# Standardised histopathologic evaluation after pancreatoduodenectomy for adenocarcinoma

## *Resection margins, tumour origin, and survival*

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*Series of dissertations submitted to the*
*Faculty of Medicine, University of Oslo*
*No. 913*

ISBN 978-82-8072-537-0



Cover: Inger Sandved Anfinsen.
Printed in Norway: AiT e-dit AS.

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# Contents

# Acknowledgements

The present work was carried out at the Surgical Department and the Pathology Clinic, Rikshospitalet University Hospital, during the years 2004-2008. Financial support was provided by the Faculty of Medicine, University of Oslo, as well as by Rikshospitalet University Hospital and by Ullevål University Hospital. Funding was also provided by a legacy administered by the Surgical Society of Oslo (Alexander Malthes legat til beste for yngre leger) and an industry research grant administered by the Norwegian Gastroenterology Society (AstraZeneca AS stipend innen gastroenterologi).

Many friends and colleagues have inspired and supported me during the work on this thesis. In particular, I am greatly indebted to my supervisor, Ivar P. Gladhaug, who helped establish this research project and encouraged me continuously. His clear guidance, characterised by genuine open-mindedness and direct, but gentle criticism, has been crucial, and indeed highly appreciated. I am also very thankful to my co-supervisor, Ole Petter F. Clausen, for letting me be a part of his research group, for introducing me to pancreatobiliary pathology, and for his enthusiasm during the many hours we spent together at the microscope and discussing manuscripts.

Among the co-authors of the included papers, I would particularly like to honour Svetlana Tafjord for her exceptional optimism and unselfishness when teaching me gastrointestinal histopathology, and Milada Cvancarova for her brilliant assistance in statistical analysis. Furthermore, Aasa R. Schjølberg, Aaste Aursjø, Vigdis Wendel, Kathrine Hagelsteen, and many other members of the staff at the Institute and Department of Pathology, as well as several staff members at the Biostatistics Unit, have taught me the methodology required and helped me on my way with this thesis.

Tor J. Eide and Inger Nina Farstad were at the time this thesis was initiated in charge of the Department of Pathology and the Section of Gastrointestinal Pathology, respectively. They had a major responsibility for establishment and implementation of the protocol for standardised histopathological reporting of pancreatoduodenectomy specimens. Anstein Bergan and Øystein Mathisen were at the time in charge of the Department of Surgery and the Section of Gastrointestinal Surgery, respectively. Without the contribution and support from these colleagues, this thesis would not have been possible.

The cooperation with Siri Larønningen, Christine Mellem, and Bjørn Møller, as well as other staff members at the Cancer Registry of Norway, was an exceptionally pleasant

experience, and very important for the completion of this thesis. Furthermore, my new colleagues at the Oncology Department at Oslo University Hospital, Ullevål, also deserve to be thanked, and in particular, Tone Ikdahl, for having given me the opportunity to start my specialisation in oncology, providing a different and complementary perspective of the present research, and for making arrangements to facilitate the completion of this thesis.

I would also like to express a sincere thanks to the editors and reviewers of the included manuscripts for providing valuable comments and suggestions for improvements.

Finally, and most importantly, I want to thank my family and friends, and in particular, my wife, Ingunn, and daughter, Elin, for their continuous encouragement and unconditional support.

Oslo, October 2009

Arne Westgaard

# Abbreviations and definitions

| | |
|---|---|
| AJCC | **A**merican **J**oint **C**ommittee on **C**ancer; established in 1959 to formulate and publish systems of classification of cancer, to be used for selecting the most effective treatment, determining prognosis, and continuing evaluation of cancer control measures |
| BilIN | **Bil**iary **i**ntraepithelial **n**eoplasia; a classification of increasing degrees of dysplasia and *in situ* neoplasia in the bile duct (BilIN1-3) similar to dysplasia in the pancreatic ducts (PanIN1-3) |
| CDX2 | caudal type homeobox 2; an intestine-specific nuclear transcription factor encoded on chromosome 13q; selectively expressed in gastrointestinal mucosa; frequently expressed in adenocarcinomas of the colon and small intestine, and more seldom expressed in pancreatic and biliary carcinomas |
| CK | **c**yto**k**eratin; cytokeratins are intermediate filaments that form part of the cytoskeleton and consist of at least 20 subtypes, which are expressed differently in epithelia during the course of terminal differentiation and may thus be used to classify different types of epithelia; cytokeratin expression is often retained during malignant transformation and may therefore be used as evidence of the cancer origin |
| CK7 | **c**yto**k**eratin **7**; an intermediate filament often expressed by simple epithelia; a large and relatively basic type II cytokeratin encoded on chromosome 12q |
| CK20 | **c**yto**k**eratin **20**; an intermediate filament often expressed by simple epithelia; a small and acidic type I cytokeratin encoded on chromosome 17q |
| IPMN | **I**ntraductal **p**apillary **m**ucinous **n**eoplasm; a grossly visible, non-invasive mucin-producing, predominantly papillary or rarely flat epithelial neoplasm arising from the main pancreatic duct or branch ducts, with varying degrees of ductal dilatation; IPMNs may be differentiated into gastric, intestinal, pancreatobiliary, and oncocytic types |
| MCN | **M**ucinous **c**ystic **n**eoplasm; a pre-neoplastic lesion consisting of ovarian-type stroma and epithelial lining with varying degrees of atypia that may occasionally progress to invasive adenocarcinoma |

| | |
|---|---|
| MUC | **muc**in; mucins are large extracellular proteins that are heavily glycosylated and function both as a barrier at epithelial surfaces and engage in signal transduction; altered mucin expression and glycosylation may be important in cancer development by influencing for example cellular growth, differentiation, adhesion, and invasion; mucins may be used both as diagnostic markers and as therapeutic targets for cancer |
| MUC1 | cell surface associated **muc**in **1**; a membrane-bound glycoprotein; encoded on chromosome 1q |
| MUC2 | oligomeric mucus/gel-forming **muc**in **2**; a secreted glycoprotein; forms an insoluble mucous barrier that protects the gut lumen; encoded on chromosome 11p |
| MUC4 | cell surface associated **muc**in **4**; a membrane-bound glycoprotein (secreted isoforms may also exist); encoded on chromosome 3q |
| PanIN | **Pan**creatic **i**ntraepithelial **n**eoplasia; a classification of increasing degrees of dysplasia and *in situ* neoplasia in the pancreatic ducts (PanIN1-3); development of most ductal pancreatic carcinomas are believed to follow a sequence of genomic changes that are reflected by increasing grades of PanIN, subsequently leading to infiltrating and/or metastasising carcinomas if left untreated |
| R | **R**esidual tumour classification; **R0**, curative resection; **R1**, microscopic evidence of residual tumour; **R2**, macroscopic residual tumour |
| study hospital | Rikshospitalet University Hospital; from January 2009, “Oslo University Hospital, Rikshospitalet”, due to the merger of three university hospitals |
| TNM | **T**umour, **N**ode, **M**etastases; the most widely used system for classifying the extent of cancer spread |
| UICC | **U**nion **I**nternationale **C**ontre le **C**ancer (International Union Against Cancer); the only international non-governmental organisation dedicated exclusively to the global control of cancer; founded in 1933; unites 262 cancer organisations in over 80 countries |

# Papers included

I. Westgaard A, Tafjord S, Farstad IN, Cvancarova M, Eide TJ, Mathisen O, Clausen OP, Gladhaug IP.
**Resectable adenocarcinomas in the pancreatic head: the retroperitoneal resection margin is an independent prognostic factor.**
*BMC Cancer*. 2008;8:5

**Publisher**: Biomed Central
**PubMed abstract**: PMID 18194510
**Permanent link to fulltext**: doi:10.1186/1471-2407-8-5

***Link to article***

II. Westgaard A, Larønningen S, Mellem C, Eide TJ, Clausen OP, Møller B, Gladhaug IP.
**Are survival predictions reliable? Hospital volume versus standardisation of histopathologic reporting for accuracy of survival estimates after pancreatoduodenectomy for adenocarcinoma.**
*European Journal of Cancer*. *Epub April 17, 2009. DOI: 10.1016/j.ejca.2009.03.019*

**Publisher**: Elsevier
**PubMed abstract**: PMID 19376696
**Permanent link to fulltext**: doi:10.1016/j.ejca.2009.03.019

***Link to article***

III. Westgaard A, Tafjord S, Farstad IN, Cvancarova M, Eide TJ, Mathisen O, Clausen OP, Gladhaug IP.
**Pancreatobiliary versus intestinal histologic type of differentiation is an independent prognostic factor in resected periampullary adenocarcinoma.**
*BMC Cancer*. 2008;8:170

**Publisher**: Biomed Central
**PubMed abstract**: PMID 18547417
**Permanent link to fulltext**: doi:10.1186/1471-2407-8-170

***Link to article***

IV. Westgaard A, Schjølberg AR, Cvancarova M, Eide TJ, Clausen OP, Gladhaug IP.
**Differentiation markers in pancreatic head adenocarcinomas: MUC1 and MUC4 expression indicates poor prognosis in pancreatobiliary differentiated tumours.**
*Histopathology*. 2009;54:337-47

**Publisher**: Wiley InterScience
**PubMed abstract**: PMID 19236510
**Permanent link to fulltext**: doi:10.1111/j.1365-2559.2009.03227.x

***Link to article***

# Synopsis

Pancreatic cancer is one of the most lethal of all common cancers, with almost equal mortality and incidence rates. Despite vast improvements in basic understanding of this disease, and improvements in surgical and medical care for these patients, there has been little improvement in relative survival over the past half century. Symptoms often present late, and most tumours are not resectable at the time of diagnosis. Pancreatoduodenectomy, i.e. surgical removal of the pancreatic head, duodenum, and distal bile duct, has since the 1980s evolved to become a relatively safe procedure when performed at specialised centres, and is the treatment of choice for clinically resectable masses in the pancreatic head. However, even after assumed margin-free pancreatoduodenectomy for adenocarcinomas, most patients die within few years due to local recurrence or systemic spread of the malignant disease. Furthermore, the use of neoadjuvant, adjuvant, or palliative chemo- and/or radiotherapy has demonstrated very limited effect on long-term survival, and new treatments are urgently needed.

Long-term survival after curative-intent pancreatoduodenectomy depends on histopathological tumour characteristics such as presence of lymph node involvement, perineural infiltration, and the particular histologic subtype, as well as completeness of surgical tumour removal. The reported rates of identified adverse prognostic factors upon histopathological assessment, for instance resection margin involvement, are often low, and this may indicate underreporting of adverse prognostic factors in pancreatoduodenectomy series and clinical trials. Furthermore, tumours that involve the pancreatic head do not always originate from the pancreatic head itself, and prognostically more favourable tumours that arise from the distal bile duct, ampulla of Vater, or peri-Vaterian duodenum may sometimes be misdiagnosed as pancreatic carcinomas. Survival estimates in clinical studies may thus be biased by insufficient quality control on histopathology, possibly due to lack of standardisation of the histopathologic assessment.

The aims of the present thesis were to evaluate to what extent standardised histopathologic assessment of pancreatoduodenectomy specimens influences on histopathologic reporting and survival estimates, and to report on prognostic factors in a setting of standardised histopathologic assessment. Particular focus has been on evaluation of the resection margins and tumour origin. The patients included were all those who underwent pancreatoduodenectomy at the study hospital (Rikshospitalet University

Hospital) 1980-2004, as well as all those who underwent pancreatoduodenectomy in Norway 1998-2004 as reported to the Cancer Registry of Norway. Four studies are included in the present thesis. In all four studies, we analysed associations between histopathological tumour characteristics and overall patient survival. In the first and third study, we performed slide review and review of histopathologic reports from resections at the study hospital. The second study was based on review of histopathologic reports, and the fourth study made use of immunohistochemistry on tissue microarrays from tumours resected at the study hospital.

Taken together, the four studies demonstrate that standardised histopathologic assessment and reporting of pancreatoduodenectomy specimens is necessary for completeness of histopathologic reporting, to avoid underestimation of resection margin and lymph node involvement, to avoid misdiagnosis of tumours originating from the ampulla of Vater (or distal bile duct or duodenum), and to obtain accurate and reliable survival estimates. Furthermore, standardisation of histopathologic reporting seems to be particularly important with respect to assessment of lymph node involvement.

