A STUDY OF MORPHOLOGICAL, IMMUNOHISTOCHEMICAL AND HISTOCHEMICAL FEATURES OF AMPULLARY CARCINOMAS.

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DECLARATION

I, Willouw de Klerk, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any other part of it has been, is being, or is to be submitted for another degree in this or any other university.

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ABSTRACT

A STUDY OF MORPHOLOGICAL, IMMUNOHISTOCHEMICAL AND HISTOCHEMICAL FEATURES OF AMPULLARY CARCINOMAS.

Carcinoma of the ampulla of Vater has been recognized as a clinically distinct entity. These are rare tumours and the treatment of choice is the Whipple procedure (pancreaticoduodenectomy). This is a formidable operation which is associated with morbidity and mortality. If reliable prognostic factors (clinical and histopathological) can be identified, then the choice of surgery – Whipple procedure or a palliative procedure – would be made easier, and such identification may even be valuable in choosing patients with poor prognostic features for adjuvant treatment. This was a predominantly retrospective study of 37 unequivocal ampullary carcinomas after Whipple resection. The aim of the first study was to examine clinical, histopathological and immunohistochemical features of ampullary carcinomas and to determine whether any of these features had significant prognostic value. The immunohistochemical panel was selected after a literature review and included p53, Ki-67, MUC1, MUC1core, MUC2 and CA19.9. The data was analyzed by multivariate analysis. The relatively small number of patients included in this study was the result of the rarity of the tumour and strict selection criteria for ampullary carcinomas. In addition, 10 patients were lost to clinical follow up. Nevertheless, the results confirmed the good prognosis of T1 and stage 1 carcinomas and suggested a possible role for MUC1 and MUC2 in the diagnosis of ampullary carcinoma in small endoscopic biopsies. None of the immunohistochemical stains could identify a subset of tumours with better or worse outcome. Vascular invasion, lymphatic invasion, perineural invasion, differentiation, Martin's classification, resection margins and histological sub-typing also did not appear to influence survival in this study. The aim of the second study was to ascertain whether the histochemical mucin profile of ampullary carcinomas correlated with the histological subclassification. The nature of the mucin produced by the carcinoma was demonstrated with the High iron diamine and the ABPAS stains. There was no pattern of mucin production which correlated with the histological subclassification and the validity of subclassifying ampullary carcinomas is questioned.

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ABBREVIATIONS

AAPC	Attenuated adenomatous polyposis coli
ABPAS	Alcian-blue periodic acid Schiff
CK7	Cytokeratin 7
CK20	Cytokeratin 20
CT	Computed tomographic
ERCP	Endoscopic retrograde cholangiopancreatography
FAP	Familial adenomatous polyposis
H&E	Haematoxylin and eosin
HID	High iron diamine
IMD	Intratumoural microvessel density
MSI	Microsatelite instability
MTS	Muir-Torre syndrome
n	Number
NF-1	Neurofibromatosis type 1
PanIN	Pancreatic intra-epithelial neoplasia
PAS	Periodic acid Schiff
PD	Pancreaticoduodenectomy
PPPD	Pylorus preserving pancreaticoduodenectomy
PSD	Pancreas sparing duodenectomy
RCPath	Royal College of Pathologists

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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

Ampullary tumours are relatively rare and represent 2% of all digestive tract tumours (1). Carcinoma of the ampulla of Vater has been recognized as a clinically distinct entity. However, the similarity in presentation to that of carcinoma of the pancreas, of the distal common bile duct, and the adjacent duodenal mucosa has in many cases resulted in failure to distinguish the clinical and pathological behavior of these tumours (2).

The treatment of choice for ampullary carcinoma is the Whipple procedure (pancreaticoduodenectomy) (3). This is a formidable operation which is associated with morbidity and mortality. If reliable prognostic factors (clinical and histopathological) can be identified, then the choice of surgery - Whipple procedure or a palliative procedure - would be made easier, and such identification may even be valuable in choosing patients with tumours with poor prognosis for adjuvant treatment (4).

1.1 ANATOMY OF THE PERIAMPULLARY REGION

The ampulla was described by the German anatomist Abraham Vater in 1720. The ampulla is strictly defined as a dilated, jug-like conduit resulting from the union of the common bile duct and major pancreatic duct, and lies within the wall of the duodenum (5). It is now commonly accepted that the *Vaterian system* is composed of the distal segments of the common bile duct and major pancreatic duct (duct of Wirsung) at the duodenum, the papilla, and the sphincteric musculature. It also includes the extra duodenal portion of the common bile duct and major pancreatic duct that join to form a common channel outside the duodenal wall (6). It is a complex structural unit composed of highly developed mucosa, musculature and nerve supply that regulates the flow of bile and pancreatic secretions. Its sphincteric function is part of the overall gastrointestinal motility system (7).

The papilla (Latin for nipple) is a cylindrical protuberance housing the terminations of the common bile duct and major pancreatic duct or a common channel, and is situated medially at the mid portion of the second part of the duodenum (8). Endoscopically the

pylorus is a major landmark for finding the papilla, although the distance is variable, ranging from 1.5 to 12 cm, especially in the case of inflammation (9).

The relationship of the common bile duct and the duct of Wirsung at the papilla is complex and has been the subject of several studies. The ducts may have separate openings into the duodenum, an interposed septum, or a common channel (Fig 1.1). In most studies, more than two thirds of these patients had a common channel. However, the frequency for separate openings into the duodenal lumen ranged from 12% to 54% and for a common channel, 36% to 88% (6, 10-16).

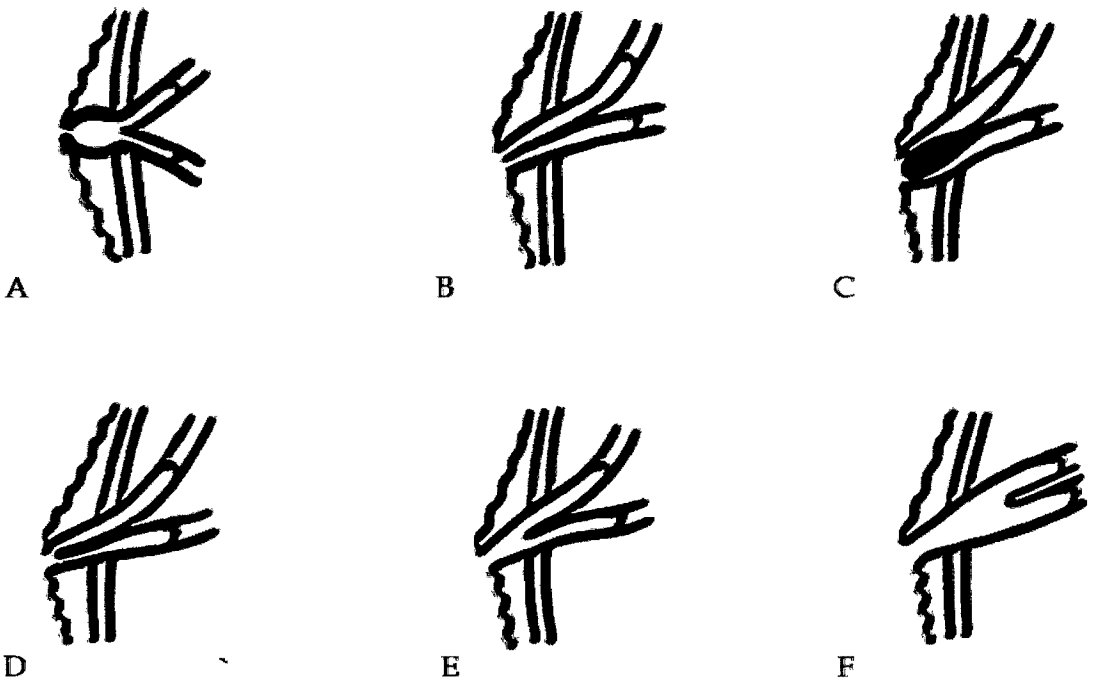

Fig 1.1. Relationship of the common bile duct and the duct of Wirsung at the papilla: A) ampulla, B) interposed septum, C) separate openings, D) short common channel, E) long common channel and F) extended common channel [adapted from Histology for Pathologists (17)].

The sphincter of Oddi consists of the intrinsic circular and longitudinal musculature of the Vaterian system, which is embryologically and functionally distinct from the muscle of the duodenal wall. It serves to inhibit flow of bile into the duodenum, pumps bile into

the duodenum when necessary, and likely precludes the entry of duodenal contents into the bile duct or major pancreatic duct (7).

Histologically, the ampulla represents a confluence of the epithelium from three different structures, namely the bile duct, the pancreatic duct and the duodenum. The epithelial lining of the pancreatic duct and the common bile duct are identical. The single layer of columnar lining cells has eosinophilic cytoplasm with basally located nuclei. Interspersed goblet cells increase closer to the ostium. The epithelium may undergo hyperplastic or metaplastic changes including mucinous cell hyperplasia, papillary hyperplasia, adenomatous hyperplasia, squamous metaplasia and pyloric gland metaplasia (18). The epithelium of the terminal portion of the common bile duct and common channel, if present, covers long slender papillary fronds or valvules, which in some respects resemble the fimbria of the fallopian tube. These formations are considerably larger than the duodenal villi, which are few or absent at the surface of the papilla (19).

Dawson and Connolly (5) highlighted the differences in mucin secretion by the normal epithelia that convene at this point. The main pancreatic duct contains predominantly sulphated acid mucins. The duodenal surface mucosa contains sialomucins and Brunner's glands neutral mucins. The common bile duct contains predominantly sialomucins and a trace of sulphated mucins. The staining of the ampulla of Vater varies. Sialomucins predominate in the lining epithelium near the opening into the duodenum. Deeper in the ampulla the lining epithelium, as well as the glands lying beneath the epithelium, secrete principally sulphated mucins (5).

1.2 PERIAMPULLARY CARCINOMAS

The term "periampullary carcinoma" refers to carcinomas which arise from the mucosa of the ampulla of Vater, the distal pancreatic duct, the distal common bile duct, or the duodenum surrounding the papilla. The importance of clearly defining the histogenic

origin of periampullary carcinomas can not be overemphasized because carcinomas originating from the different periampullary sites, exhibit different clinical behaviors (4).

The term "ampullary carcinoma" should only be employed for malignant epithelial tumours centered in the ampulla of Vater (20). Because of its location with respect to the biliary system, carcinoma of the ampulla of Vater is considered to present earlier in its course of development than carcinoma of the pancreas. The clinical course for patients with ampullary carcinoma has been recognized to be more favorable than that of patients with pancreatic carcinoma (4).

Ampullary carcinomas account for 10.2% to 36% of all surgically operable pancreaticoduodenal tumours (21). Worldwide, the resectability rate for ductal adenocarcinoma of the pancreas ranges between 5% and 15%, morbidity and mortality rates are relatively high and the 5-year survival rate is 4% to 7% (22). Researchers from the Johns Hopkins hospital reported a 36% 5-year survival rate for 19 patients with ampullary carcinoma who had undergone pancreaticoduodenectomy, whereas for 50 patients with pancreatic carcinoma who had undergone a similar procedure, the 5-year survival rate was 18% (23). In carcinomas from the biliary tract, location seems to be the most important prognostic factor. Lesions of the lower third are the most resectable and therefore associated with better results (24). In a study of distal biliary tumours at the Lahey clinic, the resectability was 100%, the median survival time was 16 months and the 5-year survival rate was 20% (25). Primary duodenal carcinoma accounts for 0.3% of all gastrointestinal malignancies. Its rarity has made the understanding of the biology of this tumour difficult. The 10 year survival rate of 20 patients treated at Kansas medical center between 1975 and 1990 was 67% (26).

There is a clear trend for duodenal cancers to have the best survival and for pancreatic cancer to have the worst survival. Survival of patients with ampullary carcinoma generally follows closely behind those with duodenal carcinomas, whereas survival of patients with bile duct tumours falls between those of ampullary and pancreatic

tumours. Howe et al. found that in their cohort of periampullary carcinomas, the highest rates of resection and overall survival were in ampullary carcinomas (27).

Whipple's observation that patients with ampullary carcinoma have improved survival relative to those with pancreatic cancer because of earlier presentation is borne out by the higher resectability rate of ampullary carcinomas. Allen Whipple stated: *"Cancer of the pancreas is usually of the infiltrating, invasive, poorly differentiated type of growth; the cancer cells invade the lymphatic and blood vessels and nerves early. Furthermore, the pancreatic tumours do not cause an early jaundice, as do carcinomas of the ampulla or the bile duct. The ampullary growths are usually of the fungating variety, the better differentiated of the adenomatous type, slower to invade the lymphatics and the blood vessels."* (28).

Howe et al. hypothesized that periampullary tumours represent a biologic spectrum of malignancies, where intestinal type tumours comprise the biologically more favourable end of the spectrum, and the pancreaticobiliary tumours the other (27).

Neither clinical presentation nor the intraoperative macroscopic appearance allows for a clear distinction between ampullary and other periampullary carcinomas. These tumours often present to the surgeon in the same way and are treated along the same surgical principles. The differentiation between them is based almost entirely on macroscopic and microscopic examination of the resected specimen (29).

1.3 EPIDEMIOLOGY AND DISEASE ASSOCIATIONS

Most individuals are over the age of 60. In a large cohort of patients studied by Klemptnauer et al., the median age was 62.2 years and the age range was 34 – 78 years (30). There is a slight male predominance and its incidence is increasing in men. A 20 year population based study revealed age-standardized incidence rates of 3.8 per 1000000 inhabitants in men and 2.7 per 1000000 inhabitants in women (31).

Several significant disease associations have been described in association with ampullary carcinoma which include: familial adenomatous polyposis (FAP), attenuated

adenomatous polyposis coli (AAPC), Gardner syndrome, neurofibromatosis type 1 (NF-1) and pancreatic intra-epithelial neoplasia (PanIN).

- FAP is an autosomal dominant familial syndrome which is characterized by numerous adenomatous polyps in the colon (a minimum of 100 polyps is required for the diagnosis) and a frequency of progression to colonic adenocarcinoma approaching 100% (32). Multiple adenomas may also be present elsewhere in the alimentary tract, including the ampulla of Vater. Offerhaus et al. found an increased relative risk for ampullary carcinoma in patients with FAP (33). Bjork et al. concluded that the lifetime risk of severe periampullary lesions in FAP patients is high, and an association between stage IV periampullary adenomas and a malignant course of the periampullary adenomatosis is strongly suggestive (34).

- AAPC (previously known as flat adenoma syndrome) which genetically represents an extremely rare variant of FAP, is characterized clinically by many, but usually fewer than 100, colonic adenomas that are typically slightly elevated and plaque-like, with a reddish surface and sometimes a central depression. Several cases of concurrent AAPC and ampullary carcinoma have been reported, suggesting that AAPC should be considered in patients with ampullary tumours (35).

- Gardner syndrome is a variation of FAP, also autosomal dominant. Patients exhibit intestinal polyps identical to those in FAP, combined with multiple osteomas, epidermal cysts and fibromatosis (36). Almost all patients develop adenomas in the ampulla of Vater and are therefore at increased risk for developing ampullary carcinomas (37).

- NF-1 (formerly known as von Recklinghausen's disease) is an autosomal dominant syndrome characterized by neurofibromas, pigmented skin lesions, iris hamartomas and a bewildering assortment of other abnormalities (38).

Periampullary tumours in patients with NF-1 are usually carcinoids or stromal tumours and, rarely, adenocarcinomas. Costi et al. reported that the ampullary and periampullary adenocarcinomas in NF-1 patients have peculiar features; the tumour may be part of a complex epithelial proliferation with associated islet cell adenomatosis of the pancreas and multiple gastric, duodenal and jejunal stromal tumours. Moreover, they occur at a younger age than those occurring in non-NF-1 patients (39). There is a strong association between somatostatin-producing carcinoids of the duodenum and NF-1 (40). All the duodenal somatostatinomas are located either at the papilla of Vater or on the medial wall of the second portion of the duodenum. Patients with these tumours seldom exhibit the somatostatin syndrome (steatorrhoea, gall stones, diabetes mellitus and hypochlorhydria). Histologically, these tumours are usually typified by glandular/acinar structures lined by uniform cells with eosinophilic, granular cytoplasm. Solid or nested foci can also be present, and psammoma bodies are found in 60.7% of cases (41). Chetty and Essa (1999) propose that the somatostatin producing cells arise from pancreatic duct epithelium or from heterotopic pancreatic tissue; they postulate that these cells eventually evolve into somatostatinomas (42).

- Pancreatic intraductal neoplasia (PanIN) is a precursor lesion for the development of pancreatic adenocarcinoma (40). Adenomas and carcinomas of the ampulla of Vater have a strong association with PanIN, and often high grade PaIN. Agoff et al. emphasized that PaIN is underreported and often unrecognized in specimens resected for ampullary carcinoma (43).

- Associations with unusual dermatological conditions have been reported which include the Leser-Trelat sign and Muir-Torre syndrome (MTS). The Leser-Trelat sign is the rapid and widespread eruption of multiple seborrhoeic keratoses. Klimopoulos et al. described a case of Leser-Trelat sign occurring in association with ampullary carcinoma (44). Matthews et al. described a case of an individual with MTS, a condition characterized by the association of multiple sebaceous

tumours and kerato-acanthomas with internal malignancies, who developed ampullary carcinoma (45).

