1. INTRODUCTION

Upper gastrointestinal cancer has seen remarkable changes in epidemiology in the last century. Although oesophageal cancer is less common than gastric cancer, its incidence is increasing rapidly especially in Westernised countries. This is largely accounted for by increased numbers of adenocarcinomas, especially those affecting the lower third of the oesophagus. Overall, gastric cancer has shown a worldwide decrease in incidence, but tumours of the proximal third of the stomach are increasing in incidence. The downward migration of oesophageal cancer and a proximal shift in gastric tumours suggest a common aetiology. These tumours of the gastro-oesophageal junction are now considered as separate entities, with different clinico-pathological and molecular characteristics.

Unfortunately, upper gastrointestinal tumours have a poor prognosis which is largely a reflection of the advanced stage of disease at presentation. Curative treatment options are limited to patients with early stage disease who are fit for major surgical intervention. Upper gastrointestinal cancer therefore remains a major clinical challenge.

Improvements in the treatment of the disease must arise from a better understanding of the molecular mechanisms which underlie progression, invasion and metastasis formation; processes which are resistant to most current treatments to date. To make the best use of current oncology treatments it is necessary to evaluate markers of prognosis which accurately predict the natural history of the disease. This will allow individualised patient therapy, targeting those patients who will derive most benefit whilst avoiding harm to those unlikely to respond. The evaluation of new biological markers may allow the development of novel therapies to specifically target invasive, angiogenic and metastatic molecules.

1.1 EPIDEMIOLOGY

Oesophageal and gastric cancer are among the most common malignancies worldwide and contribute significantly to global cancer mortality (Parkin 2001). Recent epidemiological estimates place oesophageal and gastric cancer in the top ten most common cancers in terms of incidence and mortality. Oesophageal cancer is the eighth most common cancer worldwide but the sixth most common cause of cancer mortality, while gastric cancer is the fourth most common cancer and the second most common cause of cancer deaths worldwide (Parkin et al. 2001).

1.1.1 Oesophageal cancer

Cancers of the oesophagus represented over 6,000 deaths in England & Wales in 2000 (4.5% of all cancer deaths), with a mortality rate that has increased in recent decades (Quinn et al. 2001). The incidence rises steeply with age and two thirds of patients are over the age of 65 years (Keighley 2003). The two main histological types of oesophageal cancer are squamous cell carcinoma and adenocarcinoma, which account for over 90% of cases. Other rarer histological types include mixed adeno-squamous, small cell carcinoma, leiomyosarcoma, carcinoid and lymphoma. Squamous cell carcinomas tend to be evenly distributed between the middle and lower third of the oesophagus, whereas 75% of adenocarcinomas arise in the distal oesophagus (Devesa et al. 1998).

Although squamous oesophageal cancer has had a stable or declining incidence in developed countries, there has been an alarming increase in incidence of adenocarcinomas of the distal oesophagus and gastro-oesophageal junction (Blot et al. 1991; Devesa et al. 1998). Figures for the UK showed that for cancers of the oesophagus incidence and mortality both increased by about 50% in men between 1971 and 2001, illustrating the poor prognosis associated with the disease (Figure 1.1).

Five-year survival after diagnosis with oesophageal cancer in the UK is 9%, which is slightly lower than a European average of 9.6% (Keighley 2003). The poor prognosis is largely due to advanced stage of the disease at presentation. In Europe, only about one quarter of all oesophageal cancers are operable and, for these, five-year survival is only about 20-30% (Keighley 2003).

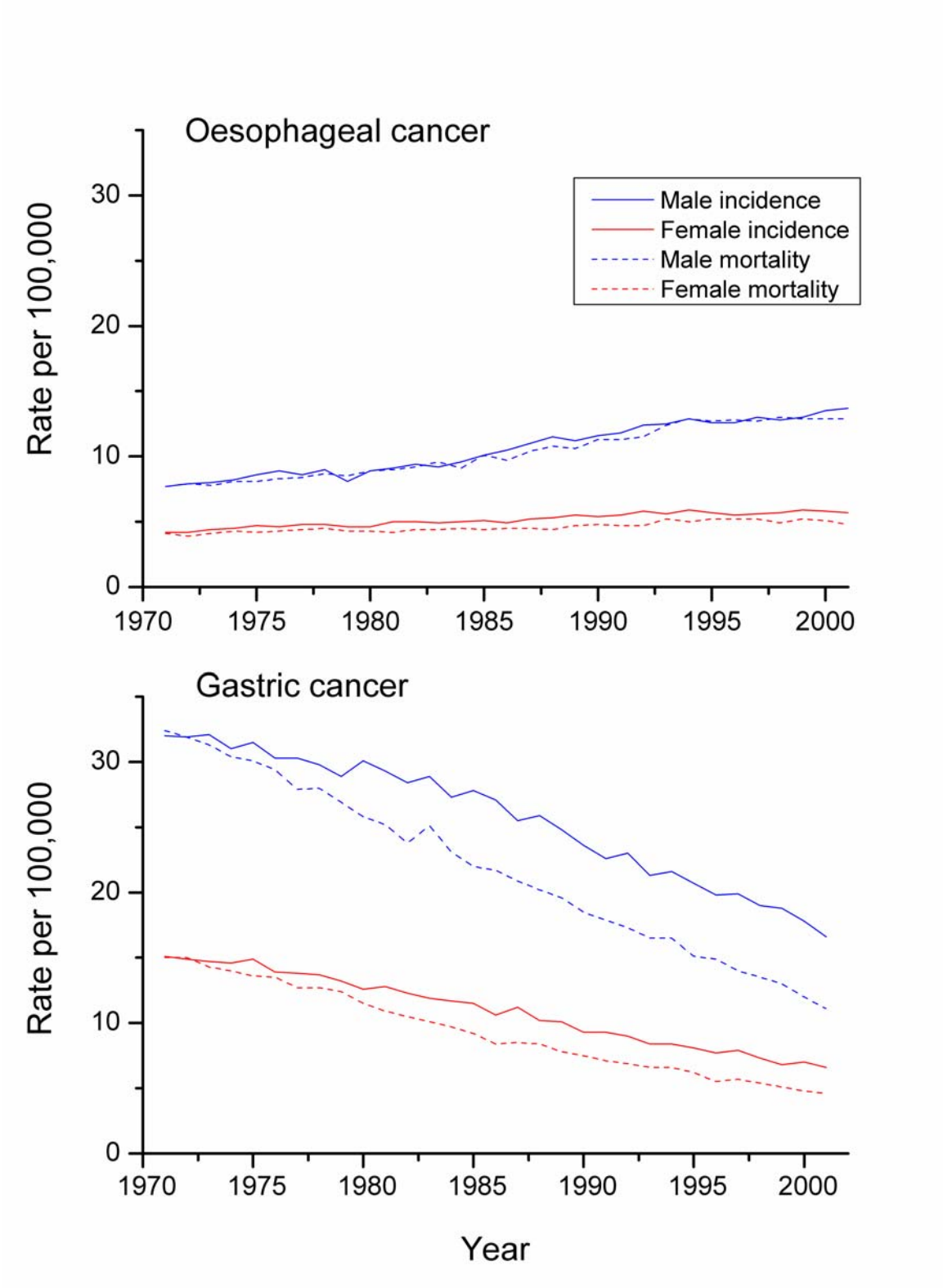

Figure 1.1 Trends in standardised incidence and mortality rates (per 100,000 population) for oesophageal and gastric cancer, among men and women in England & Wales, 1971-2001. Information source (Quinn et al. 2001)

1.1.2 Gastro-oesophageal junction cancer

Tumours around the gastro-oesophageal junction (GOJ) have increased rapidly in incidence in recent decades, especially in developed countries (Blot et al. 1991; Devesa et al. 1998; Devesa et al. 1999). Some authors think that this increase has been overstated and may simply relate to increased interest in the disease and misclassification of tumour sites (Foreman 2002). However, recent evidence showed a true increase in incidence that is unlikely to be related to either over-diagnosis or reclassification of the disease (Pohl et al. 2005).

Changes in the prevalence of commonly reported risk factors, such as gastroesophageal reflux disease (Lagergren et al. 1999a), increased body mass index (Cheng et al. 2000; Lagergren et al. 1999b), low fruit and vegetable intake (Cheng et al. 2000; Lagergren et al. 1999b) and decreasing incidence of *H. pylori* infection (Blaser 1999; Graham 2003) have been cited as possible explanations for the increasing incidence of these tumours.

Classification systems for GOJ tumours have been devised (Dolan et al. 1999; Siewert et al. 2005), but sadly they have not been widely adopted into routine clinical practice in the UK. Sub-classification is not a part of the current requirements of the Royal College of Pathologists Minium Datasets for reporting oesophagogastric cancer (Dixon 2000; Mapstone 1998). Here a carcinoma is classed as oesophageal if more than half of the tumour is above the gastro-oesophageal junction. In addition, the definition, location and extent of the gastric cardia and GOJ are controversial in much of the medical literature. Due to the different classification types used, previous studies are not comparable as they contain different patient populations with heterogenous tumour types.

The UICC classification system relies on the anatomical location of the epicentre or predominant mass of the tumour to decide whether the tumour is oesophageal or gastric in origin (Sobin 2002). With the increasing proportion of these cancers which straddle the GOJ with equal proportions on each side, it has become apparent that this system is inadequate. The Siewert classification system (Figure 1.2) was approved following a consensus conference of the International Society for Diseases of Esophagus (ISDE) meeting in 1995 (Siewert et al. 1998a) and is the most widely adopted classification system. In this system, GOJ tumours are anatomically classified into three sub-types depending on distance from the gastric cardia, which is defined as the proximal end of the typical longitudinal gastric mucosa folds (Siewert et

al. 2005). Key differences in various epidemiological, clinical and pathological characteristics between these tumour subtypes have been found and the classification system can also aid planning of surgical treatment (Table 1.1). These tumour sub-types also differ in their predilection for lymph node metastases to the mediastinal and abdominal lymph node stations (Table 1.1). Siewert's classification system is recommended for use by the British Society of Gastroenterology in the published *Guidelines for the management of oesophageal and gastric cancer* as 'it is uniform, allows data comparison from different centres, and is important for the stratification of patients in prospective studies' (Allum et al. 2002).

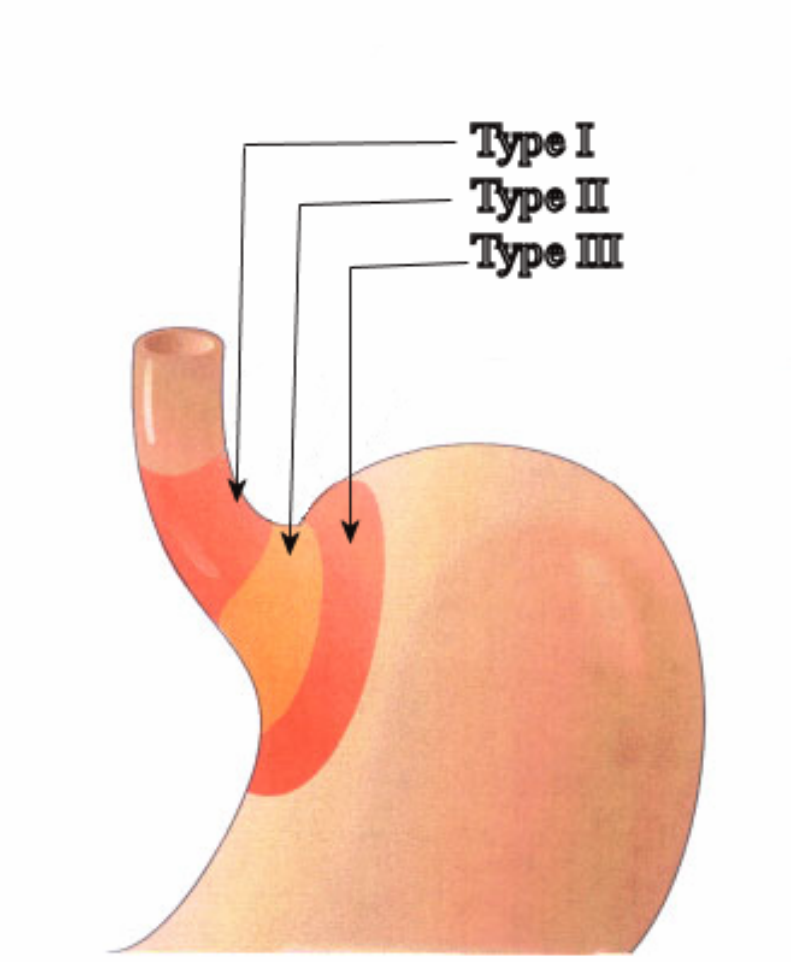

Figure 1.2 Seiwert's classification of gastro-oesophageal junctional tumours. Tumours are divided in to three types depending on their distance from the endoscopic gastric cardia: Type I – distal oesophageal cancers; Type II – true cardia carcinomas; Type III – sub-cardial carcinomas.

Table 1.1 Tumours around the gastro-oesophageal junction: Siewert's classification system and principal differences between subtypes.

GOJ subtypes	Type I (Adenocarcinoma of distal oesophagus)	Type II (True cardia carcinoma)	Type III (Sub-cardial carcinoma)
Endoscopic criteria	Tumour mass arises 1-5 cm above the endoscopic cardia†	Tumour mass arises 1cm above to 2cm below the endoscopic cardia	Tumour mass arises 2-5 cm below the area of the endoscopic cardia
Differing Characteristics	• Male predominance • Arise in association with Barrett's oesophagus (80%) • More likely to have hiatus hernia or history of GORD	• More similarities to Type 3 tumours than Type 1 • Barrett's mucosa identified in 10%	• Barrett's mucosa identified in only 2% • 60% have a diffuse growth pattern and 70% undifferentiated
Lymph node metastases	To mediastinal and abdominal lymph node stations	Mainly to abdominal lymph node stations	Mainly to abdominal lymph node stations
Precursor lesions	Barrett's oesophagus	Possible short segment Barrett's oesophagus or IM at the gastric cardia	*H. pylori* and IM of the subcardial region
Optimal surgical treatment	Transthoracic or transhiatal oesophagectomy	Controversial; may include either extended total gastrectomy, transthoracic or transhiatal oesophago-gastrectomy	Extended total gastrectomy

† defined as proximal end of the typical longitudinal gastric mucosa folds as seen at endoscopy; IM = intestinal metaplasia; Information taken from (Dresner et al. 2001; Siewert et al. 2005; Siewert et al. 2000).

Tumours involving the gastro-oesophageal junction have a worse prognosis than other locations of gastric cancer (Kajiyama et al. 1997; Kim et al. 2005; Pinheiro et al. 1999). Theoretical reasons for this include the thinness of the muscularis layer of the proximal stomach compared to other regions and the multiple routes of potential lymph node metastases. These tumours are located at the junction of three lymphatic areas (mediastinal, abdominal and retroperitoneal), with lymphatic metastases occurring early and some of these areas are not routinely resected at surgery (Aikou et al. 1987; de Manzoni et al. 2004). Studies have also shown that tumours around the gastro-oesophageal junction have high rates of poorly differentiated tumours, more advanced TNM stage, increased rates of regional lymph node metastases and distant metastatic disease. These types of tumours are thought to be more biologically aggressive and consequently are more difficult to treat.

1.1.3 Gastric cancer

Gastric cancer is the second most common cancer world-wide, with a frequency that varies greatly across different geographical regions. Incidence is highest in Japan (>40 per 100,000), Eastern Asia, South America, and Eastern Europe whereas Northern Europe, Canada, Africa and United States have the lowest incidences. Gastric cancer is rare before the age of 40, but its incidence steadily climbs thereafter and peaks in the seventh decade of life. In England & Wales in 2000, there were a total of 5,779 deaths from gastric cancers, with a corresponding mortality rate of 10.9 per 100,000 population that has fallen over time (Quinn et al. 2001).

Gastric cancer incidence rates have been declining in many developed countries (Howson et al. 1986; Newnham et al. 2003). The incidence of gastric cancer fell sharply by 40-50% in both men and women in the UK between 1971 and 1997. In addition mortality fell slightly more sharply than incidence; by about 60% in both men and women (Figure 1.1). The fall in gastric cancer incidence has been principally due to decreases in tumours arising from the antrum and body of the stomach. However, tumours arising in the proximal third of the stomach, like gastro-oesophageal junction tumours, are increasing (Devesa et al. 1999).

Classification of gastric cancer

Although Ming (Ming 1977), Goseki (Goseki et al. 1995) and the World Health Organisation have all developed histological classification systems for sub-dividing

gastric cancer, it is Lauren's classification that is most widely used (Lauren 1965). Tumours are divided into two primary histological subtypes: those which form glandular structures are known as intestinal-type whilst those which do not form glands but secrete mucin are referred to as diffuse-type. The distinction is important as each group has different characteristics (Table 1.2).

