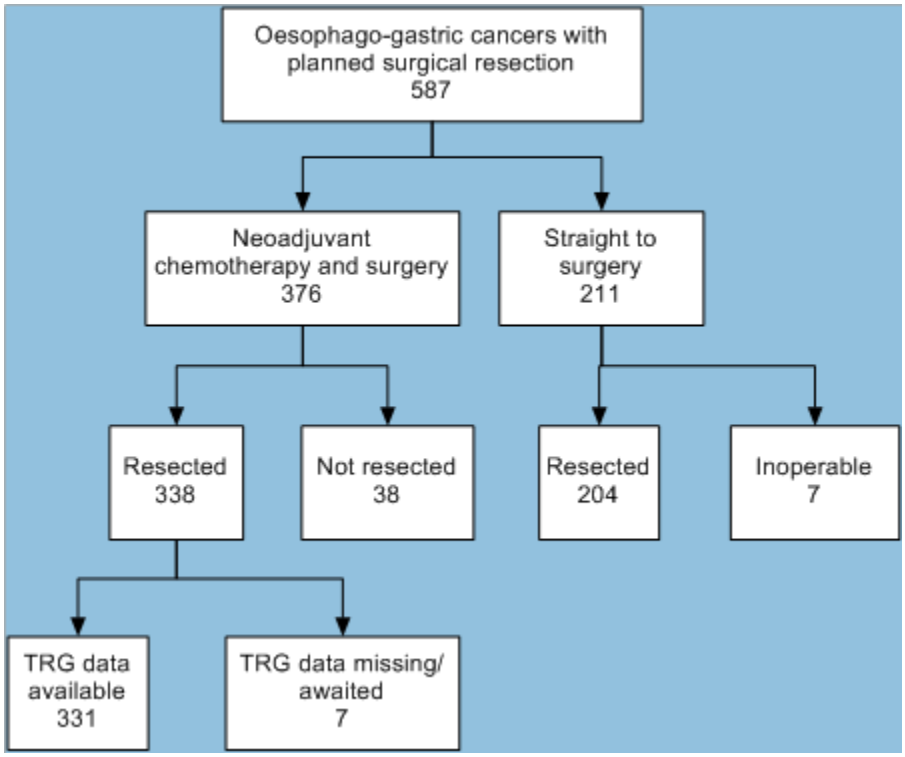

Figure 55 Patients identified from the database

Patient characteristics N=331	Non-responders N=245 (74.0 %)	Responders N=86 (26.0%)	Significance
Demographics			
Age, in years; mean (range)	64.7 (29.3-80.7)	63.9 (31.5-82.7)	0.89 (MWU)
Male, number (%)	190 (74.5)	65 (25.5)	0.82 (chi-sq)
Female	55 (72.4)	21 (27.6)	
Performance score, n (%)			
0	171 (72.2)	66 (27.8)	0.44 (chi-sq trend)
1	64 (79.0)	17 (21.0)	
2	3 (60.0)	2 (40.0)	
missing	7 (87.5)	1 (12.5)	
Referring Unit			
PHNT	68 (80.0)	17 (20.0)	0.73 (FE)
RCHT	55 (72.4)	21 (27.6)	
RDE	53 (70.7)	22 (29.3)	
SDHT	47 (73.4)	17 (26.6)	
NDDH	21 (70.0)	9 (30.0)	
Other	1 (100)	0 (0)	
Tumour characteristics			
Histological type, n (%)			
Adenocarcinoma	219 (74.0)	77 (26.0)	0.91 (FE)
Squamous cell carcinoma	24 (72.7)	9 (27.3)	
Other carcinoma	2 (100.0)	0 (0)	
Tumour location, n (%)			
Oesophagus	48 (67.6)	23 (32.4)	0.34 (chi-sq)
Gastro-oesophageal junction	165 (76.4)	51 (23.6)	
Gastric	32 (72.7)	12 (27.3)	
Tumour length, in cm median (range)			
Length, in cm, median (range)	5 (1-17)	5 (2-12)	0.56 (MWU)
Missing, n (%)	158 (64.5)	56 (65.1)	
Histological pattern			
Intestinal	140 (74.5)	48 (25.5)	0.94 (FE)
Diffuse/Mixed	10 (71.4)	4 (28.6)	
Diffuse/Signet ring	48 (75.0)	16 (25.0)	
unknown	47 (72.3)	18 (27.7)	
Differentiation grade			
Well	4 (100.0)	0 (0)	0.55 (chi-sq trend)
Moderate	89 (74.8)	30 (25.2)	
Poor	118 (73.8)	42 (26.3)	
unknown	34 (70.8)	14 (29.2)	
Pre-operative staging			
Pre-operative T stage, n (%)			
≤T2	47 (78.3)	13 (21.7)	0.46 (chi-sq trend)
T3	187 (73.0)	69 (27.0)	
T4	11 (73.3)	4 (26.7)	
Pre-operative N stage, n (%)			
N0	94 (70.7)	39 (29.3)	0.49 (chi-sq trend)
N1	129 (77.2)	38 (22.8)	
N2	22 (71.0)	9 (29.0)	
Neoadjuvant regimen, n (%)			
Cisplatin+5-FU/capecitabine	41 (82.0)	9 (18.0)	0.06 (FE)
ECX/ECF/EOX (+/- bevacizumab)	177 (72.2)	68 (27.8)	
CROSS style	3 (37.5)	5 (62.5)	
Carbotaxol +/- epirubicin/etoposide	3 (75.0)	1 (25.0)	
Missing	21 (87.5)	3 (12.5)	
Neoadjuvant therapy cycles, n (%)			
1	11 (91.7)	1 (8.3)	0.54 (chi-sq trend)
2	35 (71.4)	14 (28.6)	
≥3	104 (74.8)	35 (25.2)	
missing	95 (72.5)	36 (27.5)	

Table 59 Demographic, tumour, staging and treatment details of patients undergoing neoadjuvant therapy and surgery.

Blood biomarkers

Of 85 patients from PHNT, 84 had data available on blood markers (see Table 60). None of the haematological or biochemical biomarkers were associated with response to neoadjuvant therapy.

Patient characteristics	Non-responders (n=67)	Responders (n=17)	Significance MWU Ex 2-tailed
Haematological Markers, median (range)			
Haemoglobin	138 (75-166)	135 (117-164)	0.99
Platelets	289 (105-838)	282 (133-442)	0.50
Haematocrit	0.41 (0.26-0.49)	0.41 (0.35-0.47)	0.62
Total White cell count	8.1 (3.6-20.8)	8.3 (5.1-15.2)	0.52
Neutrophils	4.9 (1.4-15.9)	6.0 (3.2-11.8)	0.46
Lymphocytes	2.1 (0.7-3.8)	1.9 (0.7-3.1)	0.77
Neutrophil: Lymphocyte ratio	2.55 (0.82-7.67)	2.47 (1.19-16.86)	0.51
Biochemical Markers, Median (range)			
Alkaline phosphatase	78 (75-166)	67 (117-164)	0.28
Alanine aminotransferase (ALT)	18 (10-40)	20 (11-43)	0.26
Albumin	43 (36-53)	44 (40-47)	0.60

Table 60 Haematological and biochemical biomarker levels in histological responders and non-responders. Abbreviations. MWU, Mann-Whitney U test

Preliminary studies summary

A striking feature of this preliminary work, as also identified in Chapter 3, is the low proportion of patients that respond to chemotherapy - only 12.9% using the old definition (TRG 1-2) and 26.0% when considering the updated definition (TRG 1-3). This indicates the magnitude of the problem, with the great majority of patients not responding to neoadjuvant therapy and not expected to benefit. This makes it difficult to interpret results of the preliminary study. In the pilot study from the screening project data, only 2 of the 18 patients with complete TRG and biomarker data available were classified as responders to treatment using the definition at the time. These unequal sample sizes and in particular the small number of patients in the responder group limits the statistical analysis that can be performed.

From a research perspective, it is interesting to note that patients do undergo blood tests as part of routine treatment at the time periods at which blood samples would need to be taken as part of a prospective study. Currently there is no system in place to store such samples for use in future research. This appears to represent an underutilised resource and Plymouth Hospital NHS Trust Research & Development Department is investigating the feasibility of setting up a research bank which could include the storage of such samples. This would be a big step forward in maximising the potential of biological material taken (through routine diagnostic purposes) for use in future research.

Despite literature reports suggesting a link between routine haematological/biochemical parameters measured in peripheral blood and response to chemotherapy, data here do not support this. This may be because better patient selection means patients have better pre-therapy nutritional status and therefore less variation in levels of nutritional markers. It may be that there is a link between poor nutritional status and response but that this is not relevant in the context of modern patient selection.

Without any long-term storage of blood samples, a retrospective study design for investigating the use of novel biomarkers in prediction of response to neoadjuvant therapy was impossible, necessitating a prospective study.

Prospective study

Aims and objectives

The study aimed to investigate whether pre-therapy plasma/serum levels of the biomarkers M2-PK, CEA, CA19-9 and CA72-4 can be used to predict the response to neoadjuvant therapy in patients with oesophageal and gastric carcinoma as part of a curative treatment plan. Secondary aims were to investigate the performance of biomarkers in early assessment of response to neoadjuvant therapy.

Methods

Overview

A multi-centre, prospective study was set up to recruit patients with oesophageal and gastric adenocarcinoma before starting neoadjuvant therapy. Patients referred to the tertiary Peninsula Oesophago-gastric Surgery Unit from the locality and 3 other regional NHS Trusts were included in this part of the study. After collaboration with external research organisations (detailed below), further clinical patient data and samples were acquired to contribute to the sample size.

The methods of each part of the study need to be explained separately and for clarity, the local/regional and collaborative parts of the study will be simply referred to as the 'regional' study and the 'collaborative' study respectively.

Analysis of biomarker levels was performed on peripheral blood samples taken before starting neoadjuvant therapy (regional study and collaborative study) and after completion of the first cycle (regional study). Clinicopathological, demographic and radiological data were recorded in all patients and the ability of biomarkers to predict histological response to therapy was investigated.

Collaborations

Oesophageal Cancer Clinical and Molecular Stratification Study

Derriford Hospital is participating in the multicentre Oesophageal Cancer Clinical and Molecular Stratification Study (OCCAMS). This is a multicentre study established to determine predictive and prognostic biomarkers and therapeutic targets for oesophageal and junctional adenocarcinoma including whole genome sequencing. Patients from a number of UK sites are locally recruited to the study and consented. The study involves the collecting of demographic data, clinicopathological data, blood samples and resected tissue samples. These are being used to identify predictive and prognostic biomarkers alongside validating a molecular staging system and completing a DNA sequencing project as part of the International Cancer Genome Consortium (ICGC).

In 2013, the OCCAMS collaboration offered all participating centres the opportunity to set up collaborative sub-studies. As part of this arrangement, local centres could benefit from sharing centrally collected clinical data and laboratory samples for use in their own sub-studies. An application for a collaborative study was submitted and approved in August 2013. Access to plasma samples and clinicopathological data has been granted pending ethics approval which was subsequently obtained. This was the first such OCCAMS sub-study to be supported by the group.

Scottish Academic Health Sciences Collaboration

Edinburgh is one of the centres contributing to the OCCAMS study. The unit independently collects and stores blood samples for research purposes from patients with oesophago-gastric cancer as part of the Scottish Academic Health Sciences Collaboration (SAHSC) BioResource. The Edinburgh study team agreed to collaborate separately, providing anonymised plasma samples and data for this study.

Sponsorship

The project was reviewed, approved and sponsored by Plymouth Hospitals NHS Foundation Trust Research & Development Department.

Ethics

Ethical considerations

This study did not introduce an intervention or test result that altered patient management. The treating physicians did not need access to participants' research data.

The study protocol involved the taking of an additional 4ml venous blood via EDTA Vacutainer ® before and after the first chemotherapy cycle. In most cases it is expected that these would be performed alongside routine blood tests and would not involve additional skin puncture, however, approval was sought for phlebotomy specifically for the purposes of this study.

There were no specific reasons related to the study protocol that would have expected to result in informed consent not being granted.

Ethics approval

Regional study

The main protocol received a favourable ethical opinion from the Bristol Research Ethics Committee on 4th September 2013.

Study title:	Predicting the response to neoadjuvant therapy in patients with oesophago-gastric cancer
REC Reference:	13/SW/0208
Protocol number:	13/P/062

IRAS project ID: 132595

Collaborative study proportionate review

The protocol for the receipt and analysis of anonymised samples and clinical data from the OCCAMS and SAHSC collaborations received a favourable ethical opinion from the Nottingham 1 NRES Committee, East Midlands on 13th November 2013.

Study title: Predicting the response to neoadjuvant therapy in patients with oesophageal cancer– collaboration with the OCCAMS/Edinburgh studies

REC Reference: 13/EM/0437

Protocol number: 13/P/157

IRAS project ID: 139550

Sample size and recruitment planning

It was necessary to perform a sample size calculation at an early stage along with a review of the patient numbers treated in the unit to understand how long it would take to recruit patients and how many sites would need to be involved in recruitment.