- There is a greater frequency of other malignancies in survivors of ampullary tumours. This is 4 - 5 times greater than expected and not confined to gastrointestinal tract malignancies. Eriguchi et al. reported 5 cases of ampullary carcinomas associated with malignancies in other organs, including gastric, colonic and pulmonary carcinomas (46).

1.4 ETIOLOGY AND PATHOGENISIS

No definite aetiological factors such as diet, chemical or environmental causes have been directly linked to the pathogenesis of ampullary carcinomas. Much attention has been given to adenomas as precursor lesions for these tumours.

Adenomas occurring in the ampullary region, including those arising in the papilla, have been termed ampullary adenomas (47). The duodenum is the least common site for an adenoma and most duodenal adenomas occur in the vicinity of the ampulla of Vater (48). It has been postulated that carcinogens, or co-carcinogens present in bile or pancreatic secretions may play a role in the etiology of adenomas, because most of them occur near the ampulla (49). An established risk factor is the presence of genetically inheritable polyposis syndromes e.g. FAP (34). Evidence which strongly suggests that ampullary adenomas are precursor lesions for ampullary carcinomas is: (47)

- Adenomatous foci are found in adenocarcinomas
- Histological appearance identical to the appearance of adenomas of the large bowel
- Increasing grade of dysplasia during follow up
- Genetic changes are comparable to "premalignant" lesions of other gastrointestinal sites
- Median age of adenoma patients is younger than in patients with ampullary carcinomas

It is therefore reasonable to postulate that the adenoma-carcinoma sequence is as significant in the ampullary region as in the colorectal area.

1.5 MOLECULAR PATHOLOGY

- **p53:** It has been well established that disturbances in the cell cycle, with an increase in proliferative activity, occurs during carcinogenesis. For ampullary adenomas, an enhanced cellular proliferation has been reported as compared to non-neoplastic mucosa. Mutations of *p53* have been found in ampullary adenocarcinomas but not in adenomas or inflamed precursor lesions (50). In a study by Takashima et al., positivity of *p53* was 0% in pure adenomas, 36% in adenomatous areas of carcinomas with adenomatous areas, 62% in the carcinomatous areas of carcinomas with adenomatous areas, and 56% in pure carcinomas. They concluded that molecular events leading to *p53* accumulation occur relatively late during the oncogenic process (51).

- **K-ras:** *K-ras* codon 12 point mutations occur in about 40% of ampullary neoplasms at a relatively early stage in tumourogenesis. The pattern of mutations in these tumours resembles that of the adenoma-carcinoma sequence in the colon and rectum. It has been suggested that ampullary neoplasms can be detected at an early stage by searching for genetic alterations in the *K-ras* oncogene in cytological brush specimens (52).

- **Dpc4:** The *Dpc4/Smad4* gene encodes a component of the transforming growth factor (TGF) beta signaling pathway. It functions as part of various DNA binding transcriptional activator complexes (53). Loss of *Dpc4* expression occurs in approximately one third of invasive ampullary cancers but is not seen in adenomas; thus, loss of *Dpc4* expression occurs late in ampullary carcinogenesis. Although ampullary and pancreatic adenocarcinomas share histological and molecular features, ampullary carcinomas are less likely to show loss of *Dpc4* expression or *K-ras* gene mutations (54). Although *Dpc4* expression is of no

clinical relevance, its involvement in ampullary cancers gives additional weight to the hypothesis that, among all pancreatic exocrine and endocrine tumours, only ampullary carcinomas and common ductal adenocarcinomas have similar molecular fingerprints (53).

- **Microsatellite instability (MSI):** Park et al. investigated the occurrence of microsatellite instability in 64 ampullary neoplasms. Eight out of 22 adenomas, nine out of 32 carcinomas and one metastatic lesion showed MSI in 1 - 3 of the nine dinucleotide markers; those cases are categorized into microsatellite instability-low. The remaining samples were stable. None of the samples showed frame shift mutations of the poly A-tract of the factor-beta type II receptor which are frequently mutated in gastric or colorectal cancers showing MSI. To confirm this finding, the neoplasms were stained with hMLH1 and hMSH2 and all tumours were found to express mismatch repair proteins. They concluded that, in contrast to gastric and colorectal cancers, MSI does not play an important role in the carcinogenesis of ampullary carcinoma. (55)

- **Mucin carbohydrates and core proteins:** Kitamura et al. examined the expression of carbohydrate antigens which are associated with the earliest steps in mucin glycosylation (Tn and sialosyl-TN) and the expression of mucin core antigens associated with MUC1 gene product (DF3 antigen) as well as MUC2 gene product in 36 ampullary carcinomas. MUC1, Tn and sialosyl TN showed a high expression rate in carcinoma and rare or no expression in non-neoplastic epithelium around carcinoma. The expression of MUC1 and MUC2 was also a useful indicator of the prognosis of the patients (56) (see Survival and Prognosis).

- **Other:** Loss of heterozygosity of chromosome 5q occurring in the early stages of ampullary carcinoma have been demonstrated (57). Mutations of the APC gene have been found in ampullary carcinomas in patients with FAP (34).

1.6 CLINICAL PRESENTATION

Commonly, the physical findings are due to biliary obstruction early in the disease process. Jaundice, abdominal pain and significant weight loss (more than 10% of body weight) are the most frequently reported symptoms. Biliary colic, pancreatitis and haemorrhage have also been reported. The duration of symptoms ranges from 2 - 4 months (1, 58, 59). Duffy et al. described eight patients out of 55 consecutive resections who were completely asymptomatic and their periampullary lesions were discovered on work ups which had been initiated by abnormalities on routine serum liver function tests; three of these patients developed jaundice during the course of the workup. Hepatomegaly and anaemia are common clinical findings and palpable gall bladder is found in 30% of cases. Occult blood may be detected in stool samples (58).

Abnormalities in biochemical results are variable. An increase in serum alkaline phosphatase and low grade elevation of serum bilirubin are common findings. Other abnormal laboratory examinations include elevated alanine transaminase, aspartate transaminase and decreased haemoglobin levels in those patients with haemorrhage and malena (60).

1.7 DIAGNOSTIC PROCEDURES

1.7.1 RADIOLOGY

Before 1987 different diagnostic procedures were performed on indication. After 1987, diagnostic imaging was gradually standardized to include conventional ultrasonogrophy, computed tomographic (CT) scanning, endoscopic retrograde cholangiopancreaticography (ERCP), endoscopic ultrasonography, and selective angiography (61).

Duodenoscopy with ERCP indisputably seems to be the best investigation for the diagnosis of ampullary tumours (1). Not only can the tumour be visualized and

biopsied during ERCP, but biliary drainage by papillotomy and/or the insertion of an endoprosthesis may be attempted (62).

Sonography or CT scanning can be performed rapidly and easily on the jaundiced patient and each is capable of demonstrating the distal location of the obstruction and the dilatation of the common bile duct. However, visualization of the ampullary tumour is more difficult and operator dependant. In a series by Chareton et al., an ampullary tumour was suggested in only 13% of patients undergoing sonography and 23% of patients undergoing CT scanning. CT scan is recommended where staging is necessary or as ancillary work up to augment the findings of the previous examinations (1). Echoendoscopy seems to hold promise for the evaluation of lymph node involvement or mesenteric portal vein encasement (63, 64). Pre-operative angiography may be used to evaluate the resectability of tumours with regard to the vessels of the portal system (65).

1.7.2 FINE NEEDLE ASPIRATE AND BIOPSY

The diagnosis of ampullary carcinoma can be made by endoscopic biopsy, transduodenal biopsy or cytological examination.

1.7.2.1 CYTOLOGICAL EVALUATION

Endoscopic brush cytology is an effective means of diagnosing ampullary neoplasms, and it complements tissue biopsy in cases of bile duct stricture. Location, predominance of tumour stroma, an extramucosal growth pattern, sampling error, and interpretative experience influence the diagnostic evaluation. Cytological diagnosis of an adenoma does not exclude an underlying malignant neoplasm in ampullary tumours. In some instances, it may be difficult to distinguish between villous tumours with severe dysplasia and adenocarcinomas by cytology alone (66).

1.7.2.2 HISTOLOGICAL EVALUATION

Endoscopy and biopsy assume a major role in obtaining an accurate preoperative diagnosis. Pure adenomas can be locally excised, whereas adenomas that harbor invasive carcinomas require more extensive surgical procedures (67, 68). It is important to remember that in up to 50% of cases, the biopsy of a villous adenoma could miss the malignant tumour (69, 70). The biopsy should not be too superficial, or else areas of malignant change can be missed. The biopsy specimen should preferably should be in the form of multiple samples which are step sectioned, a procedure which is claimed to have a diagnostic reliability of greater than 90% (71). From a diagnostic point of view, other benign lesions also need to be excluded, which include Brunner gland hamartomas and Peutz Jeghers polyps (60). Frozen section evaluation has been reported to be inaccurate in detecting foci of adenocarcinoma of the ampulla of Vater, leading many authors to advocate pancreaticoduodenectomy as the method of treatment for these neoplasms. However, Clary et al. claim that frozen section evaluation of ampullary neoplasms is accurate. These authors advocate that ampullary resection is less morbid than pancreaticoduodenectomy and ampullary resection with frozen section is their current approach to small ampullary lesions (67).

1.8 PATHOLOGY

1.8.1 MACROSCOPIC

Grossly, ampullary carcinomas usually bulge into the duodenal lumen. The duodenal mucosa appears stretched but otherwise normal if the tumour is confined to the ampullary lumen. In other instances, the tumour presents mainly as a circumferential growth around the ampulla. In still others, a combined pattern of growth exists (72). Lechago et al. classifies ampullary tumours as 'intra-ampullary, periampullary and mixed types' according to location (73). (The term periampullary tumour in this classification based on location, should not be confused with the earlier definition where the term was used as a collective name for tumours occurring in the periampullary region.)

In a study by Howe et al. the mean size of resected ampullary carcinomas was 2.7 cm with a median size of 2.5 cm and a range of 0.3 - 10.3 cm (27). Those tumours with a prominent component of residual villous adenoma present as soft, sessile, papillary masses projecting into the duodenal lumen. The tumours may be exophytic and/or ulcerating (21).

1.8.2 MICROSCOPIC

The majority of the malignant ampullary tumours are adenocarcinomas. Many of them have a superficial papillary component with the appearance of villous adenoma or villoglandular polyp (74). The WHO classification of tumours groups ampullary carcinomas under the carcinomas of the extra-hepatic biliary tree. The intestinal type is defined as an unusual variant of adenocarcinoma (75). A modified classification of carcinomas of the extrahepatic bile ducts, published in 2000 by the World Health Organization (WHO), which is applicable to ampullary carcinomas, is as follows :(75, 76)

- Carcinoma in situ
- Papillary adenocarcinoma
- Adenocarcinoma, intestinal type
- Mucinous adenocarcinoma
- Clear cell carcinoma
- Signet ring cell carcinoma
- Adenosquamous carcinoma
- Squamous cell carcinoma
- Small cell carcinoma
- Undifferentiated carcinoma
- Carcinoid tumour
- Mixed carcinoid-adenocarcinoma
- Carcinoma, NOS
- Other (specify)

Albores-Saavedra et al. (77) classifies ampullary carcinomas into the main types and the so called "unusual types".

- The "main types" are the pancreaticobiliary and the intestinal types, which reflect the origin from two different types of mucosa at this site. The morphological criteria are as follows: The intestinal type carcinoma is composed of well formed tubular to elongate glands, complex cribiform areas and solid nests, indistinguishable from colorectal adenocarcinoma. The pancreaticobiliary type carcinomas mostly consist of simple or branching glands and small solid nests of cells surrounded by desmoplastic stroma.

- The "unusual types" include signet ring cell carcinoma, mucinous, squamous cell carcinoma, adenosquamous carcinoma and undifferentiated carcinoma. Other unusual variants described include cases with a prominent Panneth cell component, cases exhibiting hepatoid differentiation and small cell (neuroendocrine) carcinomas.

Studies have shown that the majority of ampullary carcinomas (44% - 72%) are of the pancreaticobiliary type, the second most common group of carcinoma (25% - 27%) are of the intestinal type, whilst the rest are mixed and unusual types (78, 79).

A recent study attempted to objectify the classification of ampullary carcinomas by immunohistochemical criteria. They found a distinctly different cytokeratin expression in the pancreaticobiliary types and in the intestinal types, using the cytokeratin 20 (CK20) and cytokeratin 7 (CK7) profile. Ampullary carcinomas expressing CK20 but not CK7 reflect the normal cytokeratin spectrum of intestinal mucosa, whereas carcinomas reacting positive for CK7 but negative for CK20 reflect the profile of pancreaticobiliary duct mucosa. The histological classification and the immunohistochemical characterization by cytokeratins were in good agreement (79). MUC2 apomucin expression had been used for the discrimination between the two main types of

ampullary carcinomas. Most intestinal type carcinomas were MUC2 positive while none of the panreaticobiliary type carcinomas expressed this mucin (78).

The prognostic significance of these classifications is doubtful. Kimura et al. found a significantly better prognosis for patients with intestinal type ampullary carcinomas than with pancreaticobiliary type carcinomas (80). In the collective of Memorial Sloan Kettering Cancer Center, the outcome of the intestinal type tumours was somewhat more favorable than that of the pancreaticobiliary type. However, in the collective of Zhou et al., neither the histopathological classification nor the immunohistochemical classification identified a subgroup of tumours with a better or worse outcome (79).

1.9 GRADING AND STAGING

1.9.1 HISTOLOGICAL GRADE

For non-papillary adenocarcinomas, the following grading system is suggested (76):

- GX Grade can not be assessed
- G1 Well differentiated (>95% of the tumour composed of glands)
- G2 Moderately differentiated (50-95% of the tumour composed of glands)
- G3 Poorly differentiated (5-49% of the tumour composed of glands)
- G4 Undifferentiated (<5% of the tumour composed of glands)

1.9.2 STAGING

The TNM staging system for ampullary tumours of the American Joint Committee on Cancer (AJCC) and the International Union against Cancer (UICC) is recommended (81):

Primary tumour (T)

- TX Primary tumour cannot be assessed
- T0 No evidence of primary tumour
- Tis Carcinoma in situ

- T1 Tumour limited to the ampulla of Vater or the sphincter of Oddi
- T2 Tumour invades the duodenal wall
- T3 Tumour invades 2cm or less into the pancreas
- T4 Tumour invades more than 2cm into the pancreas and/or into the adjacent organs

Regional lymph nodes (N)

- NX Regional nodes can not be assessed
- N0 No regional lymph node metastasis
- N1 Regional lymph node metastasis

Distant metastasis (M)

- MX The presence of distant metastasis can not be assessed
- M0 No distant metastasis
- M1 Distant metastasis

Stage groupings

Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage II	T2	N0	M0
	T3	N0	M0
Stage III	T1	N1	M0
	T2	N1	M0
	T3	N1	M0
Stage IV	T4	Any N	M0
	Any T	Any N	M1

Although tumour size was not included in the TNM staging system for ampullary cancers, it has been shown to have independent prognostic significance (82):

Tumour size	5-year survival rate (% + SE)
<2.5cm	65%
>2.5cm	20%

Martin's classification is as follows (59):

- Stage I: vegetating tumour limited to the epithelium with no involvement of the sphincter of Oddi.
- Stage II: tumour localized in the duodenal submucosa without involvement of the duodenal muscularis propria but possible involvement of the sphincter of Oddi.
- Stage III: tumour of the duodenal muscularis propria.
- Stage IV: tumour of the periduodenal area or the pancreas, with proximal or distal lymph node involvement.

1.10 TREATMENT

Pancreaticoduodenectomy, specifically pylorus preserving pancreaticoduodenectomy, seems to be current choice of operation, even for benign ampullary tumours. Proper patient selection and careful technique continue to be the most important factors in minimizing morbidity and peri-operative mortality (83).

1.10.1 PANCREATICODUODENECTOMY (PD)

PD has its origin in the late 1800s. Halsted performed the first transduodenal local excision of an ampullary tumour in 1898 (84). Allesandro Codivilla, in that same year, was the first to perform a PD in Imola, Italy. Allen Oldfather Whipple (1881-1963) was an American surgeon who reported the first series of PDs in 1935, and since that time the operation has been known as the "Whipple operation"(85). PD generally involves the removal of the gall bladder, common bile duct, variable lengths of the duodenum and/or stomach and the head of the pancreas. There are several variations of this procedure currently practiced. The most common procedures performed on patients

with ampullary tumours are the classic/standard PD (includes antrectomy) and the pylorus preserving pancreatico duodenectomy (PPPD) (58).

PD is performed via subcostal or midline incision. After thorough abdominal exploration the gall bladder is removed and the common bile duct is transected. The anterior aspect of the portal vein is then dissected free of the overlying pancreatic neck. Subsequently the duodenum (in the PPPD) or the stomach (in the classic/standard procedure) is transected, followed by transaction of the pancreatic neck, unicate process and the jejunum distal to the ligament of Treitz. Truncal vagotomy is performed in most patients undergoing hemi-gastrectomy. Reconstruction is undertaken with an isoperistaltic limb of jejunum in retrocolic fashion and anastomosed with an end-to-side pancreaticojejunostomy followed by an end-to-side choledochojejunostomy and an end-to-side duodenojejunostomy or gastrojejunostomy. An alternative for pancreatic-enteric reconstruction involves the use of a pancreaticogastrostomy (83).