Second, the studies demonstrate that adenocarcinomas in the pancreatic head, distal bile duct, ampulla of Vater, and peri-Vaterian duodenum may be classified by their histologic type of differentiation, pancreatobiliary versus intestinal, as an adjunct to classification by the assumed anatomic tumour origin. The true origin of adenocarcinomas involving the pancreatic head may sometimes be impossible to determine with certainty, whereas the histologic type of differentiation, which often resembles the tumour origin, is easier to determine (and does not require immunohistochemical evaluation). Moreover, the histologic type of differentiation was found to be an independent predictor of long-term survival. Third, the studies indicate that immunostaining of pancreatobiliary-type tumours targeting MUC1 and MUC4 may be used to identify a subgroup of patients with a particularly poor prognosis after curative-intent pancreatoduodenectomy.

This work confirms the need for standardising histopathologic evaluation and reporting after pancreatoduodenectomy and documents that adenocarcinomas that involve the pancreatic head should be classified by their histologic type, pancreatobiliary or intestinal (or other), in addition to classification by tumour origin (pancreas, distal bile duct, ampulla, or duodenum). Furthermore, the heavily glycosylated membrane-bound mucins MUC1 and MUC4 seem to be associated with a particularly poor prognosis for patients with pancreatobiliary-type differentiated adenocarcinomas. These proteins should be examined further in pursuit of new treatments for pancreatic cancer.

# General introduction

Pancreatic cancer has the poorest relative survival among the common cancers[1-3]. Most pancreatic cancers are ductal adenocarcinomas, and the commonest location within the pancreas is the pancreatic head[4]. Surgical resection is at present the only curative treatment option, but these carcinomas are often diagnosed at an advanced stage, and curative-intent pancreatoduodenectomy is thus only performed for 10-15% of pancreatic head adenocarcinomas[5]. Even after curative-intent pancreatoduodenectomy, most patients die within few years[6-9], due to local recurrence or systemic spread[10-13]. Extended surgical procedures and adjuvant chemo- and radiotherapy regimens have not been shown to influence considerably on long-term survival[14-17], although adjuvant chemotherapy has indeed been shown to prolong life with approximately one quality-adjusted life month[18,19]. Therefore, new treatments are urgently needed[20]. Current understanding of the biology and mechanisms of spread for these adenocarcinomas is rapidly increasing[5,20-22]. However, histopathologic evaluation of the pancreatoduodenectomy specimen is often non-standardised[23-25], and poor quality control on histopathology may thus bias the results in multi-centre studies aiming at evaluating new treatments[17,26-29].

Adenocarcinomas confined to the pancreatic head may originate from pancreatic, ampullary, distal bile duct, or peri-Vaterian duodenal tissue, and failure to reach a correct diagnosis of the cancer origin may lead to false assumptions regarding long-term survival[25,30-32]. Staging according to the TNM criteria relies on a correct diagnosis of the cancer origin, and staging criteria are different for the separate origins[33,34]. The World Health Organization's recommendations for classification of these tumours[35] also includes classification according to a morphologic diagnosis, i.e. the histologic type. In the present work, we have reported prognostic data from curative-intent pancreatoduodenectomy for adenocarcinomas originating from these four locations evaluating at the same time the importance of standardising histopathologic evaluation and reporting, with particular focus on evaluation of resection margin involvement, tumour origin, and histologic type of differentiation.

## Epidemiological aspects

Pancreatic cancer affects both sexes with a sex ratio close to one (slightly higher for men than women), and is the thirteenth commonest cancer of the world[1]. The incidence is

strongly age-dependent, affecting very few before 40 years of age, and has a peak in incidence ratios around 70-75 years[2,3]. The age-standardised incidence rate is between 4.5 and 9 per 100,000, and the estimated number of deaths per year worldwide due to pancreatic cancer is 227,000[1]. Incidence and mortality rates are higher in developed than developing countries, but this is probably best explained by differences in diagnostic capacity rather than aetiology[1,36]. Pancreatic cancer has the highest ratio of mortality to incidence (98%), but due to its relatively low incidence, it is only the eighth most common cause of death from cancer worldwide[1]. In Western countries, pancreatic cancer is the fourth or fifth leading cause of cancer-related death, and the second most frequent gastrointestinal cancer, only preceded by colorectal cancer[2,37]. Temporal studies have shown that the incidence of pancreatic cancer has been relatively stable over the last forty years, although some reports have shown an increase in incidence during the 1970s and 1980s and subsequently a decline and levelling-off during the last decades[2,38-41]. The peak in incidence occurred approximately a decade earlier for men than women, paralleling to some extent changes in smoking habits[36,42-44].

Cigarette smoking is in fact the strongest known environmental risk factor for pancreatic cancer, increasing the risk approximately two-fold and causing about 25% of the total burden of this tumour[42-44]. However, the trends in incidence of pancreatic cancer can only partly be explained by changes in smoking habits, and other factors must therefore be involved in development of pancreatic cancer[5,21,42,45]. Although risk factors associated with pancreatic cancer have been extensively explored, the causal factors for pancreatic cancer remain elusive. Studies have indicated that dietary factors, high fat intake, obesity, high alcohol consumption, and chronic pancreatitis may increase the risk of pancreatic cancer, but the results from such studies have been contradictory and inconclusive[42,45-47]. More convincingly, diabetes type II[48], and in particular recent-onset diabetes[49], has been suggested to increase the risk of pancreatic cancer. Although pancreatic cancer may induce substantial inflammation and thus cause pancreatitis and diabetes[50-52], the link between diabetes and pancreatic cancer is probably a causal rather than a consequential association[48]. However, only about 1% of patients who develop type II diabetes will be diagnosed with pancreatic cancer within 3 years of first meeting criteria for diabetes[49].

About 5-10% of pancreatic cancers are thought to be directly attributable to genetic factors[53,54]. Several genetic syndromes are associated with an increased risk of pancreatic cancer, including hereditary breast and ovarian cancer syndrome, familial atypical multiple mole melanoma syndrome (FAMMM), Peutz-Jeghers syndrome, and hereditary pancreatitis.

However, these syndromes account for very few of the total number of patients who develop pancreatic carcinomas. Most patients with hereditary pancreatic cancer have unknown genetic alterations, probably several accumulated, low-penetrant genetic alterations[54]. These genetic changes, as well as changes seen in sporadic pancreatic cancers, may increase the risk of pancreatic cancer by increasing susceptibility to environmental factors such as smoking[55]. Knowledge about molecular mechanisms and signalling pathways involved in pancreatic cancer has increased rapidly in recent years[22,56], although key features to derive effective prevention and treatment strategies for pancreatic cancer remain enigmatic.

Adenocarcinomas that involve the pancreatic head may sometimes derive from the distal bile duct, ampulla of Vater, or peri-Vaterian duodenum[32]. These tumours, particularly the ampullary and duodenal tumours, have a more favourable prognosis compared to pancreatic ductal adenocarcinomas[6,32,57,58]. In pancreatoduodenectomy series, pancreatic ductal carcinoma is generally considered the most frequent type among pancreatic, biliary, ampullary, and duodenal tumours[6,31,57-61]. However, misclassification of tumour origin has been demonstrated in up to one-third of long-term survivors[62]. The considerable variation in the reported relative incidences of tumours with respect to these four origins may thus reflect varying methods and standards in determination of the tumour origin rather than real differences in the relative incidences for pancreatic, biliary, ampullary, and duodenal tumours[23]. Importantly, reports on long-term survival after pancreatoduodenectomy for ductal pancreatic carcinomas may be biased by inadvertent inclusion of these prognostically more favourable tumours misdiagnosed as ductal pancreatic carcinomas[25,62-64].

## Surgical aspects

### Pancreatic and periampullary anatomy

The pancreas is an oval-shaped organ located in the posterior upper part of the abdomen, with a larger end (the pancreatic head) adjacent to the descending part of the duodenum, and with a tail towards the spleen (**Figure 1**, **2**, and **3**). The body (*corpus*) of the pancreas lies between the head (*caput*) and tail (*cauda*). The head represents approximately two-thirds of the pancreatic volume[65] and is, by convention, delimited from the pancreatic body by the left border of the superior mesenteric vein, whereas the left border of the aorta delimits the body from the tail[66]. The border between the head and body (anterior to the superior mesenteric vein and beginning of the portal vein) is referred to as the pancreatic neck[65]. The posterior-lateral protrusion of the pancreatic head, curving behind the superior mesenteric

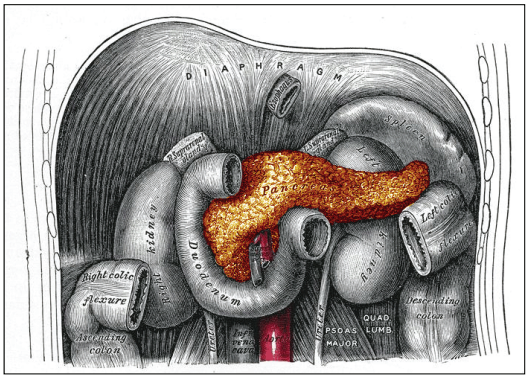


***Figure 1***

***The duodenum and pancreas.***
*Henry Gray (1821–1865), Anatomy of the Human Body, 1918 (copyright expired), slightly modified (pancreas, yellow).*

artery and vein (where there may be an indentation known as the vascular groove or pancreatic notch), is designated the uncinate process (**Figure 2**)[65].

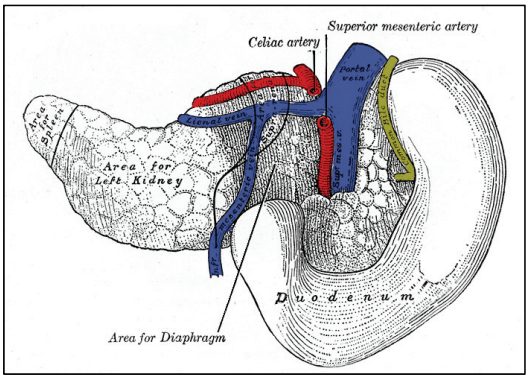


***Figure 2***

***The pancreas and duodenum from behind.***
*Henry Gray (1821–1865), Anatomy of the Human Body, 1918 (copyright expired), slightly modified (arteries, red; veins, blue; bile duct, green).*

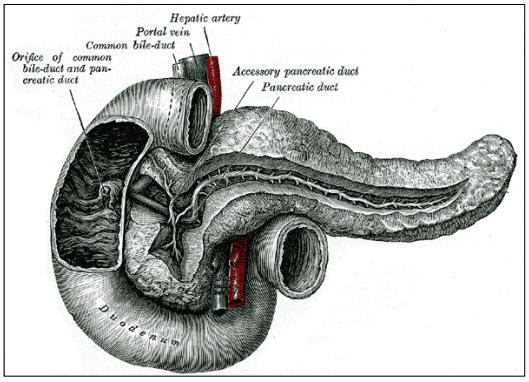


***Figure 3***

***The pancreatic duct.***
*Henry Gray (1821–1865), Anatomy of the Human Body, 1918 (copyright expired).*

Most of the pancreatic tissue mass (80-90%) is composed of exocrine acinar and ductal cells that contribute in protein and carbohydrate digestion by secretion of digestive enzymes into the gastrointestinal tract in response to stimuli from the stomach and duodenum[21,65,67]. Scattered throughout the exocrine tissue are endocrine cells gathered in

clusters called Islets of Langerhans (**Figure 4**). These endocrine cells are involved in glucose homeostasis by secretion of hormones such as insulin and glucagon into the bloodstream.

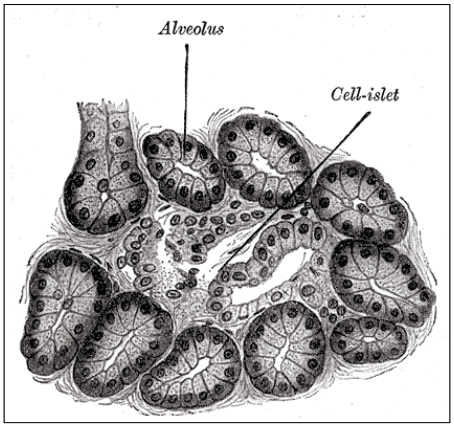


***Figure 4***

***Section of pancreas of dog (X 250).***
*Henry Gray (1821–1865), Anatomy of the Human Body, 1918 (copyright expired).*

The enzyme-secreting acinar cells (from Latin, *acinus*, grape), so named because of their organisation in bundles resembling a cluster of grapes, are arranged around a central duct and interconnected by interlobular ducts. The ductal cells add mucous and bicarbonate to the enzyme mixture and form a network of increasing size, culminating in the major pancreatic duct (of Wirsung) and the minor (accessory) pancreatic duct (of Santorini) (**Figure 3**)[68]. These two large collecting ducts empty into the duodenum at the major papilla (of Vater) and at the minor papilla, respectively[69]. (See notes on terminology, particularly regarding the use of eponymous terms, page 25.)