Table 1.2 Different characteristics of intestinal and diffuse type gastric adenocarcinoma

Characteristic	Intestinal	Diffuse
Gross pathology	Nodular, polypoid or ulcerated. Tends to be well demarcated	Plaque-like, poorly defined border
Microscopic	Well-formed glandular pattern (similar to colorectal cancer)	Single cells, small groups or cords of cells with cytoplasmic mucin. Signet ring cells common
Prevalence	More common in endemic areas	More common in low prevalence areas
Pre-malignant association	Associated with gastric atrophy, intestinal metaplasia and dysplasia	No known earlier pre-malignant cell types
Gender	Men > Women	Women > Men
Spread	Mainly haematogenous	Mainly lymphatic
Age	Increasing incidence with age	Younger age group
Prognosis	Better	Poorer

Adapted from (Munson et al. 2005)

Intestinal type gastric cancer

The intestinal type is better differentiated and is characterised by cohesive cells that form discrete glandular structures. The tumour arises from areas of gastric atrophy or intestinal metaplasia, which are thought to be pre-cancerous. Intestinal type is more common in older patients and in men. The intestinal type predominates in areas with a high incidence of gastric adenocarcinoma, whilst the diffuse type tends to occur at a

similar prevalence throughout the world (Craanen et al. 1992; Lauren et al. 1993). The world-wide decrease in gastric adenocarcinoma since the 1950's is accounted for primarily by a decrease in intestinal type disease (Craanen et al. 1992).

Diffuse type gastric cancer

Diffuse type is less differentiated and is characterised by sheets of cells without gland formation. Signet ring cells and mucin may be present. The diffuse tumours do not typically arise from any recognisable pre-cancerous lesions. It is the major histological type in non-endemic areas and has a higher association in familial types of cancer.

Survival

The five-year survival of about 12% in the UK is much lower than a European average of 21% (Keighley 2003). In Europe, only about 60% of gastric cancers are resectable when first diagnosed and surgical resection for cure is only achieved in about 40% of cases. Five-year survival after surgical resection is closely related to the stage of the tumour, and varies from 95% for early cancers to only 20% for extensive lesions (Keighley 2003). Unfortunately most patients present with advanced disease and can expect a median survival of 24 months in tumours resected with curative intent; a median survival of 8.1 months after palliative procedures; and a median survival of only 5.4 months for advanced disease without an operation (Allum et al. 2002).

Differences between Western countries and Japan

Surgical results and long term survival are consistently better in Japan compared with Western countries. Previously there has been a suggestion that the underlying disease is different between the two hemispheres, however this has largely been discounted as there is no evidence to support it (Griffin 2005; McCulloch et al. 1997). The reasons for better outcome are due to a number of reasons, including:

- Higher frequency of early stage disease detected with screening programmes
- Tumour location predominantly distal or middle third, rather than proximal lesions
- Tumour type predominantly intestinal-type, rather than diffuse
- High incidence of the disease means that most surgeons have high case volume of surgery

- Increased experience and greater use of extended lymphadenectomy techniques
- Younger patient age at initial diagnosis
- Less patient obesity and reduced rates of co-morbid disease

In addition to a better prognosis, there are striking differences in all aspects of gastric cancer management philosophy and surgical approaches between Western and Eastern countries (Table 1.3) (Siewert 2005).

Table 1.3 Main differences in gastric adenocarcinoma in Western countries and Japan

	Western countries	**Japan**
Incidence	Low	High
Prognosis	Poor	Good
Screening programme	No	Yes
Stage	Advanced (only 15% early)	Early (70%)
Tumour location	Proximal third or gastro-oesophageal junction	Distal or middle thirds
Patient age	Older	Younger
Co-morbidity	High	Low
Staging system	UICC/AJCC	Japanese staging system
Treatment	Largely palliative	Mostly curative
Neo-adjuvant therapy	Intense research interest	Minimal interest
Surgery	Limited (D0/D1)	Extended (D2/D3)
Surgeons volume	Low volume, but a move to centralise practise in specialist units	High volume, even in general units
Adjuvant therapy	'Gold standard'	In selected high risk patients

1.2 STAGING

The UICC TNM classification system is used for staging oesophageal and gastric cancer. Currently there is often confusion about whether the gastric or oesophageal staging systems should be used for specimen reporting in cancers of the gastro-oesophageal junction. Usually, Type I adenocarcinomas are staged as oesophageal cancer and Type III as gastric cancer. Type II adenocarcinomas are staged as oesophageal cancer by some authors and as gastric by others. This is not ideal as there are significant differences between the gastric and oesophageal TNM staging systems which differ in all three TNM categories (Table 1.4).

Table 1.4 Comparison between the oesophageal and gastric TNM staging

		Oesophageal staging		**Gastric staging**
T Stage	**T0**	No evidence of primary tumour	**T0**	No evidence of primary tumour
	Tis	Carcinoma in situ	**Tis**	Carcinoma in situ
	T1	Tumour invades lamina propria or submucosa	**T1**	Tumour invades lamina propria or submucosa
	T2	Tumour invades muscularis propria	**T2a**	Tumour invades muscularis propria
			T2b	Tumour invades subserosa
	T3	Tumour invades adventitia	**T3**	Tumour penetrates serosa (visceral peritoneum) without invasion of adjacent structures
	T4	Tumour invades adjacent structures	**T4**	Tumour invades adjacent structures
N Stage	**Nx**	Regional lymph nodes cannot be assessed	**Nx**	Regional lymph nodes cannot be assessed
	N0	No regional lymph node metastases	**N0**	No regional lymph node metastases
	N1	Regional lymph node metastases	**N1**	Metastases in 1 to 6 regional lymph nodes
			N2	Metastases in 7 to 15 regional lymph nodes
			N3	Metastases in more than 15 regional lymph nodes
M Stage	**Mx**	Distant metastases cannot be assessed	**Mx**	Distant metastases cannot be assessed
	M0	No distant metastases	**M0**	No distant metastases
	M1a	Metastases to coeliac or cervical lymph nodes	**M1**	Distant metastases
	M1b	Other distant metastases		

Adapted from (Sobin 2002)

There are minor differences in pT stages between the two systems with the main differences in the pN staging category. In oesophageal cancer, nodal involvement is merely classified as nodal positive (pN1) and nodal negative (pN0), irrespective of the number of lymph nodes involved. In comparison, in gastric cancer the pN category is sub-classified according to the number of involved nodes: pN1 (1-6 positive nodes), pN2 (6-15 positive nodes) and pN3 (>15 positive nodes). Metastatic lymph nodes to the coeliac axis are classified as systemic spread (pM1a) in the oesophageal system, whilst they are classified as regional in the gastric staging system. Similarly, metastases to the supra-diaphragmatic nodes or to the nodes of the lower mediastinum are considered 'non-regional' in gastric cancer, and are classified as distant metastases (pM1) in type II and type III junctional tumours. Consequently there have been calls for tumours around the GOJ to have a separate TNM staging system (de Manzoni et al. 2004); however this remains to be designed or approved by the UICC.

1.3 TREATMENT

Currently, the only curative treatment for upper gastrointestinal cancer is surgical resection. However, surgery is a major challenge due to the magnitude of the procedure and its potential for high morbidity and mortality.

In an attempt to improve prognosis in the UK, upper gastrointestinal services are being streamlined and reorganised with the development of specialist multidisciplinary teams and regional cancer networks (Guidance on Commissioning Cancer Services: Improving Outcomes in Upper Gastrointestinal Cancers. 2001; Melville et al. 2001). The overall results of surgery have improved in recent years with falling morbidity and mortality (Table 1.5). It is recommended that surgical resection for oesophagogastric cancer is performed in cancer centres with all necessary multidisciplinary services, and serving a population of at least 1 million. There is a large body of evidence indicating that hospitals which manage large numbers of patients with upper gastrointestinal cancer have better outcomes (Branagan et al. 2004; Lerut et al. 2005; Metzger et al. 2004). Improved outcomes are also due to close liaison between members of the multidisciplinary team, which include upper gastrointestinal surgeons, gastroenterologists, oncologists, histopathologists, radiologists and specialist nurses.

Table 1.5 Reasons for improved results for oesophagogastric resection

1.	Increase in specialist units
2.	Multidisciplinary approach
3.	Earlier diagnosis (Barrett's screening)
4.	Better patient selection
5.	Improved perioperative and post-operative management

1.3.1 Oesophageal cancer

Despite improvements in surgical techniques and peri-operative mortality, there has only been a slight improvement in the five-year overall survival of patients with oesophageal cancer over the last twenty years. This is mainly due to lack of effective therapeutic options in advanced stage disease and the inability to diagnose the cancer at an earlier and surgically treatable stage. Another reason for such disappointing outcomes with surgical treatment is attributed to the presence of undetected metastatic disease at presentation. As such, there has been much interest in combining surgery with either radiotherapy or systemic chemotherapy. Unfortunately, the use of either pre-operative or post-operative radiotherapy has failed to improve survival (Arnott et al. 1998). However, post-operative radiotherapy may have a role in preventing loco-regional recurrence in patients with positive surgical resection margins.

Pre-operative chemotherapy

Multimodal therapy, in particular neo-adjuvant treatment prior to surgical resection, is increasingly used in oesophageal cancer. The aims of neo-adjuvant chemo-radiotherapy are to downstage or 'sterilise' the primary to improve complete tumour resection (R0), reduce tumour recurrence, treat occult micro-metastases and ultimately to improve overall survival. Although several randomised controlled trails have shown that neo-adjuvant chemo-radiotherapy in addition to surgery can prolong survival (Naunheim et al. 1995; Vogel et al. 1995; Walsh et al. 1996), there remains considerable debate in the literature about the benefits of therapy and the definitive regime has yet to be defined. It is well documented that patients who achieve a pathological complete response (CR) after treatment have a significantly longer overall survival (Bates et al. 1996; Berger et al. 2005; Carey et al. 1993). However, a pathological CR occurs in less than 30% of patients who undergo surgery after pre-operative chemo-radiotherapy (Bosset et al. 1997; Heath et al. 2000; Kelsen et al. 1998). There is great interest in evaluating

predictive factors for patient response to neo-adjuvant chemo-radiotherapy. An accurate predictive factor would allow the targeting of therapy to patients who are most likely to achieve a benefit, while those unlikely to respond would avoid potentially toxic therapy and receive earlier surgery.

MRC OEO2 trial of pre-operative chemotherapy

In the UK the MRC have conducted a large phase III trial assessing the impact of neoadjuvant chemotherapy (MRC 2002). A total of 802 patients with operable oesophageal cancer were randomised to receive 2 cycles of cisplatin/5-FU followed by surgical resection or surgery alone. The overall survival was better in the pre-operative chemotherapy arm of the study, with a median survival of 16.8 months in the chemotherapy arm compared with only 13.3 months in the surgery alone group ($p=0.004$). As a result of the trial the regime is recommended for routine use in the UK.

A similar Intergroup trial carried out in the USA, randomised 440 patients to receive pre-operative chemotherapy (3 cycles of cisplatin/5-FU) before surgery or surgery alone (Kelsen et al. 1998). Unlike the MRC trial, however, it failed to show any benefit of pre-operative chemotherapy. It is speculated that the reasons why it failed to show a survival benefit compared with the MRC trial were:

- a longer time to definitive surgery (median of 63 days in the MRC trial compared with 93 days in the Intergroup study),
- higher doses of cisplatin and 5-FU and so higher toxicity (only 80% completed definitive surgery in the Intergroup study compared with 92% in the MRC trial).

Surgical resection

Surgical resection can be discussed in two components: resection of the oesophagus and resection of the surrounding tissue and lymphatics (Korst 2005). The surgical approach refers to the choice of incisions or technique used by the surgeon to perform the procedure. Each approach has its specific limitations and benefits (Table 1.6). There is considerable controversy regarding how radical or extensive the surrounding soft tissue resection and lymphadenectomy should be.

Standard versus radical (en-bloc) resection

A standard oesophagectomy involves resection of the oesophagus without any attempt at removing adjacent peri-tumoral tissue, lymph nodes or pleura. The radical or en-bloc oesophagectomy was initially described by Skinner (Skinner 1983), who proposed removing all peri-tumoral tissue in addition to the oesophagus in an attempt to reduce loco-regional recurrence and improve survival. In the case of the intra-thoracic oesophagus, all of the posterior mediastinal fat and lymphatics, the thoracic duct, as well as the posterior pericardium and sections of pleura bilaterally were excised with the oesophageal specimen. In cases where the tumour involved the gastro-oesophageal junction, a cuff of surrounding diaphragm was also excised.

As oesophageal cancer is characterised by extensive longitudinal submucosal spread, appropriate longitudinal clearance of the tumour is also essential. The British Society of Gastroenterology guidelines recommend that the ideal longitudinal resection margin distances should be 10 cm above and 5 cm distal to the macroscopic tumour (Allum et al. 2002).

Lymphadenectomy

The majority of patients who undergo surgery for either adenocarcinoma or squamous cell carcinoma of the oesophagus will have lymph node metastases. The aims of lymphadenectomy are to minimise staging error, reduce the risk of locoregional recurrence, increase the number of patients obtaining an R0 resection and increase the five-year survival. Three distinct regions, or fields, have been described with regards to oesophageal draining lymphatics: abdominal, mediastinum and cervical.

- The abdominal field represents the lymph node groups below the diaphragm and includes the right and left cardiac nodes, the nodes along the lesser curvature, left gastric, hepatic and splenic artery territory.
- The thoracic or mediastinal field refers to the nodal groups from the carina down towards the diaphragm and includes the para-aortic nodes along the thoracic duct, para-oesophageal nodes, right and left pulmonary hilar nodes and those at the tracheal bifurcation.
- The cervical field describes the nodal groups in the neck and includes the brachiocephalic, deep lateral, and external cervical nodes, and the deep anterior cervical nodes adjacent to the recurrent laryngeal nerve chains.

The extent of lymphadenectomy performed during oesophageal resection is also highly variable, ranging from minimal to radical. A radical oesophagectomy includes either a two-field (abdominal and mediastinal) or a three-field (abdominal, mediastinal and cervical) lymphadenectomy. Although there is considerable enthusiasm for three-field lymphadenectomy in Japan (Altorki 2005; Isono et al. 1991), this approach has not been widely adopted by Western surgeons.

Operative approaches

Operative approaches for oesophageal cancer divide broadly into either transthoracic (involving a thoracotomy) or transhiatal (not involving a thoracotomy) resections. A standard oesophagectomy can be performed using either of these approaches; however a radical lymphadenectomy can only be performed via a transthoracic approach. The operative approach is determined by the histological tumour type, tumour location, the extent of the proposed lymphadenectomy and surgeon's experience and preference.

Transhiatal oesophagectomy, as described by Orringer, involves a laparotomy for mobilisation of the stomach, dissection of the oesophagus through the diaphragmatic hiatus and oesophagogastric anastomosis performed through a cervical incision (Orringer et al. 1993).

There are three main types of transthoracic approach:

- Combination of a laparotomy and right sided thoracotomy with an intrathoracic anastomosis (Lewis 1946).
- A right sided thoracotomy, laparotomy and neck incision with a cervical anastomosis (McKeown 1976).
- Left sided approaches may involve thoracotomy alone, with the abdominal portion of the operation performed through the diaphragm, or a thoracoabdominal incision where the costal margin is divided to facilitate access to the abdomen. The anastomosis between the remaining oesophagus and stomach may be placed either in the thorax or the neck through a cervical incision (Anikin et al. 1997).

Minimal invasive techniques

Minimal invasive techniques involve laparoscopy and thoracoscopy instead of laparotomy and thoracotomy and therefore reduce the operative trauma to the patient (Nguyen et al. 2004). Many minimally invasive options for oesophagectomy have been described. These approaches include:

- Thoracoscopic oesophageal mobilisation (to avoid thoracotomy) with upper midline laparotomy for stomach mobilisation combined with a cervical incision for anastomosis
- Laparoscopic mobilisation (to avoid laparotomy) of the stomach with thoracotomy for oesophagectomy
- Laparoscopic transhiatal oesophagectomy
- Total laparoscopic and thoracoscopic oesophagectomy

Currently these techniques are only used by a minority of surgeons in the UK. As surgeons become more experienced with other advanced laparoscopic and thoracoscopic techniques and the technology become more widespread, use of these approaches is likely to increase. The most recent large series reporting the results of minimally invasive oesophagectomy are very encouraging and showed its potential compared with the open approach to lower morbidity and allow earlier hospital discharge and a quicker return to work (Luketich et al. 2003). Although several studies have shown encouraging results, the patient groups are highly selected and include a large proportion of patients with high-grade dysplasia or early carcinomas and minimal co-morbidities. Therefore, it is not clear whether these advantages would remain in clinical practice (Gossot et al. 2000). In addition, there are concerns regarding oncological adequacy of lymphadenectomy and long term survival data are currently lacking.