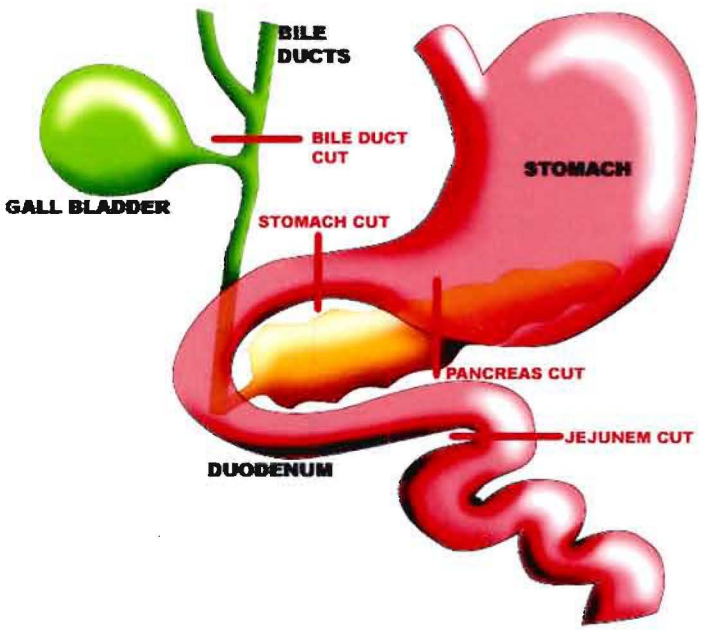

Fig 1.2. Anatomy before a Whipple resection [Adapted from Schmidt et al. (83)]

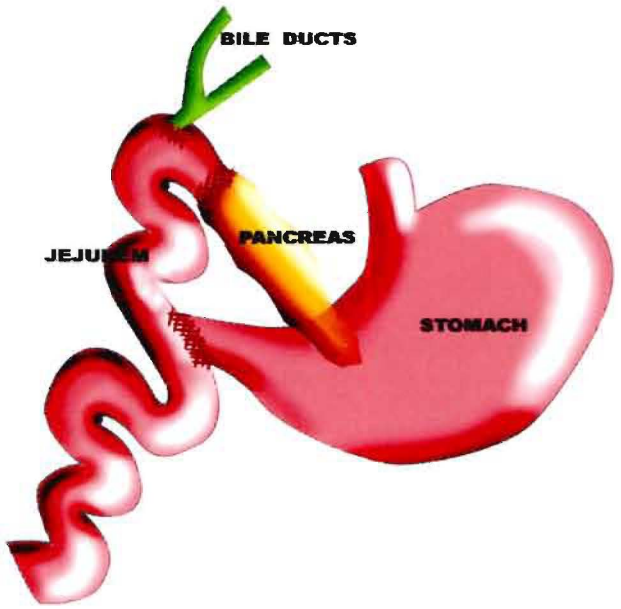

Fig 1.3. Most common anatomy after a Whipple resection [Adapted from Schmidt et al. (83)]

PD is a complex operation that previously carried a very high post-operative mortality. Numerous studies have examined the outcomes of PDs for ampullary cancer. The problem is that many of these studies include cases operated on during multiple decades by many different surgeons with significant variation in surgical technique. Furthermore, some of the reports review operative outcomes of PDs at a time when the procedure carried with it a risk of substantially higher mortality than it does today (86, 87). During the last two decades, improvements in surgical and anesthetic techniques, critical care and institutional specialization have decreased peri-operative mortality rates to below 5% in most major centers (23, 88). Brent et al. reviewed 279 patients who underwent PD from 1980 to 1989 at a single institution with a postoperative mortality of 4% (89). In a recent article, Duffy et al. reviewed an approximately 13 year experience (from 1988 through 2001) with Whipple resection, both standard and pylorus preserving types, for adenocarcinoma of the ampulla of Vater: there were no operative deaths, and all patients left the hospital (58).

Despite the decline in mortality associated with PD, postoperative morbidity continues to pose a major surgical challenge. Post-operative complications occur in roughly half

the patients (46% - 49%) after PD (83, 89). The most common complications are pancreatic fistula, delayed gastric emptying, sepsis and anastomotic leaks. Risk factors generally associated with an increased rate of complications included prolonged operations, increased operative blood loss and advanced age (89).

PPPD provides, in contrast to the standard Whipple procedure, preservation of the stomach, the pylorus and the first 2 cm of the duodenum. This is technically an easier operation than the standard Whipple procedure and preservation of the pylorus seems to improve post-operative digestive function and weight gain. There is also no reflux of bile into the stomach (90, 91). Preservation of the pylorus does not appear to alter mortality, post-operative morbidity or the hospital stay in any significant way. However, the duration of the operation is shorter and fewer blood units are transfused during the operation (1, 90, 91). In patients that underwent PPPD, gastric emptying may be prolonged during the post-operative period. Although delayed gastric emptying occurred more commonly in patients who underwent PPPD in a study by Duffy et al., the difference was not statistically significant (58).

Total PD does not seem to be indicated for ampullary tumours except when the tail of the pancreas must be or was previously resected for some reason (1). In a cohort by Allema et al., subtotal PD was the type of resection in 62 patients and in 5 patients, total PD was performed. The indications for PD was tumour involving the pancreatic resection margin with intra-operative frozen section histological evaluation, and when the remnant pancreatic duct was too friable for a safe pancreaticojejunostomy. The hospital mortality and morbidity after subtotal PD was significantly lower than the mortality and the morbidity for total PD (3).

1.10.2 AMPULLECTOMY

The first ampullary resection was performed by Halsted in 1897. The technique has been refined and standardized over the years (92). There has been much debate over the place for ampullectomy in the treatment of ampullary carcinomas. Theoretically, local

resection is only applicable for stage 0 and 1 tumours, small neuroendocrine tumours and benign lesions < 3 cm. However, it may be very difficult pre- and post-operatively, to determine whether the tumour is limited to the ampulla or whether it infiltrates adjacent structures. Mucosal spread or intestinal infiltration was frequently found even with cases with carcinoma at a relatively early stage (93). Final tumour staging is only possible after definitive histopathological examination of the resected specimen (76). Studies have found that post-operative mortality is equivalent (93, 94), or even higher (96) than that after PD; long term survival is not modified (95, 97). Ampullectomy is often associated with post-operative haemorrhage (1). Moreover endoscopic surveillance is necessary every six months and occasionally, a secondary PD is required (97). Chareton et al. reported a series of 63 ampullary tumours. Two of the patients had undergone simple ampullectomies for benign disease, both of which were classified as stage IV according to Martin's classification (one having undergone PD and the other simple exploratory laparotomy) 4 and 22 years after the local resections (1). Although this potentially curable malignancy should preferably be treated with adequate radical resection, some authors still advocate that local resection may be possible in the treatment of very small tumours (as long as the lesion is removed with intra-operative frozen section examination) and the treatment of high risk elderly patients (93).

1.10.1 PANCREAS SPARING DUODENECTOMY (PSD)

Chung et al. stated that PSD, as well as local resection, is not appropriate for duodenal (including ampullary) malignancies, early or late, because the recurrence rate is high. However, conservative procedures may have a role in the treatment of large villous adenomas (98).

1.10.2 ADJUVANT THERAPY

To limit the development of local recurrence, physicians have made use of adjuvant therapy for patients with ampullary carcinoma. Barton and Copeland reported 56 patients with ampullary carcinomas, 17 of which received post operative chemotherapy (5-fluoruracil was the most used agent). No analysis was presented but the authors

stated that no combination of drugs appeared to prolong life when used in either the adjuvant or the therapeutic setting (99). Scmidt at al. reported a 20-year experience of 295 patients who underwent PD for periampullary carcinoma. The study included 49 ampullary carcinomas. Chemotherapy, radiotherapy or combined chemo-radiotherapy for periampullary adenocarcinomas in general, without any subgroup analysis, did not seem to confer a survival advantage with those with known follow up (83). However, in a retrospective review, Chan et al. reported that 13 patients who received adjuvant chemotherapy (predominantly involving 5-fluorouracil, mitomycin-C and doxorubicin) had a significantly better survival than 16 patients who underwent resection alone (4). Yeung et al. found no residual tumour in specimens for PD performed for four patients with duodenal/ampullary carcinomas who had received neoadjuvant chemotherapy (100). The combination of intra-operative radiation and resection has not added any significant benefit to patients with ampullary and bile duct cancer when compared with resection alone (101).

Adjuvant therapy was not offered to most patients before 1990. As a result the data on patients treated with chemo-radiation is relatively small with conflicting results. Currently, adjuvant therapy is advocated mainly for patients with poor prognostic features (102). Theoretically, patient survival should be extended by adjuvant treatment. However, some studies have actually shown decreased survival rates in patients that received adjuvant therapy, compared to those who have not. The reason for this is probably that the patients who received adjuvant therapy are also the patients with the "unfavorable" tumour characteristics. This fact makes the interpretation of comparative studies difficult. More studies need to be undertaken before drawing any conclusion (58).

1.10.3 LASER TREATMENT

For unresectable tumours, several authors have proposed laser destruction after papillotomy, sometimes associated with stent replacement. Results have been

disappointing as mortality is close to 10%; and there have been no notable modifications in outcome, as the mean survival remains at approximately 6 months (103, 104).

1.10.4 BYPASS OPERATIONS

When the tumour is unresectable, bypass operations have been considered, even though their associated mortality and morbidity are high. Moreover, these operations leave a potentially haemorrhagic tumour in place. In a series reported by Divinagracia et al, palliative management with bypass procedure alone was shown to have 100% post operative mortality (60).

1.11 SURVIVAL

Different criteria for classification of ampullary carcinomas make a comparison of different collectives difficult. Additional confusion is generated by the fact that ampullary carcinomas are often grouped together with other periampullary tumours. The identification of prognostic factors is important to predict the survival probability and to draw consequences for rational and pragmatic surgical therapy (29).

The 5-year survival rates after resection is usually between 30% and 60% (1, 3-5, 19, 58, 87). Klempnauer et al. reported that survival probability increased with each year that a patient survived after resection. When a patient had already survived five years after resection, the probability to survive another 5 years was 83% (29). Prognostic factors can be divided into patient, tumour and treatment associated factors.

Tumour characteristics:

- Histogenesis: the importance of distinguishing ampullary carcinomas from other periampullary carcinomas has already been discussed. The resection rate and the prognosis are significantly higher in comparison with pancreatic adenocarcinoma and cholangiocarcinoma (27).

- TNM and stage groupings: the post resection prognosis of a patient with ampullary carcinoma is primarily determined by the anatomic extent of the disease as defined by TNM classification and stage groupings (105-110).
- Lymph node involvement: lymph nodes metastases have been shown to have independent significance as an adverse prognostic factor (106, 107, 111).
- Tumour size: although not included in the TNM system, it has been shown to have independent prognostic significance [see Grading and Staging] (82).
- Histologic grade: poor differentiation has been shown to be an adverse prognostic factor (105, 106).
- Histologic type: ampullary tumours of the papillary type have been shown to have a favourable prognosis compared to non-papillary types (105). Signet ring cell carcinomas are, by convention, classified as poorly differentiated adenocarcinomas, and poor differentiation has been shown to be an adverse prognostic factor (105, 106). As mentioned earlier, Kimura et al. found a significantly better prognosis for patients with intestinal type ampullary carcinomas than with pancreaticobiliary type carcinomas (80). In a study by Beghelli et al. only T-stage and histological type emerged as independent prognostic factors: the median actuarial survival time was 19 months for patients with pancreatobiliary cancers, whereas it was 70 months for patients with intestinal type cancers. (53) However, this could not be confirmed by Zhou et al. (79).
- Local extension: invasion of the muscle of the sphincter of Oddi has been shown to be an adverse prognostic factor (105).
- Blood/Lymphatic invasion: lymphatic and small blood vessel invasion have been shown to be an adverse prognostic factor (105, 109).
- Perineural invasion: perineural invasion by the tumour has been shown to be an adverse prognostic factor (105). In a study by Duffy et al., the major factor associated with prolonged survival was the absence of perineural invasion in resected tumour specimens (58).
- Resection margins: involvement of resection margins have been shown to be an adverse prognostic factor. Local recurrence from invasive carcinoma in the

region of the pancreatic head, most often arises in corresponding to the deep radial posterior margin of the pancreatic head. Local recurrence from intraductal tumour is most likely to occur at a ductal resection margin (i.e., the main pancreatic duct and/or the common bile duct margin) (106). Allema et al. concluded that involvement of resection margins was the strongest prognostic factor for survival and that patients with involved margins may be candidates for adjuvant therapy (3).

Special tests

- Intratumoural microvessel density (IMD): angiogenesis is required for tumour growth. Khan et al. examined the IMD in 24 pancreatic carcinomas and 23 ampullary carcinomas with CD34. In the pancreatic cancer group IMD was found to have independent prognostic significance to survival on multivariate analysis. For ampullary cancers, IMD was higher in those with lymph node metastasis (P=0.02, Mann-Whitney U-test) (110).
- Expression of mucin carbohydrates and core proteins: Kitamura et al. found that the patients with positive DF3 antigen (MUC1 gene product) expression in the carcinoma showed significantly poorer survival than those with negative DF3 expression, whereas the patients with positive intestinal-MRP (MUC2 gene product) in the carcinoma showed significantly more favourable survival than those with negative intestinal-MRP expression (56).
- MIB-1/*Ki-67* labeling index: MIB-1 has been shown to have independent prognostic significance in ampullary carcinomas. The 5-year survival rate was 40.7% for tumours with a MIB-1 index <or= 15%, and 0% for those with MIB-1 index > 15% (113).
- Ploidy: diploid tumours have been shown to have a significantly better prognosis than aneuploid tumours (113).
- Cyclin D1 expression: Yamazaki et al. found increased Cyclin D1 expression by immunohistochemistry in 17 of 30 ampullary carcinomas. Cyclin D1 expression was significantly correlated with tumour cell proliferation and disease free survival time (114).

- CA19.9 expression: there are several reports on the poor prognosis of ampullary cancers expressing CA19.9 (115, 116). Dorendeu et al. confirmed this finding. However, the patient numbers were too small for multivariate analysis (59)
- *Dpc4* expression: *Dpc4* immunohistochemical staining is not significantly associated with any clinicopathological variable, including prognosis (53).
- *p53* over-expression: Several studies concluded that accumulation of *p53* protein revealed no significant difference in prognosis (51, 59, 79).

Patient characteristics

- Age and sex: there is no significant correlation between age and survival (3). Zhou et al. found that the survival times of males were significantly shorter than females. This was probably due to the higher frequency of nodal positive carcinomas in males than in females (79). Yamaguchi et al. failed to reveal that the age of the patient at the time of operation was an independent prognostic factor (21).
- Pre-operative symptoms: there is no significant correlation between pre-operative symptoms and survival (3).

Treatment associated factors

- Adjuvant therapy: Chan et al. concluded that prognosis was better for ampullary tumours if adjuvant chemotherapy was used. Radiotherapy did not influence survival (4).
- Type of operation: although the type of operative procedure was a significant predictor of survival in one univariate analysis, prognosis depends more upon the biology of the tumour rather than the particular surgical procedure (117).

CHAPTER 2: MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL FEATURES IN AMPULLARY CARCINOMAS

This study entailed a histopathological and an immunohistochemical analysis of ampullary carcinomas in specimens following a Whipple resection.

2.1 AIM

- To examine clinical, histopathological and immunohistochemical features of ampullary carcinomas after Whipple resection.
- To determine whether any of these features have significant prognostic value.

2.2 MATERIALS AND METHODS

A thesis proposal was submitted for approval to the Groote Schuur Hospital/University of Cape Town Human Ethics Committee and ethical approval was obtained.

This was a retrospective study of 37 patients who underwent a Whipple resection for adenocarcinoma of the ampulla of Vater from 1979 through 2002. The resections were performed at Groote Schuur hospital which is an academic tertiary care hospital. Cases were only included in this study if one could confidently conclude from the macroscopic description on the original pathology report that the tumour truly arose from the ampulla of Vater. Tumours simply reported as "peri-ampullary" were excluded from this study. Patients who only had adenomas, ampullary fibrosis or other unusual tumours were excluded from this study. The expected number of cases was over fifty. Ultimately, only 37 cases could confidently be labelled as true ampullary carcinomas after review of the macroscopic description and histological evaluation. Formalin fixed, wax embedded tissue was retrieved from the archives of the Anatomical Pathology Division of Groote Schuur Hospital. For each case a single block was chosen. The selected blocks contained the most representative sample of the carcinoma, and if present, adenoma and adjacent normal epithelium. The staining of some of the sections

had faded and these blocks were re-cut and re-stained with H&E. Microscopic tumour analysis confirmed that these tumours were all adenocarcinomas.

2.2.1 HANDLING OF A RESECTED PANCREATICODUODENECTOMY SPECIMEN

[Adapted from: Royal Collage of Pathologists' Minimum dataset for the histopathological reporting of pancreatic, ampulla of Vater and bile duct carcinoma (118), College of American Pathologists' Protocol for the examination of specimens from patients with carcinoma of the ampulla of Vater (76) and Rosai and Ackerman's Surgical Pathology, 9th edition (119)].