Sample size

An initial sample size calculation was performed using the G*Power application based on an independent two-sided t-test with an effect size of 0.75 and a power of 0.8. Anticipating that 25% of patients would respond to treatment (TRG 1-3) and 75% would not respond, this generated a total sample size of 78 (19 responders and 59 non-responders).

A more sophisticated power analysis according to the proportion of patients from each group (responder/non-responder) falling above or below a range of biomarker

thresholds indicated that a sample size of 92 (estimated responder to non-responder ratio of 3:1) would identify responders with a sensitivity of 90% and a specificity of 40% for a single biomarker, equivalent to an effect size of 1.0 at a power of 0.99 and significance level of 0.05. The addition of further discriminatory biomarkers/clinicopathological features would increase the predictive accuracy of the test.

Hospital Sites

The Peninsula Oesophago-gastric Surgery Unit, based within Plymouth Hospitals NHS Trust (PHNT) at Derriford Hospital receives tertiary referrals from the Royal Devon & Exeter Hospitals NHS Foundation Trust (RDE), the Royal Cornwall Hospitals NHS Trust (RCHT), the South Devon Healthcare NHS Foundation Trust (SDHT) and the North Devon District Hospital NHS Trust (NDDH).

The Collaborative studies pledged to contribute a total of 50 patient samples. With an estimated 25% drop out rate for lack of suitability for laboratory testing or incomplete histological data, this would total 38 valid patient samples. This would leave 54 patients needing inclusion from the regional study. Data from the pilot study were available on 18 patients, leaving data from 36 patients needed from the regional study. Allowing for a 30% drop out of patients not proceeding to surgical resection after neoadjuvant treatment this would require recruitment of 51 patients in the regional study bringing the total recruitment number to 119 patients.

Over the past 2 years, The Peninsula Unit has treated on average 4.83 typically eligible patients per month. A recruitment rate of 70% total eligible patients predicts it would take 15 months (64 weeks) to recruit the required 51 patients. The recruitment period was established as October 2013 to December 2014 inclusive. It was predicted that the

70% recruitment target could be reached by recruiting mainly from the PHNT site, but also from RDE, RCHT and SDHT sites. NDDH refers very few patients each year (<5) and it was felt that the potential benefit of reaching this small number of patients would be outweighed by the significant additional burden of setting up on this site together with lengthy journeys necessary for site visits and sample transfers, therefore, NDDH was not included in the regional study. Some peripheral site patients (RCHT/RDE/SDHT) could be recruited on the local site (PHNT) when visiting for staging investigations. Other peripheral patients would need to be recruited at their local sites.

Participants, Regional Study

Patient pathway and recruitment

Patients with newly diagnosed, histologically proven oesophageal and gastric cancer were identified though multidisciplinary team meetings at Derriford Hospital and recruited after histological diagnosis had been confirmed.

As part of the usual patient management, the multidisciplinary meeting establishes the diagnosis and staging and a treatment plan is made that will include whether or not a patient is suitable for surgery. This may be after a planned course of chemotherapy with reassessment of surgical suitability after completion or without preoperative chemotherapy. Patients are normally then invited to the surgical outpatient clinic where the diagnosis and treatment options are discussed including surgery. If they wish to pursue a radical treatment plan including surgery and are offered neoadjuvant therapy they are also seen by a Consultant oncologist in the oncology clinic.

At this time, if felt appropriate, patients due to embark on a radical treatment plan including neoadjuvant chemotherapy and surgery were approached by either a

Consultant Oncologist or Consultant Surgeon and invited to discuss participating in the study. If the patient agreed to discuss it further then they were introduced to the Principal Investigator, Mr Bunting who counselled the patient regarding the study, formally offered them the opportunity to participate, gave them a patient information leaflet (see Appendix I) and took informed consent (see Appendix II).

Eligibility assessment

Inclusion criteria

- Participant diagnosed with histologically proven gastric or oesophageal cancer
- Participant willing and able to give consent to participate in the study
- Able to comply with all study requirements
- Agrees to involvement in the study being known to the study management group, treating clinicians and patient's general practitioner
- Patient planned to undergo surgical resection

Exclusion criteria

- Participant unable or unwilling to give consent
- Participant under the age of 18 years

Withdrawal criteria

- Participant withdrawing consent
- Significant deviation from the study protocol
- Adverse event effecting ability to comply with study requirements
- Participant lost to follow up

Participant involvement

After treatment decisions were made in the clinic, patients underwent routine venepuncture prior to commencing any chemotherapy or surgery. Recruited study participants would need to give two additional 4ml blood tubes for the study. It was expected that in most cases this would be done alongside the usual tests at this time. If undergoing chemotherapy prior to surgery, then blood tests were also performed after each cycle. Similarly, an additional sample was taken for purposes of the study at time.

Venepuncture was carried out by trained phlebotomy staff/the Chief Investigator.

There was no requirement for participants to make additional hospital attendances for venepuncture, clinic appointments, follow-up, investigation, procedures or operations over and above those normally required in the usual course of management of their condition outside the study.

Blood tests at peripheral sites

The first blood test taken after recruitment would be performed at the local hospital either by the Chief Investigator or the Phlebotomy Department. The second blood test (after first chemotherapy cycle) would be taken by the local hospital Phlebotomy department who would routinely be taking a test at this time.

Participants, Collaborative study

Participants recruited through the OCCAMS and SAHSC had consented to the future analysis of stored peripheral blood samples (see Appendices III and IV). In the OCCAMS study, blood samples had been taken at local sites and transported to the storage facility in Cambridge. In the SAHSC study, blood samples were stored in the SAHSC BioResource.

Data Collection

Regional study

Patient demographic details; medical history; tumour details (including anatomical location, subtype and stage); neoadjuvant treatment details; operative details and post-operative pathological detail were recorded. Radiological response to chemotherapy was assessed by Consultant radiologists with a special interest in Upper GI radiology according to the RECIST criteria[62]. Pathological details were reported by Consultant histopathologists with a special interest in Upper GI pathology. Histopathological response to therapy was reported according to the Mandard TRG Score.

Collaborative Study

Demographic details, medical history, cancer location, subtype and stage, radiological response to therapy and histopathological response to therapy were recorded as part of the OCCAMS and Edinburgh studies. Mr Bunting was given access to these data in anonymised form for use in this this study.

Sample handling

Regional study

Blood samples were taken to specimen reception at Derriford Combined Laboratories within 1 hour of taking. Samples were processed in the laboratory within 30 minutes. This consisted of centrifuging samples for 3 minutes at 3400rpm, then aspirating the serum/plasma supernatant and transferring to separate barcoded tubes. Serum samples were immediately used for alkaline phosphatase and CEA analysis. CA 19-9 tests were run twice weekly from refrigerated samples. The remaining serum was frozen at -20 degrees centigrade for subsequent analyses. Plasma samples were frozen for subsequent ELISA M2-PK batch-analysis. Serum samples were frozen for subsequent

CA72-4 batch analysis. All stored serum/plasma samples were labelled with a unique sample number, trial number and whether they contained EDTA plasma or serum.

Collaborative study

Initial blood sample handling occurred at each local centre. They were centrifuged and the plasma component separated then placed in frozen storage locally. In the OCCAMS study, samples were then transferred to a central storage facility in Cambridge where they are subjected to analysis as part of the OCCAMS study. In the Edinburgh study, samples were stored in a laboratory facility in Edinburgh. A sample of each patient's plasma was sent in anonymised form under a material transfer agreement (MTA) to Derriford Hospital for analysis in the present study. Biomarker levels were measured in plasma samples at the Clinical Biochemistry laboratory, Derriford Hospital.

Temporary sample storage

Samples were kept in frozen storage at -20 degrees centigrade.

Long-term sample storage

Plasma and serum samples remaining after analysis were kept in a storage facility frozen at −80°C for 5 years.

Sample transport

The full panel of laboratory tests required for the study were only available at the Derriford site therefore all samples taken at local sites needed to be locally processed, temporarily stored, then transferred to Derriford for analysis. Initial local processing of samples followed the same procedure as above. Samples were transferred back to Derriford in insulated ice-packs.

Biochemistry laboratory biomarker analyses

M2-PK assay

Plasma samples were analysed using the dimeric M2-PK enzyme linked immunosorbent assay (ELISA) manufactured by ScheBo Biotech, Giessen, Germany ('ELISA EDTA-Plasma Test'), see Figure 56 and Figure 57. This is a CE-marked, highly sensitive ELISA which allows the quantitative measurement of dimeric ('Tumour') M2-PK in EDTA-plasma. The test is based on two monoclonal antibodies which specifically react with the dimeric form of M2-PK and do not cross react with the other isoforms of pyruvate kinase (Type L, R, M1 and tetrameric M2). Dimeric M2-PK levels are stable in blood plasma kept at room temperature for up to 24 hours, refrigerated for 7 days and frozen for at least 6 months.

Figure 56 ScheBo Tumour M2-PK EDTA-Plasma Test kit.

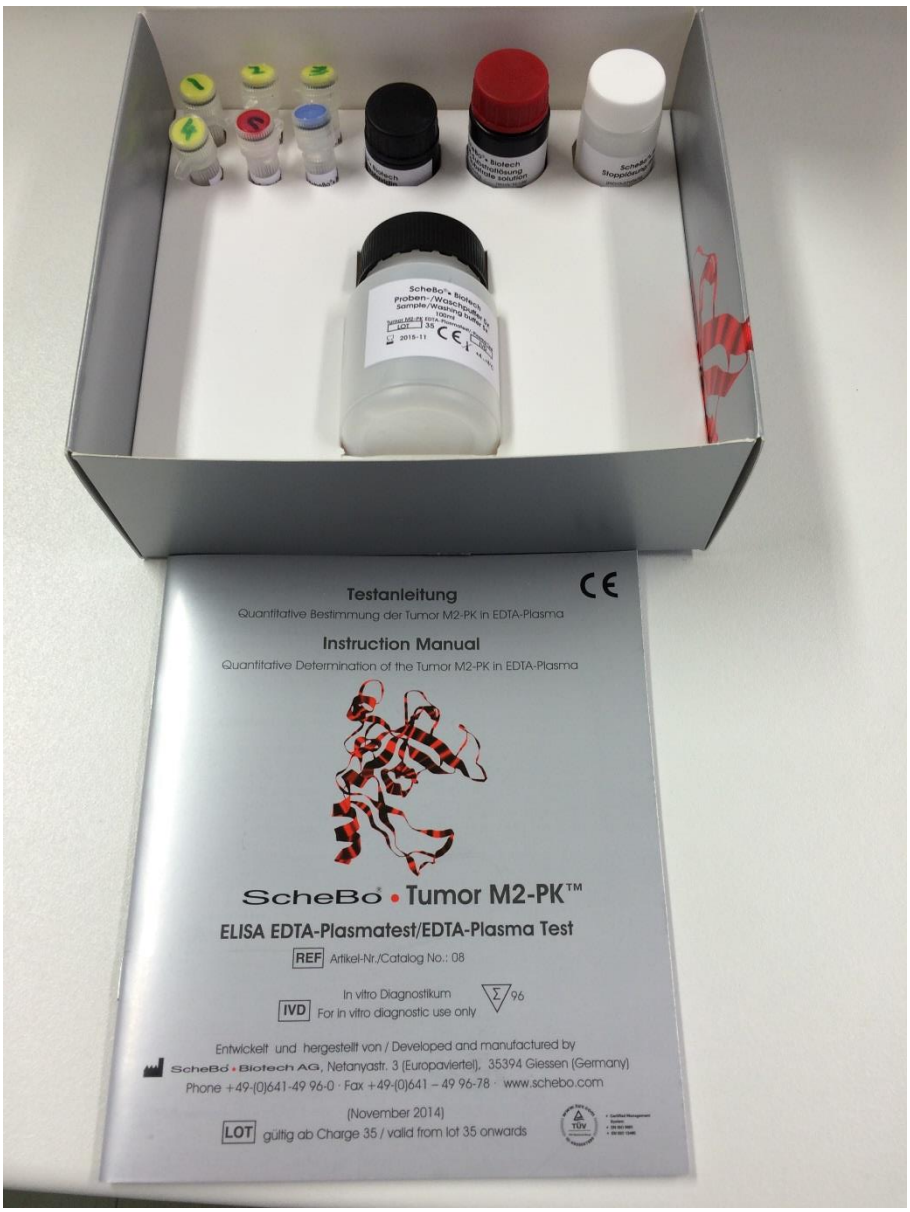

Figure 57 ScheBo Tumour M2-PK EDTA-Plasma Test kit.