Table 1.6 The advantages and disadvantages of each operative approach in the treatment of oesophageal cancer.

Approach, Reference	Advantage	Disadvantage
Transhiatal (Lin et al. 2005)	• Avoids thoracotomy • Lower morbidity and mortality • Cervical anastomosis – reduced risk of fatal leak	• Blunt dissection can potentially injure important structures • Cervical incision – potential to injure recurrent laryngeal nerve • Higher incidence of locoregional recurrence • Unable to perform radical lymphadenectomy
Left throacoabdominal (Heitmiller 1992)	• Better access to the lower oesophagus • Can be combined with cervical incision and anastomosis	• Upper limit of dissection limited by aortic arch • Limited abdominal lymph node clearance • Increased resection line involvement compared with Ivor-Lewis • Higher incidence of post-operative reflux
Ivor-Lewis (Nichols et al. 2005)	• Good access to the abdomen for abdominal lymphadenectomy • Radical mediastinal lymphadenectomy more easily achievable	• Higher incidence of post-operative cardiorespiratory complications compared with transhiatal approach
McKeown three stage (McKeown 1976)	• Allows near total oesophagectomy • Avoids intra-thoracic anastomosis • Low risk of proximal margin involvement	• Longer operating time • Risks of a neck incision • Early post-operative difficulty in swallowing
Minimal invasive techniques (de Hoyos et al. 2005)	• Range of possible techniques • May avoid thoracotomy or laparotomy (or both) • Potential to lower morbidity • Shorter time to recover and return to work	• Substantial learning curve • Expensive equipment • May compromise lymphadenectomy • Potential for port-site recurrence

1.3.2 Gastro-oesophageal junction

The surgical management of patients with tumours of the GOJ is controversial (Siewert et al. 2000). With the acceptance of the Siewert classification, surgical treatment of

these tumours is becoming more standardised. However, few randomised trials have addressed the optimal surgical approach for GOJ tumours.

Type I gastro-oesophageal tumours (distal oesophageal)

There is no great controversy surrounding the treatment of these tumours and they are treated in a similar fashion to other adenocarcinomas of the distal third of the oesophagus: with either transthoracic or transhiatal resection of the oesophagus (Section 1.3.1). A randomised controlled trial comparing transthoracic and transhiatal oesophagogastrectomy showed that the transthoracic approach was associated with a significantly higher morbidity but a trend towards a better long-term survival compared with the transhiatal approach (Hulscher et al. 2002).

Type II (true cardia tumours) and III (sub-cardial tumours) gastro-oesophageal tumours

Type II and III gastro-oesophageal tumours pose a particular problem in terms of surgical approach, extent of resection and amount of lymph node dissection required. If an adequate proximal margin can be obtained then the preferred approach is a total gastrectomy with excision of the diaphragmatic crura around the hiatus with transhiatal resection of the lower oesophagus and accompanying lower mediastinal nodes (Siewert et al. 2000; Wayman et al. 1999). As the lymph node drainage from these tumours is mainly towards the coeliac axis a formal abdominal (D2) lymph node dissection is necessary. Several other approaches to resect these tumours have been described, including, left sided thoraco-abdominal approach, proximal sub-total gastrectomy, transhiatal oesophagectomy and two-stage Ivor-Lewis oesophago-gastrectomy. However, each of these approaches have their specific limitations, such as increased cardio-respiratory morbidity, post-operative mortality and limited amount of gastric resection.

1.3.3 Gastric cancer

Currently the only curative treatment for gastric cancer is surgical resection of the primary tumour with an appropriate lymphadenectomy, as gastric cancer in general is relatively resistant to chemo and radiotherapy. Patients with early gastric cancer are in the minority and the disease typically presents at an advanced stage, which often precludes curative surgical resection (Desai et al. 2004). Even in patients who have an

apparently curative resection about a quarter progress to develop recurrent or metastatic disease (Maehara et al. 2000).

Trials of neoadjuvant therapy have for the most part been disappointing. Until recently there was also no proven role for adjuvant chemotherapy, radiotherapy or chemo-radiotherapy. However, a large randomised study showed a significant survival benefit with adjuvant chemo-radiotherapy (Macdonald et al. 2001). There is still considerable debate as to the optimal surgical resection strategy, in particular the extent of the lymph node dissection.

Extended lymphadenectomy

The lymphadenectomy for gastric cancer is divided into five different types (Table 1.7). Japanese surgeons have great experience and routinely perform a systematic (D2) dissection, to remove the nodes along the main branches of the coeliac axis, whereas many Western surgeons perform more limited (D1) lymphadenectomy, which only removes local nodal groups. It has been hypothesized that lymph node metastases occur in an ordered centrifugal pattern of dissemination and metastases to specific nodal groups can be predicted. Advocates of extended lymph node dissection argue that it provides better lymphatic staging and results in improved long-term survival, particularly in patients with minimal loco-regional lymph node involvement. The Japanese rules for gastric cancer suggest that resection is likely to afford an absolute cure if all evidence of carcinoma is resected locally and if at least one tier of nodes beyond those identified with metastases is resected (Maruyama et al. 1989). Although many high volume specialist centres can now achieve low morbidity and mortality rates, many studies have shown D2 lymphadenectomy is associated with increased postoperative morbidity and mortality.

Excellent results claimed by Japanese studies have not been substantiated in Western countries. To resolve this controversy randomised trials were conducted in the UK (Cuschieri et al. 1999) and Netherlands (Bonenkamp et al. 1999). The UK MRC trial, showed unacceptably high morbidity and mortality associated with the D2 gastrectomy with no survival advantage, when compared to D1 (Cuschieri et al. 1999). The Dutch trial reported similar results (Bonenkamp et al. 1999). The high morbidity and mortality were mainly attributed to splenectomy and pancreatic resection which were considered essential for a D2 dissection at the beginning of the trials. The two trials were criticised for various reasons: lack of surgical standardisation, including

low-case volume surgeons, failure to account for the learning curve of D2 surgery, and inclusion of patients who underwent additional splenic or pancreatic resection.

A recent meta-analysis of the literature on this subject concluded that the potential benefits of D2 surgery remain unproven (McCulloch et al. 2005). They found that D2 surgery may benefit a subgroup of patients with Stage II or III gastric cancer and recommend its use by experienced surgeons with demonstrated low operative mortality. D1 surgery was recommended for unfit patients, patients with early (Stage Ia) cancer, and by surgeons who lack training in the D2 technique.

Table 1.7 Types of lymphadenectomy for gastric carcinoma

Type of surgery		Lymphadenectomy for gastric carcinoma
D0	'incomplete'	Gastric resection and incomplete resection of local lymph nodes
D1	'limited'	Gastric resection and removal of lymph nodes within 3 cm of the tumour
D2	'systematic' or 'extended'	Gastric resection and removal of lymph nodes within 3 cm of the tumour and the nodes along the main arteries supplying the stomach
D3/D4	'super-extended'	Radical en-bloc resection including removal of lymph nodes outside the normal lymphatic pathways from the stomach, i.e. retroperitoneal nodes, para-aortic and nodes within the small bowel mesentery.

Adjuvant chemo-radiotherapy

The most promising study of postoperative adjuvant treatment for gastric cancer was published in 2001 (Macdonald et al. 2001). In the Intergroup 0116 trial, 556 patients with resected gastric cancer were randomly allocated to observation alone or adjuvant chemo-radiotherapy. The treatment regime comprised 5-fluorouracil (5-FU), folinic acid and radiotherapy (45 Gy). The median overall survival in the surgery only group was 27 months, compared with 36 months in the adjuvant treatment group. Although this regime is popular in America, it is not widely used in Europe. One major criticism of this study was heterogeneity of the surgical treatment of the patients with only 10% having a D2 lymphadenectomy. Also, 1% of patients in the Intergroup 0116 study died as a direct result of the toxicity of chemo-radiotherapy. The ability to identify those patients most likely to benefit from radiation and/or chemotherapy is of key interest to future research into multimodality treatments for gastric cancer.

MRC MAGIC TRIAL (ST02) of perioperative chemotherapy

The final results of the multi-institutional MRC trial were recently presented and suggest a benefit of perioperative chemotherapy in patients with resectable gastric, gastro-oesophageal junction and lower oesophageal adenocarcinomas (Cunningham et al. 2005a). This trial randomised 503 patients between 1994 and 2002 to surgery alone or pre-operative chemotherapy consisting of epirubicin, cisplatin and fluorouracil (ECF) followed by surgery and three further cycles of ECF post-operatively.

A statistically significant benefit was found for progression-free (hazard ratio 0.66 with 95% CI 0.53-0.81, p=0.0001) and overall (hazard ratio 0.75 with 95% CI 0.60-0.93, p=0.009) survival for patients in the chemotherapy group compared to the surgery alone group. The corresponding 5 year survival rates were 36% for the chemotherapy group compared with only 23% for the surgery alone group.

Criticisms of this trial are that the type of surgical resection was at the discretion of the local surgeon. In addition, although the trial initially enrolled patients with gastric and GOJ adenocarcinomas, the eligibility was extended in 1999 to include patients with adenocarcinomas of the lower oesophagus. Patients with gastric adenocarcinoma accounted for 74% of all patients in the trial, while patients with tumours of the GOJ and lower oesophagus accounted for 11% and 15% respectively. It also appears that not all patients were able to complete the post-operative component of the chemotherapy. The full publication of this trial is awaited with interest, especially subgroup analysis of tumour sub-site. Peri-operative ECF chemotherapy has the potential to become the 'gold' standard therapy in the United Kingdom.

1.4 CARCINOGENESIS

1.4.1 Oesophageal (adenocarcinoma) carcinogenesis

Barrett's oesophagus

Barrett's oesophagus is a pre-malignant condition that is associated with a 30-125 fold increase in the risk of developing adenocarcinoma compared with unaffected individuals (Guindi et al. 2003). There has been a lack of a universally accepted definition of Barrett's oesophagus, which has resulted in confusion and difficulties in comparing studies. Barrett's oesophagus (or columnar-lined oesophagus [CLO]) was defined by the *British Society of Gastroenterology* as an oesophagus in which any portion of the normal squamous lining has been replaced by a metaplastic columnar

epithelium which is visible macroscopically (2005). It is recommended that the diagnosis is reserved for patients with a segment of columnar metaplasia of any length visible endoscopically above the gastro-oesophageal junction and confirmed by histological assessment. The gastro-oesophageal junction is defined as the proximal limit of the longitudinal gastric mucosal folds, the distal limit of the longitudinal oesophageal vessels and the point of flaring from the tubular oesophagus into the most dilated stomach co-exist in the absence of air insufflation (2005).

Histologically, CLO represents a range of cellular types, including gastric fundic type epithelium, junctional type epithelium and intestinal metaplasia (IM) characterised by the presence of goblet cells. The malignant risk only applies to CLO with IM, as gastric fundic epithelium has no risk of developing subsequent malignancy (Spechler et al. 1996).

Pathophysiology

It is now well established that CLO is a complication of severe and long-standing reflux of bile and acid from the stomach; that is gastro-oesophageal reflux disease (GORD). CLO is found in 10-16% of patients undergoing endoscopy for symptoms of GORD (Caygill et al. 2004). Paradoxically, although studies have shown that patients with CLO are at the extreme end of the spectrum of GORD they may have none or minimal symptoms. This is thought to be a consequence of impaired sensitivity of the columnar lining to acid injury. As such many cases of CLO remain undiagnosed.

Barrett's metaplasia-dysplasia-adenocarcinoma sequence

The normal squamous oesophageal lining is transformed via the injury caused by gastro-oesophageal reflux disease, into columnar-lined epithelium with specialised intestinal metaplasia. It subsequently progresses through increasingly severe degrees of dysplasia to invasive adenocarcinoma (Figure 1.3).

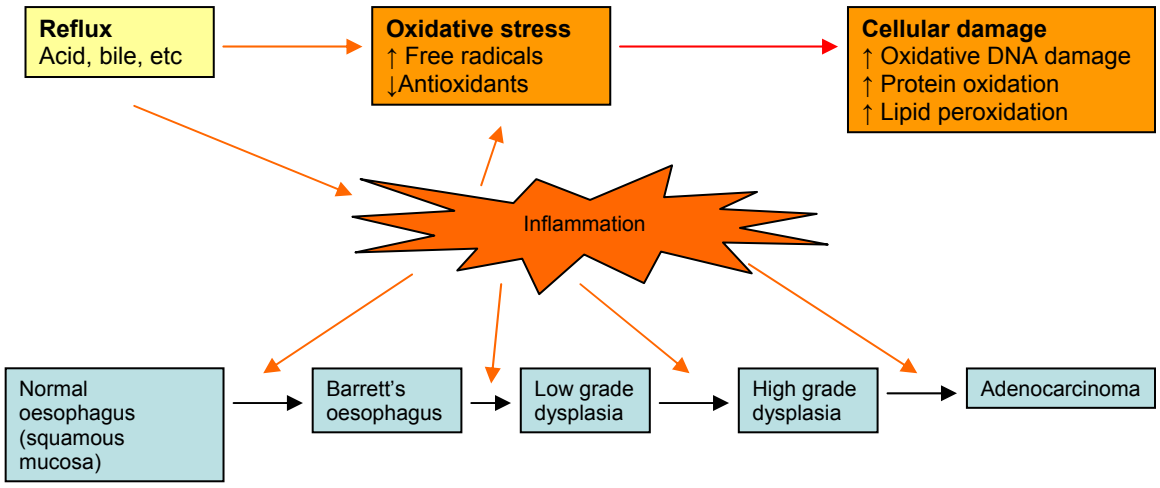

Figure 1.3 The proposed sequence of cellular changes in Barrett's adenocarcinoma development (the metaplasia-dysplasia-adenocarcinoma sequence).

The exact mechanisms through which GORD drives the carcinogenic process is largely unknown. Exposure of bile and acid combined with inflammation causes free radical damage and reduces the antioxidant capacity of the oesophageal epithelial cells. This induces oxidative stress, leading to increasing DNA and cellular damage. Adapted from (Wild et al. 2003).

Diagnosis

Accurate assessment of endoscopic biopsy material is crucial in the assessment of patients with Barrett's oesophagus, oesophageal epithelial dysplasia and adenocarcinoma. Accurate classification of these diagnostic categories often requires multiple biopsies, especially in high-grade dysplasia (HGD). The Seattle group recommend four quadrant biopsies for every 1 cm interval of Barrett's change identified at endoscopy (Levine et al. 1993).

Barrett's dysplasia

Dysplasia is defined as unequivocal neoplastic epithelium strictly confined within the basement membrane of the gland from which it arises (Riddell et al. 1983). Changes in the epithelial cells include lack of maturation, variation in nuclear size and shape, nucleolar enlargement, increased cytoplasmic ratio, hyperchromasia and presence of abnormal mitoses. Barrett's dysplasia is classified into either low grade dysplasia (LGD) or HGD by the degree of cellular changes and abnormalities present. It is of note that dysplasia is synonymous with intra-epithelial neoplasia (and HGD with carcinoma in-situ), as this terminology was not used in the original classification system (Riddell et al. 1983).

High grade dysplasia (HGD)

Appropriate care of patients with HGD in the setting of Barrett's oesophagus relies heavily on the accuracy of diagnosis of the degree of dysplasia. Early studies showed that HGD was associated with a risk of undetected adenocarcinoma on subsequent oesophagectomy specimen in up to 73% of cases (Edwards et al. 1996; Rice et al. 1993). Treatment options for HGD are controversial and range from intense endoscopic surveillance, endoscopic ablative therapy and oesophagectomy (Al-Kasspooles et al. 2002). As these cytological changes from LGD to HGD dysplasia are progressive but subtle, previous studies showed intra and inter-observer variations in the classification of the degree of dysplasia (Montgomery et al. 2001; Ormsby et al. 2002). These studies emphasize the need for a second opinion from an experienced gastrointestinal histopathologist in difficult cases, especially when the distinction is clinically important and will change therapeutic management (Allum et al. 2002). This may require further material for analysis using the 'Seattle' protocol for endoscopic biopsies to ensure sufficient tissue for accurate designation is available and to minimise the risk of missing an occult adenocarcinoma.

Risk factors for progression to malignancy

CLO with IM is thought to give rise to most, if not all, oesophageal and gasto-oesophageal junction adenocarcinomas with a rate of neoplastic change each year between 0.2% and 2% (Jankowski et al. 2000). Important clinical risk factors for progression to adenocarcinoma include male gender, age >45 years, segment >8 cm, duration of reflux history, mucosal damage (ulceration and stricture) and only rarely, family history (Table 1.8).