The common bile duct enters into the pancreas from behind (**Figure 2**) and runs through the pancreatic head to join (in 40-90% of cases[70]) with the major pancreatic duct, forming the hepatopancreatic ampulla (of Vater) (see notes on terminology, page 24). There is, however, considerable variation in the normal anatomy of these ductal structures[70]. The adult pancreas, distal bile duct, and ampulla of Vater are embryologically derived from the endodermal lining of the duodenum (**Figure 5**). The major pancreatic and common bile ducts do not always fuse to form a common channel, the length of which may vary from 0 to 3.3 cm, and the orifices of the two ducts may be divided by a septum. Furthermore, there may or may not be dilation of the common channel (*ampulla*, strictly defined, is a flask-like dilatation of a tubular structure[71]), and the normal anatomy of the Vaterian system may be distorted by carcinoma, inflammation, or fibrosis[70].

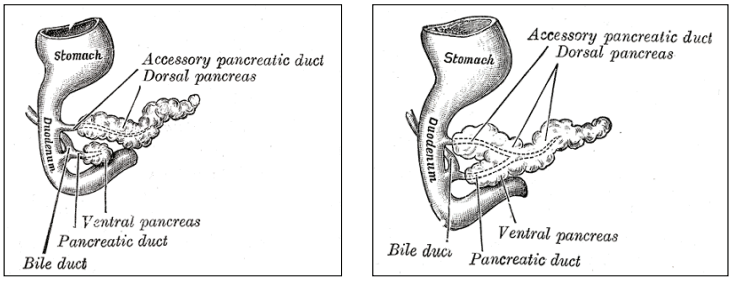


***Figure 5***

***Pancreas of a human embryo of five weeks (left) and at end of sixth week (right).***

*Henry Gray (1821–1865), Anatomy of the Human Body, 1918 (copyright expired).*

## Surgical resection of ductal adenocarcinomas in the pancreatic head

Ductal adenocarcinoma and its variants are the most common neoplasms in the pancreas (85-90% of all pancreatic neoplasms)[4], and unfortunately the most deadly[65]. About 60-70% of pancreatic ductal adenocarcinomas are found in the head of the gland[4]. This number corresponds to the size of the pancreatic head (two-thirds, see above) relative to the remaining pancreas and does not represent an actual over-occurrence of tumours at this location. Tumours in the body and tail, however, often present at a later stage and are less often resectable[4]. When preoperative work-up including imaging has demonstrated that a mass in the pancreatic head is surgically resectable[72-74], the procedure of choice is a pancreatoduodenectomy[75], often referred to as the Whipple procedure (named after Allen Oldfather Whipple, 1881-1963, who popularised this procedure during the 1930s and 1940s[76,77]). Due to the complexity of this procedure, this is most often performed as a laparotomy although some minimally invasive pancreatoduodenectomies have also been described[78-85].

A pancreatoduodenectomy (see notes on terminology, page 27) involves surgical removal of the pancreatic head, duodenum, and distal common bile duct with the gall bladder (**Figure 6**). The head of the pancreas and the duodenum share the same arterial blood supply, so both these organs must be removed. The distal stomach may or may not be resected, depending on the type of pancreatoduodenectomy employed (classical as opposed to pylorus-preserving pancreatoduodenectomy). Resectable tumours involving the pancreatic head may originate either from the pancreatic head itself or from the distal bile duct, ampulla of Vater, or peri-Vaterian duodenum[32,35,86], as mentioned previously. The origin is often impossible to determine prior to surgery, and by convenience, the four

separate tumours are often collectively referred to as "periampullary adenocarcinomas"[6,32] (see notes on terminology, page 23).

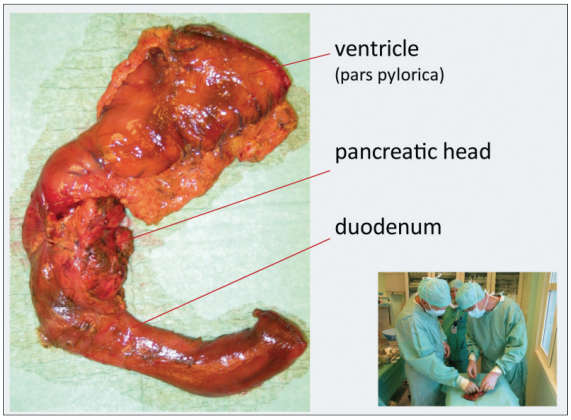


***Figure 6***

*A pancreatoduodenectomy specimen, fresh from surgery, studied by the surgeons prior to performing the final anastomoses that complete this procedure.*

Surgical resection is at present the only curative treatment option for these tumours. However, the disease often presents at an advanced stage, and only 10-15% of pancreatic head adenocarcinomas are offered potentially curative surgery, although a continuous improvement in surgical technique and intensive care has made it possible to offer surgery to an increasing number of patients[5]. Surgical mortality was considerable until the 1980s, after which operative mortality has declined to less than 5% in most high-volume and specialised centres, with acceptable although still considerable morbidity[87]. Even in the very elderly, pancreatoduodenectomy may now be performed as a relatively safe procedure[88-91].

Pancreatoduodenectomy may be performed as a standard (classical)[77] or pylorus-preserving[92] Whipple procedure, with similar morbidity and mortality[93]. The procedure may include extended lymph node dissection[14,94-96], although at a higher risk, and this should thus only be performed in clinical trials, if at all[14,97,98]. Vascular resection may be required in order to obtain free resection margins if the tumour involves the portal or mesenteric veins[98-103]. Although adding to the complexity of this already lengthy and difficult operation, pancreatoduodenectomy with venous resection has evolved as a relatively safe procedure for selected patients[104]. Venous involvement indicates local advancement of the tumour rather than a more aggressive tumour type[104], and long-term survival has been shown to depend on the depth[105] and length[106] of venous involvement. Tumour extension to the mesenteric artery or celiac axis, however, is associated with similar long-term survival as for unresected patients and is generally considered a contraindication to

pancreatoduodenectomy[98,107], although possibly feasible for selected patients following neoadjuvant preoperative radiotherapy[108].

A number of studies have demonstrated that mortality and morbidity is considerably higher for low-volume surgical institutions and surgeons, compared to high-volume surgical institutions and surgeons, respectively[109-116]. The reason for the better outcome in high-volume centres may not only be superior surgical technique, but also better intensive care, multi-disciplinary support, and administrative and financial resources in general. It could seem obvious that patients being evaluated for pancreatoduodenectomy at low-volume institutions should be referred to high-volume centres where they may undergo the best treatment[117]. However, the critics argue that community hospitals and surgeons should indeed perform a certain number of advanced abdominal operations in order to maintain sufficient competence and skills required to handle surgical urgencies when time does not allow referral to larger centres[118,119]. Moreover, the validity of studies reporting a relation between provider case volume and cancer mortality has been questioned due to the heterogeneity of results from individual studies, and a call for more direct quality measures has been advocated[120,121]. As an alternative to regionalisation of high complexity operations to high volume hospitals, attempts have been made to export surgical excellence from high to low-volume hospitals, with some success[122].

Pancreatoduodenectomy for adenocarcinomas in the pancreatic head, although intended as a curative procedure, in most cases turns out to be a palliative procedure, although some hope may be seen from recent reports[123]. Most patients die within few years even after curative-intent pancreatoduodenectomy[6-9,124], due to local recurrence or systemic spread[10-13]. A microscopically margin-free resection offers patients approximately half a year of survival benefit compared to curative-intent pancreatoduodenectomy with microscopic residual disease[8,11,24,57,58,87,124-137] (**Table 1**).

Some investigators question whether long-term survival for pancreatic cancer may at all be possible[25,138], and it has been suggested that "field cancerisation"[139,140] of the pancreatic tissue may make all pancreatic ductal carcinomas virtually unresectable due to molecular alterations in the remaining tissue even in microscopically curative resections[141]. Considerable interest has been shown in extending surgery to remove all retroperitoneal tissue that could potentially harbour neoplastic cells responsible for local recurrence, and to harvest all lymph nodes that might also represent residual disease. Such measures have not, however, been shown to increase survival after pancreatoduodenectomy[14,94-96].

.

**Table 1: Recent reports on resection margin involvement and survival after pancreatduodenectomy for ductal pancreatic adenocarcinoma**

| Reference (first & last author, year) | Location | Study period | Number of patients | Fraction R0 resections[a] | Median survival (months) R0 | Median survival (months) R1 |
|---|---|---|---|---|---|---|
| Pingpank & Eisenberg[125], 2001 | Philadelphia | 1987-2000 | 47 | 23% | - | - |
| Neoptolemos & Büchler[126], 2001 | ESPAC-1[b] | 1994-2000 | 541 | 82% | 17 | 11 |
| Katz & Moossa[58], 2004 | San Diego | 1983-2000 | 35 | 51% | - | - |
| Kuhlmann & Gouma[8], 2004 | Amsterdam | 1992-2001[d] | 160 | **50%** | 18[c] | 16[c] |
| Jarufe & Bramhall[57], 2004 | Birmingham | 1987-2002 | 133 | 51% | 17 | 12 |
| Tseng & Evans[127], 2004 | Houston | 1990-2002 | 291 | 85% | 27 | 21 |
| Raut & Evans[11], 2007 | Houston | 1990-2004 | 360 | 83% | 28 | 22 |
| | Houston | 1990-1999 | 191 | 79% | - | - |
| | Houston | 2000-2004[d] | 169 | **88%** | - | - |
| Verbeke & Anthoney[128], 2006 | Leeds | 1995-1999 | 36 | 47% | 13 | 9 |
| | Leeds | 1999-2003[d] | 26 | **15%** | 37 | 11 |
| Menon & Verbeke[129], 2009 | Leeds | 2003-2007[d] | 27 | **19%** | NA | 14 |
| Winter & Yeo[87], 2006 | Baltimore | 1970-2006 | 1175 | 58% | 20 | 14 |
| Schnelldorfer & Farnell[130], 2008 | Rochester | 1981-2001 | 338 | 82% | 18 | 15 |
| Kazanjian & Reber[124], 2008 | Los Angeles | 1987-2005 | 182 | 86% | 29[c] | 10[c] |
| | Los Angeles | 1987-1995 | 57 | 81% | - | - |
| | Los Angeles | 1996-2005 | 125 | 89% | - | - |
| Esposito & Büchler[24], 2008 | Heidelberg | 2002-2004 | 188 | 86% | 22 | 15 |
| | Heidelberg | 2005-2006[d] | 111 | **24%** | NA | NA |
| Ferrone & Allen[131], 2008 | New York | 1983-2001 | 618 | 73% | - | - |
| Smith & Neoptolemos[132], 2008 | Liverpool | 1997-2006 | 109 | 27% | 17 | 13 |
| Kato & Nakao[133], 2009 | Yokohama | 1981-2007 | 153 | 75%[e] | 15 | 9 |
| Chang & Biankin[134], 2009 | Sidney | 1990-2007 | 295 | 50% | 20 | - |
| Bilimoria & Bentrem[135], 2008 | NCDB[f] | 1998-2004 | 12101 | 84% | 17 | 12 |
| Westgaard & Gladhaug[136], 2008 | Oslo[g] | 1998-2004[d] | 40 | **55%** | 15 | 10 |
| Westgaard & Gladhaug[137], 2009 | Norway[h] | 1998-2004 | 272 | 65% | 15 | 10 |

NA, not applicable (due to small numbers and/or limited follow-up); Dash (-) indicates that numbers were not stated in the article

[a]Percentages are calculated from numbers of R0 / (R0+R1) resections; R0, margin-free; R1, margin-involved (tumour clearance > 1 mm or not stated in the article)

[b]ESPAC-1, a European multicenter study with patients recruited from 11 countries

[c]Estimated from curves in figure

[d]Study with **standardized** prospective evaluation of individual resection margins (note: varying definitions, eg., Raut & Evans restricted definition of retroperitoneal margin to superior mesenteric artery margin)

[e]Resection margin involvement defined as tumour at the resection margin (clearance of 0 mm)

[f]NCDB, National Cancer Data Base, includes reports from 1450 US hospitals (capturing ~74% of newly diagnosed pancreatic cancers)

[g]Rikshospitalet University Hospital

[h]Cancer Registry of Norway, comprising reports from all Norwegian hospitals (excluding reports from Rikshospitalet University Hospital)

## Histopathological aspects

Neoplasms of the pancreas may be divided into neoplasms with predominantly exocrine or predominantly endocrine differentiation[65,142]. Exocrine neoplasms may be further subdivided into solid and cystic tumours. Ductal adenocarcinoma is the most common type of pancreatic cancer (**Table 2**)[5,21,143-146]. These gland-forming epithelial neoplasms are believed to originate from epithelial cells lining the pancreatic ducts or smaller ductules. Morphologic features include tubular or cribriform structures, cysts, papillae, and mucin formation[145,146]. Glandular structures resembling normal pancreatic ducts are typically embedded in abundant desmoplastic stroma. This type of ductal pancreatic adenocarcinoma is sometimes referred to as pancreatobiliary-type adenocarcinoma because of its similarities to biliary carcinomas[146].