Principle of assay

The ELISA plate is coated with a monoclonal antibody only recognising dimeric M2-PK. M2-PK protein in EDTA plasma samples and calibration standards binds to the antibody and is immobilised to the plate. A second monoclonal antibody binds to the M2-PK during the next incubation. The conjugate of POD (peroxidase) and streptavidin binds to the biotin moiety. The peroxidase oxidizes 3,3'5,5'-tetra-methyl benzidine. The concentration of oxidized TMB is then determined photometrically.

The manufacturers' instructions for the assay were followed precisely. Samples, standards and controls were pipetted in duplicate and the plates were read using a microplate reader, Multiskan EX, Thermo Electron Corporation (see Figure 60). Optical densities of 450nm and 620nm are used to take measurements between 5 minutes and 30 minutes after addition of the stop solution which changes the colour of the well fluid from blue to yellow. The M2-PK level is then calculated by using a calibration standard curve based on the average values of duplicate wells. The control has to read within 15% of its expected value for the assay to be valid.

M2-PK is stable in EDTA plasma for up to three days at 4°C and for up to one year at -20°C. All M2-PK assays were performed by Mr Bunting.

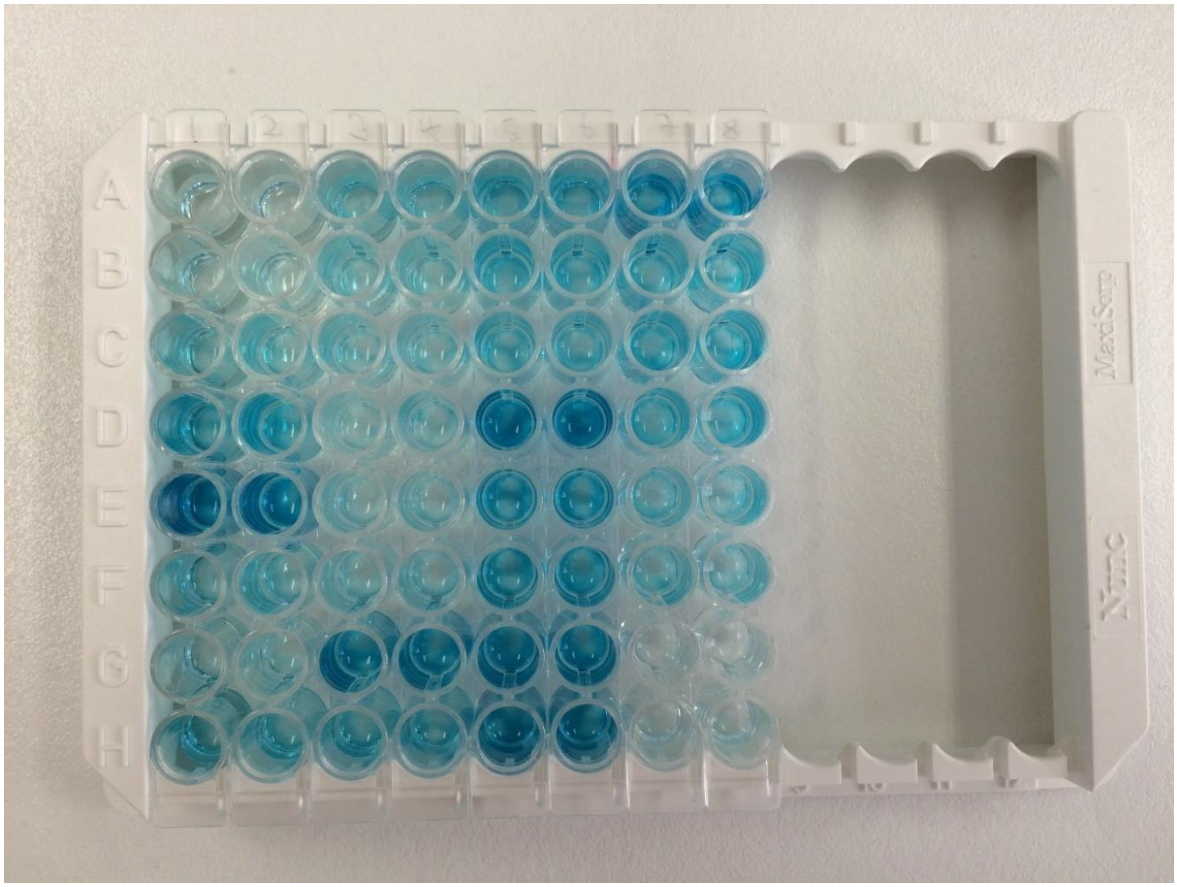

Figure 58 ELISA plate after colour reaction

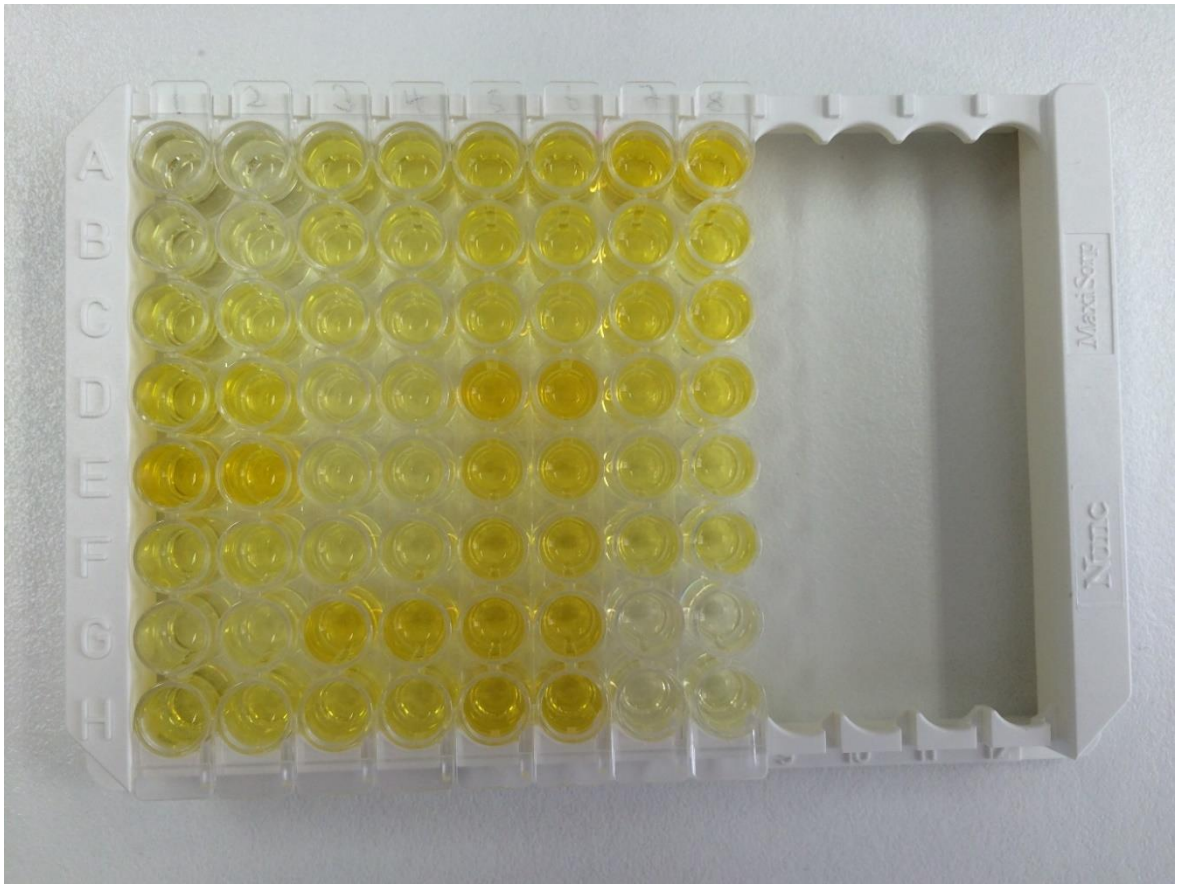

Figure 59 ELISA plate after stopping the colour reaction prior to measurement in the plate reader

Figure 60 Multiskan EX Thermo Electron Corporation plate reader in action.

CA19-9 assay

This assay is based on a sandwich ELISA technique using the 116-NS-19-9 antibody. Measurement is done by the use of a chemiluminescent reaction technique and was performed by Derriford Combined Laboratories technicians. A calibration curve was used to read off the biomarker levels.

CA72-4 assay

The CA72-4 assay is not routinely performed in the Derriford Combined Laboratories, however, although it had been through a successful validation process locally and was available for use in this study. The sandwich ELISA technique is similar to the CA19-9 chemiluminescent technique and utilises a biotinylated monoclonal antibody CC49 and the B72.3 antibody. Assays were performed by Derriford Combined Laboratories technicians.

Histopathological analysis

Response to neoadjuvant therapy was measured as part of routine staging using the tumour regression grade (TRG). According to recognised methods, TRG levels 1-3 were classified as responders and TRG levels 4-5 were classified as non-responders.

Data reporting and analysis

Demographics, pre-operative data

Patient demographics, pre-operative staging details and neoadjuvant therapy regimes were reported and compared in responders and non-responders to neoadjuvant therapy.

Resection pathology

Postoperative resection pathology details including T stage, N stage, lymphovascular invasion, presence of Barrett's oesophagus, Lauren classification, differentiation grade and R0 resection status were reported and compared in responders and non-responders to neoadjuvant therapy.

Analysis

To investigate whether pre-therapy plasma biomarker levels can predict response/resistance to chemotherapy, biomarker levels were compared in responder and non-responder groups.

The main cohort combining regional and collaborative patients was used for comparison of pre-therapy M2-PK levels in responders and non-responders to neoadjuvant therapy. It was anticipated that some patients would not have TRG data available for a number of reasons. A proportion of patients would exhibit progressive disease whilst undergoing chemotherapy and would therefore not proceed to resection where the TRG would be

reported. Similarly, it was anticipated that full pathological reporting including TRG scores may not have been available in all patients from all centres. This would lead to a number of patients not being included in the analysis and a reduction in the sample size. Although histological response (TRG) is not accurately predicted by radiological response, particularly in patients with radiological stable disease (RECIST criteria), those patients displaying progressive disease and not proceeding to resection are very unlikely to be scored as TRG1-3 if they had proceeded to resection and a partial response may be indicative of histological partial response (TRG2-3). Therefore, in order to benefit from the biomarker data in those patients without TRG data it was decided to perform a similar analysis with patients exhibiting progressive disease included in the non-responder group and those with partial response included in the responder group.

The regional patient cohort was used for comparison of CEA, CA19-1, CA72-4 and alkaline phosphatase levels in responders and non-responders since these were measured in serum samples that were only available in regional cohort patients.

Likewise biomarker levels after the first cycle of chemotherapy were only available in patients from the regional study.

For biomarkers expressing a difference between responder and non-responder groups, a sub-group analysis was used to identify differences according to chemotherapy type, histological type (Lauren classification) and recruiting centre (Regional/OCCAMS/Edinburgh).

Statistics

Statistical analysis was performed by Mr Bunting using SPSS v21 under the supervision and with the aid of Sue Ball, Research Fellow and acting lead of the Bioinformatics and Statistics Department, Plymouth University.

Continuous data, such as biomarker levels were tested for normality using the Kolmogorov-Smirnov test and equal variance was assessed using Levene's test.

For comparison of pre-operative factors in responders and non-responders, the unpaired student T-test was used for continuous variables. Fisher's exact test was used for categorical variables when the number of subjects in any cell was ≤5. The Chi square test was used for categorical variable and the Chi square test for linear trend was used for ordinal variables. The tests above were also applied to the comparison of post-operative factors in responders and non-responders.

For comparison of biomarker levels in responders and non-responders, the unpaired t-test or Mann-Whitney tests were used as appropriate.

For any biomarkers with significantly different levels in responders compared to non-responders, binary logistic regression analysis was performed to determine the performance of biomarker levels in predicting response/non-response. Fitted models were used to obtain predicted probabilities of non-response, across a range of biomarker levels. These were presented graphically, with 95% confidence intervals, as a predictive probabilities curve.

Data Storage and Management

Study data were stored and backed-up on secure, password-protected PHNT servers for five years using a password-protected database. Mr Bunting managed the database.

Case Report Forms were stored in the Postgraduate Surgical Research Office, Derriford. Standard Operating Procedures were maintained. The participants were identified by a study specific participants' code in the database. The name and any other identifying detail were not included in any electronic file. The NHS Mail email system was used for communicating any patient identifiable data.

Study Contributors

Regional study

The study protocol was written by the Principal Investigator, Mr Bunting and was reviewed by the co-investigators and the PHNT R&D department (see also Project Management). Advice on the laboratory techniques and feasibility of laboratory analyses was taken from Dr Ruth Ayling. Guidance on the study design was taken from Professor Mazurek, Justus-Liebig-University of Giessen, Germany. Mr Bunting was responsible for identifying eligible patients from the multidisciplinary team database. He was responsible for patient recruitment and taking informed consent. Mr Bunting personally performed venepuncture or liaised with the Phlebotomy department to co-ordinate this. Derriford Combined Laboratory staff trained Mr Bunting in the M2-PK ELISA technique.