Table 1.8 Clinical risk factors predisposing to Barrett's adenocarcinoma

	Highest risk	**Lowest risk**
Gender	Male	Female
Age	> 45 years	< 40 years
Length of CLO	> 8 cm	< 3 cm
Severity of reflux symptoms	Severe and frequent (> 3 times / week)	Mild and infrequent (< once / week)
Chronicity	> 10 years	< 1 year
Race	White	Black
Body mass index	Obesity	Normal weight
Family history	Gastric cancer	None
Drug therapy	Nitrates, benzodiazepines, anticholinergics, theophyllines	Non-steroidal anti-inflammatory drugs
H. pylori	Absent	Present
Cigarette smoking	Heavy smokers	Non-smoker
Mucosal damage	Ulceration or stricture in Barrett's metaplasia	Intact mucosa
Duodeno-gastro-oesophageal reflux	Markedly present	Mild or absent

Information taken from (Jankowski et al. 2000)

Helicobacter pylori and distal oesophageal and gastro-oesophageal cancer

Five studies showed that *H. pylori*, especially CagA-positive strains, exert a protective response against GOJ and distal oesophageal adenocarcinoma (Chow et al. 1998; Graham et al. 1998; Vicari et al. 1998; Vieth et al. 2000; Weston et al. 2000). It has been postulated that the reduced gastric acid production as a consequence of gastric atrophy (caused by *H. pylori* induced inflammation) reduces the injury potential of the refluxate in GORD. As described previously, long-term GORD is known to induce Barrett's changes in the lower oesophagus which is a precancerous lesion for distal

oesophageal adenocarcinoma (Falk 2001). Moreover, ammonia produced by *H. pylori* also neutralises the harmful effects of the acid reflux, independent of gastric atrophy (Raghunath et al. 2003). However, evidence that patients with achlorhydria secondary to pernicious anaemia do not have a decreased incidence of oesophageal adenocarcinoma has cast doubt over this hypothesis (Ye et al. 2003). Furthermore, another study by the same authors showed that although infection with CagA-postive stains of *H. pylori* was strongly and inversely related to the risk of oesophageal adenocarcinoma, gastric atrophy (as measured by pepsinogen I levels) was unrelated to this increased risk and there was no correlation of risk with a detailed analysis of reflux symptoms (Ye et al. 2004).

Screening of Barrett's oesophagus

Regular endoscopic surveillance is recommended for patients with Barrett's metaplasia because of their increased risk of developing adenocarcinoma. Cancers detected in such programs are frequently early stage and have a better prognosis (Corley et al. 2002; Streitz et al. 1993). However, these analyses are subject to confounding factors such as lead and length time bias, and it remains to be established conclusively if surveillance is beneficial. Cost benefit analyses suggest that the cost of detecting an oesophageal adenocarcinoma in an endoscopic surveillance program is similar to the cost of detecting breast cancer by mammography (Streitz et al. 1998).

Molecular progression

The molecular hallmarks of Barrett's oesophagus carcinogenesis are shown in Table 1.9. Although many markers have been assessed, none is currently in routine clinical use. As the vast majority of patients enrolled in surveillance programs do not progress, it calls into question the benefit of repeat endoscopies at regular intervals. The ability to further stratify the risk of progression by molecular means might permit more effective targeting of repeated endoscopy to patients at particularly high risk of progression. The Barrett's metaplastic-dysplasia-adenocarcinoma sequence is a good model to study the mechanisms of oesophageal carcinogenesis.

Table 1.9 Molecular markers associated with cancer progression in Barrett's oesophagus

	Barrett's oesophagus	Low grade dysplasia	High grade dysplasia	Barrett's adenocarcinoma
Growth self sufficiency	Cyclin D1, Ki-67, c-erbB2, TGFα/β, EGF, EGFR, PCNA			EGF
Insensitivity to anti-growth signals	p16, FHIT	APC, DCC	RB	DPC-4
Avoidance of apoptosis	p53, Fas, TNF-α, NF-κB, c-myc, c-myb, COX-2, iNOS, bax/bcl-2		K-ras, H-ras	15-Lox-1, FasL
Limitless replicative potential			hTERT	
Sustained angiogenesis	VEGF			
Invasion and metastasis			E-cadherin, catenins	

Adapted from (Morales et al. 2002)

1.4.2 Gastro-oesophageal junctional carcinogenesis

Tumour around the gastro-oesophageal junction may arise in one of three ways:

- From metaplastic columnar epithelium in the lower oesophagus (Type I)
- Glandular epithelium from the cardia of the stomach (Type II)
- Epithelium from the fundus of the stomach with proximal spread (Type III)

Type I carcinogenesis

The carcinogenesis process of Type I gastro-oesophageal carcinogenesis was discussed in section 1.4.1.

Type II carcinogenesis

In contrast to Barrett's carcinogenesis, the role of histologically detected IM at the gastric cardia and its pathogenesis in these tumours is largely unknown and

controversial. Previous studies looking at the development of GOJ tumours have been hampered because the tumours are often of an advanced stage and consequently overgrow their precursor lesions (Cameron et al. 1995). Some authors suggested that the pathogenesis is similar to Type I tumours but the tumours arise from short or ultra-short segments of Barrett's metaplasia. An analysis of small carcinomas (<2 cm) of the GOJ found associated intestinal metaplasia in 69% of cases (Ruol et al. 2000). However, a more recent study of 100 patients with Type II GOJ tumours, found gastric specialised IM in 28% of all tumours and in 41% of early (T1) cancers (Siewert et al. 2005). As Barrett's mucosa was not usually found, the authors concluded that the majority of these cancers were gastric in origin. However, other authors regard IM of the gastric cardia and Barrett's oesophagus as a continuum and the same disease process (Chandrasoma et al. 2000; von Rahden et al. 2005a).

Type III carcinogenesis

Type III GOJ tumour development will be discussed in Section 1.4.3, as it is similar to gastric carcinogenesis.

1.4.3 Gastric carcinogenesis

Risk factors

The protective and risk factors for gastric cancer are summarised in Table 1.10. The reductions seen in gastric cancer incidence are attributed to a number of environmental and dietary factors. These include a decrease in the intake of salted, pickled, smoked and chemically preserved foods and increase in consumption of fruit and vegetables. Improved housing and living standards, with a resulting reduction in *H. pylori* infection, are also associated with a reduced incidence of distal gastric cancer.

Table 1.10 Protective and risk factors for gastric adenocarcinoma

Protective factors	Risk factors
Vitamin C	*H. pylori*
Diet high in fruit and vegetables	Diet low in fruit and vegetables
Diet low in salted, smoked, or preserved foods	Diet high in salted, smoked, or preserved foods
Non-steroidal anti-inflammatory drugs	Atrophic gastritis, gastric intestinal metaplasia or dysplasia
	Pernicious anaemia
	Familial adenomatous polyposis or gastric adenomatous polyps
	Cigarette smoking
	Family history

Correa hypothesis (intestinal type)

Correa proposed that intestinal type gastric cancer occurs via a multi-step sequence of events from pre-cancerous changes to invasive disease (Correa 1988) (Figure 1.4). This theory is similar to the well described sequence of events in colorectal cancer development. However, unlike the colorectal cancer sequence where there is a strong correlation with gene sequence alterations, the precise molecular alterations in gastric cancer remain to be described. Figure 1.5 shows the genetic and molecular processes known to be involved in gastric carcinogenesis. In the proposed gastric cancer model, normal mucosa is sequentially transformed into atrophic gastritis, intestinal metaplasia, dysplasia and finally invasive adenocarcinoma. This theory is supported by the observation that both atrophic gastritis and IM are frequently seen in patients with intestinal type cancer. The sequence is believed to be initiated by *H. pylori* and affected by a wide variety of genetic and environmental factors that may act synergistically.

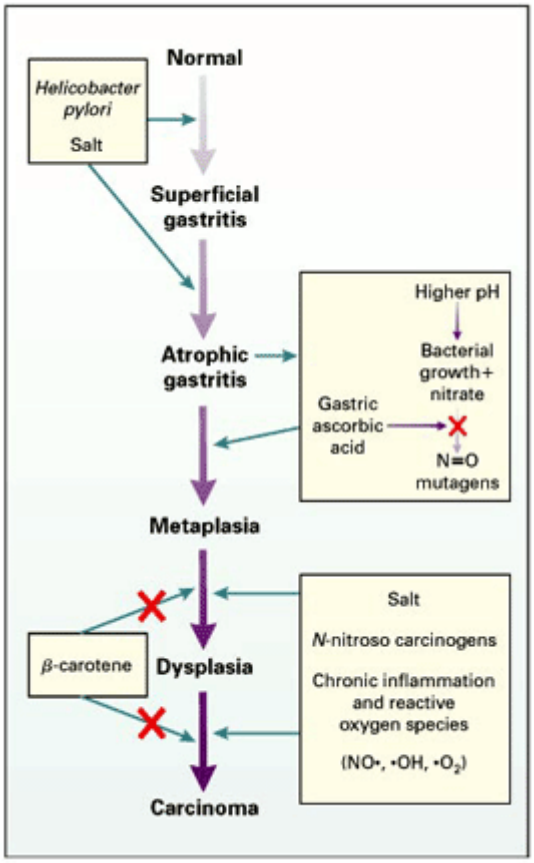

Figure 1.4 The Correa hypothesis and proposed carcinogenic mechanisms

Diffuse type carcinogenesis

Diffuse type gastric cancer has no recognisable precursor lesions and appears to arise *de novo*. A family history of gastric cancer is found in around 17-19% of younger patients, suggesting a hereditary/genetic factor may be involved (Koea et al. 2000; Ramos-De la Medina et al. 2004). Recent evidence has implicated a germ line mutation in the CDH1 gene which encodes for the E-cadherin protein (Lynch et al. 2005). Prophylactic gastrectomy has been described in at risk patients to prevent development of gastric cancer.

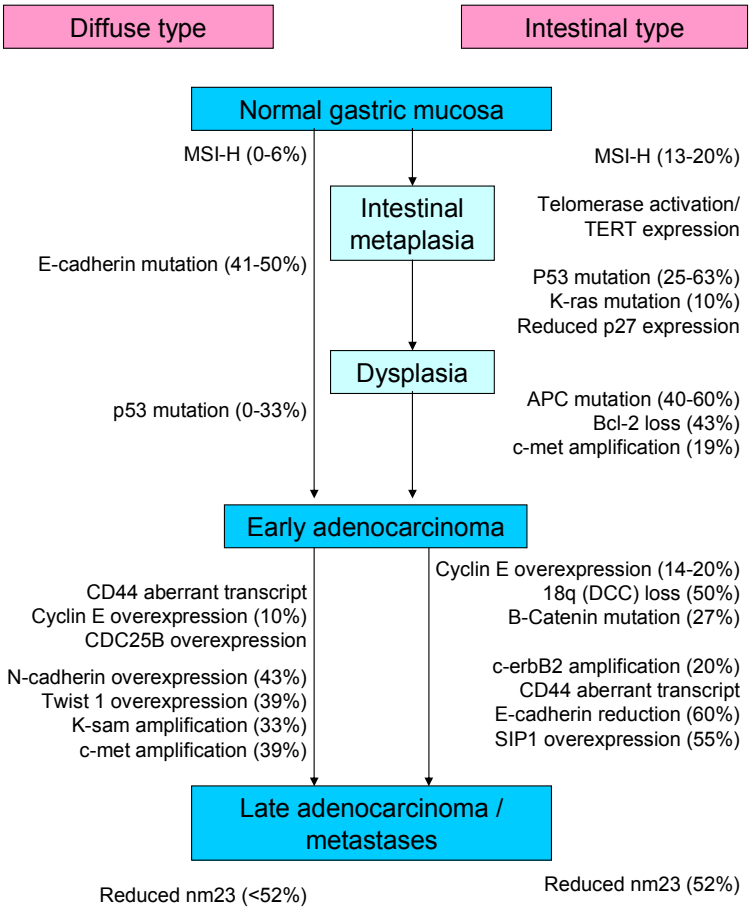

Figure 1.5 Genetic and molecular alterations in gastric cancer.

The molecular changes differ between the intestinal and diffuse-type tumours. The alterations, such as mutation, overexpression or amplification, are ordered according to the stage of cancer development. The percentages in parentheses indicate the frequencies of the alterations observed where known. Adapted from (Keller et al. 2005).

Helicobacter pylori

H. pylori is a gram-negative, spiral-shaped, microaerophilic, urease-positive bacillus (Goodwin et al. 1993). In humans it resides in the submucous layer of the stomach. Its urease enzyme splits urea into carbon dioxide and ammonia, elevating the local pH to allow its survival in the acidic environment (Marchildon et al. 1996). It was first found in association with gastric ulceration by electronmicrography of gastric biopsies in 1975 (Steer et al. 1975), but as it was impossible to culture using traditional techniques it was not formally 'discovered' until much later. Serendipitously it was cultured by Warren and Marshall using a longer than usual incubation over an extended Easter holiday

(Warren 1984). Warren and Marshall were awarded the Nobel Prize for Medicine in 2005 for this work.

Infection with *H. pylori* increases the risk of a range of disease, including gastritis, peptic ulcer disease, gastric adenocarcinoma and gastric mucosa-associated lymphoid type (MALT) B-cell lymphoma (Dunn et al. 1997; Parsonnet et al. 1994). Although infection is asymptomatic in most patients, it always induces an inflammatory response in the gastric mucosa that can be observed histologically. The most significant association of *H. pylori* is with distal gastric cancer, both intestinal and diffuse types.

Helicobacter pylori epidemiology

The principle reservoir for *H. pylori* is man and it is believed to be present in the stomach of at least half the world's population (Parsonnet 1995; Pounder et al. 1995). The prevalence of *H. pylori* is higher in developing countries than developed countries and this is believed to be related to socioeconomic factors (Suerbaum et al. 2002). Risk factors for acquiring the bacteria are low socioeconomic status, overcrowding, lack of fixed hot water supply and increased number of children in the household (Olmos et al. 2000). The infection is acquired during childhood and if not eradicated by appropriate antibiotics will usually persist throughout life. The incidence of *H. pylori* infection is declining, and this is mirrored worldwide, especially in westernised countries (Parsonnet 1995). Improved living conditions and sanitation are believed to be the main reasons for this decrease.

High prevalence of *H. pylori* is found in Asia and Eastern Europe, while North American, Western and Northern Europe have low prevalence of the organism (EUROGAST 1993). Estimates of the prevalence of *H. pylori* infection in England and Wales were calculated by Vyse in 2002 (Vyse et al. 2002). He analysed stored serum samples of 10,118 patients between 1986 and 1996 using an *H. pylori* enzyme linked immuno-sorbent assay (ELISA). The prevalence of infection varied markedly with age, with 30% of patients born in the 1940s infected, compared with only 3-4% of those born in the 1980s.

Helicobacter pylori and gastric cancer risk

The first reports of an association between *H. pylori* infection and gastric cancer were published in the early 1990s (Forman et al. 1990; Hansson et al. 1993; Parsonnet et al. 1991). By 1994 there was enough evidence from cohort and retrospective case-control studies for the International Agency for Research and Cancer (IARC) to classify *H.*

pylori as a Group 1 (definite) carcinogen (IARC monograph on the evaluation of carcinogenic risks to humans: schistosomes, liver flukes and Helicobacter pylori. 1994).

Previous epidemiological studies assessing the association between *H. pylori* and gastric cancer were subject to criticism and emerging evidence suggests the association is much stronger that was previously thought, principally due to underestimates of the prevalence of *H. pylori* (Brenner et al. 2004). Most of the initial retrospective studies assessing the association between *H. pylori* and gastric cancer only used one method of diagnosis, mainly serology. As the antibody response to *H. pylori* infection may be lost with severe gastric atrophy or IM and the development of gastric cancer, early studies probably underestimated the risk of gastric cancer (Kikuchi et al. 2000).

To overcome these biases the Helicobacter Cancer Collaborative Group conducted a meta-analysis of 12 previous case-control studies from the United States, Europe and Asia (Gastric cancer and Helicobacter pylori: a combined analysis of 12 case control studies nested within prospective cohorts 2001). They found that *H. pylori* infection was associated with a 5.9 fold increased risk of distal gastric cancer with no increased risk found for cancer of the gastric cardia. There was also a stronger association when *H. pylori* serology was analysed on blood taken more than 10 years prior to the diagnosis of gastric cancer.