- The type of specimen is recorded, e.g. a standard Whipple pancreaticoduodenectomy (PD), a pylorus-preserving PD or a total PD. The standard PD includes the head of the pancreas, duodenum, distal bile duct, gall bladder and two thirds of the stomach; the pylorus preserving PD does not include the stomach; the total PD also includes the body and the tail of the pancreas with or without the spleen and/or stomach.

- Record the length, in millimeters, of the duodenum, stomach (lesser curve and greater curve), gall bladder, cystic duct and extra-hepatic bile duct, and the maximum dimensions of the pancreas.

- The circumferential margins of the pancreas (including the anterior, medial and retroperitoneal aspects) should each be painted with a different colour ink, either when the specimen is fresh or when fixed, according to the preference of the examining pathologist, but before the blocks are taken.

- To aid fixation, the duodenum and/or stomach should be opened. The common bile duct, the pancreatic duct and the termination onto the duodenum should be probed and opened (Fig 2.1). The relationship with each other should be recorded. The presence or the absence of a stent and of a named vessel (e.g.

portal vein) should be noted. The specimen may then be pinned to a cork board, placed in a large volume of formalin and allowed to fix for 24-48 hours.

- Record the site of the ampullary tumour as being intra-ampullary, peri-ampullary or from the junction of duodenum and ampullary mucosa. The extent of the tumour can be optimally visualized either by making transverse sections along the ampulla, or by serially slicing the entire specimen.

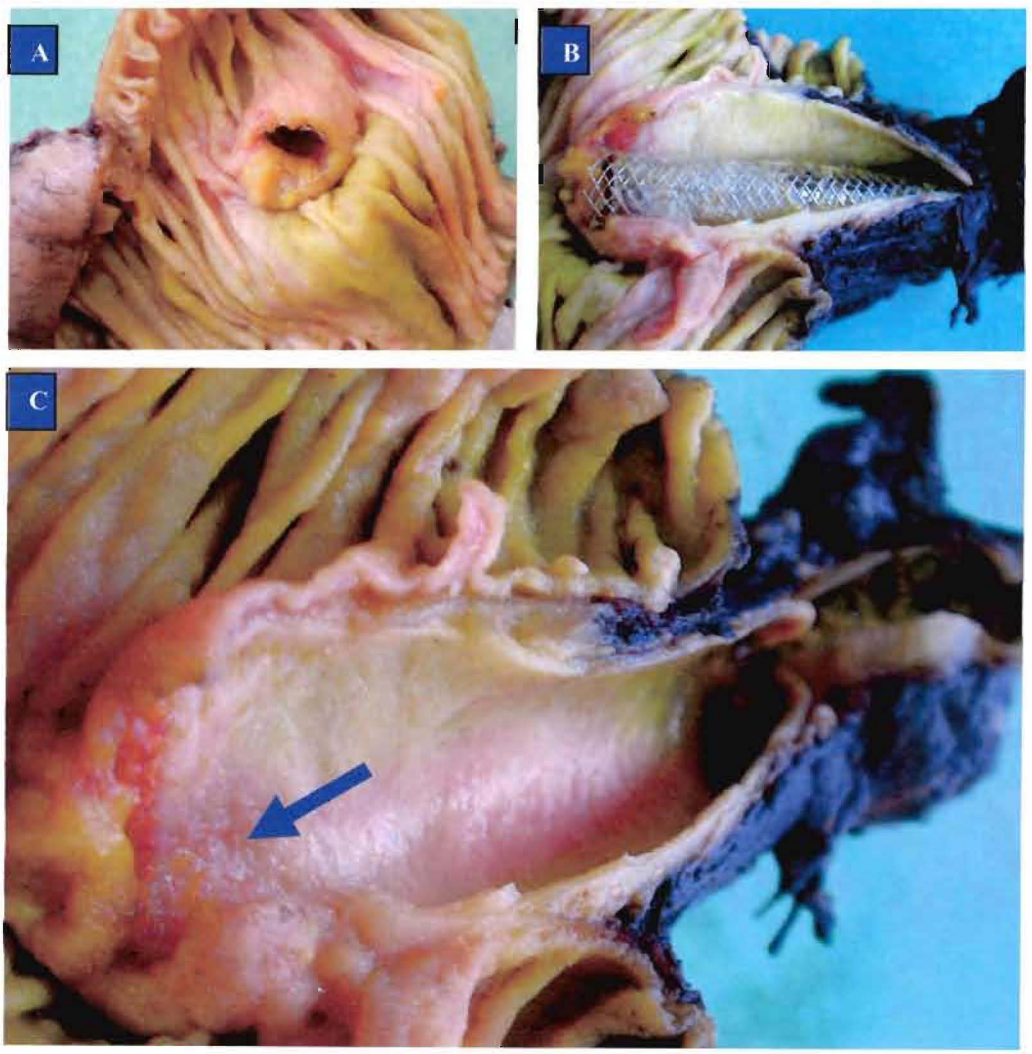

Fig 2.1 . A). In this case the ampullary carcinoma almost completely replaces the major papilla. B) The dilated ampulla is opened to reveal an in-situ stent. C) The stent is removed and a poorly defined ampullary carcinoma (arrow) can be seen at the distal end of the ampulla .

- Optimally, three dimensions of the tumour should be measured but for staging purposes, at least the maximum diameter of the tumour should be measured to the nearest millimeter.

- Adequacy of excision should be assessed by the naked eye and confirmed by microscopic examination. Potential margins (Fig 2.2) include the pancreatic transection margin (with main pancreatic duct), retroperitoneal margin (defined as the peri-pancreatic fat tissue behind the head of the pancreas), the anterior pancreatic capsule and the bile duct (common bile duct or common hepatic duct).

- The following samples should be taken:
- proximal duodenal/gastric margin
- distal duodenal margin
- pancreas: three sections, one of which includes a complete 'en face' section through the distal pancreatic margin (representing the distal margin of the main pancreatic duct)
- common bile duct: two cross sections, one from surgical margin
- tumour: at least three sections
- uninvolved duodenum
- lymph nodes: peripancreatic (superior and inferior), pancreaticoduodenal (anterior and posterior), common bile duct and peri-cystic and other groups, if present (jejunal, omental)
- other organs, if present (e.g. gall bladder)

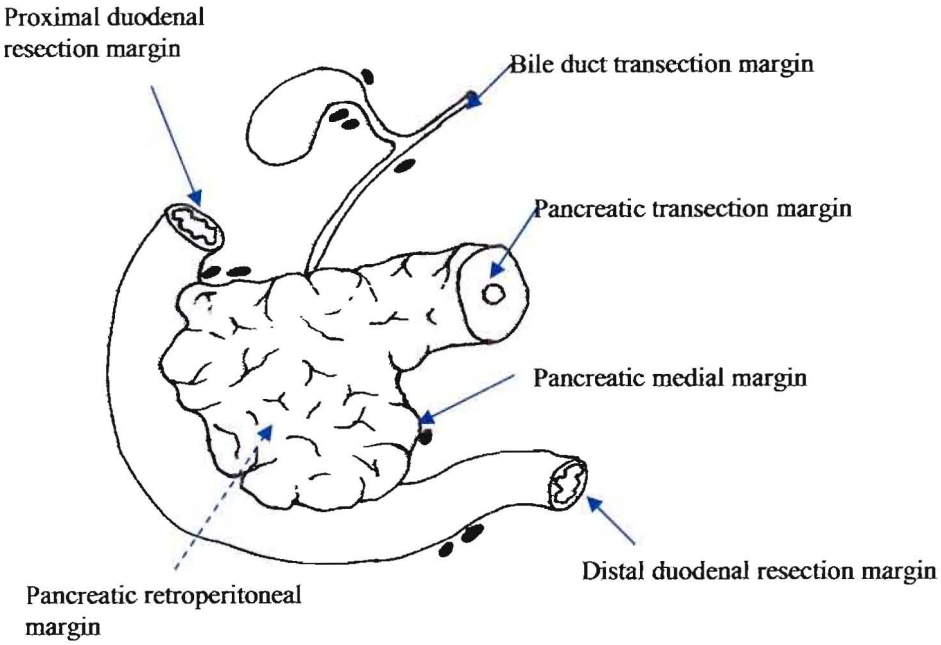

Fig 2.2. Important resection margins in a pancreaticoduodenectomy specimen. [Adapted from Royal College of Pathologists' minimum dataset for the histopathological reporting of pancreatic, Ampulla of Vater and bile duct carcinoma (118).]

2.2.2 DATA COLLECTION

With permission from Professors Bornman and Krige of the Department of Hepatobiliary Surgery at Groote Schuur hospital, access to the clinical data base of the 37 patients was obtained. Data obtained included patient demographics (age and sex), surgical data (date of operation and type of operation) and patient follow up (survival). Tumour characteristics were obtained as follows: the tumour size was recorded from the original pathology report. The degree of differentiation, perineural invasion, lymphatic invasion, vascular invasion and histological type was assessed by microscopic examination of the original haematoxylin and eosin (H&E) sections. If a carcinoma displayed more than one histological pattern, the carcinoma was classified according to the dominant histological pattern. The TNM classification, Martin's classification, and

the involvement of resection margins were obtained through a combination of microscopic evaluation and review of original histology reports.

2.2.3 STATISTICAL ANYLYSIS

Survival estimates were generated using the Kaplan-Meier survival analysis. Statistical significance was determinate if the two-sided *P*-value of a test was <0.05. Limitations of this study were the limited patient follow up and the limited sample size which is mainly due to two factors: the rarity of ampullary carcinomas as well as failure of earlier macroscopic reports to reveal the exact origin of the tumour. A limited sample size made performing quantitative analysis less than accurate. Therefore, qualitative analyses of the prognostic variables were performed. Selection of prognostic variables was based on a literature review at the time the study was commenced. Survival was calculated from the date of the operation to the date of last follow up (June 2003). If the patient died, survival was calculated from the date of the operation to the date of death. The potential prognostic variables included:

- immunohistochemical stains; *p53*, *Ki-67*, MUC1, MUC1 core antigen, MUC2 and CA19,9.
- involvement of resection margins
- histological sub-type according to the criteria published by Albores-Saavedra et al. (73)
- TNM classification and staging according to criteria published by Flemming et al. (79)
- Martin's classification (59)
- tumour differentiation (grade)
- lymphatic invasion
- perineural invasion
- vascular invasion

Patient age at time of operation and tumour size was analyzed using the Cox regression model.

IMMUNOHISTOCHEMICAL STAINS

Table 2.1. A summary of the antibodies and the conditions used for the immunohistochemical staining.

Antigen	Antibody, isotype	Dilution	Incubation time	Source
Ki-67	Clone MIB-1	1:100	30 min	DakoCytomation
Ca19.9 (Sialyl Lewis)	C241: 5:1:4	1:100	30min	Novacastra
p53 protein	DO-7	1:50	30 min	DakoCytomation
MUC1 glycoprotein	Ma552	1:100	30 min	Novacastra
MUC1 core glycoprotein	Ma695	1:100	30 min	Novacastra
MUC2 glycoprotein	Ccp58	1:100	30 min	Novacastra

Pressure cooking was used for antigen retrieval. The retrieval technique was as follows: Sections were cut 2 micrometer thick, mounted on slides coated with APES and incubated overnight at 60 degrees Celsius. The sections were de-waxed in xylol, rehydrated in graded alcohols and washed in water. Endogenous peroxidase activity was blocked using 2% hydrogen peroxide in water for 10 minutes. Antigen retrieval was performed by bringing to the boil 1.5l of 0.01M sodium sitrate buffer (pH 6.0) in a pressure cooker. Once the buffer had boiled, slides were placed in metal racks and placed in the buffer, ensuring slides were completely covered. The lid was then locked. Once the cooker had reached full pressure, the sections were boiled for a further two minutes. The cooker was removed from the heat source and immersed in cold water. Sections were then washed in PBS (pH 7.6). Non-specific staining was blocked by incubating sections in normal goat serum (DakoCytomation X0907) for 10 minutes at room temperature. The sections were drained and incubated with the primary antibodies at optimal dilution for the recommended incubation times at room temperature. Sections were then washed well in PBS. Sections were then treated with a horseradish peroxidase labeled polymer Envision [Dakocytomation K40001 (monoclonal) or K4002 (polyclonal) for 30 min at room temperature. Sections were then well washed in PBS. Positivity was developed by applying 3.3'-diaminobenzidine

(Dakocytomation K3466) for 5-10 minutes at room temperature. Sections were washed in water and then counterstained in aqueous haematoxylin solution, followed by dehydrating using graded alcohols, cleared in xylol and mounted in Entellan.

2.2.6 IMMUNOHISTOCHEMICAL SCORING SYSTEM

The staining of *p53*, CA19.9, MUC1, MUC1c and MUC2 was recorded and scored semiquantitavely based on the scoring system of Zhou et al. (76). The percentage of tumour cells which stained was scored as follows:

- 0 = no staining reaction
- 1 = <10 % positive-stained tumour cells
- 2 = 10-50% positive-stained tumour cells
- 3 = >50-80% positive-stained tumour cells
- 4 = >80% positive-stained tumour cells

Final analysis was performed by using a simplified scoring system: scores 0 and 1 were regarded as negative, and scores 2 - 4 as positive.

The *Ki-67* labeling index was calculated based on the scoring system used by Dorandeu et al. (59): the mean of 10 fields with a 400x magnification was expressed as a percentage of labeled cells. For analysis, a score of more than 15% was recorded as being positive.

2.3 RESULTS

2.3.1 DESCRIPTIVE ANALYSIS

There was a total of 37 patients - 18 (49%) of who were male and 19 (51%) female. Calculated from the time of operation, the age range was from 27 to 83 years, with a mean age of 58.8 years and a median age of 59 years (SD 16.02). Nine patients (24%) underwent a standard Whipple procedure and 28 (76%) patients underwent a PPPD. Macroscopically, the tumour size ranged from 0.7 to 6 cm with a mean of 2.5 cm and a median of 2 cm (SD 1.3).

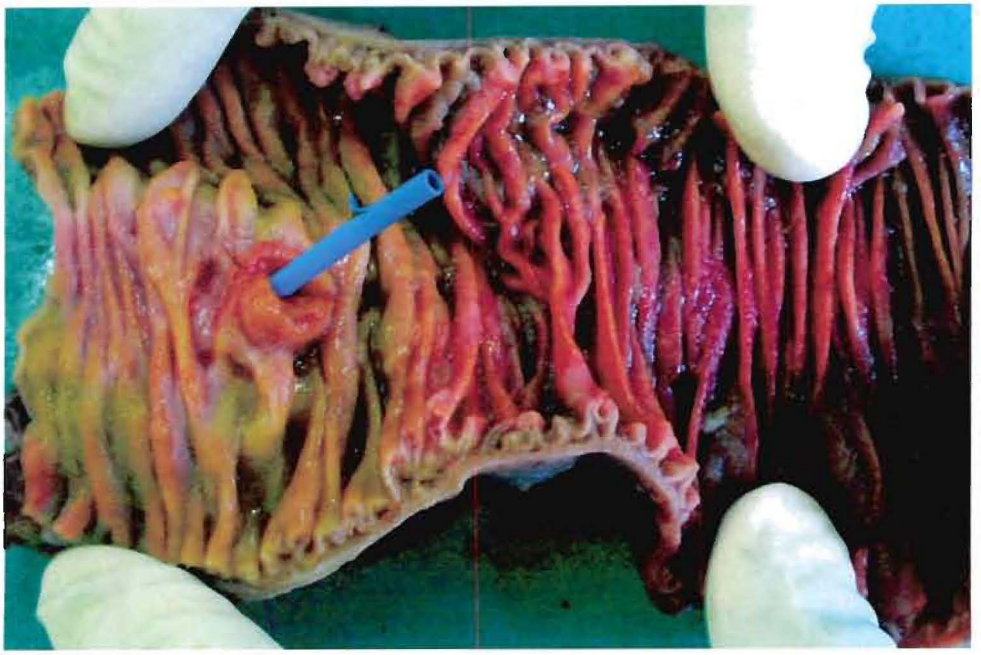

Fig 2.3. An ampullary tumour bulging into the duodenal lumen. A stent extending into the ampulla of Vater temporarily relieved the obstruction caused by the tumour.

In 13 (35%) patients, residual adenoma was detected in the specimen. Five (14%) tumours were well differentiated, 24 (65%) tumours were moderately differentiated and 8 (22%) tumours were poorly differentiated. No tumours were regarded as being undifferentiated. With histological typing, 19 (51%) tumours were classified as intestinal type carcinomas (Fig 2.4), 16 (43%) as pancreaticobiliary type carcinomas (Fig 2.5) and the 2 (6%) tumours classified as "other type" were both mucinous (colloid) carcinomas (Fig 2.6). In both cases of mucinous carcinoma, more than 80% of the tumour displayed a striking mucinous component.

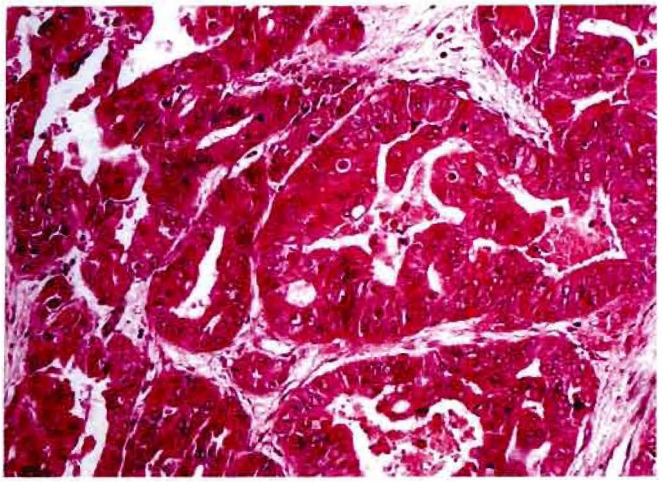

Fig 2.4. Intestinal type carcinoma.