Collaborative study

The Collaborative study protocol was approved by Professor Rebecca Fitzgerald, Chief Investigator of the OCCAMS study together with OCCAMS collaborative partners, as an OCCAMS-linked collaborative study. Mr Rob O'Neill is the Principal Investigator and Collaborator in the SAHSC-linked study and approved the protocol.

Informed Consent

Regional study

Consent for the study was obtained by Mr Bunting. It was taken at either of the opportunities described in the subject recruitment section above. A recent diagnosis of oesophageal or gastric cancer can have significant psychological impact on patients. Investigators therefore needed to express sensitivity in all matters including participation in the study. The appropriateness of introduction to the study was considered and the timing of invitation decided accordingly. Consent was only taken after a full verbal explanation and written information leaflet has been given to the patient outlining the exact nature of the study and its implications including potential risks in taking part. The participants understood that they were able to withdraw from the study at any time with no prejudice to future care and no obligation to give an explanation for the withdrawal. If patients agreed to participate, written consent was taken by means of participant dated signature and dated signature of the investigator. A copy of the signed Informed Consent was given to participants. The original was retained in the Research office.

Collaborative study

Patients had been consented under the OCCAMS and Edinburgh studies. These included agreements that stored blood samples may be used for future ethically approved research studies. Samples were anonymised and coded prior to storage.

Discontinuation/Withdrawal of Participants from the Study

Each participant had the right to withdraw study at any time. In addition, the investigator was able to discontinue a participant from the study at any time if the investigator considers it necessary for any reason including:

- Ineligibility (either arising during the study or retrospective having been overlooked at screening)
- Significant protocol deviation
- Significant non-compliance with study requirements
- An adverse event which resulted in inability to continue to comply with study procedures
- Disease progression which resulted in an inability to continue to comply with study procedures
- Consent withdrawn
- Lost to follow up

Source Data

Source documents included the patients written hospital notes; the digital whiteboard of patient information kept on a PHNT information technology server and electronic records in the case of radiological scans, radiological reports and laboratory results. These sources were be used to create CRF entries. The CRF was used as the source document for clinical information not held on the above records.

All documents were stored safely in confidential conditions. On all study-specific documents other than the signed consent, the participant was referred to by the study participant code, not by name.

Quality control, quality assurance procedures and study regulation

The study was conducted in accordance with the latest approved protocol. The investigator ensured that it was conducted according to principles of the following: the Declaration of Helsinki, the International Conference on Harmonisation (ICH) guidelines for Good Clinical Practice (CPMP/ICH/135/95, July 1996), the Data Protection Act, the

NHS Research Governance Framework for Health and Social Care (2nd edition) and PHNT standard operating procedures.

The study was considered low risk and as such the researchers monitored the study themselves. Specific reviews with input as required were performed by the Plymouth Hospitals NHS Trust Research Governance Manager, Chris Rollinson.

Data were evaluated for compliance with the protocol and accuracy in relation to source documents by the Principal Investigator.

Participant Confidentiality

The trial staff ensured that participants' anonymity was maintained. The participants were identified only by initials and a participant ID number on the CRF and any electronic database. All documents will be stored securely and only accessible by trial staff and authorised personnel. The study will comply with the Data Protection Act which requires data to be anonymised as soon as it is practical to do so.

Project management

Principal Investigator

- Mr David Bunting, Speciality Registrar in General Surgery and Clinical Research Fellow, (PHNT)

Supervisors/co-investigators

- Mr Grant Sanders, Consultant Upper GI Surgeon, PHNT (MD Supervisor)
- Dr Ruth Ayling, Consultant Clinical Chemical Pathologist, PHNT (MD Supervisor)
- Mr Tim Wheatley, Consultant Upper GI Surgeon, PHNT (Co-investigator)

Director of Studies

- Professor Janusz Jankowski, Associate Dean for Research, Plymouth University, Consultant Gastroenterologist, PHNT

Peripheral site Lead Investigators

- Dr Liz Toy (Royal Devon & Exeter NHS Foundation Trust)
- Dr Charlotte Thomson (South Devon Healthcare NHS Foundation Trust)
- Dr Richard Ellis (Royal Cornwall Hospitals NHS Trust)

Statistical Advice

- Andrew Bailey, Statistician
- Sue Ball, Statistician

Advisor

- Mr Steve Hornby, Speciality Registrar

Financing and insurance

Funding Sources

Research Funding of £8828.08 was awarded by Plymouth Hospitals General Charity.

Publication policy

Articles submitted for scientific publication will be reviewed by at least one of the study supervisors. All members of the study management team involved in the product of scientific work will appear as named authors. Acknowledgements will be made to others involved in the project but not directly contributing to the articles.

Results

Patients

62 patients were recruited from the regional study and 103 patients were recruited from the collaborative study, see

. This gave a total recruitment of 165 patients. 22 of these did not undergo neoadjuvant therapy for reasons detailed in

and were excluded from the study. Of these 22 patients, 19 were from the OCCAMS part of the collaborative study and 3 were from the regional study. Of 143 patients undergoing neoadjuvant therapy, 16 did not proceed to surgical resection and were excluded from the study (7 regional, 9 collaborative). Of the remaining 127 patients undergoing resection following neoadjuvant therapy, a further 22 patients were excluded due to lack of sufficient resection pathology data, all from the collaborative study. 1 patient had no chemo-naïve blood sample and the remaining 21 did not have a TRG score recorded. This left 105 patients eligible for analysis.

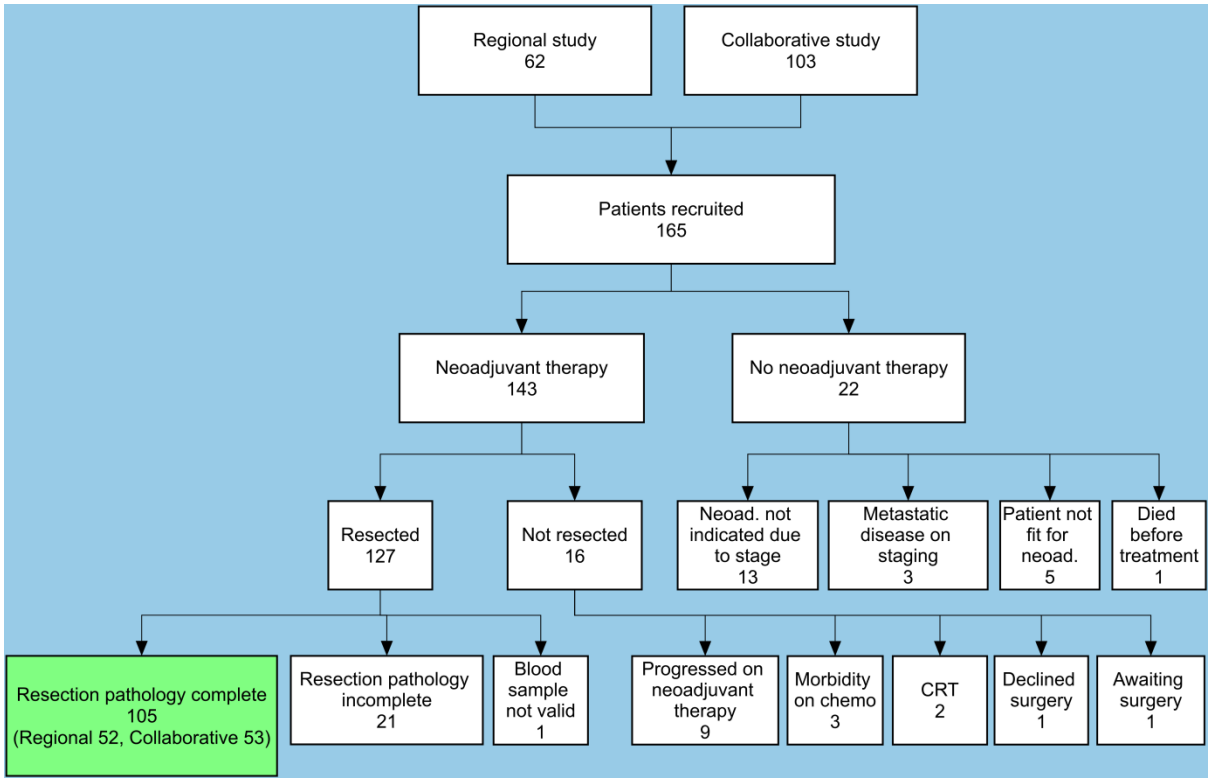

Figure 61 Patient recruitment and eligibility (abbreviations: Neoad., Neoadjuvant therapy; CRT, chemoradiotherapy).

Demographics and preoperative variables

Patient demographics, pre-operative staging details and neoadjuvant therapy regimes in responders and non-responders are shown in Table 61. There were 27 responders and 78 non-responders to neoadjuvant therapy.

There were no differences in pre-therapy demographic, pathological or treatment factors between the two groups.

Pre-operative factors, n=105		Responders (n=27)	Non-responders (n=78)	*P* value	*test*
Age, years	**mean (range)**	59.1 (33.2-78.0)	61.6 (34.2-79.0)	0.37	T-test
	missing, n (%)	3 (11.1)	13 (16.5)		
Gender	**M:F**	23:4	65:13	1.00	FE
BMI	**mean (range)**	26.4 (19.2-32.7)	26.5 (18.6-36.3)	0.98	T-test
	missing, n(%)	17 (63.0)	43 (55.1)		
Performance status	**0**	22 (29.3)	53 (70.7)	0.44	FE
	1	5 (19.2)	21 (80.8)		
	not reported	0 (0.0)	4 (100.0)		
Geographical site	**PHT**	10 (19.2)	42 (80.8)	0.20	FE
	REI	3 (21.4)	11 (78.6)		
	AHC	14 (35.9)	25 (64.1)		
Tumour type	**AC**	26 (26.0)	74 (74.0)	1.0	FE
	SCC	1 (20.0)	4 (80.0)		
Tumour Site	**Oesophagus**	4 (15.4)	22 (84.6)	0.26	FE
	Junction	19 (27.5)	50 (72.5)		
	Gastric	4 (40.0)	6 (60.0)		
Pre-treatment T Stage, n (%)	**1**	1 (100.0)	0 (0.0)	0.51	Chi-sq trend
	2	4 (36.4)	7 (63.6)		
	3	17 (20.0)	67 (79.8)		
	4	2 (50.0)	2 (50.0)		
	not reported	3 (50.0)	3 (50.0)		
Pre-treatment N Stage	**0**	7 (29.2)	17 (70.8)	0.625	Chi-sq trend
	1	11 (20.4)	43 (79.6)		
	2	5 (27.8)	13 (72.2)		
	3	0 (0.0)	2 (100.0)		
	not reported	3 (42.9)	4 (57.1)		
Prognostic Stage Group	**1**	1 (100.0)	0 (0.0)	0.34	Chi-sq trend
	2	8 (26.7)	22 (73.3)		
	3	15 (21.7)	54 (78.3)		
	missing	3 (60.0)	2 (40.0)		
Neoadjuvant regime	**Cisplatin/5FU**	5 (19.2)	22 (80.8)	0.46	FE
	ECX/EOX	21 (27.3)	56 (72.7)		
	CROSS	1 (50.0)	1 (50.0)		

Table 61 Preoperative demographic, clinical and pathological factors in responders and non-responders.

Percentages may not add to 100 due to rounding. (Abbreviations: 5FU, %-fluorouracil; ECX, epirubicin, cisplatin, capecitabine; EOX, epirubicin, oxaliplatin, capecitabine; CROSS, chemoradiotherapy as used in the CROSS trial; AC, adenocarcinoma; SCC, squamous cell carcinoma; FE, Fisher's exact test; Chi-sq trend, Chi square linear-linear association test)

Postoperative resection pathology

Postoperative resection pathology details are shown in Table 62. Pathological T stage and N stage were both lower in responders (P<0.001 and P=0.001 respectively). There was less lymphovascular invasion in responders (P=0.004) and the R0 resection rate was higher in responders (P=0.03).