In a long-term prospective study by Uemura et al (Uemura et al. 2001), 1525 patients with non-ulcer dyspepsia, active gastric or duodenal ulceration and gastric hyperplastic polyps were followed for a mean of 7.8 years. Patients received upper gastrointestinal endoscopy at 1 and 3 years. *H. pylori* status was assessed by three methods: histological assessment, rapid urease testing and serology. Only patients negative for all three tests were considered to be *H. pylori* negative. Thirty six of the 1246 patients (2.9%) who were *H. pylori* positive developed gastric cancer compared with none of the 280 patients who were *H. pylori* negative. They also found that the presence of severe gastric atrophy, corpus-predominant gastritis and IM increased the subsequent risk of gastric cancer development. More recently Ekstrom et al showed that the presence of CagA antibodies as markers of past *H. pylori* infection had a stronger relationship to the development of gastric cancer (Ekstrom et al. 2001).

Brenner et al re-analysed a large German case-control study looking at the association of *H. pylori* infection with the development of gastric cancer (Brenner et al. 2004). They addressed the possible underestimation of *H. pylori* associated disease due

to clearance of the organism with disease progression by excluding certain sub-groups of patients. These subgroups were chosen to reflect situations where the *H. pylori* test results would not adequately reflect prior *H. pylori* infection (false-negative testing). They were patients with gastric cancer whose serological testing was carried out more than 3 months after surgery, patients with T4 gastric cancer, and patients who were seronegative for *H. pylori* antibody using ELISA assay but positive for CagA antibodies. These exclusions increased the odds ratio for the development of distal gastric cancer from 3.7 (95% CI 1.7-7.9) to 18.3 (95% CI 2.4-136.7) with any strain of *H. pylori*. Cag-A strains of the infection had an even higher odds ratio of gastric cancer development. As all 32 patients who developed distal gastric cancer had evidence of previous *H. pylori* infection, they concluded that *H. pylori* infection is probably a pre-requisite for the disease.

H. pylori mediated carcinogenesis

The precise molecular mechanisms of gastric cancer development remain largely unknown. *H. pylori* induced chronic inflammation caused by reactive oxygen and nitrogen species leading to genetic damage to DNA is a strong factor in carcinogenesis (Wu et al. 2005). Proliferation of epithelial cells in the environment of oxyradical-induced injury results in conversion of DNA damage to mutations (genetic alteration) or non-mutational silencing of genes at the transcriptional level (epi-genetic alteration). In addition, inflammatory alterations may also promote growth and invasion of tumours without either genetic or epi-genetic changes (peri-genetic alteration). For example, cyclooxygenase-2 (COX-2) protein is frequently upregulated in gastric cancer, but the gene coding COX-2 is neither mutated or epigenetically altered (Saukkonen et al. 2003). The importance of the interaction between the surrounding microenvironment of stromal cells and cytokines has recently been recognised as important (Cheng et al. 2003).

Why do only a proportion of patients with H. pylori develop gastric cancer?

Only approximately 1% of patients with *H. pylori* infection develop gastric cancer. This can be explained by differences in the host genetic susceptibility, environmental factors and bacterial virulence. El-Omar et al demonstrated that polymorphisms in the pro-inflammatory cytokine interleukin-1β predispose to atrophic gastritis and increase the risk of developing gastric cancers in susceptible individuals (El-Omar et al. 2000).

1.5 PROGNOSTIC FACTORS

The definition of a prognostic marker is a 'variable that provides prospective information on patient outcome by which therapeutic decisions can be guided'. This is in contrast to predictive factors which give information on a likely tumour response to a particular therapeutic treatment. To be of value clinically, prognostic markers must be widely applicable and should ideally be:

- Better than those currently available
- Sensitive and specific
- Reproducible
- Able to be implemented within current technology
- Cost effective

Ideally, a prognostic marker will give novel information that does not overlap or replicate those derived from existing clinico-pathological factors. However, those giving the same information may be of use if they a) represent potential targets for new therapies or b) provide earlier prognostic data, for example from initial diagnostic biopsy.

1.5.1 Current clinico-pathological prognostic factors in oesophagogastric cancer

Many studies assessing prognostic markers in oesophagogastric cancer have revealed conflicting results and are inconsistent. Current putative clinico-pathological prognostic factors can be categorized into clinical factors, tumour-related factors and treatment-related factors. Table 1.11 summarises current prognostic markers for oesophageal and gastric cancer. The lack of reproducibility may be related to size of the patient group, heterogeneous patient population, differences in therapeutic treatment or surgical technique and differences in prognostic marker evaluation. Potential molecular prognostic factors will be discussed separately (Section 1.6.1).

Table 1.11 Current established and putative prognostic factors for oesophageal, gastro-oesophageal junction and gastric cancer

	Oesophageal and gastro-oesophageal cancer	References	Gastric cancer	References
Clinical factor	Patient Age	(Poon et al. 1998)	Patient age	(Cuschieri et al. 1999)
	Co-morbidity	(McCulloch et al. 2003)	Patient gender	(Cuschieri et al. 1999)
	Nutritional status	(Nozoe et al. 2002)	Co-morbidity	(McCulloch et al. 2003)
			Emergency presentation	(Blackshaw et al. 2004)
Tumour related	T stage	(Rice et al. 2003)	T stage	(Yu et al. 1995)
	N stage	(Eloubeidi et al. 2002)	N stage	(Siewert et al. 1998a)
	M stage	(Christie et al. 1999)	M stage	(Kim et al. 1998)
	Overall TNM stage	(Kunisaki et al. 2005)	Overall TNM stage	(Kim et al. 1998)
	Tumour differentiation	(Langley et al. 2002)	Tumour differentiation	(Zhang et al. 2004)
	Histological subtype	(Siewert et al. 2001)	Lauren classification	(Cunningham et al. 2005b)
	Residual disease	(Holscher et al. 1995)	Serosal invasion	(Kooby et al. 2003)
	Tumour length	(Eloubeidi et al. 2002)	Residual disease	(Siewert et al. 1998a)
	LRM involvement	(Law et al. 1998)	Tumour size	(Siewert et al. 1998a)
	CRM involvement	(Dexter et al. 2001)	Eosinophilic infiltration	(Cuschieri et al. 2002)
	Vascular invasion	(Zafirellis et al. 2002)	Lymphocytic infiltration	(Songun et al. 1996)
	Lymphatic invasion	(von Rahden et al. 2005b)	Maruyama index	(Peeters et al. 2005)
			Lymphatic invasion	(Yokota et al. 2000)
			Vascular invasion	(Yokota et al. 1999)
Treatment related	Neo-adjuvant treatment	(MRC 2002)	Adjuvant chemotherapy	(Macdonald et al. 2001)
	Surgeon volume	(Metzger et al. 2004)	Surgeon volume	(Meyer 2005)
	Surgical technique	(Hulscher et al. 2002)	Lymphadenectomy	(McCulloch et al. 2005)
			Additional splenectomy or distal pancreatectomy	(Nanthakumaran et al. 2005)

LRM = longitudinal resection margin; CRM = circumferential resection margin

1.5.2 Prognostic factors in oesophageal and gastro-oesophageal cancer

Important histopathological factors are summarised in Table 1.11. The most important predictors of prognosis appear to be the overall TNM stage (Sobin 2002), completeness of resection (R classification) (Hofstetter et al. 2002) and presence of lymph node metastases (Eloubeidi et al. 2002; Langley et al. 2002). The residual tumour classification is one of the strongest prognostic factors after surgical resection (Hofstetter et al. 2002; Siewert et al. 2000). R0: complete microscopic and macroscopic resection; R1: residual microscopic disease; R2: residual macroscopic disease (Hermanek et al. 1994). Both the pathologist and the surgeon have roles in defining the R status during and after surgery.

Circumferential resection margin (CRM) involvement

CRM involvement is a controversial prognostic factor. Although it has long been established that involvement of the proximal or distal resection margin is associated with a poor prognosis (Law et al. 1998; Mulligan et al. 2004), the relevance of the CRM status in oesophageal cancer is unclear and few studies have addressed this issue. In contrast, the pathological reporting of CRM in rectal cancer is an important predictor of local disease recurrence, survival and an indicator for post-operative oncological therapy (Adam et al. 1994; Nagtegaal et al. 2002). The first study carried out in oesophageal cancer involved only a small number of patients but suggested that CRM positivity was a significant prognostic factor (Sagar et al. 1993). However, only two subsequent papers assessed the effect of CRM status on patient survival and have produced conflicting results (Dexter et al. 2001; Khan et al. 2003).

Lymph node metastases

The presence of lymph node metastases is one of the most important adverse prognostic factors in oesophagogastric cancer surgery and is often one of the most significant independent factors on multivariate analysis (Eloubeidi et al. 2002; Roder et al. 1994; Siewert et al. 2000; Siewert et al. 2001). There is no consensus regarding the minimum number of lymph nodes to be included in a curative resection for accurate pathological staging. The current UICC guidelines (2002) recommend a minimum examination of 6 lymph nodes to classify a patient N0 (TNM Supplement: A Commentary on Uniform Use 2003). However this falls short of the 15 recommended by a consensus conference

of the International Society for Diseases of the Esophagus (ISDE) (Fumagilli 1996). As mentioned previously, the current oesophageal staging criteria simply divides patients into lymph node metastases present (pN1) and or absent (pN0). This system is crude and does not allow for the total number of resected/examined nodes. There is strong evidence that a lymph node ratio (number of nodes involved/number nodes examined) may be a better system. The significance of metastatic lymph node ratio has been described in oesophageal adenocarcinoma in Western patients (ratios of 0.2 and 0.3) (Holscher et al. 1995; Siewert et al. 2000; Zafirellis et al. 2002), squamous cell carcinoma in Western patients (ratio of 0.2) (Roder et al. 1994) and squamous cell carcinoma in Japanese patients (ratio of 0.1) (Tachibana et al. 2000). The differences in ratios for each of these studies may reflect the differences in nodal yields obtained from two-field oesophago-gastrectomy for adenocarcinoma and three-field oesophago-gastrectomy for squamous cell carcinoma. Noticeably, in all of these five studies, the lymph node ratio was of greater prognostic significance than the N stage. As there is no consensus on the best lymph node ratio, it is important that the pathologist accurately reports the number of nodes examined with the total number of nodes involved.

Immunohistochemically detected lymph node micrometastases

Immunohistochemical techniques can identify micrometastases which are missed by standard haematoxylin and eosin staining. Cytokeratin, a component of the cytoskeleton of epithelial cells, is not found in normal nodes enabling monoclonal antibodies to certain cytokeratin markers (such as AE1/AE3) to be used to detect micrometastases. These techniques may detect single tumour cells or cell clusters in lymph nodes that have been staged tumour free on routine examination. The prognostic outcome of the detection of micrometastases detected by immunohistochemistry is controversial as some studies have found an association with increased risk of tumour recurrence and decreased survival (Heeren et al. 2005; Izbicki et al. 1997; Komukai et al. 2000), but others have not (Nakamura et al. 2002; Sato et al. 2001). The viability of the tumour cells and their potential to from true metastases has been questioned. As such, these techniques remain research tools and are not currently used in daily clinical practice.

1.5.3 Prognostic factors in gastric adenocarcinoma

A variety of prognostic factors have been reported for surgically treated patients with gastric adenocarcinoma. Table 1.11 summarises some of the important clinico-pathological prognostic factors. Strong prognostic factors which are often independent on multivariate survival analysis include complete (R0) resection (Siewert et al. 1998a), overall TNM stage (Eloubeidi et al. 2002) and the presence of lymph node metastases (Rodriguez Santiago et al. 2005).

Tumours involving the gastric cardia have a worse prognosis than non-cardia gastric cancer (Kajiyama et al. 1997; Kim et al. 2005; Pinheiro et al. 1999). Reasons for this include high rates of poorly differentiated tumours, advanced TNM stage, increased regional lymph node involvement and distant metastatic disease. In addition to different clinicopathological characteristics, tumours of the gastric cardia appear to have different gene expression profiles (Kim et al. 2005).

Size of the primary tumour (as measured by greatest dimension) has been identified in several retrospective studies to be of prognostic significance (Adachi et al. 2000; Msika et al. 2000; Yokota et al. 1999). Increasing tumour diameter is associated with increased number of lymph node metastases and reduced 5-year survival. A prospective, randomised trial observed that tumour size was an independent prognostic factor on multivariate analysis in patients who underwent an R0 resection (Siewert et al. 1998a).

There are differences between histopathological classification systems. The WHO, Lauren, Ming and Goseki classification systems have been shown to yield some prognostic information (Cuschieri et al. 2002; Davessar et al. 1990; Songun et al. 1999), but very few studies have shown them to be independent predictors of prognosis. Ming's infiltrative type was an independent predictor of prognosis in one study (Roy et al. 1998). Although Lauren's diffuse type appears to have a worse prognosis than intestinal type tumours, this was not an independent predictor of prognosis in one of the largest studies in gastric cancer (Kim et al. 1998). The Goseki classification has been shown to give prognostic information above TNM staging (Songun et al. 1999), however in other recent studies it was an unreliable prognostic factor (Fontana et al. 2003; Monig et al. 2001).

The presence of tumour deposits within surrounding microvessels and lymphatics may have potential as a prognostic factor. Yokota et al showed that lymphatic invasion was significant on multivariate analysis in lymph node negative

patients (Yokota et al. 1999). In addition, tumour microvessel invasion was also shown to be an independent prognostic factor in two recent studies in lymph node negative patients (Hyung et al. 2002; Kooby et al. 2003). A recent study confirmed these findings and showed that lymphovascular invasion appears to lose its prognostic significance in patients with lymph node metastases and advanced T stages (Dicken et al. 2004).

1.5.4 Molecular prognostic factors

It is hoped that the most accurate prognostic information will be achieved by combining clinico-pathological and molecular marker data. Although there is great interest in molecular predictors of prognosis in oesophageal and gastric cancer no molecular marker has entered routine clinical practice. There are a wide variety of methods for assessing molecular markers; each with advantages and disadvantages (Table 1.12)

Table 1.12 Comparison and applicability of different methods for assessing potential molecular prognostic markers

	PCR	**RT-PCR**	**Northern blotting**	**ISH**	**IHC**	**Tissue micro-array**	**cDNA micro-array**
Cellular constituent examined	RNA	mRNA	mRNA	mRNA	Prot	Prot	RNA
Used in formalin-fixed tissue	Y	N	N	Y	Y	Y	N
Microdissection needed	Y	Y	Y	N	N	N	N
Cellular localisation possible	N	N	N	N	Y	Y	N
Used in routine diagnosis	Y	N	N	Y	Y	N	N

PCR = polymerase chain reaction; RT = reverse transcriptase; ISH = in situ hybridisation; IHC = immunohistochemistry; Prot = protein; Adapted from (McLeod et al. 1999).

Many potential immunohistochemical molecular markers have been assessed in oesophageal and gastric cancer. Table 1.13 shows a selection of molecular markers which have been shown to be statistically significant on survival analyses. They are divided into the six 'Hallmarks of Cancer' as first described by Hanahan and Weinberg (Hanahan et al. 2000), which are: (1) limitless replicative capacity; (2) insensitivity to growth inhibition signals; (3) self-sufficiency from growth signals; (4) sustained angiogenesis; (5) evasion of apoptosis and (6) tissue invasion and metastasis.

Table 1.13 Promising molecular markers of prognosis in oesophagogastric cancer

	Oesophageal and gastro-oesophageal adenocarcinoma	Reference	Gastric adenocarcinoma	Reference
Limitless replicative capacity			Cyclin-D2 Telomerase	(Takano et al. 2000) (Usselmann et al. 2001)
Insensitivity to growth inhibition signals			Rb myc	(Feakins et al. 2003) (Songun et al. 1996)
Self-sufficiency from growth signals	c-erbB-2	(Flejou et al. 1994)	EGFR PTEN	(Gamboa-Dominguez et al. 2004) (Lee et al. 2003)
Sustained angiogenesis	VEGF Endoglin (CD105) COX-2	(Saad et al. 2005) (Saad et al. 2005) (Buskens et al. 2002)	VEGF iNOS COX-2	(Maeda et al. 1996) (Li et al. 2005) (Shi et al. 2003)
Evasion of apoptosis	Bcl-2	(Raouf et al. 2003)	Survivin Caspase-3	(Meng et al. 2004) (Li et al. 2004)
Tissue invasion and metastasis	E-cadherin Beta-catenin MMP	(Krishnadath et al. 1997) (Krishnadath et al. 1997) (Murray et al. 1998)	E-cadherin Beta-catenin c-Met MMP	(Zhou et al. 2002) (Zhou et al. 2002) (Huang et al. 2001) (Zhang et al. 2003)

1.6 TUMOUR HYPOXIA

Hypoxia, a reduction in the normal tissue oxygen tension, occurs when cellular oxygen needs are outstripped by supply and is associated with several pathophysiological processes including malignancy. In 1955, Thomlinson and Gray showed that tumour chords, with a radius greater than 200 μm invariably contained necrosis, whereas chords with a radius less than 160 μm did not (Thomlinson et al. 1955). They proposed a diffusion gradient of oxygen tension between the well oxygenated cells adjacent to blood vessels and areas of necrosis. The presence of hypoxic, but viable, cells immediately adjacent to necrosis was proposed. It is now thought that there are two principle types of tumour hypoxia: chronic diffusion-limited and acute perfusion-limited hypoxia (Figure 1.6). Diffusion-limited hypoxia occurs when cells are simply too far away from the nutrient-supplying capillaries for oxygen to diffuse. In acute/intermittent hypoxia, cells are rendered hypoxic for a variable amount of time. The intermittent type is thought to be due to abnormal tumour vasculature, such as out-pouchings, compressed vessels, arterio-venous malformations and tortuous sinusoids leading to altered and erratic blood flow (Konerding et al. 1999). Temporarily occluded vessels may re-establish flow leading to reperfusion effects which include an increase in free-radical concentration, tissue damage and activation of stress-response genes.