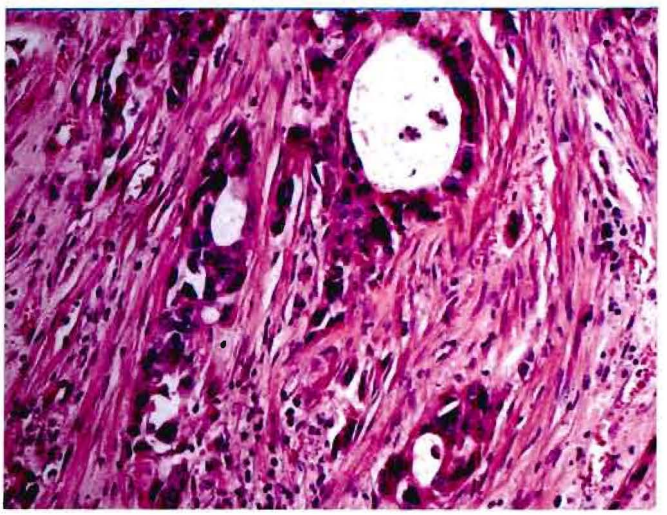

Fig 2.5. Pancreaticobiliary type carcinoma

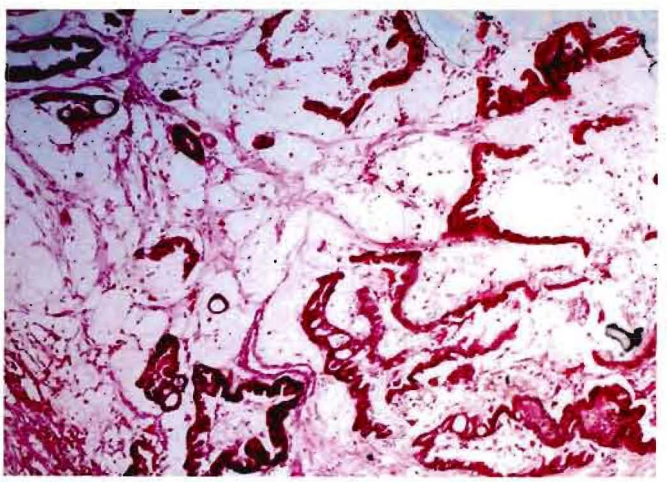

Fig 2.6. Mucinous carcinoma.

Carcinoma involved the pancreatic resection margin in 2 cases (5%). Ten patients (27%) were lost to follow up. Follow up ranged from 2 days to 216 months with a mean follow up time of 65 months and a median follow up time of 38 months (SD 66.1). The patient who was followed up for 216 months was still alive 18 years after the operation. Perineural invasion (Fig. 2.7) was noted in 9 patients (24%). Vascular invasion (Fig. 2.8) was noted in 8 cases (22%) and lymphatic invasion was noted in 12 cases (32%).

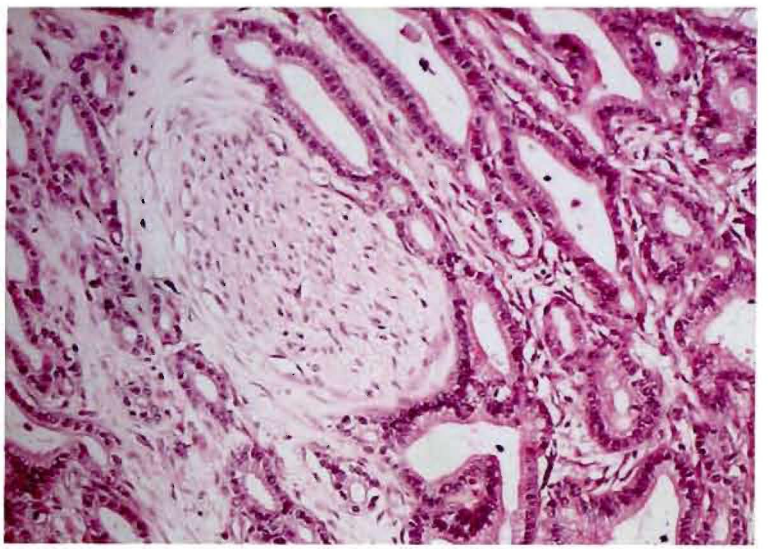

Fig 2.7. Perineural invasion.

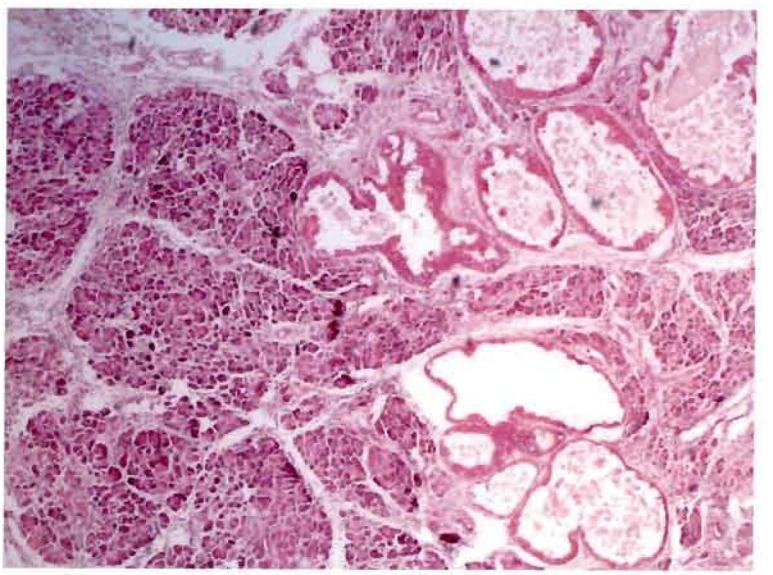

Fig 2.8. Pancreatic invasion.

The association between NF-1 and periampullary somatostatinomas is well described (40). The current series included a patient with ampullary adenocarcinoma as well as a second periampullary neuroendocrine tumour, which was proven to be a somatostatinoma with immunohistochemical staining. Although not indicated in the original clinical information, the patient was later confirmed to have NF-1 after this was suggested by the reporting pathologist. The tumour had a distinctive packeted appearance with some glandular features. The cytoplasm was eosinophilic, granular and voluminous. No psammoma bodies were noted. There was no connection with the ampullary adenocarcinoma (Fig 2.9).

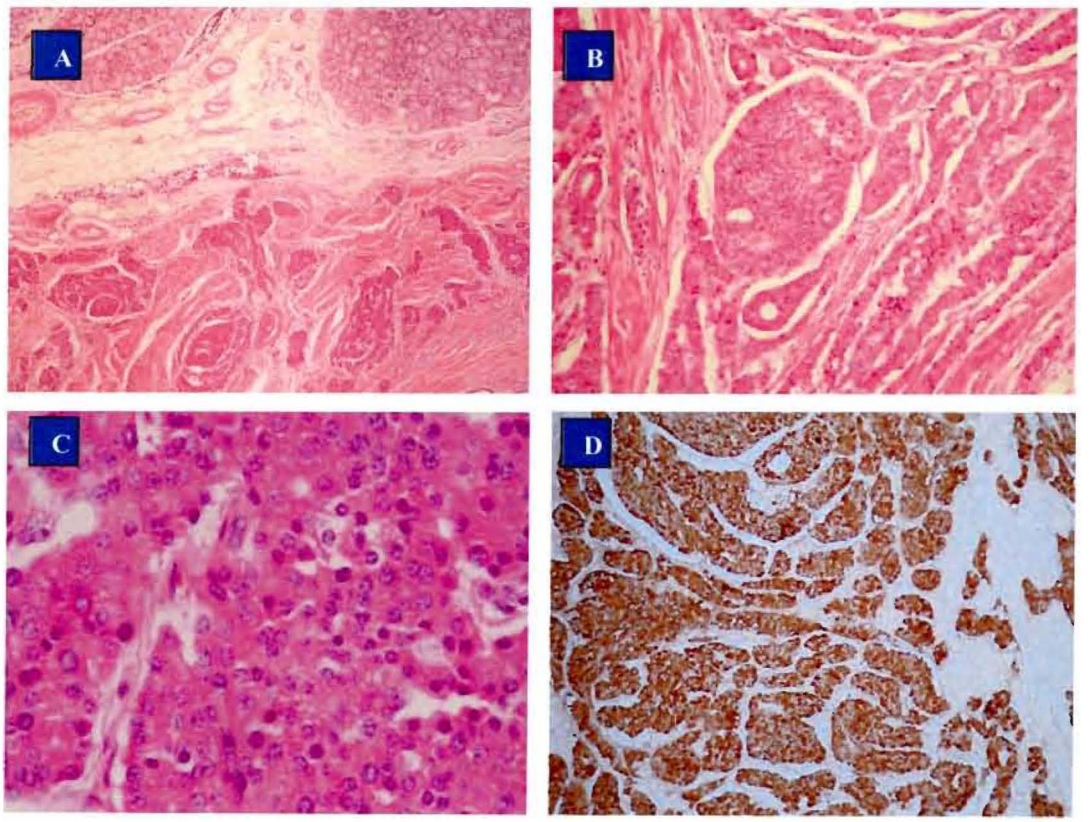

Fig 2.9. A & B) The somatostatinoma is composed of islands, glandular structures and trabeculae and can be seen to infiltrate muscle. C) The cells have voluminous eosinophilic cytoplasm and round nuclei with a stippled appearance. D) The positive somatostatin immunohistochemical stain.

Another patient who had a Whipple resection for ampullary adenocarcinoma was a 53 year old female. Sections from the pancreas of the resected specimen showed extensive

fatty replacement. She was also known to have asthma and a cardiomyopathy. Lipomatous pseudohypertrophy of the pancreas is associated with chronic pulmonary disease (120). One is unable, given the pancreatic duct obstruction, to make a confident diagnosis in this patient but the co-existent asthma and cardiomyopathy was an interesting finding. Other interesting findings were carcinomas with squamous differentiation and carcinomas with numerous tumour giant cells.

Table 2.2. A summary of the TNM classification and staging.

T	Number	Percentage
T1	9	24%
T2	11	30%
T3	14	38%
T4	3	8%

N	Number	Percentage
N0	30	77%
N1	7	23%

No distant metastases were recorded.

Stage	Number	Percentage
I	9	24%
II	19	51%
III	7	19%
IV	2	5%

Table 2.3. A summary of Martin's classification.

Martin classification	Number	Percentage
I	5	49%
II	7	19%
III	7	19%
IV	18	49%

Table 2.4. A summary of the positive immunohistochemical stains.

	Carcinoma (n=37)	Adenoma (n=7)	Normal epithelium (n=33)
p53	13 (35%)	3 (42%)	0 (0%)
Ki-67	29 (78%)	3 (42%)	11 (33%)
MUC1	19 (51%)	2 (28%)	0 (0%)
MUC1c	15 (40%)	2 (28%)	0 (0%)
MUC2	8 (21%)	5 (71%)	33 (100%)
Ca19.9	31 (84%)	5 (71%)	10 (30%)

The *p53* staining was strong nuclear positivity. Thirteen (35%) carcinomas stained positively while the normal epithelium was negative. *Ki-67* staining was strong and nuclear. The highest percentage of staining (78%) was seen in the carcinomas (Fig 2.10).

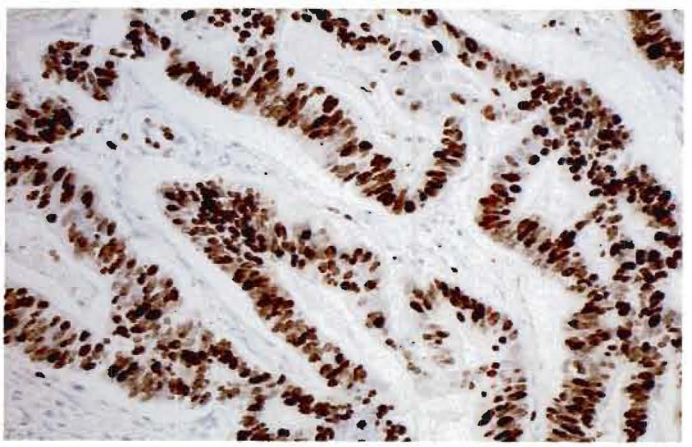

Fig 2.10. The strong nuclear staining of Ki-67 in a carcinoma with a labeling index of >15%.

MUC1 and MUC1 core antigens had a similar staining pattern - strong luminal and cytoplasmic staining. The extra-cellular mucin was also positive. Normal epithelium, including the accessory pancreatic ducts, was negative with both anti-bodies. MUC1 was positive in just over half of the carcinomas (51%) and MUC1 core in 40% (Fig 2.11 and 2.12).

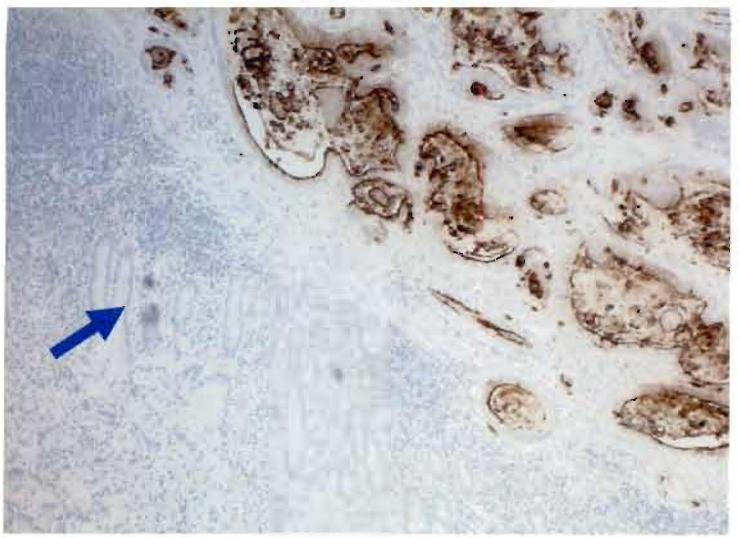

Fig 2.11. A) Strong MUC1 positivity in a carcinoma. The overlying mucosa (blue arrow) is negative.

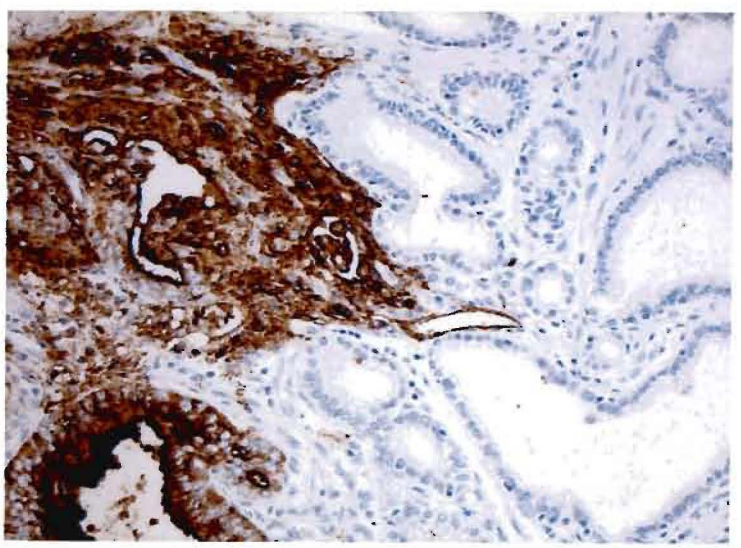

Fig 2.12. This picture contrasts the strong MUC1 positivity in a carcinoma with the negative adjacent accessory pancreatic ducts.

The goblet cells in the duodenum were positive with MUC2 in all cases (Fig 2.13). Only 21% of the carcinomas stained positively with MUC2, the rest were negative (Fig 2.14).

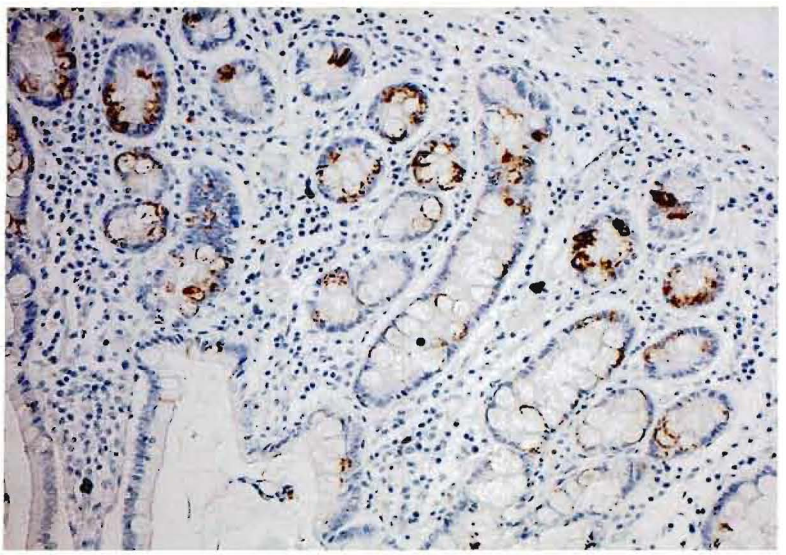

Fig 2.13. The photograph demonstrates MUC2 positivity in the non-neoplastic mucosa.