Less frequent variants of ductal adenocarcinoma comprise adenosquamous carcinoma, colloid (or mucinous non-cystic) carcinoma, hepatoid carcinoma, medullary carcinoma, signet ring cell carcinoma, undifferentiated (anaplastic) carcinoma, as well as undifferentiated carcinoma with osteoclast-like giant cells[143,144].

Malignancies of the exocrine pancreas that are normally categorised as non-ductal malignancies comprise serous cystadenocarcinoma, mucinous cystadenocarcinoma, invasive intraductal papillary-mucinous neoplasm (IPMN), acinar cell (cystadeno)carcinoma, solid-pseudopapillary carcinoma, and pancreatoblastoma[5,145,147].

**Table 2: Histological variants of malignant tumours of the pancreas**

| **Malignant neoplasms of the exocrine pancreas** |
|---|
| *Ductal adenocarcinomas* |
| - pancreatobiliary-type adenocarcinoma (typical type of ductal adenocarcinoma; also called *duct cell adenocarcinoma, tubular-type adenocarcinoma, duct cell carcinoma,* or simply *pancreatic cancer*) |
| - intestinal-type adenocarcinoma |
| - adenosquamous carcinoma |
| - colloid (mucinous non-cystic) carcinoma |
| - hepatoid carcinoma |
| - medullary carcinoma |
| - signet ring cell carcinoma |
| - undifferentiated (anaplastic) carcinoma |
| - undifferentiated carcinoma with osteoclast-like giant cells |
| *Ductal adenocarcinomas?* |
| - mucinous cystadenocarcinoma |
| - invasive intraductal papillary-mucinous neoplasm (invasive IPMN) |
| - mixed acinar-ductal carcinoma |
| - mixed ductal-endocrine carcinoma |
| - mixed acinar-endocrine-ductal carcinoma |
| - clear cell carcinoma |
| *Non-ductal malignancies of the exocrine pancreas* |
| - serous cystadenocarcinoma |
| - invasive mucinous cystic neoplasm |
| - acinar cell (cystadeno)carcinoma |
| - pancreatoblastoma |
| - solid-pseudopapillary neoplasm |
| **Malignant neoplasms of the non-exocrine pancreas** |
| *Endocrine neoplasms* |
| *Other (rare) primary malignancies of the pancreas* |
| *Secondary neoplasms* |

However, mucinous cystadenocarcinoma and IPMN may in fact have ductal origin[146], and recently, intestinal-type adenocarcinoma has been recognised a separate type of ductal adenocarcinoma of the pancreas[148]. In addition, mixed ductal-endocrine carcinoma and clear cell carcinoma are sometimes classified as tumours of ductal origin[5,145].

Secondary tumours may occasionally present as pancreatic masses, due to either metastatic or direct spread (for example from the biliary tract or adjacent intestine). Pancreatic tumours may derive from cells of the non-exocrine pancreas, particularly from the endocrine cells in the islets of Langerhans[22,147]. Small cell carcinoma is considered an exceedingly uncommon pancreatic tumour of non-ductal origin[146]. The existence of this type has in fact been debated due to the possibility that these tumours may represent metastasis from for example an occult primary tumour in the lung. Small cell carcinomas are now classified as endocrine carcinomas (in addition to large cell endocrine carcinomas)[149]. Finally, cases of primary pancreatic lymphoma[150,151] and connective tissue tumours such as pancreatic carcinosarcoma[152] (very uncommon) have occasionally been reported.

As mentioned previously, primary adenocarcinomas that involve the pancreatic head and may be removed by curative-intent pancreatoduodenectomy, do not necessarily derive from the pancreatic tissue itself. Some of these tumours originate from the adjacent duodenum, distal common bile duct (where it passes through the pancreatic head), or the hepatopancreatic ampulla (the common orifice of the bile duct and main pancreatic duct towards the duodenal lumen; present in 40-70% of the normal population[70]). Although these tumours have a common embryologic ancestry (**Figure 5**), duodenal (and ampullary) adenocarcinomas have a far better prognosis compared to pancreatic (and biliary) adenocarcinomas[31,145,153-156]. Interestingly, the rate of cancer development is similar in pancreatic, extrahepatic biliary tract, ampullary, and duodenal carcinomas, taking into consideration the relative surface area of the ductal system in these sites[157]. This may possibly indicate a field effect of cancerisation of these epithelia[157,158].

The particular tissue of cancer origin may in fact be difficult to determine, by strict anatomic considerations (**Box 1**)[30,31,136,153,159-162], especially when the tumour involves several of the potential origins. For example, ampullary tumours larger than 1-2 cm are most likely to invade the duodenum, distal bile duct, and/or pancreas, and such relatively large ampullary tumours may be impossible to discriminate from invasive tumours originating from either of these other periampullary tissues. Furthermore, ampullary adenocarcinomas may have histopathological characteristics of either intestinal or pancreatic/biliary

pancreatic/biliary tissue[31,155] – sometimes even both lines of differentiation within the same tumour (these carcinomas may be classified according to the predominant type[30,155]).

**Box 1: Determination of the tumour origin**

Determination of the origin of adenocarcinomas that involve the pancreatic head may be difficult or even impossible, in some cases.

*Adequate macroscopic handling and sectioning* of the specimen includes preparation of a whole-mount block that includes the distal bile duct, the pancreatic duct, and the ampulla of Vater, and the duodenum. Slides from this block should then be prepared by sectioning parallel to these structures of potential tumour origin. The tumour's relation to each of these structures may then be evaluated on the same slide.

*Slide reevaluation* of the tumour origin has limited value unless the slides were originally prepared as described above, since considerable variation in normal anatomy and tumour involvement of these periampullary structures may make it difficult in retrospect to distinguish between the separate ductal epithelia.

Irrespective of how the slides are prepared, microscopic assessment of the tumour origin should be based on (1) identification of the main localization and centre of the tumour, as well as (2) the pattern of growth relative to periampullary anatomy, and (3) whether there are tumour associated changes such as dysplasia of adjacent epithelia.

In particular, the following criteria apply:

- **Duodenal adenocarcinoma**: The tumour is mainly localised in the duodenum. Dysplasia of duodenal epithelium is often present. Infiltration to the ampullary region and/or pancreas may occur.
- **Ampullary adenocarcinoma**: The tumour is mainly localised in the ampullary region. Dysplasia in the ampullary epithelium is often present, and an associated intraampullary adenoma component may be present. Some tumours fill the entire ampulla, and there may be dilatation of the bile and/or the pancreatic ducts proximally.
- **Distal bile duct adenocarcinoma**: The tumour grows inside or alongside the distal bile duct epithelium, often with a fusiform growth pattern. Dysplasia of the adjacent biliary epithelium is often present (BilIN). There is almost always infiltration into the pancreas, and sometimes also into the ampullary region and/or the duodenum.
- **Ductal pancreatic adenocarcinoma**: The tumour is mainly localised in the pancreas. PanIN-changes are often present. Infiltration into the ampullary region, the distal bile duct and/or the duodenum may occur.

Typically, the pancreatobiliary-type adenocarcinomas are composed of simple or branching glands with cuboideal epithelium and surrounded by abundant desmoplastic tissue, whereas intestinal-type carcinomas consist of solid nests with cribriform areas and taller, often

pseudostratified epithelium with basally located nuclei and often presence of mucin[31,155]. Whereas the tumour origin may be difficult to determine, the histologic type of differentiation may give an indication of the cancer origin[30], although some tumours possibly derive from metaplastic or transdifferentiated epithelia.

### Molecular characterisation of neoplasms; premalignant lesions

Molecular studies of ductal pancreatic adenocarcinomas have revealed associations with genetic mutations that involve activation of K-ras and inactivation of p53, p16, DPC4, as well as dysregulation of growth factors and growth factor receptors, and upregulation of matrix metalloproteinases and regulators of tumour angiogenesis[163,164]. Several of these genetic alterations have also been identified in precursor lesions of pancreatic cancer[164,165]. Three distinct precursors have been recognised[165-167]. First, most ductal pancreatic carcinomas evolve from a sequence of genetic and histologic alterations that are designated pancreatic intraepithelial neoplasia (PanIN) 1-3[143,144,168-170]. Second, intraductal papillary mucinous neoplasm (IPMN), which is a grossly visible, non-invasive mucin-producing, predominantly papillary or rarely flat epithelial neoplasm arising from the main pancreatic duct or branch ducts, with varying degrees of ductal dilatation, may progress to either mucinous non-cystic (colloid) carcinoma or ductal adenocarcinoma[165,171,172]. Third, mucinous cystic neoplasm (MCN), which consists of ovarian-type stroma and epithelial lining with varying grades of atypia, may progress to invasive adenocarcinoma[173]. Although these precursor lesions harbour many of the same genetic alterations, they are believed to represent distinct pathways to development of invasive pancreatic cancer[165,166].

Similarly, at least two major precursor lesions have been associated with the development of biliary tract carcinomas. Biliary intraepithelial neoplasia (BilIN) 1-3[174,175] is a microscopic lesion of flat or low-papillary dysplastic epithelium that may be considered the biliary counterpart of PanIN[175-177]. Second, intraductal papillary neoplasm of the bile duct (biliary IPN) is considered the biliary counterpart of pancreatic IPMN[175,176]. Ampullary carcinomas are believed to derive from intestinal-type mucosa or from pancreatic duct-type ampullary mucosa[178]. Ampullary carcinomas may often derive from pre-existing ampullary adenomas[31,179] or distended glands (so-called overreplacement of the ampullary mucosa)[180]. Similar to the adenoma-carcinoma sequence theory for pancreatic (and colorectal) carcinoma development, ampullary and papillary carcinomas may result from a stepwise accumulation of genetic alterations[181-187].

Although there are many similarities in the molecular pathogenesis and characteristics of pancreatic and (other) periampullary adenocarcinomas, several molecules are to some degree differentially expressed on the cells of pancreatic, biliary, ampullary, and duodenal cancers. Immunohistochemical evaluation of pancreatoduodenectomy specimens is sometimes used to discriminate between tumours of these separate origins, and to discriminate between histologic subtypes for each origin, for example between intestinal and pancreatobiliary subtypes of ampullary carcinomas[31,155,188,189]. The most commonly used markers of pancreatobiliary-type and intestinal-type differentiation were evaluated in paper IV. In particular, cytokeratins (for example CK7 and CK20) are intermediary filaments that are often selectively expressed in certain tissues, and the expression is often retained in neoplastic transformation[190]. The caudal-type homeobox transcription factor CDX2 regulates axial development and intestinal differentiation and may be used as a marker of intestinal morphology[148,191,192]. Finally, the membrane-bound mucins MUC1 and MUC4[193-195] and the secreted gel-forming mucins MUC2, MUC5AC, and MUC6[195] have been considered potential markers of differentiation in the gastrointestinal tract[196]. These mucins are large glycoproteins that function both as epithelial barriers and engage in signal transduction[195,197,198]. They are important in cancer development with respect to cellular growth, differentiation, adhesion, and invasion[195]. Preclinical studies have demonstrated that mucin expression reduces intracellular uptake and response to chemotherapy[199]. MUC1 and MUC2 were originally used by Kitarmura and colleagues[200] and Matsubayashi and colleagues[188] to discriminate between intestinal- and pancreatobiliary-type ampullary carcinomas. MUC4 has been found to be overexpressed in ductal pancreatic carcinoma[201-205] and extrahepatic bile duct carcinoma[206,207]. Due to low expression of MUC4 in the normal[208-210] and cancerous intestine[211], MUC4 might also be used to differentiate between pancreatic, biliary, duodenal, and ampullary pancreatobiliary-type versus intestinal-type adenocarcinomas[189].