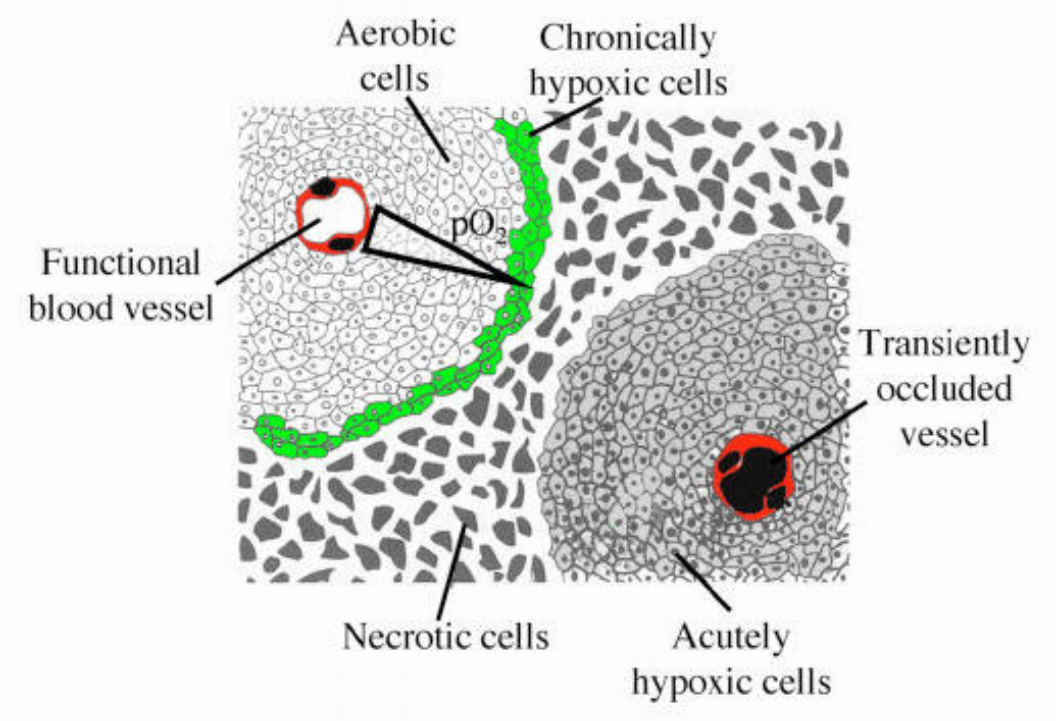

Figure 1.6 Schematic representation of diffusion limited (chronic hypoxia) and perfusion limited (acute hypoxia). Modified from (Horsman et al. 1992)

1.6.1 Measuring tumour hypoxia

A number of approaches are being investigated for the measurement of hypoxia in human cancers (Table 1.14). Tumour oxygenation can be measured directly or indirectly. Direct measurements of oxygen tension within tumours can be obtained by polarographic needle electrodes and this is the current 'gold standard'. Exogenous drugs, such as pimonidazole and EF5, which bind to hypoxic cells are also being explored for their usefulness in the assessment of levels of hypoxia within tumours. Hypoxia-inducible proteins such as HIF-1α, carbonic anhydrase-9 (CA-9) and Glut-1 which can be studied using immunohistochemistry are also being investigated as potential surrogate markers of tumour hypoxia. Non-invasive imaging techniques, such as dynamic contrast enhanced MRI and PET are in their infancy but have obvious potential clinical benefits regarding routine clinical use. Table 1.14 summarises the advantages and disadvantages of some of the approaches under investigation for measuring tumour hypoxia in patients.

1.6.2 Oxygen electrode measurements of tumour hypoxia

Oxygen tension can be assessed directly using polarographic needle electrodes to measure the partial pressure of oxygen in tissues (pO_2). The early oxygen electrode studies provided evidence that hypoxia was present in human cancers, with hypoxia demonstrated in human rectal, uterine cervix and head and neck tumours (Gatenby et al. 1985; Wendling et al. 1984). The oxygen electrodes used in these earlier studies were larger than the newer models and as such were associated with artefacts due to tissue compression and bleeding and considered unreliable for routine clinical use.

Subsequent studies used the Eppendorf pO_2 histograph incorporating a computerised polarographic needle electrode with the advantage of having a finer tip controlled by an automatic stepper motor. This enabled multiple measurements to be taken rapidly while minimising tissue compression and bleeding artefacts. Studies carried out using the Eppendorf equipment involved a range of human tumours, including uterine cervix, head and neck, breast, soft tissue sarcoma, brain, prostate and melanoma (Adam et al. 1999; Brizel et al. 1994; Hockel et al. 1991; Lartigau et al. 1997; Movsas et al. 2001; Rampling et al. 1994; Vaupel et al. 1991). Measurements with Eppendorf pO_2 have been largely confined to accessible tumours which are ≥3 cm in diameter. Median pO_2 values of 10-30 mmHg in tumour tissue have been reported, in contrast to median values in normal tissues of 24-66 mmHg (Brizel et al. 1999; Hockel

et al. 1993; Vaupel et al. 1991). In one study, 82% of all readings taken in human tumours were less than 2.5 mmHg (Hockel et al. 2001). Intra-tumour variability in oxygen tension is accounted for by taking multiple measurements from the tumour (Nordsmark et al. 1994). Readings are typically taken in four tracks within a tumour, with 40 measurements per track. Necrosis is a confounding factor, leading to zero or negative oxygen electrode readings.

Tumour pO_2, was first shown to be a prognostic factor in 1993 by Hockel et al (Hockel et al. 1993). In a study of 31 cervical cancer patients who received radiotherapy, with or without concomitant chemotherapy, those with hypoxic tumours (defined as median $p0_2$ <10 mmHg) had a significantly shorter overall and recurrence-free survival time compared with those with well-oxygenated tumours. This finding has been confirmed in further studies and is irrespective of whether surgery or radiotherapy was the primary treatment modality. Tumour hypoxia has been shown to be a predictor of a poor outcome in several tumour sites and appears to be independent of pathological variables such as tumour size, grade, and extent of necrosis (Brown 1999; Nordsmark et al. 2005).

There have been no published studies using direct measurements of oxygen tension in patients with oesophagogastric cancer. This may be because of fear of potential tumour implantation whilst using the oxygen electrode intra-operatively. Although most direct measurement studies have come from easily accessible tumours, such as cervical and head and neck, one small study did directly measure oxygenation in pancreatic tumours intra-operatively immediately prior to resection (Koong et al. 2000). This study has shown that it is feasible to take measurements of oxygen tension within the abdominal cavity during surgery. However, the study only included a few patients with measurements taken prior to surgical resection with an additional disadvantage that the technique added an extra 15 to 30 minutes to the already lengthy surgical procedure.

Table 1.14 Advantages and disadvantages of various methods of measuring tumour hypoxia

Method	Principal of measurement	Advantages	Disadvantages
Eppendorf polarographic electrodes	Needle electrode advances through tissue; current through probe is proportional to oxygen tension in adjacent cells.	• Current 'gold standard' • Provides a direct measure of tumour oxygenation • Direct comparison possible with adjacent normal tissue	• Invasive; potential risks of bleeding and injury • Only suitable for accessible tumours • Tumour generally has to be greater than 3 cm diameter • Cannot differentiate between extreme hypoxia and necrosis • Cannot distinguish between acute and chronic hypoxia • Possible sampling errors
2-nitroimadazole compounds; e.g. pimonidazole or EF5	Visualisation of nitroimidazole reduced product using an antibody	• Safe • Can be used on tumours inaccessible to polarographic electrodes (biopsy or resection specimens and small tumours) • The possible bias of sampling necrosis can be corrected for	• Can only be used 'prospectively'; infusion of drug up to 24 hours prior to biopsy/resection is required; thus precluding the possibility of studying archival specimens • Possible sampling errors (biopsies may not be representative of the whole tumour)
Hypoxia related markers; e.g. HIF-1*α*, CA-9, Glut-1.	Immunohisto-chemical staining of tumour biopsy or surgical specimen	• Can be used on tumours inaccessible to polarographic electrodes (biopsy or resection specimens and small tumours) • Archival specimens can be used	• Possible sampling errors (biopsies may not be representative of the whole tumour) • Not hypoxia specific, i.e. other factors may upregulate transcription of these factors
Non-invasive methods; e.g. contrast enhanced dynamic CT, dynamic contrast-enhanced MRI and PET	Rate of magnitude of uptake of various contrast agents thought to reflect oxygenation status; visualisation of hypoxia marker	• The whole tumour is imaged • Hypoxic tumour physiology and metabolism can be correlated with the cross-sectional image (i.e. tumour volume) • Non-invasive • Repeatable	• Least studied • Low spatial resolution

1.6.3 Exogenous markers of hypoxia

The binding of 2-nitroimidazole drugs, such as pimonidazole and EF5, to hypoxic cells is being exploited as a method of measuring tumour hypoxia. The drugs bind selectively to hypoxic cells by forming covalent bonds with intracellular thiols (Raleigh et al. 1990). Bioreduction occurs at pO_2 levels below approximately 10 mmHg (Koch et al. 1995), with binding proportional to the degree of hypoxia. A single dose of the drugs is given intravenously prior to surgical resection or tumour biopsy. Formalin-fixed tissue is subsequently stained using immunohistochemistry techniques with monoclonal antibodies that recognise the chemically reduced adducts (Woods et al. 1996). Although pimonidazole and EF5 have the same mechanism of hypoxic binding, they differ in their pharmacokinetic and pharmacodynamic properties. In a study involving only a small number of patients with head and neck cancer, patients with hypoxic tumours (as revealed by pimonidazole staining) were found to have a higher incidence of locoregional recurrence compared with those with well-oxygenated tumours (Kaanders et al. 2002). This approach, unlike oxygen electrodes, is not limited to accessible tumours and therefore is applicable to a wider range of tumour types, including oesophagogastric carcinoma. There have been no published studies of pimonidazole assessment of hypoxia in oesophagogastric cancer.

1.6.4 Non-invasive measurements of tumour hypoxia

Non-invasive imaging approaches are also being developed for measuring tumour hypoxia eg PET, MRI and single photon emission computed tomography (SPECT). The principle advantages of these methods are that they allow the assessment of hypoxia throughout the whole of the tumour and avoid the general anaesthesia and potential morbidity of direct tumour measurements. However, most of these methods are currently in their infancy and the ideal non-invasive imaging method is yet to be developed.

Non-invasive imaging of hypoxia in gastric cancer: FDG Positron Emission Tomography

PET scanning has proved useful in several studies assessing the stage of gastric cancer (McAteer et al. 1999; Yeung et al. 1998). The uptake of ^{18}F-label glucose analogue 2-fluro-2-deoxy-D-glucose (FDG) compound reflects in part the enhanced glycolysis of the primary tumour. Glut-1 expression (a related hypoxia marker), as assessed by

immunohistochemistry, has been shown to correlate with the in vivo uptake of FDG (Brown et al. 2002).

In a recent study, Mochiki et al assessed the utility of FDG PET in the staging of gastric cancer (Mochiki et al. 2004). In 85 patients the staging of CT, FDG PET and final histology were compared. The primary tumour was visualised in 75% of patients, with increased FDG uptake associated with increased depth of invasion, tumour size and number of lymph node metastases. The failure of FDG PET to detect the primary tumour was mainly in early stage gastric cancers. FDG PET scanning also provided additional information relating to tumour aggressiveness and prognosis, as patients with high FDG uptake had a significantly shorter survival when compared with those with low FDG uptake.

FDG PET has also been shown in one small study to be useful in predicting which patients with locally advanced gastric cancer will respond to neo-adjuvant chemotherapy (Ott et al. 2003). Baseline scans were compared with scans 14 days after commencing neo-adjuvant chemotherapy (leucovorin, cisplatin and 5-FU). A reduction in 35% of FDG uptake was defined as a metabolic response. There was a significantly lower overall survival in non-responders compared to patients who had a metabolic response to the chemotherapy. The potential future benefits of this approach may be that it will allow non-responders to avoid ineffective and potentially harmful treatment and get earlier surgical resection, whilst in responding patients a full course of neo-adjuvant therapy can be administered.

1.6.5 Hypoxia and cancer treatment resistance

Hypoxia and radiotherapy resistance

Hypoxic tumours are associated with a poor prognosis and resistance to cancer treatments. The response of cells to ionizing radiation is dependent upon the availability of oxygen. It has been known since 1953 that well oxygenated tumour cells have an approximately three fold greater sensitivity to sparsely ionising radiation compared with hypoxic cells (Gray et al. 1953). The mechanism by which greater radiation damage occurs in the presence of oxygen is generally referred to as the oxygen fixation hypothesis. Briefly, the interaction between radiation and tissues produces free radicals that damage molecules, particularly DNA. The free radicals have a longer half-life in

the presence of oxygen and cause more DNA damage, ie oxygen is said to 'fix' the damage produced by radiation (Vaupel 2004).

Hypoxia and chemoresistance

Hypoxic cells in tumours can also be resistant to chemotherapy. Reasons for this are multifactorial, but include the impaired drug diffusion (Jain 1994), reduced cell proliferation (Bedford et al. 1974), decreased cytotoxic drug activity (Durand et al. 1994) and induction of stress proteins (Sakata et al. 1991) that occur in a hypoxic environment. 5-FU, doxorubicin, bleomycin, procarbazine, etoposide and vincristine are examples of drugs which are dependent on cellular oxygenation for their maximal efficacy (Grau et al. 1992; Teicher 1994).

Hypoxia and poor prognosis after surgical treatment

Hypoxia is important not only in the prognosis of patients undergoing chemo- and/or radiotherapy but also for those treated with surgery alone. Studies in patients with uterine cervix carcinoma (Hockel et al. 1996) or soft tissue sarcoma (Brizel et al. 1996) showed that hypoxia, measured using Eppendorf polarographic electrodes, predicted a poor outcome in patients who had primary surgery with or without radiation. The papers hypothesised that the poor prognosis of surgically treated hypoxic cancers was related to hypoxia induced changes that made the tumour intrinsically more aggressive. Emerging evidence has supported this suggestion and shown that hypoxia plays a key role in promoting tumour progression by stimulating angiogenesis, invasion and metastasis formation (Hockel et al. 2001). In order to increase oxygen availability and decrease oxygen consumption, hypoxic cells exhibit an adaptive response by increasing the transcription of a wide range of genes including those involved in the control of angiogenesis, pH, glucose transport, oxygen transport and cellular proliferation (Semenza 1999). The key factor involved in the adaptive response of a tumour to cellular hypoxia is HIF-1α.

1.7 HYPOXIA-INDUCIBLE FACTOR-1

HIF-1 is a hetero-dimer consisting of α and β subunits. HIF-1α expression is related to cellular oxygen status whereas the HIF-1β subunit is constitutively expressed independently of cellular hypoxia (O'Rourke et al. 1997). HIF-1α dimerises with HIF-1β in the nucleus and transcriptionally activates a number of genes through binding to

hypoxia-responsive elements (HREs). The HIF-1α subunit is stabilised during hypoxia but degrades rapidly in normoxia via the ubiquitin pathway (Figure 1.7) (Pugh et al. 1997). This process is primarily regulated by proline hydroxylation. The presence of functional von-Hippel-Lindau (VHL) protein is required for ubiquitination. In tumours with VHL mutations, such as renal cell carcinoma and cerebellar haemangioblastomas, accumulation of HIF-1α is found (Maxwell et al. 1999).