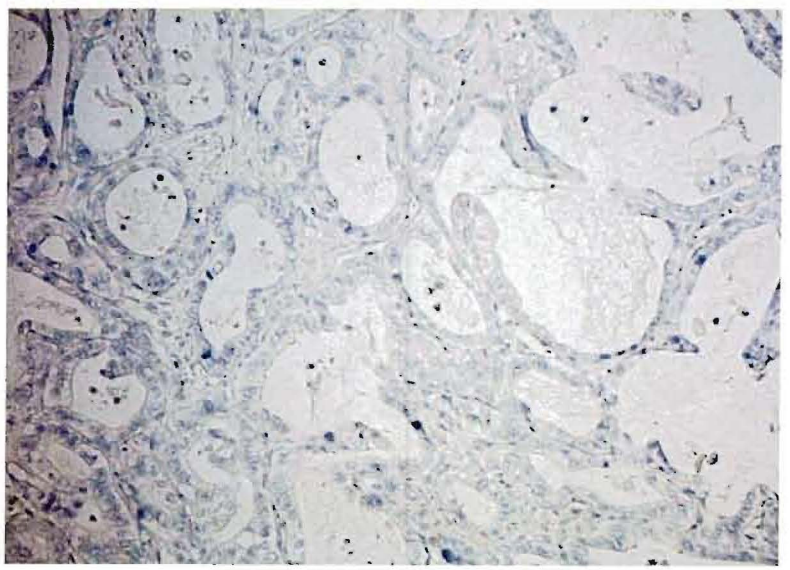

Fig 2.14. This carcinoma stained negatively with MUC2.

The staining pattern for Ca19.9 was luminal and cytoplasmic. A total of 31 (84%) carcinomas stained positively with this stain (Fig 2.15). Five adenomas (71%) were positive and in 10 (30%) cases there was weak staining of the normal epithelium.

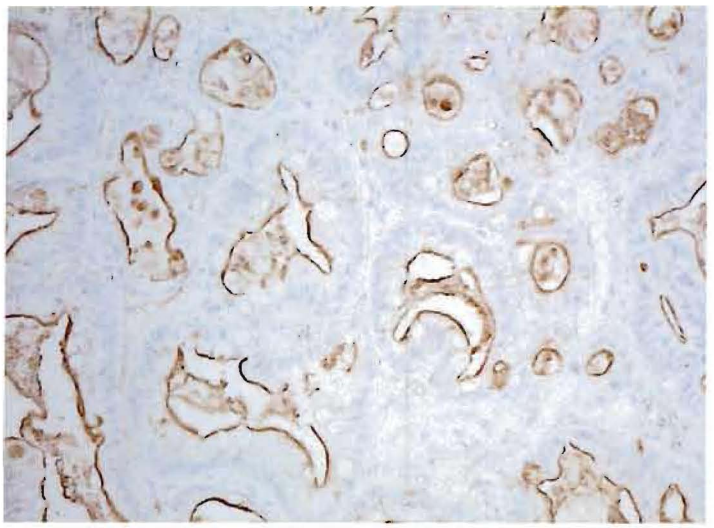

Fig 2.15. An example of CA19.9 staining in a carcinoma which is luminal and weak cytoplasmic.

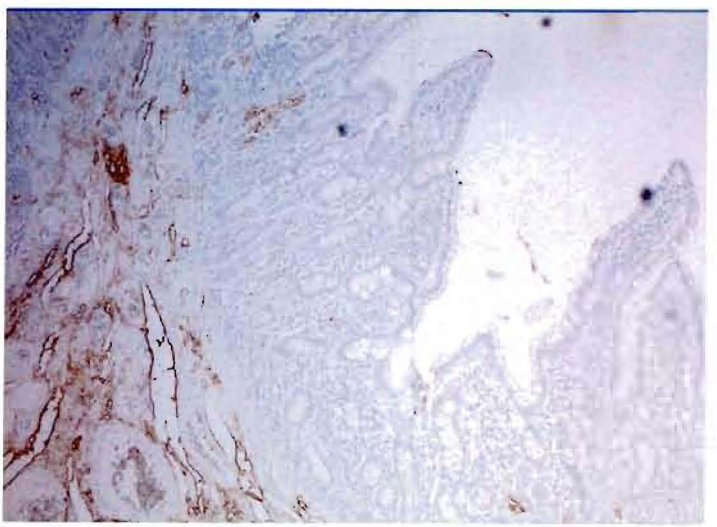

Fig 2.16. Ca19.9: positive staining can be seen in the carcinoma in the left lower corner of this picture. The normal mucosa overlying the carcinoma is negative.

2.3.3 STATISTICAL RESULTS

The detailed Kaplan Meier survival estimates for patients after Whipple resection for ampullary adenocarcinoma can be seen in Appendix 9. The 1 year survival estimate was 78% (SE 0.08). The 5 year survival rate was 44% (SE 0.1) (Fig 2.17).

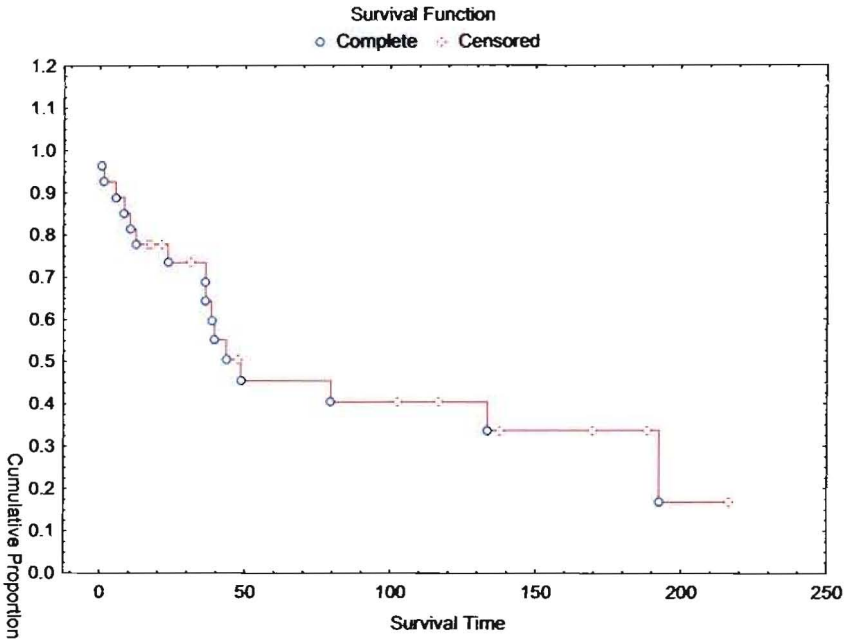

Fig 2.17. Kaplan Meier overall survival estimate

Using the Kaplan Meier method, patients with T1 tumours had a distinctly better survival compared to patients with T2, T3 and T4 tumours as a group (P = 0.0009). However, there was no statistical significance in the survival estimates between patients with T2, T3 and T4 tumours (Fig 2.18).

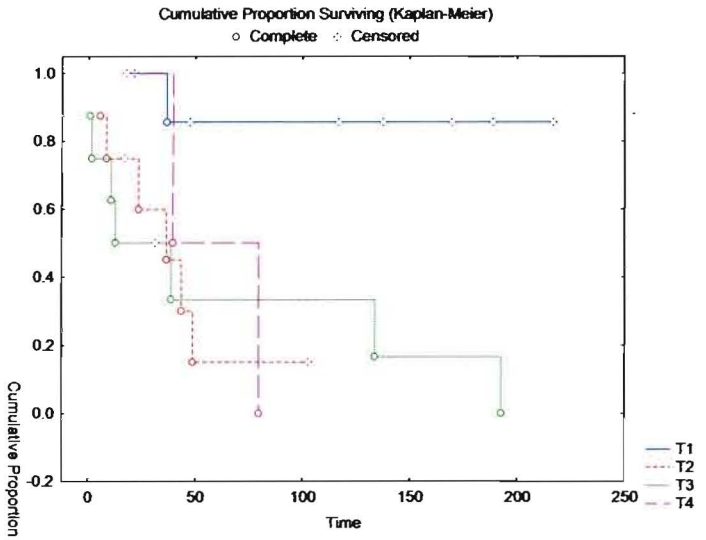

Fig 2.18. Kaplan Meier survival estimate influenced by T-stage.

The overall stage is a variable which is co-linear with T-stage but is influenced by the N-stage (M-stage was constant since no metastases were recorded). Patients with stage I tumours had a significantly better survival compared to patients with stage II, III and IV tumours (P = 0.012). However, there was no significance in the estimates between patients with stage II, III and IV tumours (Fig 2.19).

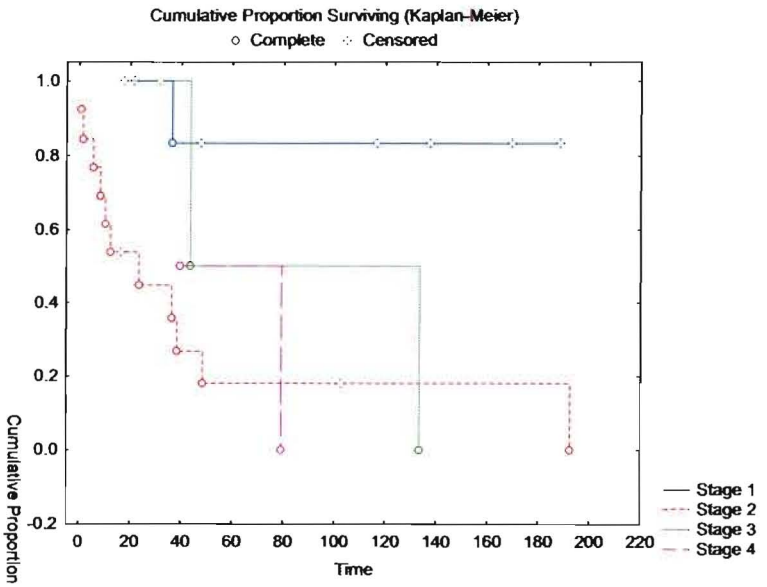

Fig 2.19. Kaplan Meier survival estimate influenced by stage

The immunohistochemical stains, including *p53*, *Ki-67*, MUC1, MUC1c, MUC2 and CA19.9 did not show any significant influence on survival, nor did perineural invasion, vascular invasion, lymphatic invasion, resection margins, differentiation, or histological type (Table 2.5).

Table 2.5. Summary of variables with insignificant P-values.

Variable	**P-value**
p53	0.283
Ki-67	0.6
MUC1	0.97
MUC1c	0.897
MUC2	0.361
Ca19.9	0.072
Martin classification	0.2
Perineural invasion	0.72
Vascular invasion	0.9
Lymphatic invasion	0.7
Histological sub-type	0.41
Resection margins	0.43
Differentiation	0.573

2.4 DISCUSSION

The age range in this study (27 - 83 years) is very similar to that of a large study from the Memorial Sloan-Kettering Cancer Center (28.3 - 87 years) (27). The mean age of 58.8 years is also similar to previous reports (27, 31). The youngest patient in the collective reported by Howe et al. (27) had FAP. None of the younger patients in this study had any known disease associations. In the present study, there was a slight female predominance (51%), whereas previous studies recorded a definite male predominance (31, 55). The mean tumour size of 2.5 cm is similar to the mean size reported in previous studies (27).

Intestinal type tumours (51%) were slightly more common than the pancreaticobiliary subtype (46%). This finding is in keeping with the study of Howe et al. (27) who found that most of the ampullary cancers (70%) in their cohort of 123 patients had intestinal type morphology. They suggest that ampullary tumours are biologically more similar to intestinal than pancreatic carcinomas. However, other collectives found the pancreaticobiliary subtype to predominate over the intestinal subtype (76, 77). A recent study (2004) by Zhou et al. found a good correlation between CK7 and CK20 expression, and histological sub-classification. The pancreaticobiliary type carcinomas were CK7+ and CK20- whilst the intestinal type carcinomas were CK20+ and CK7-. In addition, they found all the intestinal type carcinomas in their cohort to be positive with MUC2 (79). In the present study, only 8 (50%) of the intestinal type tumours stained positively with MUC2. This finding is difficult to explain and may be due to discrepancies in the histological classification based on examination of the H&E sections.

Despite the fact that adenocarcinoma of the ampulla of Vater is relatively uncommon, there have been numerous reports on these tumours in the literature. The number of patients included in these studies range from 31 - 459 patients: the largest multi-institutional study consisted of 459 patients collected from 57 centers in Japan between 1949 and 1974 (121). The largest single institutional series consisted of 120 patients and was reported from Johns Hopkins (1969 - 1996) (86).

The current series consists of 37 patients who had a Whipple resection for ampullary adenocarcinoma at a single institution from 1979 - 2003. The histopathological and the immunohistochemical features, most of which have been claimed to have prognostic significance, were examined.

Previous studies attempting to define important prognostic variables for patients with ampullary carcinomas have been limited in several ways. Most have limited numbers of patients, operated on over several decades. Many have not performed multivariate analyses to identify independent prognostic factors. Perhaps the most significant problem with these studies is the lack of review to confirm that tumours originate from the ampulla of Vater. Accidental inclusion of tumours of the pancreas, bile duct or duodenum could dramatically alter the results of survival analysis. This is often a difficult task and may be subject to some interpretation on the part of the pathologist (27). Monson et al. found that only 74 of 104 tumours initially thought to be ampullary carcinomas at the Mayo Clinic could be confirmed to have arisen from the ampulla. Seven were reclassified as having originated from the pancreas, 6 from the bile duct, 2 from the duodenum, and in 15, the origin could not be determined (122).

The current study had several limitations which made the interpretation of data challenging.

- Firstly, due to the rarity of this tumour a limited number of cases were available for evaluation.
- These cases stretched over several decades and some of the earlier reports on patient outcome were from a time when the Whipple procedure carried with it a risk of substantially higher mortality than it does today. The result is that relatively few of these procedures were performed in the past. During the last two decades, improvements in anesthetic techniques, critical care and institutional specialization have decreased mortality rates (23).
- The macroscopic description at the time of dissection is one of the most important factors in the precise localization of a periampullary tumour. Therefore, the original macroscopic description in the pathology report was

crucial for patient selection. Before the prognostic significance of the exact localization of these tumours was described, many tumours in this area were simply described as "periampullary carcinoma" or "adenocarcinoma, not otherwise specified". Also, in advanced disease, identifying the exact origin of the tumour is sometimes just not possible. If there was any doubt as to the origin of the tumour, the case was excluded from this study. Consequently, more than 15 potential cases had to be omitted.

- Very few of the original reports included a block key in order to be certain where the specimen was sampled. This is especially important in the sampling of resection margins.
- Lastly, a problem hampering prognostic studies in South Africa is clinical follow up of patients, especially those from rural areas and previously disadvantaged communities. A total of 10/37 (27%) patients were lost to clinical follow up in this study.

Due to the problems encountered as mentioned above, the number of patients which could be confidently included in this study, was much smaller than the expected number when this study commenced. Optimally, a cohort of at least 50 patients is necessary for accurate multivariate analysis. Despite the relatively small number of cases, multivariate analysis was performed on the potential prognostic variables and the 5 year survival rate of the patients with follow up data was calculated. The overall 5-year survival rate in the present study was 44%, which is comparable to post operative survival in cohorts stretching over a similar time frame: Howe et al. (27) (1983 - 1995) reported a 5 - year survival rate of 46% and Chareton et al. (1) (1970 - 1992) reported a 5 - year survival rate of 38%. It is clear that the studies which include patient data from long ago have not benefited from the improvements made in patient care and improved surgical technique over the past two decades. There are wide variations in the 5-year survival rates reported in the different collectives, with the lowest survival rates coming from series dating back many decades. The lowest 5-year survival rate (6%) was reported by Nakase et al. (121). Their cohort of patients stretched from 1949 - 1974. The highest 5-year survival rate (67%) was reported by Duffy et al. (58) who studied 55

consecutive patients who had a Whipple resection for ampullary carcinoma from 1988 through 2001.

The only prognostic variable which significantly influenced survival with multivariate analysis in this study was the T-stage: T1 tumours, which are co-linear with the stage 1 tumours, had statistically significantly better survival compared to the higher stages (T2, T3 and T4) (Fig 2.18). However, there was no difference in survival between the T2, T3 and T4 tumours. Neoptolemus et al. found tumour stage to be significant by multivariate analysis in their study (123). Several other studies confirmed the prognostic value of TNM and stage groupings (105-110). Surprisingly, multivariate analysis did not reveal any prognostic significance for the following variables: nodal metastases, tumour grade, lymphatic/vascular invasion, resection margins, tumour size, Martin's classification or histological sub-classification (Table 2.5). Previous studies confirm the significant association of nodal metastases (106, 107, 111), tumour grade (105, 106, 123), lymphatic/vascular invasion (105, 109), resection margins (106), Martin's classification and tumour size (82). Kitamura et al. (80) claimed that histological sub-typing had prognostic value, but neither Zhou et al. (79) nor the present study could confirm this finding. Previous authors claimed that the absence of perineural invasion was a major factor associated with prolonged survival in patients with ampullary carcinomas (57, 105). The current study could not confirm perineural invasion to be an adverse prognostic factor (Table 2.5). On the contrary, perineural invasion was noted in the patient who was still alive at 216 months follow up. The *Ki-67* labeling index was also >15% in this case, which was found to be a poor prognostic indicator by other authors (113). However, this tumour displayed several of the well accepted good prognostic indicators including a T1N0M0, stage 1 tumour, clear resection margins and no lymphatic invasion.