## Notes on terminology

### Periampullary adenocarcinoma: a subtype of ampullary adenocarcinomas?

The term "periampullary"* is used in an inconsistent manner in the medical literature[213]. Many authors use the term pragmatically to denominate a primary adenocarcinoma that may be removed by pancreatoduodenectomy, irrespective of whether the cancer originated in the pancreas, distal bile duct, ampulla or duodenum[214]. This may seem reasonable due to the aforementioned difficulties in determination of the true cancer origin for these tumours. However, other investigators restrict the use of this term to denominate the area directly surrounding the ampulla, for example in the sense "periampullary duodenal carcinomas", which is thus a *duodenal* tumour located close to the ampulla[31]. However, *ampullary* tumours are sometimes subclassified as "intra-ampullary" and "peri-ampullary" carcinomas, although the latter may be impossible to distinguish from periampullary duodenal carcinomas[31]. In some research articles, it may seem unclear whether periampullary adenocarcinoma refers to a subgroup of ampullary or duodenal adenocarcinomas (or both)[215]. Occasionally, investigators exclude duodenal adenocarcinomas from reports on pancreatoduodenectomies without accounting for the possible difficulties in discrimination between periampullary *ampullary* and periampullary *duodenal* tumours[128].

The disagreement on terminology, and inconsistencies in classification and reporting, may cause considerable confusion and adds to difficulties in comparison between pancreatoduodenectomy series. For example, peri-ampullary (intestinal-type) ampullary tumours might have been excluded from reports on pancreatoduodenectomies for adenocarcinomas if the intention was to exclude (periampullary) duodenal tumours. On the other hand, pancreatobiliary-type ampullary tumours may have been misdiagnosed as pancreatic or biliary tumours. Thus, reports on pancreatoduodenectomy series must include tumours of all four origins, as well a clear description of the method used to determine the tumour origin and the histologic type of differentiation (pancreatobiliary-type, intestinal-type, or other histologic type). Importantly, if studies use different methods to classify tumours by origin and histologic type (i.e. studies have different sensitivity and specificity for *diagnosing* for example pancreatic versus periampullary tumours), survival statistics might be biased in a similar way as commonly referred to as the Will Rogers phenomenon (i.e. changes in diagnostic techniques mislead survival statistics due to stage migration[216]).

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* Periampullary, around an ampulla (Dorland's Medical Dictionary for Health Consumers)[212].

Such an error was simulated on the data obtained at the study hospital (1998-2004) to see the effect on median survival for ampullary and pancreatic carcinomas if periampullary *duodenal* carcinomas would have been misclassified as periampullary *ampullary* carcinomas, and if at the same time pancreatobiliary-type *ampullary* carcinomas would have been misclassified as *pancreatic* carcinomas. Since duodenal and intestinal-type ampullary carcinomas have similar prognosis, the "erroneous" inclusion of duodenal tumours among ampullary carcinomas had a substantial effect on the estimated median survival for ampullary carcinomas (increased from 65 months to 94 months upon misclassification). Furthermore, the estimated median survival for pancreatic carcinomas was almost unchanged (increased slightly from 15.0 to 15.1 months), due to the fact that pancreatic adenocarcinomas and *pancreatobiliary*-type ampullary adenocarcinomas have similar median survival (i.e., 15.2 versus 16.7 months, respectively, confirming the original data presented in paper III, figure 4A).

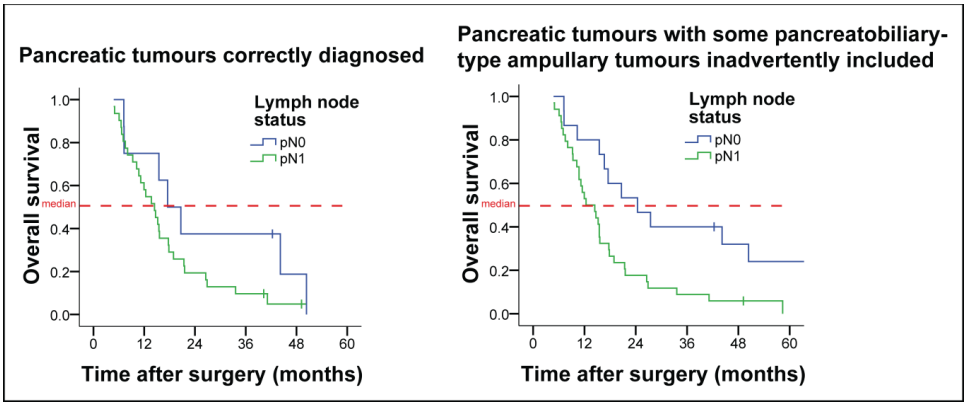


***Figure 7.*** *Effect of misdiagnosis on subgroup survival analysis for node-free (N0) versus node-involved (N1) resections. Survival curves to the right simulate erroneous inclusion of pancreatobiliary-type ampullary tumours among resected pancreatic tumours (study hospital, 1998-2004). Estimated median survival increased from 18 to 24 months for N1 resected patients, and decreased from 14 to 12 months for N0 resected patients, when pancreatobiliary-type ampullary tumours were "erroneously" included.*

However, the insignificant increase in estimated median survival seen among pancreatic cancers by introducing this error was reflected in a much higher effect in subgroup analysis, analogous to the Will Rogers phenomenon (**Figure 7**). In particular, estimated median survival for N0 resected patients increased erroneously by 50% (from 18 to 24 months) due to misclassification with respect to ampullary or pancreatic origin.

The inconsistencies regarding the term "periampullary adenocarcinoma" evoked many discussions among authors and reviewers of the papers included in the present thesis. In the papers I and III, we defined "periampullary" in the pragmatic sense, i.e. including all (four origins of) adenocarcinomas potentially resectable by pancreatoduodenectomy. Due to the controversies and criticism expressed by several co-authors and reviewers, the use of "periampullary" was largely avoided in the papers II and IV.

### Ampulla or papilla – of Vater, Santorini, or Bidloo?

Petros Mirilas and colleagues[69] reviewed the origin of the terms "ampulla", "papilla", "ampulla of Vater", and "papilla of Vater" in an article about "benign anatomical mistakes". According to Mirilas, citing the canonical work on "The origin of medical terms" by Henry Alan Skinner[217]: "In anatomy, the term ampulla is applied to a dilated part of a duct or other channel." Dr William S. Haubrich[218] stated that an "ampulla is known to us as a small, sealed glass container used to preserve medicines in a sterile, stable condition. The word comes from Latin *ampulla*, 'flask'. ... Interestingly, the Latin *ampulla* also means bombast or inflated discourse, as a 'blowing out.' Glass flasks were and are fashioned by blown air." *Papilla*, according to Mirilas (citing Skinner), "comes from Latin, meaning a nipple or teat, and is probably a variant of *papula*, meaning a small swelling, and is related to *papare*, meaning to suck or feed in an infantile manner." The hepatopancreatic ampulla should therefore, according to Mirilas, refer to "the dilation at the confluence of the bile and main pancreatic ducts", while the major duodenal papilla should refer to the nipple-like opening of this structure as seen from the duodenum.

In English literature, the term "hepatopancreatic ampulla" is not commonly used. The eponymous term "ampulla of Vater" is usually preferred, although according to Mirilas, the anatomists Vesalius (1543) and Collins (1685) made account of this structure before Abraham Vater (1720)[69]. Moreover, the structure that Vater described was in fact not an ampulla, but an elevation of the mucosa representing what is now denominated the perivaterian diverticula[69]. According to Mirilas (citing Velasco Suárez[219]), "all the anatomists referred to the confluence of the two ducts as a simple union" until Santorini, the same year Vater made his discoveries (1720), gave a very precise description of the vesicular dilatation that characterises the hepatopancreatic ampulla. Thus, although "ampulla of Vater" is still widely accepted in the English literature, this term is wrong and should be abandoned in favour of the official anatomic term "biliaropancreatic ampulla" or, more simply, "hepatopancreatic ampulla"[69]. Adding to the confusion, the term "papilla" is

often mistakenly used in the medical literature when "ampulla" is meant, and likewise, "ampulla" is sometimes used when "papilla" is meant[69]. Mirilas and colleagues conclude that the eponymous term "papilla of Vater" is wrong even when used in the correct context, since "it was Gottfried Bidloo who first noted the structure in 1685" [69]. Due to frequent anatomical misattribution and controversy, other approaches than the continued use of eponyms should be considered to honour those pioneers of pancreatic, ampullary, and periampullary anatomy[68,69,219] and surgery[77,220].

### Pancreatobiliary or pancreaticobiliary?

The most common and "typical" histologic type of adenocarcinoma originating from the exocrine pancreatic tissue is the ductal type referred to as the pancreat**o-**biliary[30,31,156,165,191,192,221-228] or pancreat**ico**biliary [155,156,178,188,224,229-234] histologic type.

Early investigators such as Kimura and Matsubayashi both denoted this histologic type "pancreat-**ico**-biliary"[155,188], while others such as Albores-Saavedra have used the term "pancreat-**o**-biliary", for example in the authoritative *Atlas of Tumour Pathology*[31]. Fingerhut[75] argued that pancreat**ico**- should be used when referring specifically to the pancreatic duct and pancreat**o**- when referring to the gland in general. Thus, a tumour classified according to the histologic type of differentiation as *pancreat**ico**biliary* should have histological features resembling pancreatic ducts, possibly indicating a **ductal** pancreatic origin. As already mentioned, the *tissue* of origin (pancreas, ampulla, distal bile duct, duodenum, see **Box 1**) may be intriguingly difficult to determine. However, determination of the true *cell of origin* may be even more difficult, since ductal pancreatic carcinomas may also originate from acinar (or endocrine) cells that have undergone metaplasia (or transdifferentiation) to a ductal cell form[235-237]. Furthermore, recent evidence points at the possibility that pancreatic cancer may arise from cancer stem cells[238-243], which may be one reason why these tumours are so resistant to chemo- and radiotherapy[244-246]. As we have shown[30,189], the histological *phenotype* (with or without immunohistochemical characterisation of the tumour) may independently predict survival after pancreatoduodenectomy for adenocarcinoma. Since such phenotypic classification does not rely on determination of the initial cell of origin, we believe that the preferred term should be *pancreat**o**biliary histologic type*, indicating an origin *pertaining* to the pancreas, but without restricting the definition to tumours originating from pancreatic duct cells. However, both terms, *pancreat**o**biliary* and *pancreat**ico**biliary*, are used synonymously and interchangeably in the literature, with few or no practical consequences.

### Pancreatoduodenectomy or pancreaticoduodenectomy?

The terms pancreat**ico**-duodenectomy and pancreat**o**-duodenectomy refer to the same surgical procedure, i.e. the Whipple procedure in either of its variants (for example classical, extended, pylorus-preserving, etc.). As mentioned in the previous section, the strict meaning of pancreatico- is somewhat different from the meaning of pancreato-. The removal of pancreatic tissue is certainly not restricted to excision of the pancreatic ducts, and the term pancreatoduodenectomy should thus be the preferred term[75]. However, the inaccurate use of pancreaticoduodenectomy is of trivial importance, since these terms are used interchangeably, with few practical consequences.

# Aims

Using a standardised, systematic protocol for histopathologic evaluation and reporting after pancreatoduodenectomy for adenocarcinomas in the pancreatic head, ampulla of Vater, distal bile duct and peri-Vaterian duodenum, the aim of this thesis was to answer the following specific questions with respect to curative-intent resections for such tumours:

1. How often is the retroperitoneal margin involved in non-curative resections? Is resection margin involvement, and retroperitoneal margin involvement in particular, an independent prognostic factor after curative-intent pancreatoduodenectomy?

2. What is the relative importance of standardised histopathology and institutional volume for completeness of histopathologic reporting and accuracy of survival estimates?

3. What is the interobserver variability in classification of the cancer origin of pancreatoduodenectomy specimens? May classification by the histologic type of differentiation, pancreatobiliary versus intestinal, be used as an adjunct to classification of these tumours by their assumed anatomic origin?

4. Does immunostaining for molecular markers of the histologic type of differentiation improve classification of the histologic type? May such markers identify patients with particularly poor prognosis after pancreatoduodenectomy for adenocarcinoma?

# Summary of results

## Paper I

**Resectable adenocarcinomas in the pancreatic head: the retroperitoneal resection margin is an independent prognostic factor.**
*BMC Cancer*. 2008;8:5.