HIF-1α expression is common in human cancers. Zhong and colleagues studied HIF-1α expression using immunohistochemistry in 179 tumour specimens and found that it was overexpressed in 13 out of 19 common tumour types (Zhong et al. 1999). These included colon, breast, lung, skin, ovarian, pancreatic, prostate, renal and gastric carcinomas. Positive HIF-1α staining was found in premalignant tissue, such as colonic adenoma, breast ductal carcinoma in situ and prostate intraepithelial neoplasia. In contrast, most benign tissue showed no evidence of HIF-1α expression, although weak HIF-1α staining was reported in some tissue, such as adrenal cortical cells and pancreatic acinar cells. The localisation of HIF-1α expression in the malignant tissue was predominantly nuclear. Areas immediately adjacent to necrotic tumour and the invading tumour margins revealed the most intense staining. Occasionally other localised or more diffuse staining patterns were found, and considered to be the result of genetic alterations and local microenvironmental factors other than hypoxia.

Although hypoxia is the main regulator of HIF-1α, there is emerging evidence that it is stabilised by several non-oxygen dependent mechanisms. Various tumour specific genetic alterations involving oncogenes (PAS and MYC) and tumour suppressor genes (TP53, PTEN and VHL) have been associated with HIF-1α stabilisation (Semenza 2002). Cytokines, such as insulin like growth factor (IGF), epidermal growth factor (EGF), and interleukin-1 stimulate receptor tyrosine kinases which also influence HIF-1α levels (Semenza 1999).

1.7.1 HIF-1α expression as a prognostic factor

Table 1.15 summarises the findings from studies examining the prognostic significance of HIF-1α expression in tumours. Most have shown that the immunohistochemical expression of HIF-1α is associated with a poor prognosis. Differences in the scoring systems used along with the small number of patients included may explain the lack of significance in some of the studies. In three studies, in head and neck, oral cavity and non-small cell lung cancer, HIF-1α expression predicted a good prognosis (Beasley et

al. 2002; Fillies et al. 2005; Volm et al. 2000). Evidence is emerging that suggests HIF-1α has both pro and anti-tumour properties, especially with regard to apoptosis (Greijer et al. 2004). In support of this suggestion one of the studies reporting HIF-1α expression as a good prognostic factor showed a strong correlation between HIF-1α and the expression of pro-apoptotic factors such as caspase-3, Fas, and Fas ligand (Volm et al. 2000). The balance between the pro or anti-apoptotic effects of HIF-1α are likely to be determined by the associated genetic alterations, such as TP53 or members of the Bcl-2 family of genes.

1.7.2 HIF-1α expression as a predictive factor for response to radiation and chemotherapy

The measurement of HIF-1α expression by immunohistochemistry has been shown in some studies to predict response to both chemotherapy and radiation. In a study of 98 patients with squamous cell oropharyngeal cancer treated with external beam radiation (median total dose 74 Gy), the expression of HIF-1α was highly predictive of tumour response (Aebersold et al. 2001). Tumours with high versus low HIF-1α expression were three times less likely to achieve a complete response to radiotherapy. Moreover, the degree of HIF-1α expression correlated with disease-free ($p=0.008$) and overall ($p=0.006$) survival. The predictive power of HIF-1α expression remained after multivariate analysis corrected for co-variables.

In a recent retrospective study of 65 patients with oesophageal cancer who underwent chemo-radiotherapy, HIF-1α expression predicted treatment response (Sohda et al. 2004). The treatment regime was 40 Gy external beam radiation to the primary tumour, mediastinum and neck, with an additional 20-30 Gy boost in the oblique position. The chemotherapy comprised one course of 5-FU and cisplatin or nedaplatin. The expression of HIF-1α was assessed in pre-treatment biopsies using immunohistochemistry. Only 8% (3/38) of patients with HIF-1α expressing tumours showed a complete response to chemo-radiotherapy compared with 44% (13/27) of patients with HIF-1α negative tumours. The difference was statistically significant ($p=0.009$). In another study in 37 patients with early oesophageal cancer, high HIF-1α expression was associated with a poor response to combined radiotherapy and photodynamic therapy (Koukourakis et al. 2001)

Table 1.15 HIF expression and prognosis in different tumour sites

Cancer site	Subtype	Stage	Treatment	Pts	% HIF exp	Exp Pat	Cut offs	Surv	p-value Uni	p-value Multi	Author, Ref
Astrocytoma	N/A	Operable	Surgery ± ART	83	92%	N	< or > 30%	Poor	0.0227	0.03 [A]	(Korkolopoulou et al. 2004)
Bladder	TCC	All	TUR ± BCG ± CRT	93	75%	N	Quartiles	Poor	0.009	0.02	(Theodoropoulos et al. 2004)
Bladder	TCC	Ta/T1	TUR ± BCG	140	47%	N + C	Low vs high	TP	0.058	N/S	(Theodoropoulos et al. 2005)
Breast	A	Lymph node +	Surgery ± CT ± tamoxifen	206	76%	N + C	Quartiles	Poor	0.0454	0.003	(Schindl et al. 2002)
Breast	A	Lymph node +	Surgery ± CT ± tamoxifen	77	56%	N	Quartiles	Poor	0.04	NS	(Gruber et al. 2004)
Breast	A	All	Surgery ± CT ± tamoxifen	150	75%	N	< or ≥ 5%	Poor [B]	0.008 [B]	0.021 [B]	(Bos et al. 2003)
Breast	A	Early	Surgery	745	100%	N + C	< or > 10%	Poor	0.019	0.03	(Dales et al. 2005)
Cervix	S (63) A (15)	Advanced	Radical RT	78	94%	N	<1%, 10-50, >50%	Poor	0.04	0.02	(Burri et al. 2003)
Cervix	S	Early	Surgery ± ART	91	81%	N	> 4 points	Poor	<0.0001	0.0129	(Birner et al. 2000)
Cervix	S (33) A (3) AS (7) O (2)	Locally advanced	RT	45	n/a	N	Median (2%)	NS	NS	N/A	(Haugland et al. 2002)
Cervix	S	Ib-IVa	Radical RT	99	67%	N + C	< or >10%	NS [C]	0.56	N/A	(Hutchison et al. 2004)
Cervix	S	Ib-IVb	Surgery ± RT ± CT	38	N/A	N + C	Median	TG	0.11	N/A	(Mayer et al. 2004)

[A] independent prognostic factor when HIF-1α expression was analysed in association with grade; [B] overall there was only a borderline significance (P=0.059), however HIF-1α expression was significant in predicting a poor prognosis in 81 patients without lymph node metastases; [C] although overall survival was not significant, high HIF-1α expression was associated with a poor outcome for small tumours but good outcome in large tumours; [D] HIF-2α and the combination of HIF-1α and HIF-2α were significant in multivariate analysis; [E] HIF-2α expression was a statistically adverse prognostic factor in multivariate analysis

Table 1.15 (continued)

Colorectal	A	All	Surgery ± CT	139	58%	N + C	< or > 10%	TP	0.077	N/A	(Kuwai et al. 2003)
Colorectal	A	Curatively treated	Surgery	87	45%	N + C	< or > 5%	NS [D]	>0.05	N/A	(Yoshimura et al. 2004)
Endometrial	A	All	Surgery ± RT	81	49%	N + C	Low vs High	Poor	0.03	0.01	(Sivridis et al. 2002)
GIST	N/A	All	Surgery	53	32%	N	< or > 10%	Poor	<0.05	NS	(Takahashi et al. 2003)
GIST	N/A	All	Surgery	62	56%	N + C	< or > 10%	Poor	0.009	N/A	(Chen et al. 2005b)
Head and Neck	S	Locally advanced	CRT	75	48%	N + C	Mean value	Poor	0.05	NS [E]	(Koukourakis et al. 2002)
Head and Neck	S	Early	Surgery	79	87%	N	Positive or negative	Good	0.027	N/A	(Beasley et al. 2002)
Oesophageal	S	All	Surgery ± CRT	82	39%	N + C	Mean %	TP	0.078	N/A	(Kimura et al. 2004)
Oesophageal	S	Early	PDT ± RT	37	65%	N + C	Unknown	TP	0.08	N/A	(Koukourakis et al. 2001)
Oesophageal	S	All	Surgery	130	68%	N	< or > 10%	Poor	0.0007	NS	(Kurokawa et al. 2003)
Oligodendroma	N/A	Operable	Surgery ± CRT	51	80%	N	> 4 points	Poor	0.0434	0.0187	(Birner et al. 2001a)
Oral cavity	S	Early	Surgery ± RT	85	36%	N	< or > 5%	Good	<0.01	0.01	(Fillies et al. 2005)
Oropharygneal	S	All	RT and CT	98	98%	N + C	0,1-10,10-50%	Poor	0.001	0.0009	(Aebersold et al. 2001)
Ovarian	A	I-IV	Surgery and ACT	102	69%	N	Quartiles	NS	0.6848	N/A	(Birner et al. 2001b)
Nasopharyngeal	S	Locally advanced	RT	90	58%	N	Quartiles	TP	0.06	N/A	(Hui et al. 2002)
Upper urinary tract	TCC	All	Surgery ± RT ± CT			N	Positive vs negative	Poor	0.0001	<0.0001	(Nakanishi et al. 2005)

A = adenocarcinoma, SCC = squamous cell carcinoma, AS = adenosquamous; N = nuclear, C = cytoplasmic; TP = trend poor prognosis; TG = trend good prognosis; N/A = not applicable NSCLC= Non small cell lung cancer; GIST=Gastrointestinal stromal tumour; CT=Chemotherapy; RT=Radiotherapy; CRT=Chemoradiotherapy; PDT= Photodynamic therapy; TUR=transurethral resection of bladder tumour; BCG= intra-vesical bacille Calmette-Guerin; NS =Not significant

1.7.3 HIF-1α regulated products

Around 50 genes with HREs have been identified (Table 1.16) (Maxwell et al. 2001; Park et al. 2004). Known HIF-1 inducible proteins which might be important in gastric cancer include carbonic anhydrase-9 (CA-9), glucose transporter 1 (Glut-1), erythropoietin (Epo), inducible nitric oxide synthase (iNOS), and vascular endothelial growth factor (VEGF). CA-9 is a transmembrane glycoprotein which is involved in the maintenance of intracellular pH in the hypoxic environment (Beasley et al. 2001). Glut-1 plays an important role in the survival of tumour cells by ensuring an adequate energy supply (Newsholme et al. 1991). Epo is the principal regulator of red blood cell production, and its expression in tumours is thought to contribute to survival of tumour cells in the hypoxic environment by stimulating angiogenesis (Acs et al. 2001). iNOS catalyses the formation of nitric oxide (NO), a regulator of vascular permeability and promoter of tumour growth (Maeda et al. 1998). VEGF is the principal pro-angiogenic growth factor and its stimulation under hypoxia plays a key role in promoting the survival of malignant cells, in local tumour growth and invasion, and in the development of metastases (Folkman 1990). The expression of HIF-1α has been shown to correlate not only with VEGF but also with the level of angiogenesis in tumours, measured as microvessel density (MVD) (Koukourakis et al. 2004).

1.7.4 HIF-2α and other HIF isoforms

A number of proteins have been identified recently that are closely related to HIF-1α and control the transcription of hypoxia-regulated genes in a similar way to HIF-1α (HIF-2α and HIF-3α). A study in non-small cell lung cancer showed that HIF-2α expression was related to a poor outcome whereas HIF-1α was not (Giatromanolaki et al. 2001). Another study showed a predominant role of HIF-2α over HIF-1α in the regulation of the transcriptional response to hypoxia in renal cell carcinoma (Sowter et al. 2003). These findings raise the possibility of tissue specific differences in the relative importance of HIF proteins in determining tumour progression and prognosis. Different isoforms of the various HIF proteins have also been identified (Gothie et al. 2000; Kondo et al. 2002; Maynard et al. 2003; Tanimoto et al. 2003). There is some evidence that HIF polymorphisms have a role in generating differences in the potential for tumour progression between individuals (Tanimoto et al. 2003).

Table 1.16 HIF-1 target genes

Gene Function	Gene Product
Angiogenesis	VEGF VEGF receptor-1 (flt-1) Transforming growth factor beta3 Plasminogen activator inhibitor 1
Glucose metabolism	Glucose transporter 1 Glucose transporter 3 Glyceraldehyde-3-P-dehydrogenase Hexokinase 1 Hexokinase 2 Aldolase A Aldolase C Enolase 1 Lactate dehydrogenase A Phosphofructokinase L Phosphoglycerate kinase 1 Triose phosphate isomerase Pyruvate kinase M
Iron metabolism	Ceruloplasmin Transferrin Transferrin receptor
Vascular tone	Alpha-adrenergic receptor Adrenomedullin Endothelin-1 Heme oxygenase Nitric oxide synthase 2
Cell proliferation and survival	GF-binding protein 1 GF-binding protein 2 GF-binding protein 3 Insulin-like growth factor (IGF-2) p21 NIP3
Nucleotide metabolism	Adenylate cyclase
pH regulation	Carbonic anhydrase IX and XII
Erythropoiesis	Erythropoietin

Information taken from (Maxwell et al. 2001) and (Park et al. 2004).

1.7.5 HIF-1α expression in benign and malignant gastric tissue and its possible role in Helicobacter pylori induced carcinogenesis.

HIF-1α expression in benign gastric tissue

HIF-1α is not generally expressed in normal tissue (Talks et al. 2000; Zhong et al. 1999). Zhong and colleagues reported no expression in normal gastric mucosa, but only examined one specimen (Zhong et al. 1999). Ito and colleagues studied 71 endoscopic biopsies of normal gastric mucosa from Japanese patients with gastric cancer, peptic ulcer or dyspepsia and found low expression of HIF-1α in benign gastric mucosa (Ito et al. 2003). They observed the expression of HIF-1α to be greater in patients receiving non-steroidal anti-inflammatory drugs (NSAID) compared with those not taking NSAIDs (Ito et al. 2003). The authors hypothesised that this was related to acute ischaemic/hypoxic damage of mucosal cells *in vivo*. A similar situation of acute alcohol injury in a rat model has also been shown to increase HIF-1α stabilisation, especially in the mucosa bordering areas of necrosis (Szabo et al. 2001).

The possible role of HIF-1α in Helicobacter pylori induced gastric carcinogenesis

The molecular mechanisms of distal intestinal type gastric cancer development remain largely unknown (Section 1.4.3). Several cell signalling pathways have been implicated, including those involved in the control of apoptosis and proliferation, and the activation of tyrosine kinase signalling pathways (EGFR, Her2-Neu and c-Met) (Naumann et al. 2004). There is emerging evidence that HIF-1α may be involved in the aetiology of gastric cancer (Figure 1.7). Infection with *H. pylori* is the major initiating and driving factor. The proposed mechanim is that the formation of reactive oxygen species (ROS), due to neutrophil infiltration in response to *H. pylori* infection, causes epithelial cell injury and progressive DNA damage (Obst et al. 2000). There is evidence that gastric epithelial ROS, both endogenous and *H. pylori*-induced, may lead to HIF-1α expression under normal oxygen conditions (Park et al. 2003). A recent cell line study showed that ROS from the more virulent cagA bearing strains of *H. pylori* cause HIF-1α stabilisation and accumulation in gastric cancer cells under normoxic conditions (Park et al. 2003). In cells under the influence of ROS produced by *H. pylori*, HIF-1α is continuously present regardless of cellular oxygen status.

In addition to ROS, another important mediator in the chronic inflammatory process is nitric oxide (NO) which, in response to *H. pylori* infection, is produced by gastric epithelial and non-epithelial cells from L-arginine via iNOS. Increased iNOS expression is seen in *H. pylori* infected gastric mucosa (Mannick et al. 1996; Pignatelli et al. 1998). NO has been shown to interfere with HIF-1α prolyl hydroxylases under normoxia, preventing degradation and resulting in HIF-1α accumulation and activation (Metzen et al. 2003).