Several immunohistochemical markers, including certain mucin core antigens, have been claimed to have prognostic significance in ampullary carcinomas. Prior to commencement of this study, a literature review produced reports on the prognostic value of the following immunohistochemical stains: *p53* (51, 59), MIB1/*Ki-67* (113),

MUC1, MUC1core, MUC2 (56) and CA19.9 (115,116). These immunohistochemical stains were therefore included in this study.

The insignificant prognostic value of *p53* in this study (Table 2.5) is in keeping with earlier observations that *p53* over expression is not useful as a prognostic biomarker for ampullary carcinomas (51, 59). Neither CA19.9 nor the *Ki-67* labeling index had any significant prognostic value in the present study (Table 2.5). There are several reports on the poor prognosis of ampullary carcinomas expressing CA19.9 (115, 116) and of carcinomas with a *Ki-67* labeling index of > 15% (113).

A previous study concluded that MUC1 expression in ampullary carcinomas correlated with a significantly poorer survival whilst patients with tumours expressing MUC2 showed significantly more favorable survival (56). This study did not show any significant prognostic value for any of these mucin core antigens (Table 2.5). However, a striking observation was the expression of MUC1 and MUC1c in respectively 51% and 40% of ampullary carcinomas and in 28% of adenomatous areas adjacent to carcinoma. No staining was observed in the normal epithelium. These findings are in concordance with the findings of Kitamura et al. (54) who found a high expression rate of MUC1 in ampullary carcinomas but rare or no expression in the adjacent normal epithelium. MUC2 antibody expression was the reverse of MUC1 and MUC1c expression; 100% of the adjacent normal epithelium expressed MUC2 in the goblet cells, 71% of adenomas and only 28% of carcinomas expressed MUC2. These findings suggest that the combination of MUC1 and MUC2 antibodies could be helpful in the diagnosis of ampullary carcinomas in small endoscopic biopsies. If the endoscopist is convinced that the lesion is localized in the ampulla of Vater, a positive MUC1 stain and a negative MUC2 stain would support the diagnosis of an ampullary carcinoma or dysplastic epithelium in an adenoma. Conversely, a negative MUC1 stain and the presence of MUC2 positive goblet cells are in keeping with non-neoplastic ampullary or duodenal epithelium.

In conclusion, the results of this study do not support a role for any immunohistochemical stains as part of the routine work-up for ampullary carcinomas. Despite the small number of patients, the credibility of this study lies with the strict case selection in order to be certain that only patients with unequivocal ampullary carcinomas were included in this study. This cohort consisted of a relatively small number of patients mainly due to the strict selection criteria and patients lost to follow up.

At present the Royal College of Pathologists (RCPath) only includes TNM and stage grouping, resection margins, perineural invasion, vascular invasion and maximum tumour diameter as prognostic variables in the pathology data sheet for ampullary carcinomas (118). They do not consider the use of special techniques to assess DNA ploidy, proliferation markers, oncogenes, nuclear morphometry or mucin core antigens justifiable in a minimum data set. Immunohistochemistry to detect micro metastases in H&E stained tumour-free nodes is also not currently recommended. The current RCPath minimum pathology data sheet for ampullary carcinomas is attached as Appendix 12. (118).

CHAPTER 3: HISTOCHEMICAL MUCIN PROFILE OF AMPULLARY CARCINOMAS

The study entailed the examination of the histochemical mucin production by ampullary carcinomas. The hypothesis was that certain histochemical mucin profiles may correlate with the histological subtyping of ampullary carcinomas.

3.1 AIM

- To ascertain whether the histochemical mucin profile of ampullary carcinomas correlates with the histological sub-classification suggested by Albores - Sevaadra et al. (77).

3.2 MATERIALS AND METHODS

This research proposal was submitted to the Groote Schuur Hospital/ University of Cape Town Ethics committee and approval was obtained.

This was a retrospective study comparing the histochemical mucin profiles of 37 ampullary adenocarcinomas from patients who had a Whipple resection at Groote Schuur Hospital. Formalin fixed wax embedded tissue was retrieved from the archives of Anatomical Pathology Division of Groote Schuur hospital. For each case a single representative block was chosen. The block contained the most representative sample of the carcinoma. The ampullary carcinomas were classified histologically according to the criteria of Albores - Saavedra et al. (77). The two histochemical stains used to elucidate the nature of the mucin secreted were high iron diamine (HID) and the combined alcian blue PAS technique for acid and neutral mucins (For technique and solutions please see appendix 10 and 11)(124). The mucin profile of the different histological types of ampullary carcinomas was compared.

3.2.1 BACKGROUND ON MUCINS

The different types of mucins can be divided as follows:

- Neutral mucins: there are no subdivisions to this group. They are periodic acid Schiff (PAS) positive but do not stain with alcian blue.
- Acidic mucins: this group is divided into sulphated mucins, carboxylated mucins and sulphated sialomucins. The carboxylated mucins include the sialidase-labile sialomucins (present mainly in salivary and bronchial glands), the sialidase-resistant sialomucins (present mainly in the gastrointestinal tract) and hyaluronic acid (found mainly in the umbilical cord).
- The sulphated sialomucins is a controversial group and there has been some debate as to whether this group constitutes a separate entity or merely occurs in situations in which there is a mixture of a sulfomucin and a sialomucin (124).

Sulphated mucins are clearly and specifically demonstrated by the HID stain. The rationale is that, by treating sections with a mixture of certain diamine salts and ferric chloride, a black brown cationic complex is formed that bonds to sulphate containing moieties. Thus, with the HID stain, sulphated mucins stains black/brown, and carboxylated mucins stain blue (124).

3.2.2 HISTOCHEMICAL ANALYSIS

The HID stain was interpreted as follows:

- Mucin staining only black/brown = sulphated mucin
- Mucin staining predominantly black/brown with significant blue staining = predominantly sulphated mucin
- Mucin staining equally with black/brown and blue = mixed
- Mucin staining predominantly blue with some black/brown staining = predominantly sialomucin
- Mucin staining only blue = sialomucin

The AB PAS stain was interpreted as follows:

- Mucin staining only aquamarine = sulphated or carboxylated mucin
- Mucin staining predominantly aquamarine and significant magenta staining = predominantly sulphated or carboxylated mucin
- Mucin staining equally with aquamarine and magenta = mixed
- Mucin staining predominantly with magenta and significant aquamarine staining = predominantly neutral mucin
- Mucin staining only magenta = neutral mucin

3.3 RESULTS

Table 3.1. A summary of the mucin secreted in the different histological types of ampullary carcinoma.

	Su	PSu	Mi	PSialo	Sialo	NS	Total
Int type	1	5	5	3	3	2	19
PB type	0	6	3	4	3	0	16
Muci	0	0	1	1	0	0	2
All grps	1	11	10	7	6	2	37

Abbreviations used in table 3.1:

Su = Sulphated mucin

PSu = Predominantly sulphated mucin

Mi = Mixed

PSialo = Predominantly sialomucin

Sialo = Sialomucin

Int type ca = Intestinal type ampullary carcinoma

PB type = Panreaticobiliary type ampullary carcinoma

NS = No significant staining

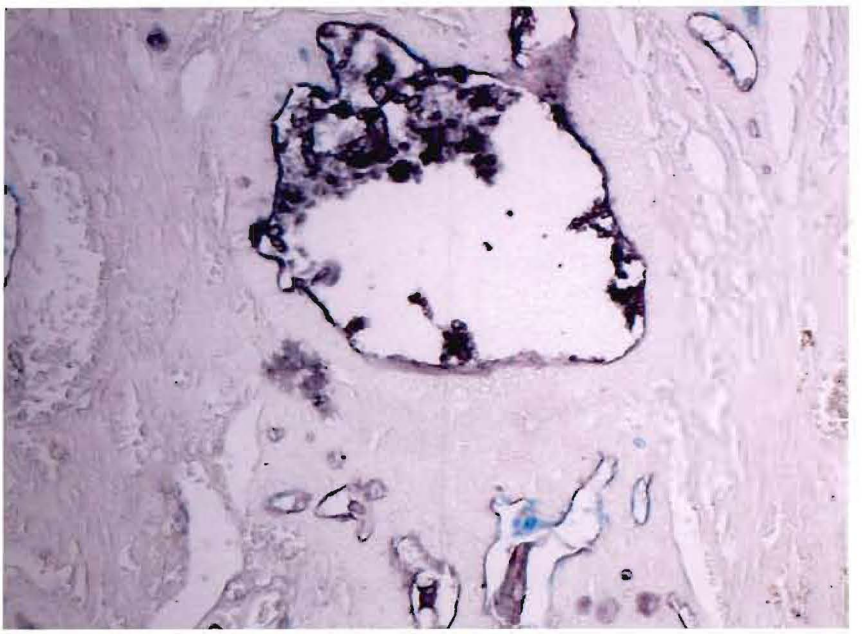

Fig 3.1. HID stain demonstrating predominantly sulphated mucins (black staining) and a trace of sialomucins (blue staining) in a carcinoma.

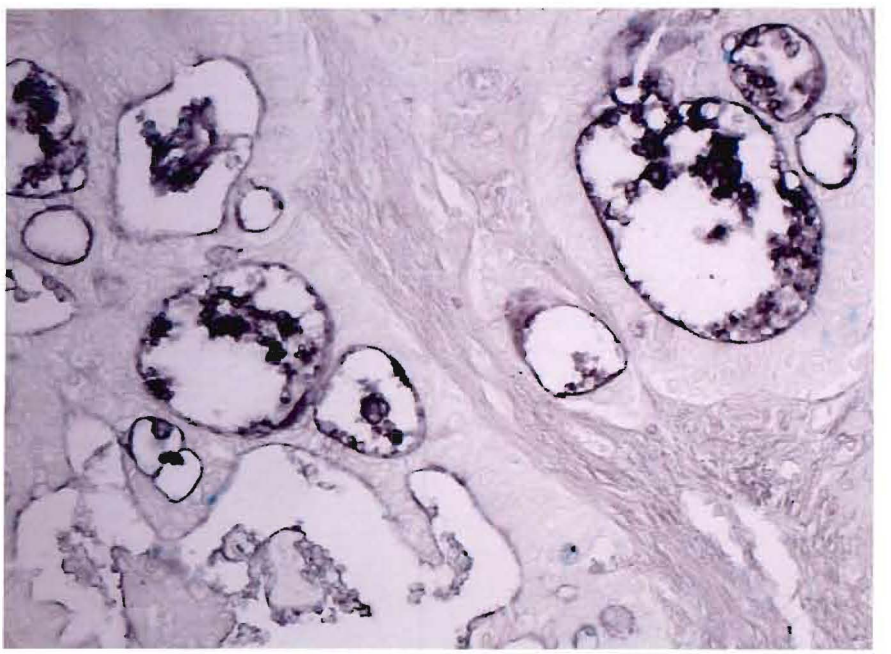

Fig 3.2. HID stain demonstrating only sulphated mucins in this carcinoma.

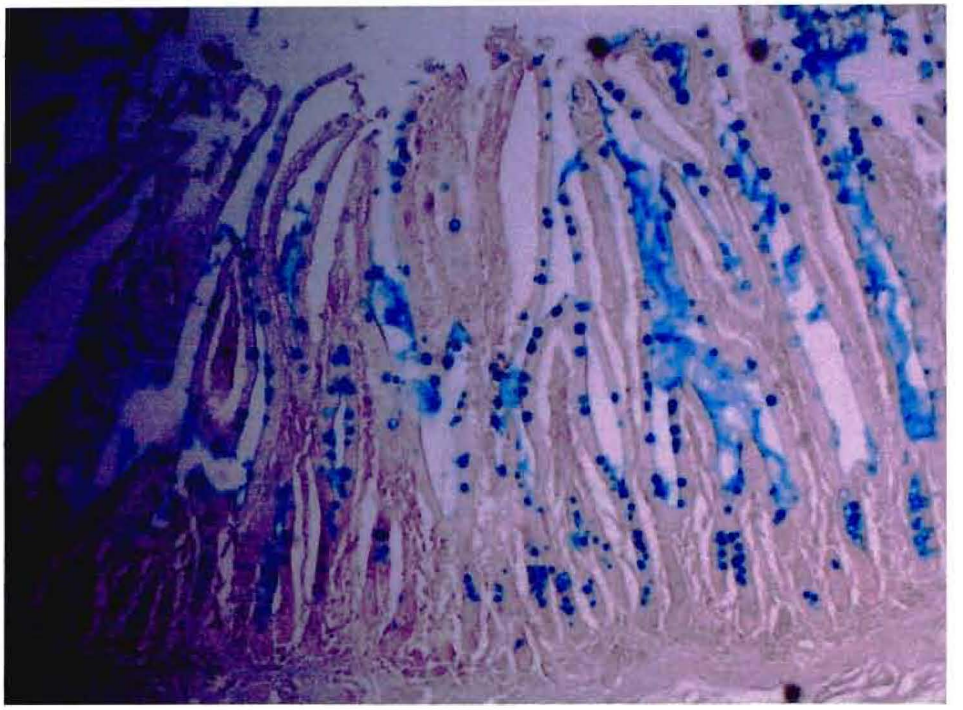

Fig 3.3. HID stain demonstrating sialomucin in the goblet cells of the normal overlying epithelium.

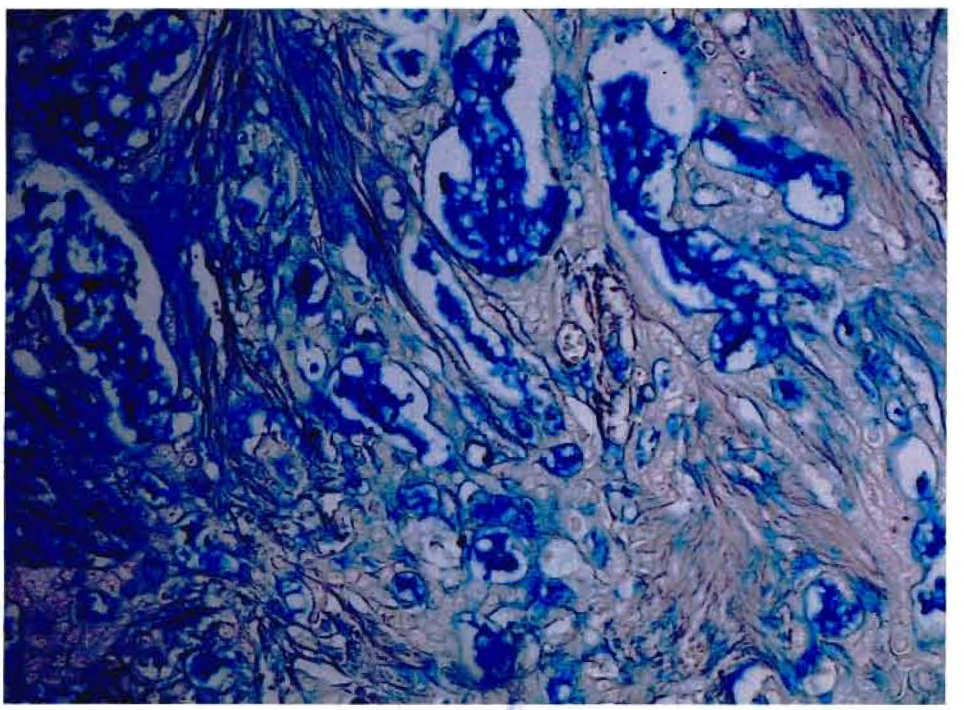

Fig 3.4. This carcinoma secreted only sialomucin as seen on the HID stain.

3.3 DISCUSSION

In the ampulla of Vater, the different types of epithelium that converge at this point secrete different types of mucin. The main pancreatic duct contains predominantly sulphated mucin, as does the epithelium of the gall bladder (125). The duodenal surface mucosa contains sialomucin and Brunner's glands neutral mucins, and the common bile duct contains predominantly sialomucin. The staining of the ampulla of Vater varies: sialomucin predominates in the lining epithelium near the opening into the duodenum. Deeper in the ampulla the lining epithelium, as well as the glands lying beneath the epithelium, secrete principally sulphated mucin (5).

In the current study the possibility of a correlation between the histological subtyping and the histochemical mucin profile of ampullary carcinomas were investigated (77). The hypothesis was that a histochemical mucin profile, which is a relatively inexpensive additional test, may aid in the histological subclassification of ampullary carcinomas. This study was a byproduct of the study termed "Morphological and immunohistochemical features of ampullary carcinomas".