This paper describes the standardised protocol for histopathologic assessment of the pancreatoduodenectomy specimen. The study confirmed that the retroperitoneal resection margin, a margin previously often not evaluated in reports from pancreatoduodenectomy series, is the margin most often involved in microscopically non-curative resections for adenocarcinomas. Furthermore, we found that the retroperitoneal margin is an independent prognostic factor after pancreatoduodenectomy for adenocarcinoma. The study also focused on the difficulties in determination of the tumour origin (pancreas, distal bile duct, ampulla, or periampullary duodenum), and described how the tumour origin might be adequately evaluated. Evaluating resection margins and tumour origin by our standardised protocol for evaluation of pancreatoduodenectomy specimens, we found that obtaining a free margin had significantly less impact on survival after resection of pancreatic tumours than after resection of ampullary tumours. In a subgroup analysis of curative (R0) resections, tumour origin was found to be the only histopathologic factor independently associated with long-term survival. However, the sample size was rather small in this subgroup analysis, and larger studies might demonstrate statistical significance of additional histopathologic tumour characteristics besides tumour origin, as the results from the following papers indicate.

## Paper II

**Are survival predictions reliable? Hospital volume versus standardisation of histopathologic reporting for accuracy of survival estimates after pancreatoduodenectomy for adenocarcinoma.**

*European Journal of Cancer*. *Epub April 17, 2009. DOI: 10.1016/j.ejca.2009.03.019*

In this paper, we evaluated completeness of histopathologic reporting and accuracy of prognostic estimates comparing the study hospital with all other Norwegian institutions reporting on pancreatoduodenectomies for adenocarcinoma between 1998 and 2004. We evaluated the importance of standardised histopathologic evaluation and reporting at the study hospital with evaluation at other medium-volume and at low-volume institutions.

The study demonstrates that standardisation of histopathologic reporting is more important than hospital volume for completeness of histopathologic reporting and for accuracy of prognostic estimates, particularly with respect to lymph node evaluation. The study included all pancreatoduodenectomies reported during this time period to the Cancer Registry of Norway, a population-based database that by law receives mandatory reports from all Norwegian surgical and pathological institutions. This registry includes information about the morphologic and topographic tumour diagnosis (i.e. histologic type and anatomic location of tumour origin, respectively), as well as nodal status, and degree of differentiation. These prognostic factors were retrieved from the histopathologic reports and prospectively registered for all reported pancreatic head adenocarcinoma resections. Completeness in reporting of histopathologic prognostic factor was significantly higher in reports from the study hospital compared to reports from other institutions of medium or low institutional volume. Lymph node status was the histopathologic prognostic factor that seemed to depend most on standardisation of histopathologic examination. The number of lymph nodes retrieved was significantly higher at the study hospital, and the ability to discriminate between favourable (N0) and poor (N1) prognostic groups was significantly higher at the study hospital compared to the other institutions. Standardisation of histopathologic reporting may be less important for accuracy of prognostic estimates with respect to resection margin evaluation, estimation of tumour size, and evaluation of the cancer origin.

## Paper III

**Pancreatobiliary versus intestinal histologic type of differentiation is an independent prognostic factor in resected periampullary adenocarcinoma.**
*BMC Cancer*. 2008;8:170.

Although the tumour origin (pancreas, distal bile duct, ampulla, or duodenum) may be difficult to determine with certainty, these adenocarcinomas are considered separate entities in TNM classification and staging according to the recommendations by WHO, UICC and AJCC. However, the pancreas, ampulla of Vater, and distal bile duct are all embryologically derived from the duodenum, and each of these tumours may sometimes have histopathologic features that make them morphologically indistinguishable from colonic adenocarcinomas. Acknowledging this, ampullary tumours have often been subdivided in intestinal and pancreatobiliary types, according to their histologic type of differentiation, and the intestinal type of ampullary tumours has consistently been shown to be associated with more favourable long-term survival. In this study, we hypothesised that the phenotype, either intestinal or pancreatobiliary, might thus be more important than the assumed cancer origin in terms of tumour biology, and patient prognosis after resection. Among all patients who underwent pancreatoduodenectomy at the study hospital between 1998 and 2004, we compared determination of the tumour origin with classification of the histologic type, pancreatobiliary versus intestinal. For the first time in the medical literature, to our knowledge, we presented individual frequencies for each of these two histologic types in a series of consecutive pancreatoduodenectomy resections for adenocarcinoma. As expected, exceptionally few pancreatic and distal bile duct tumours were intestinal-type, whereas approximately two thirds of ampullary tumours and all duodenal tumours were intestinal-type adenocarcinomas. However, the histologic type was easier to determine and a better predictor of survival after curative-intent pancreatoduodenectomy for adenocarcinomas. Among tumours with pancreatobiliary-type differentiation, lymph node involvement, vessel involvement, and increasing tumour size were independent adverse prognostic factors. The main results from this study were validated using resections performed at the study hospital 1980-1997 as an historical control.

## Paper IV

**Differentiation markers in pancreatic head adenocarcinomas: MUC1 and MUC4 expression indicates poor prognosis in pancreatobiliary differentiated tumours.**
*Histopathology*. 2009;54:337-47.

In the fourth study, we investigated whether immunohistochemical characterization using molecular markers associated with the histologic type, pancreatobiliary versus intestinal, could be useful in classification of these tumours. For the purpose of this study, we included pancreatoduodenectomies performed at the study hospital 1980-2004, and evaluated immunoreactivity for several molecular markers in archived tissue material. The immunohistochemical analyses were performed on small samples of tumours aligned in tissue microarrays (TMA), which allows simultaneous evaluation of several molecular markers with minimal use of expensive antibodies and valuable tissue material. In this study, we found that immunohistochemical characterisation using antibodies directed at molecular markers of the histologic type of differentiation, pancreatobiliary versus intestinal, did not discriminate better between the two histologic types than classification of the tumours after haematoxylin and eosin staining of ordinary sections. Furthermore, such immunohistochemical classification of the histologic type did not discriminate better between prognostically favourable versus poor subgroups. However, immunostaining with MUC1 and MUC4 identified a subgroup of patients with pancreatobiliary-type differentiation that had a particularly poor prognosis. The finding might be useful in future development of treatments targeting these molecules or signalling pathways in which these molecules are involved.

# Discussion

## Methodological considerations

The methods have been described and discussed in the included papers. The following is an additional overview and reflection on strengths and limitations to the methodology applied.

### Patient selection, follow-up, censoring, and completeness

The patients included in the present work comprise all patients who underwent pancreatoduodenectomy at Rikshospitalet University Hospital (study hospital) between 1980[†] and 2004, as well as all patients who underwent pancreatoduodenectomy in Norway between 1998 and 2004 identified through histopathology reports to the Cancer Registry of Norway (**Figure 8**).

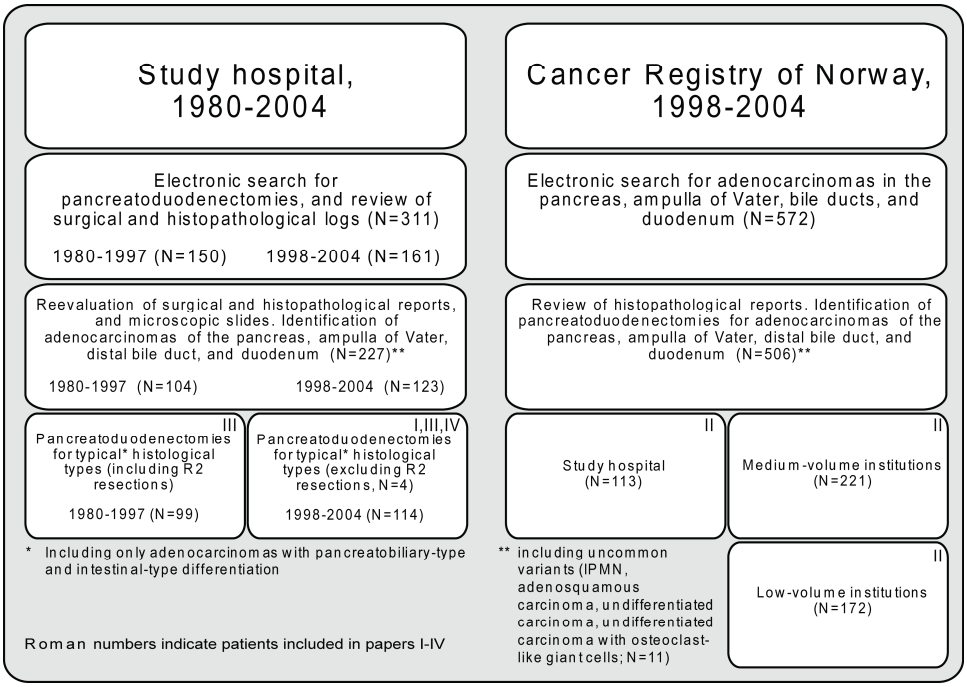


***Figure 8.*** *Patient selection for studies included in the present thesis.*

The number of pancreatoduodenectomies performed at the study hospital per year increased steadily from < 10 in the 1980s to 20-40 in the last decade (**Figure 9**). A

[†] Registration of patients who underwent pancreatoduodenectomy started during the spring of 1980. There may have been a few more pancreatoduodenectomies earlier this year. These few, if any, patients who may have undergone resection in 1980 before registration started, have thus not been included in the present work.

standardised protocol for histopathologic assessment of the pancreatoduodenectomy specimen was implemented at the study hospital in 1998. The study hospital is a tertiary referral hospital serving approximately one million inhabitants in the South-Eastern region of Norway. There is one other tertiary hospital in the same region, with similar numbers of pancreatoduodenectomies per year. Patients are referred from a number of different institutions to either one of these two hospitals, depending on the address of each patient, with no probable bias on age, gender, social status, previous health record, or tumour characteristics. There were no national or regional guidelines for standardisation of histopathologic assessment and reporting during the study period. Furthermore, national guidelines did not recommend adjuvant treatment as routine practice prior to 2006.

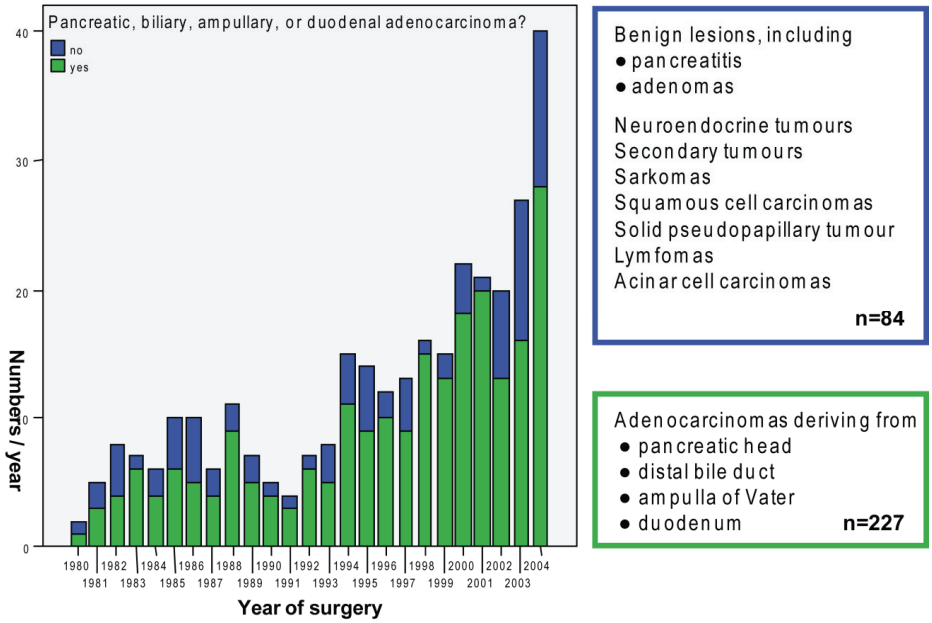


***Figure 9.*** *Pancreatoduodenectomies performed at Rikshospitalet University Hospital (study hospital), 1980-2004. Total number of resections, 311 (minimum 2, maximum 40).*

For the purpose of papers I, III, and IV, patients were retrieved by prospective registration of pancreatoduodenectomies, by review of operation protocols and pathology reports, as well as by electronic searches in the patient administrative system at the study hospital. A few patients might have been missed early in 1980 since prospective registrations of pancreatoduodenectomies started during the spring and not at the beginning of the year. However, the number of missed patients is probably less than five. Follow-up was complete for all patients except for censoring due to end of the study period. Follow-up

was limited to five years, whereas most patients were dead within two years (minimum follow-up for survivors, 1.6 years; paper I). Censoring may thus not significantly have biased the results of the presented analyses.