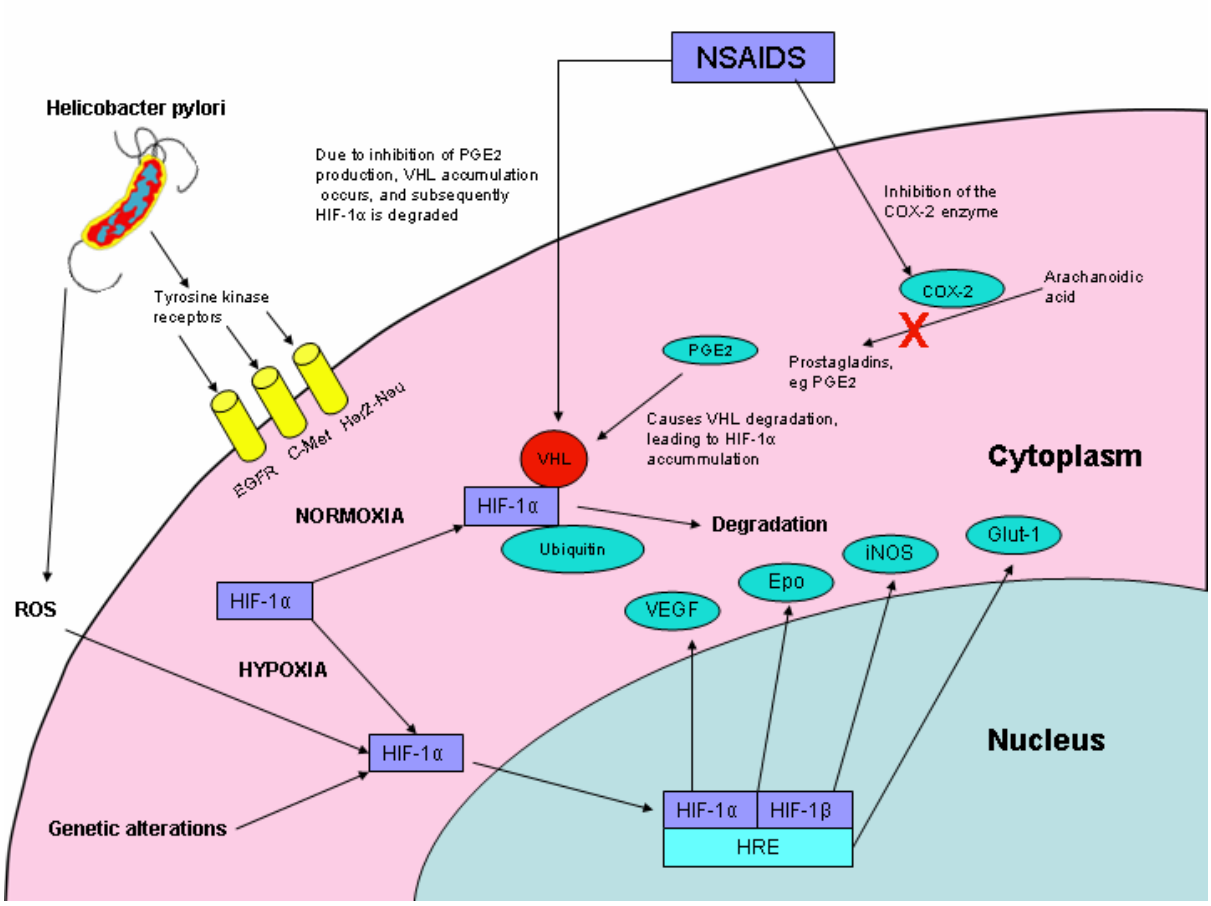

Figure 1.7 The hypoxia-inducible factor (HIF) pathway.

Hypoxia, reactive oxygen species from *H. pylori* and specific genetic alterations (such as TP53, PTEN and pVHL mutations) can cause HIF-1α to be stabilised. When activated, HIF-1α forms a dimer with HIF-1β and binds to hypoxia responsive elements within the nucleus. This initiates the transcription of a number of hypoxia inducible genes products, such as VEGF, Epo, iNOS, and Glut-1. During normoxia, HIF1-α is rapidly degraded by the ubiquitin pathway in conjunction with VHL. This is a process that involves prolyl hydroxylation. This degradation pathway may be disrupted by PGE_2, which is a product of the COX-2 enzyme. Non-steroidal anti-inflammatory drugs inhibit the production of prostaglandins, including PGE2, and are known to result in increased HIF-1α degradation.

The possible role of HIF-1α in non-steroidal anti-inflammatory drug (NSAID) induced protection of gastric carcinogenesis

In contrast to the study showing the expression of HIF-1α in NSAID related gastritis (Ito et al. 2003), there is some evidence that NSAIDs can also decrease HIF-1α expression. NSAID use is associated with a decreased risk of gastric cancer (Akre et al. 2001; Wang et al. 2003). The anti-cancer effects of NSAIDs relate to their ability to inhibit the expression of cyclooxygenase-2 (COX-2), which is upregulated in malignancy. Although the exact mechanism of action has yet to be completely defined, there is some evidence that HIF-1α has a role.

COX-2 functions to convert arachidonic acid to prostaglandins in inflamed and neoplastic tissue. Expression of COX-2 is induced by pro-inflammatory cytokines, oncogenes, growth factors and hypoxia. Overexpression is common in human cancers, including gastric, and is associated with advancing tumour progression and the development of metastases (Shi et al. 2003). COX-2 expression induces the synthesis of prostaglandins, especially PGE_2 (Liu et al. 2002). PGE_2 causes the degradation of VHL protein, thereby increasing HIF-1α expression (Figure 1.7) (Wang et al. 2004).

A cell line study by Jones and colleagues (Jones et al. 2002), showed that NSAIDs (both non-selective and COX-2 specific types) inhibited angiogenesis in a model of rat gastric cancer. Decreased HIF-1α levels were seen in the cells exposed to NSAIDs. A mechanism was proposed where the NSAIDs increased the expression of the VHL tumour suppressor protein (Figure 1.7), resulting in HIF-1α ubiquitination and inhibition of hypoxia-induced angiogenesis. NSAIDs have also been shown to reduce both HIF-1α and HIF-2α levels in prostate cancer cells (Palayoor et al. 2003). In the latter study, the HIF inhibition was independent of the COX expression. A recent cell line study has confirmed the COX-2/PGE_2/HIF-1α/VEGF pathway to play an important role in gastric cancer angiogenesis (Huang et al. 2005). Both high levels of HIF-1α and VEGF were observed in cells that over-expressed COX-2.

1.8 HIF-1α AND HYPOXIA INDUCIBLE PRODUCTS AS PROGNOSTIC MARKERS IN GASTRIC CANCER

There is a paucity of data on HIF-1α and gastric cancer. In the large immunohistochemical study of HIF-1α expression in human cancers by Zhong and colleagues, expression was found in gastric cancer (Zhong et al. 1999). However, only two specimens with gastric adenocarcinoma were stained for HIF-1α, both of which

were positive (one with 1% staining and the other with over 50% staining). Unfortunately, unlike other cancers (Table 1.15), few published studies have described HIF-1α expression in gastric adenocarcinomas.

Two studies have assessed HIF-1α as a prognostic marker in gastrointestinal stromal tumours (GIST) of the stomach. Takahashi and colleagues (Takahashi et al. 2003) examined the expression of HIF-1α, VEGF, anti-CD31 (to score MVD) and Ki-67 using immunohistochemistry in 53 patients with GIST affecting the stomach. HIF-1α expression was shown in 32% of the specimens and correlated significantly with tumour size, liver metastasis and overall prognosis. HIF-1α expression correlated well with VEGF expression and the level of angiogenesis measured histologically as MVD. A further study, which assessed 62 patients with GIST, confirmed these findings and also found that high HIF-1α was associated with a high incidence of tumour recurrence and distant metastasis (Chen et al. 2005b).

Several tumour suppressor genes (VHL, PTEN), which are relevant to the HIF pathway, are frequently inactivated in gastric cancer (Lee et al. 2003; Yang et al. 2003). The inactivation of these genes are known to cause the upregulation of HIF-1α in normoxic conditions (Bardos et al. 2004). It will be of interest in future studies to correlate HIF-1α expression with these proteins. Several studies have used immunohistochemistry to evaluate hypoxia inducible proteins in gastric cancer (Table 1.17).

1.8.1 Carbonic anhydrase-9 (CA-9) and gastric cancer

Overexpression of CA-9 has been reported in various cancer types. However, expression is low or even lost in most gastric cancers (Leppilampi et al. 2003; Pastorekova et al. 1997), indicating that the biological function of CA-9 in gastric cancer might be more complex than in other types of cancer. The gastric mucosa is one of the most predominant sites of physiological CA-9 expression as biologically it is involved in the production of gastric acid. Leppilampi and colleagues (Leppilampi et al. 2003) performed an immunohistochemical study of CA-9 expression in normal gastric mucosa, gastric adenomas and different grades of gastric carcinomas. CA-9 expression was high in normal and hyperplastic mucosa and lower in dysplasia and gastric malignancy. This loss of expression of CA-9 may be related to neoplastic alteration, including dedifferentiation during gastric carcinogenesis. CA-9 may also be involved in

early gastric carcinogenesis as CA-9 deficient mice show increased cellular proliferation and develop gastric hyperplasia (Gut et al. 2002).

CA-9 was expressed in the invasive edge of gastric cancer resection specimens (Chen et al. 2005a). Although high CA-9 expression was associated with shorter post-operative survival (P=0.028), only 23 patients were included in the survival analysis. In the same paper, an in vitro study revealed that cells expressing CA-9 had increased proliferation rates and were much more invasive than cells which lacked CA-9 (Chen et al. 2005a).

1.8.2 Glut-1 and gastric cancer

Glut-1 is expressed in gastric cancer (Kawamura et al. 2001; Kim et al. 2000; Noguchi et al. 1999; Yamamoto et al. 1990). Noguchi and colleagues found high (>25%) Glut-1 expression was associated with a poor post-operative survival in 70 gastric cancer patients (Noguchi et al. 1999). A more detailed study by Kawamura and colleagues (Kawamura et al. 2001) investigated 617 gastric carcinomas and found 182 (30%) were positive for Glut-1. Staining was mainly localised in the central part of tumour nests, with a preferential association with central necrosis. Staining varied between histological sub-type: papillary (44%), tubular (32%), poorly-differentiated (28%), signet-ring (1%), and mucinous (1%). The expression of Glut-1 increased progressively with increasing tumour stage. There were also significant associations between Glut-1 positivity and adverse tumour features and survival (Table 1.17).

1.8.3 Erythropoietin receptor (Epo-R) and gastric cancer

Ribatti and colleagues (Ribatti et al. 2003) studied Epo-R expression immunohistochemically in 40 patients with gastric adenocarcinoma. They found that Epo-R expression correlated with angiogenesis, measured as MVD, and progression of disease. Moreover, Epo-R expression increased with advancing tumour grade.

1.8.4 Inducible nitric oxide synthase (iNOS) and gastric cancer

Several papers have examined iNOS expression in gastric cancer specimens and found associations with adverse pathological features and reduced survival (Feng et al. 2002; Rajnakova et al. 2001; Song et al. 2002; Song et al. 2004). iNOS expression is found in both diffuse and intestinal types of the disease (van der Woude et al. 2003). Increased expression correlated with increasing tumour stage and number of lymph node metastases (Feng et al. 2002; Rajnakova et al. 2001; Song et al. 2002). In a recent paper

by Song and colleagues, the level of expression of iNOS was 59% and correlated with VEGF expression (Song et al. 2004).

1.8.5 Vascular endothelial growth factor (VEGF) and micro-vessel density (MVD) in gastric cancer

VEGF expression is associated with an increased risk of metastatic disease and reduced survival in patients with gastric cancer (Liu et al. 2001; Song et al. 2002). In three studies, VEGF expression was also found to be an independent adverse prognostic factor for survival (Fondevila et al. 2004; Maeda et al. 1996; Maeda et al. 1999). Du and colleagues (Du et al. 2003) studied VEGF expression and MVD in 80 patients with gastric cancer. VEGF expression was found in 68% (54/80) and was closely related to the degree of tumour differentiation, presence of lymph node metastases and MVD. In addition, positive staining for VEGF tended to be located at the centre of the tumour or at the edge of areas of necrosis, consistent with hypoxia-induced expression.

Koukarakis and colleagues (Koukourakis et al. 2000) studied MVD in a range of human tumours including 98 patients with locally-advanced inoperable gastric cancers. They all received chemotherapy with a median follow up of 9 months (range 2–60 months). When survival was plotted against MVD the results revealed a 'U-shaped' distribution. Patients with low and high tumour MVD had a poor prognosis, whilst patients with an intermediate MVD survived longer. In the low MVD group, poor oxygenation and reduced drug delivery were believed to be the underlying reason for poor outcome. The highly vascularised tumours or the high MVD group were thought to have a poor prognosis due to their highly aggressive, angiogenic phenotype and early metastatic capabilities.

Table 1.17 The expression of HIF-1 inducible genes in gastric adenocarcinoma

Marker	Patients	Exp	Cut offs	Expression correlated with	Other comment	Author , Ref
Glut-1	617	30%	0-1%, 2-30% and >30%	Increasing tumour stage, lymphatic and vascular invasion, peritoneal and hepatic metastases. Shorter survival (P=0.0001)	Perinecrotic expression	(Kawamura et al. 2001)
Glut-1	70	19%	< or > 25%	Tumour invasion, lymphatic and vascular invasion, and lymph node metastases	Significant association with overall survival in univariate (P=0.0009) and multivariate (P=0.03) analysis	(Noguchi et al. 1999)
CA-9	74	N/A	N/A	CA-9 expression was high in normal and hyperplastic mucosa and lower in dysplasia and gastric malignancy. There was no correlation with tumour stage.	CA-9 has a physiological role producing gastric acid and high expression occurs in benign tissue.	(Leppilampi et al. 2003)
CA-9	59	N/A	IRS < or > 3	Associated with reduced post-operative survival (P=0.03) (assessed in 23 patients)	Staining observed in the invasive tumour edge	(Chen et al. 2005a)
Epo-R	40	N/A	N/A	Grade of tumour and extent of angiogenesis		(Ribatti et al. 2003)
VEGF	206	74%	Positive vs Negative	Associated with shorter disease free survival (P<0.02) and overall survival (P<0.01)	Statistically significant in multivariate analysis	(Fondevila et al. 2004)
VEGF	76	39%	< or > 10%	Lymph node metastasis (P=0.009) Shorter overall survival (P<0.05)		(Ichikura et al. 2001)
VEGF	50	50%	N/A	Not assessed	Staining observed more often in the invasive margin of the tumour rather than the centre	(Liu et al. 2001)
VEGF	129	43%	< or > 5%	Lymphatic invasion (P<0.05), venous invasion(P<0.05), lymph nodes metastases (P<0.01) and liver metastases (P<0.01)	Poor overall prognosis in multivariate analysis	(Maeda et al. 1996)
VEGF	195	31%	Positive or Negative	VEGF expression was observed more frequently in the patients who developed recurrence	Assessed on pre-operative biopsies in patients with early gastric cancer. Poor overall prognosis in multivariate analysis	(Maeda et al. 1999)
VEGF	80	67%	IRS 0-2 vs 3-6	Degree of differentiation (P<0.01), lymph node metastases (P<0.01) and MVD (p<0.05)		(Du et al. 2003)
iNOS	55	44%	< or > 10%	Lymph node metastases (P=0.014) p53 expression (P=0.005)	Increased expression of iNOS, VEGF and p53 also found in intestinal metaplastic tissue	(Feng et al. 2002)
iNOS	55	53%	Median score	Advanced tumours (P=0.015), size > 5cm (P=0.025) and metastases (P=0.002)	P53 expression was correlated (P=0.018)	(Rajnakova et al. 2001)
iNOS	46	59%	Positive or Negative	Advanced tumour stage (P=0.019) and lymph node metastases (P<0.05), but not associated with MVD	Reduced 5-year survival (P<0.05)	(Song et al. 2002)
iNOS	85	59%	IRS 0-1,2,3-4, 5	Associated with advanced stage and lymph node metastasis	VEGF expression and iNOS expression correlated (P=0.018)	(Song et al. 2004)

1.8.6 Targeting HIF-1α as a therapeutic approach in gastric cancer

There is interest in HIF-1α as a cancer therapeutic target (Semenza 2003). This stems in part from studies implicating HIF-1α in tumour resistance to chemotherapy and radiation (Aebersold et al. 2001; Unruh et al. 2003). Blocking HIF-1α activity has potential to inhibit cancer progression by depriving cancer cells of the means to adapt to hypoxia and a nutrient depleted environment. Several compounds are currently undergoing assessment in murine gastric tumour models.

YC-1 [3-(5'-hydroxymethyl-2'-furyl1)-1-benzylindazole] is a soluble guanylyl cyclase stimulator developed for its ability to inhibit platelet aggregation and vascular contraction, and for the treatment of circulation disorders. Yeo and colleagues studied its therapeutic use in vivo in gastric tumours induced subcutaneously in nude mice (Yeo et al. 2003). They found that YC-1 blocked angiogenesis and inhibited tumour growth, resulting in fewer blood vessels, lower expression of HIF-1α and HIF-1 regulated genes. They concluded that YC-1 is a potent inhibitor of HIF-1 with the potential to become the first anti-angiogenic anticancer agent to target HIF-1α.

A recent study by Stoeltzing and colleagues assessed a gene therapy approach for inhibiting HIF-1α in nude mice transfected with human gastric cancer cell lines both subcutaneously and orthotopically (Stoeltzing et al. 2004). Inhibition was carried out by overexpressing a dominant-negative construct of HIF-1α that dimerised with endogenous HIF-1β to produce non-transcriptionally active HIF-1. Inhibition of HIF-1α reduced VEGF expression, angiogenesis and tumour size. They concluded that HIF-1α was a valid target for the treatment of gastric cancer.