The results for the different histological types of ampullary carcinomas were as follows:

- 32% of the intestinal-type ampullary carcinomas secreted predominantly sulphated mucin, 42% secreted predominantly sialomucin and 26% secreted mixed sulphated and sialomucin. Two of the intestinal-type tumours had no significant staining.
- 38% of the pancreaticobiliary-type ampullary carcinomas secreted predominantly sulphated mucin, 44% secreted predominantly sialomucin and 19 secreted mixed sulphated and sialomucin.
- One of the mucinous carcinomas secreted predominantly carboxylated mucin whilst the other secreted mixed sulphated and sialomucin

In comparison with the intestinal type and the mucinous tumours, a slightly higher percentage of pancreaticobiliary type tumours secreted sulphated mucins. The majority

14. Newman HF, Weinberg SB, Newman EB, et al. The papilla of Vater and distal portions of the common bile duct and the duct of Wirsung. Surg Gynecol Obstet 1958;106:687-694.

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Appendix 1

sex	diagnosis	adenoma	operation	tumour dif	age	tumour size
M	AC	y	st whip	mod	59	2.5
F	AC	n	st whip	mod	83	2.7
M	AC	n	st whip	mod	76	5
M	AC	y	st whip	mod	45	2.5
F	AC	n	st whip	mod	41	2
F	AC	n	st whip	mod	62	1
M	AC	y	pppd	mod	53	2
M	AC	n	pppd	mod	52	n/s
F	AC	y	pppd	mod	65	n/s
F	AC	y	st whip	poor	72	1
F	AC	n	pppd	poor	27	3
M	AC	n	pppd	mod	66	n/s
F	AC	n	pppd	mod	56	1.5
M	AC	y	st whip	mod	66	5
F	AC	n	pppd	poor	57	3
F	AC	y	pppd	poor	62	6
M	AC	n	pppd	mod	62	n/s
F	AC	y	pppd	mod	62	4
F	AC	n	pppd	mod	52	1.5
F	AC	y	pppd	poor	45	1.3
F	AC	n	pppd	well	74	n/s
M	AC	n	pppd	mod	45	n/s
F	AC	n	pppd	poor	53	2
M	AC	n	pppd	mod	71	1.4
M	AC	n	pppd	mod	44	2.5
M	AC	y	pppd	mod	71	1.7
F	AC	n	pppd	poor	70	2
M	AC	n	st whip	mod	48	2
F	AC	n	pppd	well	53	1
M	AC	n	pppd	well	60	n/s
F	AC	n	pppd	well	37	2.5
M	AC	y	pppd	well	42	0.7
M	AC	n	pppd	mod	37	n/s
F	AC	y	pppd	poor	59	2
M	AC	n	pppd	mod	36	3.5
M	AC	n	pppd	mod	64	2.4
F	AC	y	pppd	mod	78	4

Appendix 2

Res marg	Perineur inv	Vasc inv	lymphatic
clear	n	n	n
involved	n	n	n
clear	n	n	n
clear	n	n	n
clear	n	n	n
clear	n	n	n
clear	y	n	n
clear	y	y	y
clear	n	n	n
clear	n	y	y
clear	n	n	n
involved	y	n	y
clear	n	n	n
clear	n	n	n
clear	n	n	y
clear	n	n	n
clear	y	y	n
clear	n	n	y
clear	n	n	n
clear	n	n	y
clear	y	n	n
clear	n	n	n
clear	n	n	n
clear	n	n	n
clear	n	n	n
clear	n	y	y
clear	n	n	n
clear	y	y	y
clear	n	n	n
clear	y	y	y
clear	n	n	y
clear	y	y	y
clear	n	n	y
clear	n	n	n
clear	y	y	n
clear	n	n	n
clear	n	n	n

Appendix 3

HID	PAB	mucin type	histological type
bk++am+	am++	psu	it
bk++am++	am+++	m	col
bk+++am+	am++ma+	psu	it
bk+am+	am+ma+p+	m	it
bk+am++	am+ma+p+	psi	it
bk++am+	am++ma+	psu	it
bk++am++	am++ma+p+	m	it
am++	am+ma+	si	it
bk+am+	am+	m	pb
bk+am++	am++	psi	it
bk+am+	am+ma+	m	pb
bk+++am++	am++ma+	psu	pb
bk+am+	am+	m	it
bk++am++	am++p+	m	it
bk++am+	am++p+	psu	pb
am+	am++ma+	si	pb
am+	am+	si	pb
bk+++am+++	am+++	psu	it
bk++am+	am+	psu	pb
am+	am+p+	si	it
bk+am+++	am+	psi	pb
no staining	no staining	ns	it
bk+	am+	su	it
bk+am+	am+ma+	m	pb
bk++am+++	am+++	psi	col
am+	am+ma++	si	it
bk+am+++	am++ma++	psi	pb
bk+am++	am+	psi	it
bk+am+++	am++ma+	psi	pb
bk+++am+	am+++ma+	psu	pb
bk+++am++	am++p++	psu	it
bk+++am++	am++ma+	psu	pb
bk+++am+	am++	psu	pb
am+++	am+++p+	si	pb
bk+am+++	am++p+	psi	pb
bl+am+	ma+++	m	it
no staining	no staining	it	it

Appendix 4

P53 ca	MIB1 ca	MUC1 ca	MUC1c ca	MUC2 ca	Cerb B2 ca	Ca 19.9 ca
neg	neg	pos	pos	neg	neg	pos
pos	neg	pos	pos	neg	neg	pos
neg	pos	pos	pos	neg	neg	pos
neg	neg	pos	pos	pos	neg	pos
neg	pos	pos	pos	neg	neg	pos
neg	pos	neg	neg	neg	neg	pos
neg	pos	neg	neg	neg	neg	pos
pos	pos	pos	pos	neg	neg	pos
pos	pos	neg	neg	pos	neg	neg
neg	pos	pos	pos	neg	neg	pos
pos	pos	neg	neg	neg	neg	pos
pos	neg	neg	neg	neg	neg	neg
pos	pos	pos	neg	neg	neg	pos
neg	pos	neg	neg	pos	neg	pos
pos	pos	neg	neg	neg	pos	pos
neg	pos	pos	pos	pos	neg	pos
pos	neg	pos	pos	neg	neg	neg
neg	pos	neg	neg	pos	neg	pos
neg	neg	pos	pos	neg	neg	pos
pos	pos	pos	pos	neg	neg	pos
neg	neg	neg	neg	neg	neg	pos
neg	pos	pos	pos	neg	neg	pos
pos	pos	pos	neg	neg	neg	pos
pos	pos	neg	neg	neg	neg	pos
neg	pos	neg	neg	pos	neg	pos
pos	pos	neg	neg	neg	neg	pos
neg	pos	pos	pos	neg	neg	pos
neg	pos	neg	neg	neg	neg	neg
neg	pos	pos	neg	neg	neg	pos
neg	pos	neg	neg	neg	neg	neg
neg	pos	pos	pos	neg	neg	pos
neg	pos	neg	neg	neg	neg	pos
neg	pos	pos	pos	pos	neg	pos
neg	neg	neg	neg	neg	neg	neg
neg	pos	neg	neg	neg	neg	pos
pos	pos	neg	neg	neg	neg	pos
neg	pos	neg	neg	pos	neg	pos

Appendix 5

P53 ad	MIB1 ad	MUC1 ad	MUC1c ad	MUC2 ad	CerbB2 ad	CA19.9 ad
neg	neg	neg	neg	pos	neg	pos
a	a	a	a	a	a	a
a	a	a	a	a	a	a
neg	neg	pos	pos	pos	neg	pos
a	a	a	a	a	a	a
a	a	a	a	a	a	a
neg	pos	pos	pos	pos	neg	pos
a	a	a	a	a	a	a
pos	neg	neg	neg	pos	neg	neg
pos	pos	neg	neg	pos	neg	pos
a	a	a	a	a	a	a
a	a	a	a	a	a	a
a	a	a	a	a	a	a
neg	neg	neg	neg	pos	neg	pos
a	a	a	a	a	a	a
a	a	a	a	a	a	a
a	a	a	a	a	a	a
a	a	a	a	a	a	a
a	a	a	a	a	a	a
a	a	a	a	a	a	a
a	a	a	a	a	a	a
a	a	a	a	a	a	a
a	a	a	a	a	a	a
a	a	a	a	a	a	a
a	a	a	a	a	a	a
pos	pos	neg	neg	pos	neg	pos
a	a	a	a	a	a	a
a	a	a	a	a	a	a
a	a	a	a	a	a	a
a	a	a	a	a	a	a
a	a	a	a	a	a	a
a	a	a	a	a	a	a
a	a	a	a	a	a	a
a	a	a	a	a	a	a
a	a	a	a	a	a	a
a	a	a	a	a	a	a
a	a	a	a	a	a	a

Appendix 6

P53 n	MIB1 n	MUC1 n	MUC1c n	MUC2 n	CerbB2 n	CA19.9 n
neg	neg	neg	neg	pos	neg	pos
a	a	a	a	a	a	a
neg	neg	neg	neg	pos	neg	neg
neg	neg	neg	neg	neg	neg	pos
neg	neg	neg	neg	pos	neg	neg
neg	neg	neg	neg	pos	neg	pos
neg	pos	neg	neg	pos	neg	neg
neg	pos	neg	neg	pos	neg	neg
neg	neg	neg	neg	pos	neg	neg
neg	neg	neg	neg	pos	neg	pos
neg	pos	neg	neg	pos	neg	pos
neg	neg	neg	neg	pos	neg	pos
neg	neg	neg	neg	neg	neg	pos
a	a	a	a	a	a	a
neg	pos	neg	neg	pos	neg	neg
neg	neg	neg	neg	pos	neg	neg
neg	neg	neg	neg	pos	neg	pos
a	a	a	a	a	a	a
neg	neg	neg	neg	pos	neg	neg
neg	neg	neg	neg	pos	neg	pos
neg	neg	neg	neg	pos	neg	neg
a	a	a	a	a	a	a
neg	neg	neg	neg	pos	neg	neg
neg	neg	neg	neg	pos	neg	neg
neg	neg	neg	neg	pos	neg	pos
neg	pos	neg	neg	pos	neg	neg
neg	neg	neg	neg	pos	neg	neg
neg	neg	neg	neg	pos	neg	neg
neg	neg	neg	neg	pos	neg	neg
neg	pos	neg	neg	pos	neg	neg
neg	neg	neg	neg	pos	neg	neg
neg	pos	neg	neg	pos	neg	pos
neg	pos	neg	neg	pos	neg	neg
neg	pos	neg	neg	pos	neg	pos
neg	neg	neg	neg	pos	neg	neg
neg	pos	neg	neg	pos	neg	neg
neg	pos	neg	neg	pos	neg	neg

Appendix 7

T	N	M	S	Martin
3	0	0	2	4
4	0	0	4	4
3	0	0	2	4
1	0	0	1	2
1	0	0	1	2
3	0	0	2	4
1	0	0	1	1
4	0	0	4	4
2	0	0	2	2
3	1	0	3	4
3	0	0	2	3
3	0	0	2	4
1	0	0	1	1
1	0	0	1	1
2	1	0	3	4
1	0	0	1	1
3	0	0	2	4
3	1	0	3	4
3	0	0	2	4
2	0	0	2	2
3	0	0	2	4
1	0	0	1	1
2	0	0	2	2
2	0	0	2	3
2	0	0	2	3
2	0	0	2	3
2	0	0	2	3
3	1	0	2	4
2	0	0	2	3
3	1	0	3	4
2	1	0	3	4
1	0	0	1	2
3	1	0	3	4
3	0	0	2	4
4	0	0	3	4
2	0	0	2	3
1	0	0	1	2

Appendix 8

date of op	status	date of death	survival 7/2003 or dod	follow up
5/9/1979	died	22/5/1996	192 m	286m
22/12/1980	died	23/3/1984	39m	271m
14/1/1981	died	3/2/1981	1m	270m
21/01/1981	died	1984	36m	270m
13/7/1981	lost		lost	lost
20/10/1983	died	22/10/1983	2d	237m
28/06/1985	alive		216m	216m
14/10/1985	died	26/05/1992	79m	213m
22/07/1987	lost		lost	lost
25/05/1987	lost		lost	lost
23/12/1987	lost		lost	lost
26/11/1987	died	unknown	unknown	188m
30/11/1987	alive		188m	188m
25/5/1989	alive		169m	169m
2/4/1991	lost		lost	lost
19/2/1992	alive		137m	137m
29/4/1992	lost		lost	lost
12/6/1992	died	1995	133m	133m
11/9/1992	lost		lost	lost
9/3/1993	died	1/4/1997	48m	124m
3/3/1993	died	21/05/1996	38m	124m
10/11/1993	alive		116m	116m
17/8/1994	died	20/08/1997	36m	107m
30/1/1995	alive		102m	102m
11/12/1995	died	1996	5m	91m
30/8/1995	lost		lost	lost
17/7/1996	died	1997	8m	84m
15/5/1997	died	1998	10m	74m
1/9/1999	died	5/8/2001	23m	46m
29/9/1999	lost		lost	lost
29/9/1999	died	12/5/2003	43m	45m
3/12/2001	alive		21m	21m
4/1/2001	alive		31m	31m
21/7/2001	died	21/07/2002	12m	24m
20/2/2002	alive		17m	17m
18/3/2002	alive		16m	16m
11/8/1999	alive		47m	47m

Appendix 9

	SUIRVIVAL	SE
0.0600	0.962963	0.036345
1.0000	0.925926	0.050401
5.0000	0.888889	0.060481
8.0000	0.851852	0.068367
10.0000	0.814815	0.074757
12.0000	0.777778	0.080009
16.0000		
17.0000		
21.0000		
23.0000	0.734568	0.086448
31.0000		
36.0000	0.688657	0.092436
36.0000	0.642747	0.097007
38.0000	0.596836	0.100356
39.0000	0.550926	0.102602
43.0000	0.505015	0.103816
47.0000		
48.0000	0.454514	0.105002
79.0000	0.404012	0.104778
102.0000		
116.0000		
133.0000	0.336677	0.106782
137.0000		
169.0000		
188.0000		
192.0000	0.168339	0.130459
216.0000		

Appendix 10

Combined alcian blue-PAS technique for acid and neutral mucins (Mowry 1956)

Preperation of stains

a) **Alcian blue:**

Alcian blue	1g
3 percent acetic acid	100ml

b) **Schiff's reagent**

Basic fuchsin	1g
Distilled water	200ml
Potasium or sodium metabisulphate	2g
Analar conc. Hydrochloric acid	2ml
Decolourizing charcoal	2g

Method

1. Dewax sections and bring to water.
2. Alcian blue solution, 5min.
3. Wash in water, then distilled water.
4. 1 per cent aqueous periodic acid, 5 min.
5. Rinse well in distilled water.
6. Schiff's reagent, 15 min.
7. Wash in running tap water, 5-10 minutes.
8. Stain nuclei lightly with one of the usual haematoxylin solutions; differentiate as appropriate and blue.
9. Wash in water.
10. Rinse in absolute alcohol.
11. Clear in xylene and mount as desired.

Appendix 11

High iron diamine (Spicer 1965)

Solutions

1. *High iron diamine:*

N, N-dimethyl-meta-phenylenediamine dihydrochloride	120mg
N, N-dimethyl-para-phenylenediamine dihydrochloride	20mg
Distilled water	50ml
Ferric chloride (60% BDH solution)	1.4ml

Dissolve the two diamine salts simultaneously in the distilled water and then add the ferric chloride solution and mix.

2. *1% alcian blue in 3% acetic acid*
3. *0.5% aqueous neutral red*

Technique

1. Positive control and the test sections to distilled water.
2. Treat all sections with the high iron diamine solution for 18-24 h.
3. Wash well in running water.
4. Stain with alcian blue solution for 5 minutes.
5. Wash in water, stain nuclei with the neutral red solution for 2-3 minutes
6. Wash in water, dehydrate, clear and mount as desired.

Appendix 12

NATIONAL MINIMUM DATASET

AMPULLARY CARCINOMA HISTOPATHOLOGY REPORT

Hospital ..... Hospital no ..... NHS no .....

Date of request ..... Date of reporting..... Report no.....

Pathologist ..... Surgeon .....

Type of specimen: .....

Specimen dimensions
Length of duodenum ..... mm
Length of lesser curve ..... mm
Length of greater curve..... mm
Length of gall bladder ..... mm
Length of bile duct ..... mm
Size of pancreas ..... x ..... x ..... mm

Maximum tumour diameter ..... mm
Other organs .....
Named vessel identified Yes No
Which vessel? .....
Stent in place Yes No

Type of tumour Adenocarcinoma Other (see Table 3) .....

Differentiation: Well Moderate Poor

Maximum depth of invasion (T)
- Tis: Carcinoma *in situ*
- T1: Tumour limited to ampulla of Vater or sphincter of Oddi
- T2: Tumour invades the duodenal wall
- T3: Tumour invades 20 mm or less into pancreas
- T4: Tumour invades more than 20 mm into pancreas and/or into other adjacent organs

Perineural invasion Yes No

Named vessel involved Yes No

Margins
Resection margin involvement Yes No If yes, which margin .....

Lymph node involvement (N)
Total number of nodes .....
Number of nodes involved .....
- NX: Cannot be assessed
- N0: Regional lymph nodes* not involved
- N1: Regional lymph nodes* involved

Distant metastasis (M)
- MX: Cannot be assessed
- M0: No distant metastasis
- M1: Distant metastasis

** Please see text and Figure 2 for definition of regional nodes*

Comments

Pathological staging pT pN pM Completely excised at all margins? Yes No

Signature:..... Date:..... SNOMed codes:.....