Post-operative factors		Responders (n=27)	Non-responders (n=78)	*P* value	*test*
Pathological T stage	**T0**	7 (100.0)	0 (0.0)	<0.001	Chi-sq trend
	T1	5 (45.5)	6 (54.5)		
	T2	4 (26.7)	11 (73.3)		
	T3	11 (18.3)	49 (81.7)		
	T4	0 (0.0)	9 (100.0)		
	Tx	0 (0.0)	3 (100.0)		
Pathological N stage	**N0**	16 (50.0)	16 (50.0)	0.001	Chi-sq trend
	N1	7 (20.6)	27 (79.4)		
	N2	3 (13.0)	20 (87.0)		
	N3	1 (7.1)	13 (92.9)		
	Nx	0 (0.00)	2 (100.0)		
Lymphovascular invasion	**Y**	5 (12.2)	36 (87.8)	0.004	FE
	N	19 (40.4)	28 (59.6)		
	Unknown	3 (17.6)	14 (82.4)		
Barrett's oesophagus	**Y**	9 (21.4)	33 (78.6)	0.49	Chi-sq
	N	14 (32.6)	29 (67.4)		
	Unknown	3 (17.6)	14 (82.4)		
Signet ring cells	**Yes**	2 (15.4)	11 (84.6)	0.50	FE
	No	20 (29.4)	48 (70.6)		
	Missing	5 (20.8)	19 (79.2)		
Differentiation Grade	**Well**	1 (33.3)	2 (66.6)	1.0	Chi-sq trend
	Moderate	4 (17.4)	19 (82.6)		
	Poor	15 (21.7)	54 (78.3)		
	not reported	7 (70.0)	3 (30.0)		
R0 resection status	**R0**	22 (32.4)	46 (67.6)	0.03	FE
	R1	4 (12.1)	29 (87.9)		
	Not reported	1 (25.0)	3 (75.0)		
Lauren classification	**Intestinal**	20 (29.4)	48 (70.6)	0.50	FE
	Diffuse/mixed	2 (15.4%)	11 (84.6)		
	Missing	5 (20.8)	19 (79.2)		

Table 62 Postoperative histopathological factors and response to neoadjuvant therapy.

Percentages may not add to 100 due to rounding. (Abbreviations: FE, Fisher's exact test; Chi-sq, Chi-square test; Chi-sq trend, Chi square linear-linear association test)

Biomarker results

Pre-therapy M2-PK

In the cohort combining regional and collaborative patients, pre-therapy M2-Pyruvate kinase levels were measured in 102 patients because 3 patients did not have stored frozen plasma available. Two of these patients were the first two patients recruited in the regional study and modifications of the initial laboratory processing ensured that future samples were saved and stored in the correct way. One further patient sample could not be located. Pre-therapy M2-PK levels were lower in responders compared to non-responders (P=0.037), see Table 63.

Pre-therapy M2-PK in cohort including radiological response definition

There were 13 patients without TRG data but who were shown to have radiological progressive disease. 3 of these patients were radiological partial responders and 10 patients had progressive disease. Of the latter, one had radiological progressive disease and was resected but did not have TRG data available and the remaining 9 did not proceed to resection. There was a total of 118 patients available in this cohort when these additional 13 patients were included (see Table 64). 4 patients had missing biomarker data, leaving 114 patients for the analysis. M2-PK levels were higher in non-responders compared to responders (P=0.037).

CA19-9, CA72-4, CEA, alkaline phosphatase

In the regional cohort alone, pre-therapy CA19-9, CA72-4, CEA and alkaline phosphatase levels were measured. Of 52 patients from the regional cohort, 17 were recruited through the screening study at a time when CEA and alkaline phosphatase were not being measured, therefore fewer patients had CEA/alkaline phosphatase estimation compared to CA19-9 and CA72-4. 1 patient had a missing CA19-9 level and 13 patients

had missing CA72-4 values. The CA72-4 assay was not run weekly and batches were processed later from frozen stored samples. A number of serum samples were not found in storage and others had insufficient sample volume. None of the biomarkers had significantly different levels in responders and non-responders, see Table 65.

Blood test	N=105		M2-PK levels						P-value (MWU)
	N valid (responders: non-responders)	Missing, n(%)	Responders			Non-responders			
			Mean	Median	Range	Mean	Median	Range	
Test 1 (pre-therapy)	102 (26:76)	3 (2.9)	27.4	27.6	8.1-50.2	36.4	33.9	12.4-111.9	0.037

Table 63 M2-PK levels in responders and non-responders to neoadjuvant thearpy from the combined collaborative and regional cohort.
(abbreviations: MWU, Mann-Whitney U test)

Blood test	N=118		M2-PK levels						P-value (MWU)
	N valid (responders: non-responders)	Missing, n(%)	Responders			Non-responders			
			Mean	Median	Range	Mean	Median	Range	
Test 1 (pre-therapy)	114 (29:85)	4 (3.4)	29.0	29.1	8.1-60.5	37.0	34.9	12.4-111.9	0.037

Table 64 M2-PK levels in responders and non-responders to neoadjuvant therapy from the combined collaboration and regional cohort where non-responders include radiological progressive disease in additional to histological non-response
(abbreviations: MWU, Mann-Whitney U test)

Blood test	Marker	N=52		Biomarker levels						P-value (MWU)
		N valid (responders: non-responders)	Missing, n(%)	Responders			Non-responders			
				Mean	Median	Range	Mean	Median	Range	
Test 1 (pre-therapy)	CEA	33 (7:26)	19 (36.5)	4.9	3.4	1.1-11.8	10.3	2.3	0.5-185.8	0.67
	CA19-9	51 (10:41)	1 (1.9)	23.1	20.0	2.0-69.0	37.0	14.0	1.0-428	1.0
	CA72-4	39 (8:31)	13 (25.0)	3.7	1.6	0.9-11.1	5.4	3.6	0.8-18.7	0.35
	Alk phos	30 (6:24)	22 (42.3)	70.8	62.0	48.0-123.0	78.0	80.0	20.0-130	0.35
Test 2 (post 1st cycle)	M2-PK	20 (4:16)	32 (61.5)	17.8	17.1	11.7-25.1	24.0	20.0	6.7-68.5	0.55
	CEA	19 (3:16)	33 (63.4)	4.5	4.6	1.8-7.0	8.5	2.2	0.7-94.4	0.32
	CA19-9	18 (3:15)	34 (65.4)	36.3	34.0	14.0-61.0	29.2	16.0	1.0-100.0	0.53
	CA72-4	8 (2:6)	44 (84.6)	16.7	16.7	6.2-27.2	5.6	3.9	1.6-12.9	0.25
	Alk phos	20 (3:17)	32 (65.4)	76.7	73.0	66.0-91.0	82.9	78.0	19.0-146.0	0.70

Table 65 Biomarker levels in responders and non-responders to to neoadjuvant thearpy from the regional cohort.
(abbreviations: MWU, Mann-Whitney U Test; alk phos, alkaline phosphatase)

Sub-group analysis

Chemotherapy type

Levels of M2-PK differed in responders compared to non-responders in patients undergoing triple agent chemotherapy (ECF/ECX), P=0.028 but the difference in patients undergoing dual agent therapy (cisplatin/5-FU) was not significant, see Table 66. When radiotherapy patients were excluded, the difference was significant, P=0.03. There were only two patients in the chemoradiotherapy group so no statistical analysis was possible.

Histological type (Lauren classification)

When patients were subdivided according to histological type, M2-PK levels did not differ between responders and non-responders, see Table 67.

Recruiting centre

When patients were subdivided according to the recruiting centre, none of the sub-groups alone had significantly different M2-PK levels in responders compared to non-responders, Table 68. With the Edinburgh patients, M2-PK levels were remarkably similar in responders and non-responders. When Edinburgh patients were excluded, M2-PK levels were significantly lower in non-responders compared to responders (P=0.02).

Chemotherapy type	N (Resp:nonR)	M2-PK levels						P-value (MWU)
		Responders			Non-responders			
		Mean	Median	Range	Mean	Median	Range	
Type 1	26 (5:21)	29.6	25.3	12.6-50.2	34.9	33.3	17.1-74.3	0.61
Type 2	74 (20:54)	26.7	27.6	8.1-37.6	37.3	35.1	12.4-111.9	0.028
Type 3	2 (1:1)	-	-	-	-	-	-	n/a
Radiotherapy excluded (Types 1 and 2)	100 (25:75)	27.2	26.1	8.1-50.2	36.1	33.4	11.7-111.9	0.030

Table 66 M2-PK levels in responders and non-responders to neoadjuvant thearpy divided according to chemotherapy type.
Type 1 dual agent therapy (Cisplatin/5-FU), Type 2 triple agent therapy (ECF/ECX), Type 3 chemoradiothearpy.

Histological type (Lauren Classification)	n (Resp:nonR)	M2-PK levels						P-value (MWU)
		Responders			Non-responders			
		Mean	Median	Range	Mean	Median	Range	
Intestinal	66 (20:46)	28.2	30.1	8.1-41.8	38.0	35.7	12.4-111.9	0.09
Diffuse/mixed	12 (1:11)	-	-	-	32.6	32.3	17.4-54.1	n/a

Table 67 M2-PK levels in responders and non-responders to neoadjuvant thearpy divided according to histological type.

Recruitment centre	n (Resp:nonR)	M2-PK levels						P-value (MWU)
		Responders			Non-responders			
		Mean	Median	Range	Mean	Median	Range	
Regional	49 (9:40)	29.9	32.2	11.1-41.8	39.2	36.0	12.4-101.3	0.23
Collaborative-Edinburgh	14 (3:11)	29.3	25.3	12.6-50.2	28.7	22.6	17.1-53.3	1.00
Collaborative-OCCAMS	39 (14:25)	25.4	26.0	8.1-34.9	36.9	32.3	12.9-111.9	0.24
Regional and OCCAMS (Edinburgh excluded)	88 (23:65)	27.1	29.1	8.1-41.8	37.7	35.2	12.4-111.9	0.02

Table 68 M2-PK levels in responders and non-responders to neoadjuvant thearpy divided according to Recruitment Centre.

M2-PK and pre-operative, demographic, clinical or pathological factors

In order to demonstrate that M2-PK levels are not simply a surrogate for other known demographic factors or tumour characteristics, the relationship between M2-PK levels and such factors was investigated, see Table 69 and Figure 62. No factors were significantly associated with M2-PK levels with the exception of differentiation grade, P=0.04. Moderately differentiated tumours appeared to have higher M2-PK levels compared to well and poorly differentiated tumours.

N=102		N	M2-PK median (range)	*Statistical Test*	*Significance*
Age		102		Pearson Correlation	R=0.16 P=0.31
Gender	**Male**	85	32.3 (8.1-111.9)	M-W	P=0.20
	Female	17	31.1 (16.7-50.2)		
Body mass index		88		Pearson Correlation	R=0.06 P=0.58
	missing	14			
Pre-treatment T Stage	**1**	1	n/a	K-W	P=0.19
	2	10	33.9 (21.5-101.3)		
	3	83	31.4 (8.1-111.9)		
	4	3	25.6 (18.1-28.4)		
	Not reported	5			
Pre-treatment N Stage	**0**	22	29.9 (15.8-74.3)	K-W	P=0.46
	1	53	31.1 (8.1-101.3)		
	2	18	35.4 (12.6-111.9)		
	3	2	21.4 (20.9-22.0)		
	Not reported	7			
Performance status	**0**	72	28.7 (8.1-111.9)	K-W	P=0.08
	1	26	36.8 (11.1-101.3)		
	Not reported	4			
Tumour Site	**Oesophagus**	24	28.8 (11.1-101.3)	K-W	P=0.85
	Junction	68	33.2 (8.1-111.9)		
	Gastric	10	29.5 (12.4-61.4)	K-W	
Lymphovascular invasion	**Yes**	40	32.3 (8.1-101.3)	M-W	P=0.95
	No	45	32.4 (12.4-111.9)		
	Unknown	17			
Barrett's oesophagus	**Yes**	40	31.2 (11.1-101.3)	M-W	P=0.50
	No	42	32.6 (8.1-111.9)		
	unknown	18			
Differentiation Grade	**Well**	3	27.6 (23.4-38.8)	K-W	P=0.04
	Moderate	23	39.2 (20.2-74.3)		
	Poor	66	27.1 (8.1-111.9)		
	unknown	10			
Geographical site	**PHT**	49	34.6 (11.1-101.3)	K-W	P=0.12
	REI	14	23.6 (12.6-53.3)		
	AHC	39	28.3 (8.1-111.9)		
Tumour type	**AC**	97	31.4 (8.1-111.9)	K-W	P=0.40
	SCC	5	33.3 (20.9-82.2)		

Table 69. Relationship between M2-PK levels and demographic/tumour characteristics.
(Abbreviations: R, Pearson Correlation Coefficient; M-W, Mann Whitney test; K-W, Kruskal-Wallis test; PHT, Plymouth Hospitals NHS Trust; REI, Royal Edinburgh Infirmary; AHC, Addenbrooke's Hospital Cambridge.