In paper II, all reported pancreatoduodenectomies in Norway between 1998 through 2004 were identified by a search in the Cancer Registry of Norway, a nationwide, population-based database. A strength of this study was that it evaluated population-based and nationwide data on resected pancreatic head adenocarcinomas, with high level of completeness[247], no patients missed to follow-up, and minimum 3.4 years of follow-up time for the surviving patients. Thus, few patients were censored, and inclusion bias was reduced to an absolute minimum. The patients identified by the search in the Cancer Registry did not reveal any missed patients from the original recordings performed at the study hospital. However, nine patients that had been identified by the prospective registration, manual review, and electronic searches at the study hospital were missed by the applied search strategy in the Cancer Registry. The explanation for this is that identification of patients at the Cancer Registry was limited to review of pancreatoduodenectomy pathology reports, and that some reports had not been sent to the Cancer Registry from the reporting institutions. A search in the Cancer Registry to include a review of reports from all biopsy-proven pancreatic head adenocarcinomas (in addition to the reports from pancreatoduodenectomy specimens) would have required review of reports from 4803 patients[‡]. This was considered far too extensive, and unnecessary for the purpose of this study. Since some reports might also be missing from other hospitals, the missing reports from the study hospital were not included. Importantly, we do not believe that the missing data may have biased the results in a direction contrary to the conclusions presented in this study[§].

### Histopathology

Most of the present work has been accomplished by evaluation of ordinary haematoxylin and eosin stained sections, a method developed as early as in the 1870s and perhaps not so "modern" although still fundamental to modern surgical pathology[248]. In an age of advanced

[‡] In addition, the Cancer Registry receives clinical reports, and making use of all available information at the Cancer Registry would have identified all except one pancreatoduodenectomy at the study hospital.

[§] Missing reports are not likely to have been of superior quality compared to those received at the Cancer Registry. In particular, reports missing and received from the study hospital were of similar quality. Insufficient logistics, rather than insufficient pathology, is the likely reason for why reports were not received.

technology, with a plethora of opportunities in genetics and nanotechnology, the power of such a simplistic methodology should be neither over- nor underestimated[**]. The ability of the pathologist to identify on ordinary stains a distinct pattern reflecting the severity and underlying biology of the disease is astonishing to a non-pathologist. Furthermore, immunohistochemistry is invaluable to characterise morphologic details that may not be visible on ordinary stained sections, as well as give a more precise structural, molecular, and functional idea of the tumour characteristics.

### Immunohistochemistry

Immunohistochemical procedures and histopathologic evaluation performed as part of the present study were principally carried out by experienced technicians and pathologists at the pathology department of the study hospital, in which there are established routines for immunohistochemistry (including quality control). The following is therefore only a brief description of some important methodological limitations and considerations regarding immunohistochemical evaluation of pancreatoduodenectomy specimens[249,250].

Problems in interpretation of immunostains may arise from varying methods for tissue fixation over time and between institutions. The present work included evaluation of some specimens that were over 25 years old. In ordinary haematoxylin and eosin stained sections, aging of the wax blocks does not represent a problem[249], whereas for some immunohistochemical staining protocols this may be more challenging. Not only aging of the waxed specimen itself, but variations in fixation protocols over time (for example in the pH of the applied diluent buffer)[251], may result in inadequate staining unless antigen retrieval and immunostaining protocols are optimised[249].

Most of the immunohistochemistry in the present work was performed on sections from tissue microarrays[252-256] (**Figure 10**), although immunostains from these arrays were also compared with ordinary sections for selected tumours. The major strength of tissue microarray technology is that a large amount of specimens may be evaluated under the same conditions, since the inevitable variability

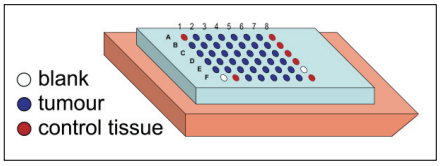


***Figure 10.*** *Tissue microarray*

[**] Genetic and preclinical studies are certainly necessary to reveal the true nature and principle mechanisms of pancreatic and periampullary cancers, in order to develop effective treatments for patients with these cancers. However, application of such techniques was beyond the scope of this thesis.

seen when staining individual whole-sections is reduced by applying the exact same methodology to all the samples processed on a single microscopic slide. Furthermore, reducing the number of slides to be stained, and evaluated, saves time, reduces the amount of antibody needed for analyses, and saves invaluable tissue that may then be used for further analyses. However, adjustment to varying fixation protocols and age of specimens is not so straight-forward in tissue microarrays, in which a large number of tumours that may have been subject to different fixation protocols (and storage time), are processed simultaneously, without the possibility to adjust antigen retrieval and staining protocols individually, as could be necessary. Furthermore, tissue microarrays are composed of small biopsy cores from each tumour, and results may be biased by selective sampling if tumour markers are heterogeneously expressed. In the present study, such variability due to possible heterogeneousness was avoided by use of rather large cores (1.0 and 1.5 mm diameter) and by evaluating more than one core from most tumours.

In general, results obtained by immunohistochemistry must be interpreted in light of possible variations in fixation and antigen retrieval techniques, specificity of primary and secondary antibodies, differences in use of chromogen detection and signal amplification procedures (for example use of, and blocking for, endogenous biotin), adequate use of internal controls (which are difficult to quantify), as well as other differences in staining protocols (for example time of exposure to antigens)[257-261]. Furthermore, peptide binding sites may be hidden by glycosylation, which represents a particular problem when evaluating the mucin proteins studied in the present work. And finally, identification of prognostic groups by combined expression and/or absence of several different molecular markers might and might not be biologically relevant.

Although the adequate threshold to define a true positive sample relies on methodology (for example antigen retrieval[249]) which in addition to disease prevalence may vary from one dataset/institution to another, optimisation of cut-points to maximise the difference between “positive” and “negative” samples increases the risk of reporting significant results by chance[262]. The same risk applies to optimising the combination of markers used to define a “positive” sample, unless such optimisation is used to prove that no “optimal” combination is in fact an adequate discriminator between positive and negative samples[189]. Efforts to standardise reporting on prognostic markers[250,263-265] have proven difficult to implement in practice[266]. Selective publication of positive results also adds to the concerns about the discriminating ability of reported prognostic markers[267]. Reports on tumour markers thus need confirmation by use of different methodological approaches and

by validation in separate datasets before applying results in clinical studies. Although a powerful tool to see structural and physiological characteristics and generate new hypotheses, the ultimately qualitative nature of immunohistochemistry (although some quantification methods do exist) must be recognised and acknowledged.

In the present work (paper IV), each of the issues mentioned above were considered separately for each antibody, in addition to routine quality control performed at our institution (including testing of antibody specificity by Western blotting). Furthermore, for selected stains we tested interobserver variability with respect to scoring of the stained specimens. However, we did not validate the results from immunohistochemistry using alternative methodology (for example genetic or *in situ* hybridisation studies), nor validate the results in separate datasets. These results thus need validation in separate studies.

## Standardised histopathologic evaluation, synoptic reporting

Standardisation of histopathologic reporting is important to avoid bias and make results applicable to clinical guidelines[29]. Prospective registration of important histopathologic prognostic factors should thus be standardised between pathologists, between institutions, and between continents[268,269]. In particular, protocols should be standardised between institutions in order to improve multi-centre comparisons[28]. There have been numerous suggestions for standardisation of histopathological evaluation and synoptic reporting of pancreatoduodenectomy specimens[23,24,33,161,270-280]. Substantial improvement of the quality of histopathologic reporting for pancreatic, biliary, ampullary, and duodenal adenocarcinomas may be achieved (1) by standardising *macroscopic* evaluation, preparation, and sectioning, (2) by standardising *microscopic* evaluation, and (3) by use of template-based, synoptic *reporting* of pancreatoduodenectomy specimens. The various published protocols on histopathologic evaluation and reporting are, however, inconsistent[23,136].

## Resection margin involvement

### *Retroperitoneal resection margin – varying definitions*

In the present work, we defined the "retroperitoneal margin" as "the area of sharp dissection in the peripancreatic fatty tissue behind the pancreatic head and lateral to the mesenteric vessels" (paper I). A resection margin is a margin of sharp surgical dissection[††217,281,282].

[††] Skinner[217]: "**Resection** (latin *resectio*), a cutting off or trimming, from *resecare*, to cut loose, or cut off."

The posterior and medial peripancreatic soft tissue margins (posterior to the pancreatic head and medial to the uncinate process adjacent to the superior mesenteric artery, with some peripancreatic tissue lying in between[283]) is referred to by different names in the scientific literature[136,283,284]. In the lack of international consensus on terminology, we chose to use the term "retroperitoneal resection margin" because it was the term most frequently used at the time the present work was initiated. However, some authors[23,284] consider "retroperitoneal margin" a misnomer due to the fact that the entire pancreatic head, not only the posterior aspect, is located retroperitoneally. Moreover, the evaluated "retroperitoneal margin" may not be the same in reports from different centres[11,136,283-285], or from different pathologists. In the present work, we have extended the definition of the "retroperitoneal margin" applied in for example the AJCC Cancer Staging Manual[33], by including a wider area representing the peripancreatic posterior (surgical) dissection margin, as described in more detail in paper I (see figure 1, paper I). Some investigators have advocated examination of the entire "circumferential resection margin"[24,128,286]. The anterior surface of the circumferential margin is in fact not a *resection* margin[287], and although it does make sense to determine the distance of tumour growth relative to the entire surface of the excised surgical specimen[23], we did not routinely examine the anterior margin in the present work. However, the retroperitoneal resection margin is the margin most often involved in R1 resections, and this margin is often simultaneously involved in cases where tumour invasion is seen at other margins[24,128,129,133,134,136,288].

***Residual tumour (R) classification***

Residual tumour classification was incorporated into the fourth edition of the *TNM Classification of Malignant Tumors* in 1978 and in the corresponding third edition of the *AJCC Manual for Staging of Cancer* (see detailed description of the current R classification in the TNM Supplement[289]). According to the last edition of the staging manual[33], the following margins should be routinely evaluated in pancreatoduodenectomy specimens: "common bile (hepatic) duct, pancreatic neck, retroperitoneal margin, other soft tissue margins (such as posterior pancreatic), duodenum, stomach". In addition, the anterior serosal surface of the pancreatic head is sometimes considered a "resection margin"[24,128,277], although this surface is not actually a surgical margin[23,290]. As mentioned above, there is considerable variation between studies with respect to evaluation of the resection margins, and this may be one reason for the wide range of reported R1 rates in different studies (**Table 1**)[23].

Moreover, differences in reported R1 and R0 rates between studies may be explained by variations in

- marking and inking of resection margins
- determination of the tumour origin (eg., the rates of ampullary versus pancreatic adenocarcinomas varies considerably between studies, which may be attributed to misdiagnosis rather than real differences in the relative incidences for these tumours)[25]
- method of sectioning of resection margins; some evaluate the retroperitoneal margin by a single "shave section" (parallel to the resection margin), whereas others evaluate serial sections perpendicular to the resection margin
- number of sections evaluated from each resection margin

In the current version of TNM residual tumour classification[291], R1 is defined as tumour involvement *at* any of the resection margins (clearance 0 mm), whereas R0 is defined as tumour clearance at *any distance* (> 0 mm) from the resection margins. However, pancreatic adenocarcinomas may spread in the form of isolated ducts and cells situated in the midst of normal pancreatic tissue or in peripancreatic fat and connective tissue[143,292]. Thus, some investigators have defined an R0 resection as tumour clearance > 1 mm, and correspondingly, defined an R1 resection as tumour involvement within 1 mm from the resection margins[24,128,272]. Some investigators have suggested that a tumour clearance more than 1 mm (eg. 1.5 mm) could even be more appropriate to define a curative resection[134,285]. However, most articles on pancreatoduodenectomies for adenocarcinoma do not state the exact distance used to discriminate a margin-free versus a margin-involved resection, a fact that adds difficulties to comparisons between series.

Wittekind and colleagues recently proposed to expand the R classification[293], in an attempt to eliminate confusion (with particular focus on differences in definitions for R classification and circumferential resection margin involvement in colorectal cancer, but suggesting that the same classification should be used for other cancers, including pancreatic head carcinomas). According to this revision[293], R1 should now be defined as tumour clearance > 1 mm from the resection margins. **Figure 11** summarises the current criteria for R classification encompassing this revision.