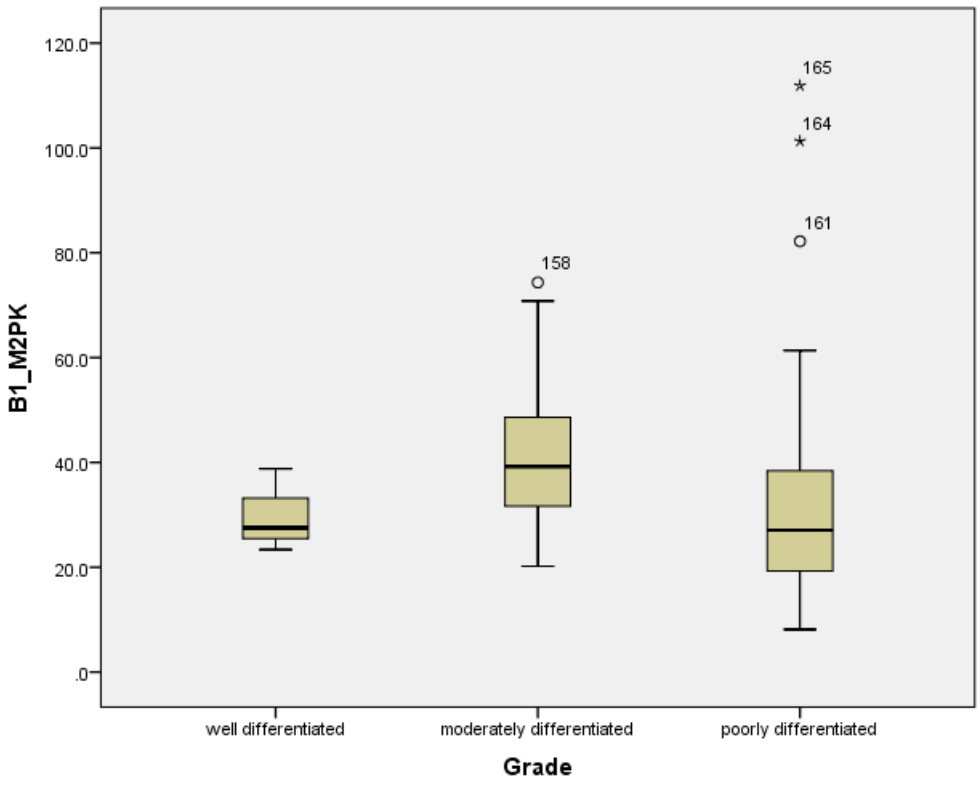

Figure 62. Relationship between pre-therapy M2-PK levels and tumour differentiation grade.

Logistic regression analysis

Binary logistic regression showed that pre-therapy M2-PK levels were able to predict response with each unit increase in the biomarker level being associated with a 4.1% (95% CI, 0.5%-7.6%) decrease in the likelihood of response (P=0.027), see Table 70.

Binary logistic regression	n	Exp (B)	Exp (B) CI	Sig
M2-PK	102	0.959	0.924-0.995	0.027

Table 70 Binary logistic regression showing M2-PK level is predictive of response to neoadjuvant therapy.

Figure 63 shows the predicted probability of not responding to neoadjuvant therapy for any given level of M2-PK with 95% confidence intervals. Given that currently we know that 26% patients will respond to neoadjuvant therapy, from this graph, it can be stated that to be 95% certain a patient will have a greater than 26% chance of responding to therapy, the M2-PK level would need to be <15 and to be 95% certain a patient will have a smaller than 26% chance of responding to therapy, the M2-PK level would need to be >65. The numbers of patients within our 105 patient cohort with such extreme values were 8 and 5 respectively, together representing 12.4% of the cohort.

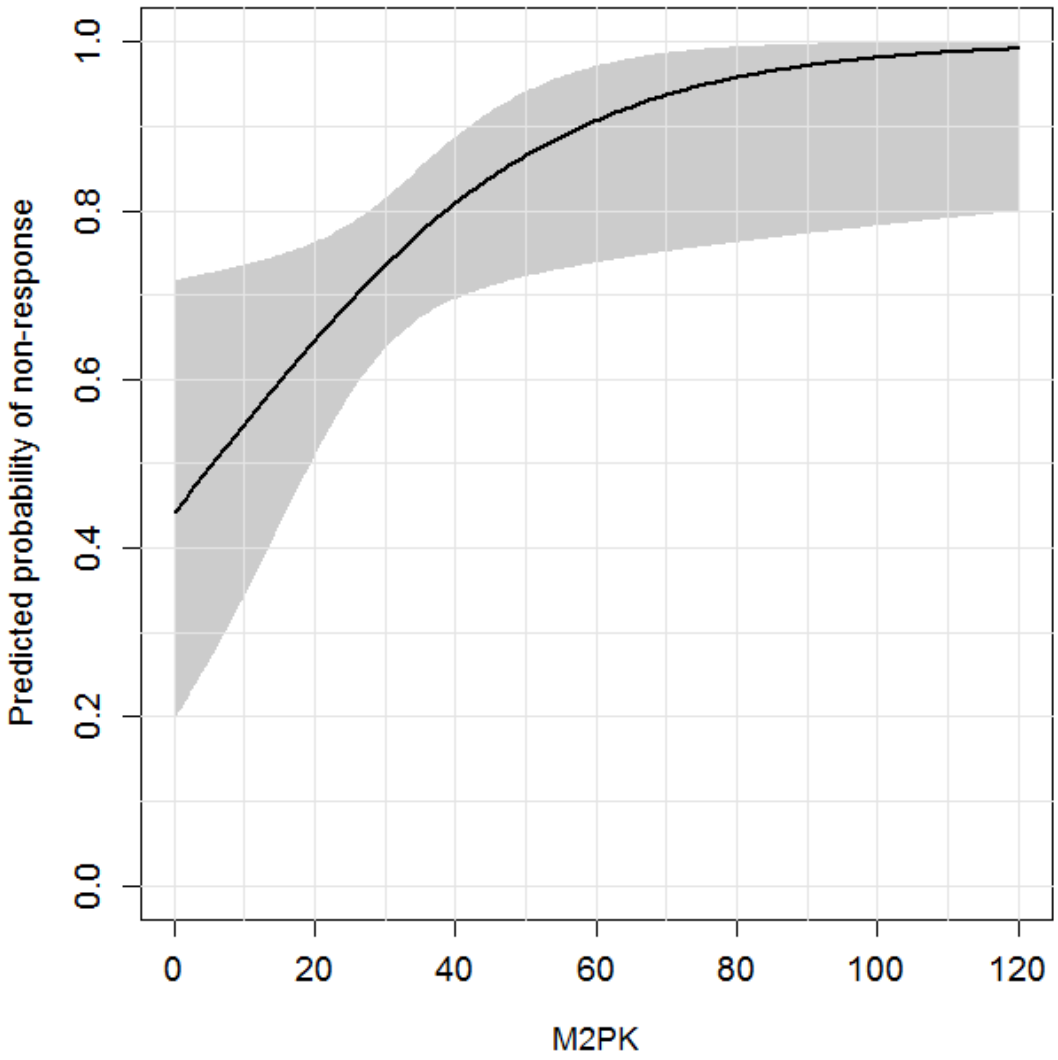

Figure 63 Predicted probability of non-response based on M2-PK level. 95% Confidence interval shown by shaded area.

Discussion

Patients

Of the 165 patients initially recruited, only 105 were ultimately eligible for inclusion in the study. Of the 60 ineligible patients, the majority (50) were from the collaborative studies and illustrates one of the limitations of working in collaboration. Many of these patients were not suitable because they did not undergo neoadjuvant therapy. In many, neoadjuvant therapy was not indicated on the basis of staging and in fact, many had early tumours that were treated with endoscopic resection techniques. These samples were identified by the collaborative centre as potentially appropriate but it was only when clinical data became subsequently available that their ineligibility was identified. Following this realisation, the system for identification of further patient samples by the OCCAMS team was modified. In addition, the 'pick list' identified by the OCCAMS team was second-screened by Mr Bunting against the clinical data to check eligibility before transportation of samples. Since the TRG is essential to the definition of response to neoadjuvant therapy, the 21 patients without TRG results available were ultimately ineligible for analysis. The majority were from the Edinburgh cohort with the remainder from the OCCAMS cohort. This simply results from differences in routine pathology reporting between individual centres and demonstrates another limitation of collaborative working. One patient was excluded from analysis when it was identified that their blood sampling data was after the date of initiation of neoadjuvant therapy and therefore did not represent a chemo-naïve sample. Not being able to control the timing of blood tests is another limitation of collaborative working and this also demonstrates the importance of carefully examining data provided from external centres to check eligibility and compliance with the study methodology. 16 (10%) of the

165 patients did not undergo resection which is similar to the proportion identified in the historical cohort of patients undergoing neoadjuvant therapy from Chapter 3.

Demographics and preoperative variables

None of the demographic factors or preoperative variables was associated with response to neoadjuvant therapy which is in concordance with the data from the historical cohort presented earlier in this chapter. Patient numbers are smaller than in the historical study and this study was not powered to detect such differences which may be subject to type II error, however, from this work there is no evidence to suggest that any preoperative factors can be used in a model to predict response to therapy.

Postoperative resection pathology

Response to therapy was associated with lower postoperative lymph node stage. In Chapter 3, T and N down-staging was observed in histological responders therefore this is expected and has been reported elsewhere as discussed in Chapter 3. R0 resection rates were higher in responders which is also expected and indeed this is one of the mechanisms through which neoadjuvant therapy is thought to carry a survival advantage. 29.4% patients with intestinal type histology responded to therapy compared to 15.4% with diffuse or mixed types, however, this trend was not significant. This was reported as a part of the post-operative histology rather than pre-operatively because the classification was often not reported on the preoperative biopsies although since histological type is not thought to be influenced by neoadjuvant therapy affect, this should reflect preoperative histological type. In the historical cohort presented earlier in this chapter, response rates were similar in the different histological types and the prospective study provides no evidence to contradict this.

Biomarker results

The evidence presented in Chapter 6 suggests that there may be a link between M2-PK activity/M2-PK levels and sensitivity to chemotherapy [260-262, 302]. Specifically, a low enzyme activity, or high levels of the inactive dimeric form may be associated with resistance. This is the only study to have investigated this phenomenon in patients with oesophago-gastric cancer and has shown higher levels of pre-treatment dimeric M2-PK in non-responders to neoadjuvant therapy.

The sub-group analyses showed significantly different M2-PK levels in responders compared to non-responders in patients undergoing triple agent chemotherapy (as per MAGIC protocol); in all patients undergoing chemotherapy (chemoradiotherapy excluded) and in patients recruited regionally or via the OCCAMS collaborative. In patients undergoing dual agent chemotherapy (without epirubicin), differences did not reach significance which may be due to smaller patient numbers. It is not known whether there is any relationship between M2-PK and sensitivity to anthracycline agents, however, the presence of this agent within the triple therapy regime seems to enhance rather than diminish the expected relationship. Patients recruited from Edinburgh did not show any difference in M2-PK levels between responders and non-responders. This was a small group of patients, due in part to lack of TRG reporting, so significance would not likely be achievable with a similar effect size to that seen in patients from other recruiting centres. However, the observed M2-PK levels were very similar in responders and non-responders. The reasons for this lack of difference are unclear but may be due to very small responder numbers (3), or differences in specimen preparation/storage. Whilst the M2-PK levels are generally stable in plasma under normal laboratory processing conditions, there are many variables that can potentially

influence the measured M2-PK level that are difficult to control on multiple sites, particularly when samples have been processed prior to inclusion in this study. These include, time to centrifugation/separation, storage time and temperature, number and duration of freeze thaw cycles and sample transport conditions.

Pre-treatment levels of markers analysed in the regional patient cohort (CEA, CA19-9, CA72-4 and alkaline phosphatase) did not differ between responders and non-responders. As discussed in Chapter 5, others have suggested better responses to chemotherapy are found in in patients with normal baseline levels of CEA and CA19-9[226]; however, there are many differences between this study and ours. Theirs was a study in palliative patients, included pancreatic and biliary malignancy, response was measured radiologically and chemotherapy regimens included docetaxel. The present study gives no evidence to suggest that levels of these tumour cell antigens are associated with response to neoadjuvant therapy.

Biomarker levels taken after completion of the first cycle of chemotherapy were available only in patients recruited in the regional study. Subject numbers were small for a number of reasons. It was often difficult to co-ordinate this blood test with a planned patient visit to hospital. This was particularly problematic at peripheral sites where a successful biomarker level measurement relied on a number of factors falling into place, which included: the patient having a hospital appointment within the right time frame, the patient having the correct sample form in possession, the correct blood tubes being collected, the specimen being transferred to the local laboratory in a timely manner, correct sample labelling and storage, transport of the samples to Derriford and correct labelling, storage and analysis at Derriford. In reality, this was very difficult to achieve without dedicated staff on site at peripheral centres and any further similar

studies would need to bear this in mind. From the small sample numbers available, there did not appear to be any trends worthy of further investigation. In any case, the value of a marker of early response to therapy at this point in time is likely to be less useful than a truly predictive marker measured before initiation of treatment.

Investigating the relationship between M2-PK levels and pre-operative demographic/clinical variables showed that only differentiation grade was associated with M2-PK levels. This relationship demonstrated no clinically meaningful trend because compared to moderately differentiated tumours, levels in both well-differentiated and poorly-differentiated tumours were lower. This may represent a type I error, whereby no true relationship exists. There is no clinical explanation for this statistical finding. It could be hypothesised that more aggressive, poorly differentiated tumour types would have higher dimeric M2-PK expression but this is certainly not demonstrated in the results.

The main significant finding that M2-PK levels were higher in non-responders was further investigated using binary logistic regression and confirmed that levels were significantly associated with response.

Predicted probabilities of non-response, obtained from the fitted logistic regression model give an idea of how biomarker levels could be used in a clinical environment to give some indication of the likelihood of responding to chemotherapy. Currently clinicians have no means to estimate an individual's chance of a beneficial response and can only inform patients that they have a 26% chance of responding adequately. By knowing this baseline response rate, it was possible to identify from the graph, M2-PK thresholds above and below which the response rate would be different from baseline with a 95% certainty. The numbers of patients in these distribution tails represents those

in whom the M2-PK level would be clinically useful in modifying with some certainty the predicted response rate. These patients represent 12.4% of the cohort. Therefore 1 in 8 patients would stand to benefit from this test.

Limitations

By their very nature, serum/plasma markers have obvious limitations. A single snapshot of the marker level is estimated in each blood sample. The marker level represents a surrogate marker or cellular biochemistry, however, levels may depend on a number of factors other than expression within the primary tumour. These include the presence of different cancer cell clones within the same tumour; circadian variation in tumour cell metabolism; rate of clearance of the marker from the bloodstream and other features of peripheral blood such as hyperbilirubinaemia.

Blood sample handling introduces sources of error wherever there may be inconsistencies such as centrifuge time/velocity, haemolysis, storage temperature, storage duration and freeze-thaw cycles.

The involvement of peripheral sites produces inconsistency in the timing of samples and the way specimens are processed, transported and stored.

Working with collaborations enabled the recruitment numbers to be increased. However, there are clear limitations when compared to the methodology of a purpose designed, single-centre study. Blood specimens had been collected as part of the parent studies prior to the commencement of this study which means the timing of blood samples could not be controlled. Samples were inevitably stored for longer prior to analysis. There tended to be more missing clinical and pathological data items and the accuracy of such data was difficult to determine.

Steps were made along the way to minimise the effects of the limitations above, however, the use of such markers in a clinical environment often involves the same potential inconsistencies in sample timing, collection, processing and storage. Therefore, a marker that is robust in spite of these limitations is more likely to succeed in clinical use.

CONCLUSIONS

In this biomarker discovery study, pre-therapy M2-PK levels can predict the likelihood of responding to neoadjuvant therapy in a cohort of patients with oesophago-gastric cancer. This test is likely to be useful for 1 in 8 patients undergoing the test. If this biomarker could be used in conjunction with other predictive markers as they become available, then a combined model could be built that may prove more clinically useful.

CHAPTER 8 - THESIS SUMMARY

In Chapter 1, the overall poor prognosis in oesophago-gastric cancer was highlighted and whist there is some evidence that the addition of neoadjuvant therapy regimes has improved survival, gains are modest and limited by toxicity. Response rates to neoadjuvant therapy are low. There is no evidence that response rates and overall survival can be improved simply by adding more therapeutic agents to existing regimens. Authors have called for further research into predicting response to therapy so that such treatment is tailored to individual patients accordingly. This problem formed the basis of the thesis and the general idea of adopting a personalised medicine approach in the management of patients with oesophago-gastric cancer continued as an overall theme of the thesis. Chapter 1 described the numerous staging investigations that patients being worked up for oesophago-gastric cancer go through. These can be, costly, invasive and associated with a potential delay in starting curative therapies, often adding little to an individual's overall management. It was decided to explore the idea that staging could be streamlined, offering certain investigations only to those in whom they were likely to change management.

In Chapter 3, a historical cohort of patients from our unit was used to redefine response to neoadjuvant therapy, to assess pre-treatment clinical staging accuracy, to investigate neoadjuvant therapy toxicity and to explore neoadjuvant therapy efficacy.

Response to chemotherapy according to histopathological regression was re-defined by undertaking a survival analysis. Staging accuracy was not sufficiently accurate for T or N down-staging to be included in this definition and true T/N down-staging was restricted to those with a histological response.

16.2% patients suffered severe life-threatening or fatal adverse events associated with neoadjuvant therapy. Overall survival was poorer in those suffering adverse events. Over 10% patients starting neoadjuvant therapy did not proceed to resection, and chances of this were greater in patients with adverse events. Even within patients proceeding to resection, those suffering adverse events from chemotherapy showed a trend towards poorer overall survival.

Only histological responders to neoadjuvant therapy stand to gain any benefit from this additional treatment. Those with more advanced disease stage appear to have greater potential to benefit; however, these benefits may be evident at earlier stages in responders. Thus response to therapy may be more important than disease stage when considering whether the addition of neoadjuvant therapy is beneficial. Non-responders to therapy may even have poorer outcomes compared to patients undergoing surgery only. These findings, together with poor pre-treatment clinical staging accuracy suggests that if response to therapy can be predicted, this should be used in addition to, or instead of clinical staging to determine which patients should be offered neoadjuvant therapy. This gives further support to the need for a clinically useful means of predicting response to neoadjuvant therapy.

In Chapter 4, a proposed method of streamlining staging, whereby the use of PET-CT and staging laparoscopy could be limited to those in whom management was most likely to be altered was investigated. In each case, criteria based on endoscopy and CT findings were able to accurately stratify patients according to whether PET-CT/laparoscopy would be likely to change management.

Chapter 5 reviewed the published literature on predicting response to neoadjuvant therapy in patients with oesophago-gastric cancer. While there are some promising

molecular markers, further validation studies are needed before any marker would be useful in a clinical context.

In Chapter 6, the tumour M2-PK biomarker is introduced, its role in cancer metabolism is explained and the rationale behind its potential as a predictive marker of response to neoadjuvant chemotherapy in patients with oesophago-gastric cancer is described.

Main findings

Chapter 7 presents the prospective, multicentre, collaborative study set up to investigate whether a panel of biomarkers including the novel marker M2-PK is able to predict response to neoadjuvant therapy in patients with oesophago-gastric cancer. Of all the markers investigated, only M2-PK was predictive of response and could be expected to modify the baseline chance of response in 1 in 8 patients.

Study relevance and wider implications

Non-responders may have a reduced survival compared to those undergoing surgery alone due to the toxicity and delay to surgery. However, a poor response to neoadjuvant therapy may simply reflect adverse tumour biology in patients who would have otherwise done poorly with surgery alone; therefore, we need to be cautious in drawing any conclusions when directly comparing non-responders with patients undergoing surgery alone. Predicted non-responders (based on an accurate prediction of response and a validated definition of response) would need to be randomised to surgery alone or neoadjuvant therapy followed by surgery in order to definitively answer the question of whether undertaking neoadjuvant therapy and not achieving a response is associated with poorer survival than undergoing surgery alone. This reiterates the importance of accurately re-defining and standardising response to neoadjuvant therapy and the need for further research into predicting the response to chemotherapy. Mr Bunting

continues to work with the OCCAMS collaboration on agreeing a unified definition of response to neoadjuvant chemotherapy and identification of predictive biomarkers.

Diagnosis and screening

There has already been some research aimed at investigating M2-PK as a diagnostic or screening biomarker. Data in oesophago-gastric cancer are due to be published this year. The quantification of chemo-naïve plasma M2-PK levels in patients with oesophago-gastric cancer from the present study could be used to support further research in this area.

Prognostic marker

There is evidence that M2-PK may be a useful prognostic indicator in biliary tract and colonic cancers[307, 308]. This study will involve collecting data that has the potential to assess whether M2-PK is associated with disease progression and survival in oesophageal and gastric cancers.

Other cancers

Since many other epithelial tumours are treated with platinum, 5-fluorouracil and anthracycline-based chemotherapy, the results of this study may be applicable to other common cancers including breast, lung and colon cancer.

Appendix I Patient information leaflet, regional study

Patient Information Sheet – Part 1

Predicting the response to neoadjuvant therapy in patients with oesophago-gastric cancer

Invitation

You are being invited to take part in a research study alongside about 80 other patients which forms part of an educational project. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully, and discuss it with others such as family, friends or your GP if you wish.

Part 1 tells you the purpose of this study and what will happen to you if you take part.

Part 2 gives you more detailed information about the conduct of the study.

Ask us if there is anything that is not clear, or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

The purpose of this study is to analyse the levels of a naturally produced substances present in a patient's blood. The results will help to find out whether the substance levels can be used to predict how well chemotherapy will work or whether it can be used to monitor the effects of treatment. This information may in the future help individual patients and doctors to decide whether or not they should undergo pre-operative chemotherapy.

Why have I been chosen?

You have been chosen as you have a diagnosis of cancer of the oesophagus or stomach.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form to confirm that you understand what is involved when taking part in this study. If you decide to take part you are free to leave the study at any time and without giving a reason. If you withdraw, unless you object, we will still keep records relating to the treatment given to you, as this is valuable to the study. A decision to withdraw at any time, or a decision not to take part, will not affect the quality of care you receive.

What will happen to me if I take part?

You will be asked a short series of questions, for example 'Do you smoke?' or 'Is there a history of cancer in the family?' You will be asked to provide a sample of blood before any treatment is started, after the first and last cycles of chemotherapy and after surgery. A set of bloods is taken routinely at these times therefore another needle is usually not required. No extra hospital visits will be required for any part of the study.

What are the side effects of any investigation performed as part of the study?

Blood testing is a very safe procedure with minimal side-effects limited to local discomfort and occasionally minor bruising. If you do decide to take part in the study, you must report any problems you have to your study nurse or doctor. There is also a contact number given at the end of this information sheet for you to phone if you become worried at any time. In the unlikely event of an emergency occurring during the conduct of the study, we may contact your nominated next of kin.

What are other possible disadvantages and risks of taking part?

There are no disadvantages to taking part in the study and it will not affect your treatment in any way.

What are the possible benefits of taking part?

There are no benefits to you for taking part in the study.

What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your question. If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital.

In the event that something does go wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence, then you may have grounds for a legal action for compensation but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

Will my taking part in this study be kept confidential?

Yes. All the information about your participation in this study will be kept confidential. The details are included in Part 2.

Contact Details

Chief investigator (Clinical Research Fellow): Mr David Bunting, Tel. No: 01752431486

Specialist Nurse: Marlyn Bolter, Tel. No: 01752517905

If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

Patient Information Sheet – Part 2

Predicting the response to neoadjuvant therapy in patients with oesophago-gastric cancer

What if new information becomes available?

Sometimes during the course of a clinical trial, new information becomes available on the tests that are being studied. If this happens, we will tell you about it and discuss with you whether you want to or should continue in the study. If you decide to withdraw, we will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

On receiving new information, we might consider it to be in your best interests to withdraw you from the study. If so, we will explain the reasons and arrange for your care to continue. If the study is stopped for any other reason, you will be told why but this will have no impact on your continuing care will be arranged.

What will happen if I don't want to carry on with the study?

You are welcome to withdraw your consent from the study at any stage without giving reason. This will not have any impact on your on-going care.

Will my part in this study be kept confidential?

If you consent to take part in this study, the records obtained while you are in this study as well as related health records will remain strictly confidential at all times. The information will be held securely on paper and electronically at your treating hospital under the provisions of the 1998 Data Protection Act. Your name will not be passed to anyone else outside the research team or the sponsor, who is not involved in the trial. You will be allocated a trial number, which will be used as a code to identify you on all trial forms.

Your name will only appear on your consent form. All other records related to the research will have your name removed and will only feature your initials and date of birth. There is the possibility that one of the documents will contain your hospital number, however this will not appear on the same sheet as any clinical results.

Your records will be available to people authorised to work on the trial but may also need to be made available to people authorised by the Research Sponsor, which is the organisation responsible for ensuring that the study is carried out correctly. A copy of your consent form may be sent to the Research Sponsor during the course of the study. By signing the consent form you agree to this access for the current study and any further research that may be conducted in relation to it, even if you withdraw from the current study.

The information collected about you may also be shown to authorised people from the UK Regulatory Authorities and Independent Ethics Committee; this is to ensure that the study is carried out to the highest possible scientific standards. All will have a duty of confidentiality to you as a research participant.

If you withdraw consent from further study treatment, unless you object, your data and samples will remain on file and will be included in the final study analysis. In line with the Trust's procedures, at the end of the study, your data will be securely archived for a minimum of 5 years. Arrangements for confidential destruction will then be made. With your permission, other doctors who may be treating you will be notified that you are taking part in this study.

Informing your General Practitioner

Your GP will not be routinely informed of your participation in the study, however if the need arises or you would wish it to be so then a letter along with a summary of the project will be sent to them.

What are the study methods?

78 patients will have blood samples taken before starting chemotherapy, after the first and last cycles of chemotherapy and after surgery. The levels of chemical markers in the blood will be studied in relation to the response to chemotherapy which is measured in a sample of the tissue removed during surgery in all patients as a routine part of the analysis. The ability of biomarker levels to accurately predict the response to chemotherapy will be investigated. This will tell us whether in the future, we may be able to safely withhold chemotherapy from those are not likely to benefit or perhaps switch to a second-line, more effective treatment earlier.

What will happen to any samples I give?

Samples of serum and plasma (the liquid part of blood not containing cells) will be kept for up to 5 years for further testing should new investigations become available. Further tests would only be performed for a separate study after approval by a Regional Ethics Committee has been granted.

Will any Genetic testing be done?

There is currently no plan perform genetic tests on the stored samples. Should this occur then you would be contacted in writing to affirm your consent.

What will happen to the results of this clinical **t**rial?

The results of the study will be published in a medical journal and presented at a scientific conference. The data will be anonymous and none of the patients involved in

the trial will be identified in any report or publication. If you wish to be sent a copy of the results, please indicate this by ticking the box on the consent form.

Will this affect my insurance policies (critical illness, mortgage protection and health insurance)?

You should consider whether this will affect insurance policies and seek advice if necessary.

Who is organising and funding this clinical trial?

The study has been organised Mr David Bunting, Clinical Research Fellow. External funding has been secured from the Plymouth Charitable Trust Small Grants Scheme. Researchers are not being paid for conducting the trial.

Who has reviewed the study?

The study has been reviewed and is sponsored by Plymouth Hospitals NHS Trust. It has also been reviewed by the Plymouth University Peninsula School of Medicine and Dentistry. Favourable ethical opinion for conduct in the NHS has also been granted by the South West Research Ethics Committee.

Contact for further information

You are encouraged to ask any questions you wish, before, during or after your treatment. If you have any questions about the study, please speak to your study nurse or doctor, who will be able to provide you with up to date information about the procedures involved. If you wish to read the research on which this study is based, please ask your study nurse or doctor.

If you or your relatives have any concerns about any aspect of research please speak to the researchers using the contact details you will have been provided with. Alternatively, you may wish to contact the hospital's Patient Advice and Liaison

Service (PALS). PALS offers support, information and assistance to patients, relatives and visitors and will:

• Provide information about hospital services.

• Offer advice on where to go to get health information.

• Help with problems that you haven't been able to sort out with staff on a ward or in a clinic.

• If you want to make a complaint - advise you how to do so.

• Tell you about independent organisations that can help you with a complaint.

• Listen to your views on how we can improve our services, and pass this on to the appropriate people for action.

PALS can be contacted at:
Patient Advice & Liaison Service

Level 7, Derriford Hospital

Plymouth

PL6 8DH

Email: plh-tr.PALS@nhs.net

If you decide you would like to take part then please read and sign and date the consent form. You will be given a copy of this information sheet and the consent form to keep. A copy of the consent form will be filed in your patient notes, one will be filed with the study records and one may be sent to the Research Sponsor.

You can have more time to think this over if you are at all unsure.

Thank you for taking the time to read this information sheet and to consider this study.

Appendix II Consent form regional study

Plymouth Hospitals NHS
NHS Trust

V2.1 20/08/2013

Consent Form

Title: Predicting the response to neoadjuvant therapy in patients with oesophago-gastric cancer

Name of Researchers: Mr David Bunting, Dr Ruth Ayling, Mr Tim Wheatley, Mr Grant Sanders, Professor David Wright.

Please initial the boxes

1. I confirm that I have read and understand the information sheet (Version 2.2) for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without my medical care or legal rights being affected. ☐

3. I am willing to allow access to my medical records by authorized people but understand that strict confidentiality will be maintained. The purpose of this is to ensure that the study is being carried out correctly. ☐

4. I agree to take part in the above study ☐

I would like to receive a copy of the study results (please tick)

Yes ☐ No ☐

Name of patient	Date	Signature
Name of person taking Consent (if not researcher)	Date	Signature
Researcher	Date	Signature

(one copy for patient, one for researcher, one for medical notes)

Appendix III Consent form OCCAMS

Plymouth Hospitals NHS NHS Trust

OCCAMS

MRC

Consent for the OCCAMS Study

Patient Identification Number: **Please initial boxes**

1.	I confirm that I have read and understand the ***OCCAMS*** patient information sheet (version 5 dated 23rd April 2013) and have had the opportunity to ask questions and discuss the trial.	☐
2.	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	☐
3.	I understand that any of my medical notes may be looked at by responsible individuals or by regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.	☐
4.	I agree that my, hospital number and date of birth be securely stored by the trial office	☐
5.	If necessary, I give permission for information about my progress to be obtained from my GP or through the Office for National Statistics, and understand that this will be done using my full name and/or NHS number.	☐
6.	I understand that the doctors in charge of this trial may close the trial, or stop my participation in it at any time, without my consent.	☐
7.	I agree to the collection and storage of additional tumour tissue for future ethically approved research studies. Samples will be coded to protect my identity.	☐
8.	I agree to the collection and storage of additional blood samples. I agree that these samples can be used for the Personalised blood biomarkers research study and confirm that I have read and understood the related information sheet version 2 dated 05.05.2011.	☐
9.	I agree that my stored blood samples may be used for future ethically approved research studies. Samples will be coded to protect my identity.	☐
10.	I understand this research may include looking at the whole DNA sequence of my tissue, and blood as a reference, and that this information will not be used to alter my clinical care	☐
11.	I understand that my anonymised coded information may be held on an International database with controlled access	☐
12.	I agree that any of my tissue which has been collected for previous research studies may be accessed for use in the OCCAMS study	☐
13.	I understand that participating in this study may involve additional biopsies but that there will be no additional procedures	☐
14.	If I am having an Endoscopic Ultrasound (EUS), I understand that this may involve taking lymph node samples.	☐
15.	I agree that if I am having lymph node samples taken as part of my routine investigation at EUS that the lymph node may be marked with dye as described in the information sheet (optional)	☐
16.	I agree to the transfer of my anonymised clinical data to the OCCAMS research team and that this data may be used for future ethically approved research.	☐
17.	I agree that the research staff can re-contact me in the future if necessary	☐
18.	**I agree to participate in the *OCCAMS* trial for the parts I have initialled above** **PTO**	☐

Plymouth Hospitals NHS
NHS Trust

OCCAMS

MRC

1. **OPTIONAL**: I would like to receive a summary of the main results of the ***OCCAMS*** trial when they are published. (*initial box only if you agree*).	☐
2. **OPTIONAL**: I give permission for my full name to be given to the trial office when I am registered on the ***OCCAMS*** trial (*initial box only if you agree*).	☐

Patient

Signed .....

Print name ..... Date:

.....

Researcher

Signed .....

Print name ..... Date:

.....

Appendix IV Consent form SAHSC

Document Name	QF-TGU-A-CONSENTF VERSION 1.2	Review date	25-Jul-2014

NHS Lothian

CHIEF SCIENTIST OFFICE

South East Scotland SAHSC BioResource

The collection of Tissue, Biospecimens and Data for Research

PARTICIPANT CONSENT FORM

Explanation of consent procedure

You are being invited to donate tissue to the South East Scotland SAHSC BioResource and should have had time to read the Participant Information Sheet (Version 1.2) and discuss it with your healthcare team, GP, family and friends.
If you decide to participate, please initial the boxes for numbers 1 to 7, circle YES or NO for numbers 8 and 9, and sign at the bottom (overleaf).
All of your information will be treated strictly confidentially, and all BioResource staff will follow the principles of the Data Protection Act 1998.

Please Initial

1. I confirm that I have read this consent form and Participant Information Sheet (Version 1.2) and have had the opportunity to ask questions about them. ☐
2. I understand that some surplus tissue or body fluids may be left over during the course of my operation(s) / investigation(s) within NHS Lothian and I agree to donate this surplus material to the South East Scotland BioResource for future research use. ☐
3. I declare that I have given my consent voluntarily to the storage of this surplus tissue and understand that I am free to withdraw at any time without giving any reason, and that my medical care will not be affected. ☐
4. I agree that my donated sample(s) may be used by clinical, academic or commercial researchers, and may be used abroad. ☐
5. I give the SAHSC BioResource permission to access and store information about my general physical health, past, present and future illness, diagnosis and treatment from my medical records. I understand that all information collected will be kept strictly confidential by the BioResource research team. ☐
6. I understand that the information obtained through any research conducted is unlikely to have any direct medical benefit to me, but I agree to my clinical care team being informed if research tests on my samples could have direct impact on my care during the course of my treatment ☐
7. I understand that I will not benefit financially if this research leads to the development of a new treatment or medical test or product. ☐

Author	: Frances Rae	Date : 01-Sep-2011
Authority for Issue	: Craig Marshall	Date : 01-Sep-2011
Quality Checked	: Craig Marshall	Date : 01-Sep-2011

1. I agree that my surplus tissue or other biospecimen may be stored and used for future DNA testing including genetic analysis.
   Please clearly circle YES or NO

   YES / NO

2. I agree to provide an extra blood sample, by venepuncture, and/or urine sample if necessary.
   Please clearly circle YES or NO.

   YES / NO

Name of patient (please print)	**Signature**	**Date**
.....	.....	.....

Name of person witnessing consent	**Signature**	**Date**
.....	.....	.....

Thank you for agreeing to take part in this research

3 Copies:
1 to Participant, 1 to South East Scotland SAHSC BioResource, Original to be stored in Patient File

Contact details for BioResource team:

The Tissue Governance Manager
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Study ID label:

List of abbreviations

AC	adenocarcinoma
AC	adenocarcinoma
AJCC	American Joint Committee on Cancer
ALDH-1	aldehyde dehydrogenase-1
ALT	Alanine transaminase
AMBP	Alpha-1-Microglobulin/Bikunin Precursor.
AUGIS	Association of Upper Gastrointestinal Surgeons of Great Britain and Ireland
BASO	British Association of Surgical Oncology
BSG	British Society of Gastroenterology
CEA	Carcinoembryonic antigen
Chi-sq	Chi-square test
CHK2	Checkpoint kinase 2
CI	confidence interval
CR	clinical response
CRT	chemoradiotherapy
CRT	chemoradiotherapy
CT	computed tomography
CT	chemotherapy
CTCAE	Common Terminology Criteria for Adverse Events
DPD	dihydropyrimidine dehydrogenase
DPD	Dihydropyrimidine dehydrogenase
EBUS	endobronchial ultrasound
ECF	epirubicin, cisplatin & fluorouracil
ECX	epirubicin, cisplatin & capecitabine
EGFR	epidermal growth factor receptor
ELISA	enzyme-linked immunosorbent assay
EOX	epirubicin, oxaliplatin & capecitabine

ER	endoscopic resection
EUS	endoscopic ultrasound
FDG	fluorodeoxyglucose
FE	Fishers exact test
FNA	fine needle aspiration
GC	gastric cancer
GIST	gastrointestinal stromal tumours
GST	glutathione S-transferase
GST	glutathione-S-transferase
Hb	haemoglobin
HER-2	human epidermal growth factor receptor 2
IHC	immunohistochemistry
JCC	Japanese Joint Committee
MAGIC	Medical Research Council Adjuvant Gastric Infusional Chemotherapy
MDCT	multidetector CT
MDT	multidisciplinary team
MRC	Medical Research Council
MRI	magnetic resonance imaging
MRP1	multidrug resistance protein 1
MST	Median survival time
MT	metallothionein
MTHFR	methylene-tetrahydrofolate reductase
MWU	Mann-Whitney U test
NACRT	neoadjuvant chemoradiotherapy
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NLR	neutrophil lymphocyte ratio
OC	oesophageal cancer
OCCAMS	Oesophageal Cancer Clinical and Molecular Stratification Study (OCCAMS)
OS	overall survival

PallCT	palliative chemoradiotherapy
PCNA	Proliferating cell nuclear antigen
PCNA	Proliferating cell nuclear antigen
PCR	polymerase chain reaction
PCR	polymerase chain reaction
PET	positron emission tomography
PFS	progression-free survival
PHN	Plymouth Hospitals NHS Trust
PR	pathological response
PR	pathological response
RCHT	Royal Cornwall Hospitals NHS Trust
RDE	Royal Devon and Exeter NHS Foundation Trust
RECIST	Response Evaluation Criteria in Solid Tumours
SAHSC	Scottish Academic Health Sciences Collaboration (SAHSC) BioResource.
SC	squamous carcinoma
SC	squamous cell carcinoma
SIGN	Scottish International Guidelines Network
SNPs	single nucleotide polymorphisms
SUV	standardized uptake value
TMA	tissue microarray
TP	thymidine phosphorylase
TPA	tissue plasminogen activator
TPS	tissue polypeptide-specific antigen
TRG	tumour response grade
Trx	thioredoxin
TS	Thymidylate synthase
TS	Thymidylate synthetase
UICC	International Union Against Cancer
VATS	video-assisted thoracosopic surgery

VEGF	vascular endothelial growth factor
WHO	World Health Organisation

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