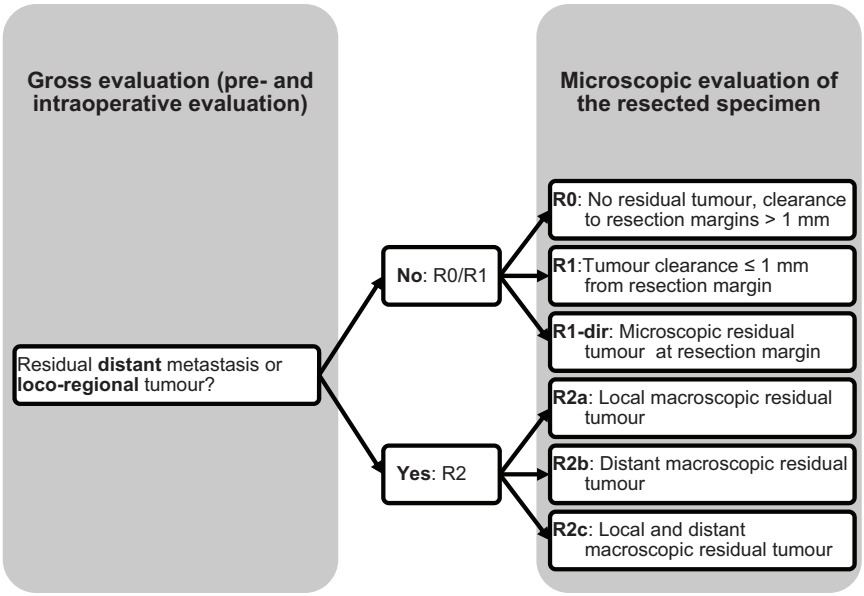


***Figure 11.*** *Expanded residual tumour (R) classification (adapted from TNM Supplement, 2003, and Wittekind and colleagues, 2009)*[289,293]

Some points of note[289,291,293]:

- In R classification, both local-regional residual tumour and distant residual tumour (remaining distant metastases) should be taken into consideration.
- Cases with distant metastases (M1) that (in addition to the primary tumour) have been removed completely are thus classified as R0.
- Cases should be classified as RX if the pathologist cannot make a reliable topographical orientation and assessment of the resection lines, for example if the tumour is removed in two or more parts (not *en bloc*).
- R classification corresponds to *detectable* residual tumour, and R0 does not necessarily represent a curative resection.
- According to the TNM Supplement recommendations, a carcinoma *in situ* lesion at the resection margin (i.e. PanIN3) should be classified as R1 with the suffix "is", i.e. R1(is). However, there is no uniform agreement, and such lesions are sometimes classified as R0[11]. Data on the clinical importance of PanIN3 at the resection margins is scarce[144]. In the present work, there were only a few cases with PanIN3 at the resection margins, and these were classified as R0.

## Statistical considerations

### *Sample size, heterogeneity; stratification or multivariable analysis*

Papers I, III, and IV evaluate prognostic factors for overall survival among 114 consecutive pancreatoduodenectomies for 40 pancreatic, 41 ampullary, 17 distal bile duct, and 16 duodenal adenocarcinomas. Tumours of separate origins may be heterogeneous with respect to histopathologic and biologic characteristics[32]. The number of patients evaluated in the present work was limited, for each of the separate tumour origins. However, due to the aforementioned inherent difficulties in distinguishing between pancreatic head tumours of separate origins, tumours of all four origins were included in a multivariable Cox regression analysis, in which these differences can be adjusted for adequately with respect to their association with overall survival. The feasibility of this analysis was evaluated by examination of log minus log plots. Furthermore, we proposed that this should be the preferred method for reporting on prognostic factors after pancreatoduodenectomy, rather than only presenting the results from separate subgroups, since these subtypes with respect to tumour origin may not be clearly distinguishable.

The primary outcome variable in all four studies included in the present work was death from any cause; i.e., we studied overall and not disease-specific survival (discriminating between perioperative death and conditioned long-term survival). Most patients who undergo curative-intent pancreatoduodenectomy for adenocarcinoma die from recurrence of this disease within few years[130]. Accordingly, median survival was only 1-2 years in each of the four studies in this thesis, similar to other studies (**Table 1**). We therefore believe that evaluation of disease-specific death would not have altered the conclusions substantially and was thus not necessary in the current context.

In paper II, we compared institutions with different protocols and standards for histopathologic evaluation and reporting. The study hospital was compared with other medium-volume institutions and with low-volume institutions. The actual numbers of pancreatoduodenectomies for adenocarcinoma performed at the study hospital and at the three medium-volume institutions were 113 (study hospital), and 94, 80, and 47 (medium-volume institutions), respectively. The medium-volume institution with least volume performed less than half the number of pancreatoduodenectomies compared to the study hospital. However, inclusion of only two hospitals in the medium-volume category would increase the possibility of obtaining differences by chance. The mean number of operations at the 21 low-volume institutions was much lower than the number of

pancreatoduodenectomies performed by the medium-volume institution with the lowest volume (8.2 versus 47). Thus, we considered the comparisons appropriate with respect to hospital volume. However, it should be recognised that the study hospital not only differed from the other medium-volume institutions in terms of standardisation of histopathologic evaluation and reporting, but also in terms of volume of surgery (and pathology).

The cutpoints defining "high-volume" and "medium-volume" institutions in pancreatic surgery vary considerably in the published literature[121]. In general, the number of patients in "high-volume" centres is considerably lower for pancreatoduodenectomies than for operative procedures in for example heart or vascular surgery[109,294]. The threshold for a possible improvement on survival estimates due to increasing hospital or surgeon volume may be higher than the cutpoint of 47 resections per 7 years (~7 resections per year) set in the present study. Furthermore, volume of surgery is not entirely the same as volume of pathology, which we did not measure in our study. However, the main conclusion in this paper was that prognostic accuracy for lymph node evaluation depended more on standardisation of histopathology than on volume of surgery. To evaluate whether the threshold for obtaining an effect of institutional volume on accuracy of histopathologic reporting could have been set too low, we also compared the three medium-volume institutions with respect to prognostic validity for lymph node staging (data not published, for confidentiality reasons). Among these three medium-volume institutions other than the study hospital (mean number of pancreatoduodenectomies for adenocarcinoma per year: 13, 11, and 7, respectively, compared to 16 at the study hospital), there was no indication that the institution with the *highest* work-load had more accurate survival estimates based on lymph node evaluation. In fact (by coincidence), we found a trend indicating that the medium-volume hospital with the *lowest* workload had the highest hazard ratio for lymph node involvement, although non-significant. Thus, long-term survival could not be predicted more accurately at institutions with higher workload based on lymph node evaluation in the present study. However, although our data indicate that standardisation of histopathologic evaluation and reporting is more important than institutional volume, we cannot rule out that an effect of increasing workload on quality of histopathology, irrespective of measures to standardise histopathologic reporting, might be seen at institutions with even higher work-load than those evaluated in this study.

***Validity, accuracy, completeness, correctness***

We believe that the main conclusions drawn from the four included papers are valid, i.e. that the conclusions are likely to be true in view of the measures used and in the applied research design (internal validity)[29,295]. For example, statistical tests and *predefined* cut-off levels to define positive immunostaining were chosen in order to avoid inflation of arbitrary (false positive) results. External validity must be confirmed by independent studies, i.e. studies that apply the same methods on a separate sample of subjects. However, some further validation was also performed in the present work, particularly in paper III.

In this paper (paper III), we validated the results from our study on an historical control cohort from the same institution. Results from historical cohorts may inevitably be biased by changes in treatment practice over time, as well as by changes in for example methods for processing pathology specimens. In spite of these limitations, evaluation with an historical control group may provide additional proof of concept, and confirm or contradict results from the main study analysis. We believe that evaluation of the results from the main analysis using an historical control group in paper III adds strength to the conclusions drawn in this study, although we acknowledge the limitations of this approach. The results from this study, as well as the other three studies, should thus be evaluated in independent studies. Importantly, external validity should be established before applying the results to clinical studies[28].

The use of terms such as *validity*, as explained above, as well as *completeness*, *accuracy*, *adequacy*, and *quality* may sometimes be confusing. The following is a brief discussion on these and related terms in the context of paper II. *Accuracy* is the degree of closeness of a measured or calculated quantity to its actual (true) value. Based on previous studies and our own experience, we assumed that standardised (evaluation and) reporting may improve the level of details (*completeness*), which in turn might affect the *diagnostic accuracy*. The results from our study do indicate that diagnostic accuracy may improve, although we did not evaluate diagnostic accuracy in particular. For example, with respect to tumour origin classification, non-standardised reporting led to significant underreporting of ampullary and duodenal tumours. We hypothesised that completeness of histopathologic reporting might in turn be important for accuracy of prognostic estimates (i.e. *prognostic accuracy*, or *prognostic validity*). Analogous to our approach for evaluation of the prognostic accuracy in paper II, Tomlinson and colleagues[296] evaluated "accuracy of staging node-negative pancreas cancer" by determining the minimum number of lymph nodes required to optimally discriminate between prognostic favourable versus prognostic poor

groups. However, Tomlinson and colleagues only evaluated the prognostic accuracy for lymph node staging, whereas paper II compares the effect on survival estimates for each reported histopathologic factor independently, in a setting of standardised versus non-standardised histopathology reporting. Finally, since the *purpose* of histopathologic reporting is to provide information about the patient prognosis after resection, we conclude in paper II that standardised reporting increases *quality* or in fact *adequacy* of histopathologic reporting, as stated in the article.

## Discussion of results

This thesis evaluates the importance of standardising histopathologic assessment and reporting after pancreatoduodenectomy for adenocarcinomas in the pancreatic head, distal bile duct, ampulla of Vater, and peri-Vaterian duodenum. The included papers focus particularly on the importance of standardised evaluation of the resection margins and tumour origin, on histopathological and immunohistochemical classification of the histologic type versus the tumour origin, and on the importance of standardised reporting for accuracy of prognostic estimates. Most patients who are diagnosed with an adenocarcinoma in the pancreatic head die within few months after diagnosis[1,2,5,21]. Even when the tumour is surgically resectable, complete removal of a typical ductal pancreatic adenocarcinoma offers only approximately 1-2 years of prolonged survival (**Table 1**). The effect of adjuvant treatment is limited, and new treatments are urgently needed[3,15,297]. Standardised evaluation of resected specimens should be an important measure to avoid bias in clinical studies[17,28,29,250]. However, histopathologic assessment and reporting of the pancreatoduodenectomy specimen is often non-standardised[23,25]. In particular, clinical studies might fail to prove new treatments effective if determination of resection margin or lymph node involvement is insufficient[17,26-28], or if tumour types that respond dissimilarly to the offered treatments should be included simultaneously, inadvertently, in such studies[32,35,86].

Taken together, the four papers included in the present thesis demonstrate that standardised histopathologic assessment after pancreatoduodenectomy for pancreatic, ampullary, distal bile duct, and duodenal adenocarcinomas is necessary for complete, consistent, and correct classification of tumours and for reliability of prognostic estimates. Standardisation of histopathologic evaluation and reporting was found to be more important than institutional volume, and standardisation of lymph node evaluation was particularly important to discriminate adequately between favourable versus poor prognostic groups.

Non-curative resection is most often due to involvement of the retroperitoneal margin. Discrimination between curative (R0) and non-curative (R1) resections had a larger impact on long-term survival for ampullary than for pancreatic carcinomas. Although the tumour origin (pancreas, distal bile duct, ampulla, or duodenum) was an independent prognostic factor in curative (R0) resections, accurate determination of the tumour origin may be impossible in some cases, and the histologic type of differentiation, pancreatobiliary versus intestinal, was a better prognostic indicator than the tumour origin. In multivariable survival analysis, the histologic type of differentiation (pancreatobiliary versus intestinal) was the strongest adverse predictor of survival (hazard ratio 3.1, 95% confidence interval [1.8-5.1]), followed by lymph node involvement (hazard ratio 2.5, 95% confidence interval [1.5-4.4]).

In pancreatobiliary-type adenocarcinomas of comparable size, survival did not differ for patients depending on whether the tumour originated from the pancreas, ampulla, or distal bile duct. Furthermore, nodal involvement, vessel involvement, and increasing tumour size were independent adverse prognostic factors. The histologic type of differentiation may be determined with almost perfect agreement between two independent observers, and tumours with mixed-type differentiation may be classified by the most prominent histologic type. Immunohistochemical characterisation targeting markers of the histologic type of differentiation (including cytokeratins, mucin proteins, and the homeodomain protein CDX2) is often used as evidence of the line of differentiation, although such immunohistochemical characterisation only partly corresponds to subclassification (pancreatobiliary versus intestinal) using ordinary haematoxylin and eosin stained sections. Immunohistochemical subclassification of tumours did not discriminate better between prognostically poor versus favourable subgroups of patients than subclassification based on evaluation of ordinary haematoxylin and eosin stained sections. However, immunostaining directed at identification of MUC1 and MUC4 may be used to identify patients with particularly poor prognosis among those who have pancreatobiliary-type tumours.

The systematic protocol for standardised evaluation and reporting of histopathologic prognostic factors described in papers I and II differs in some respects from recently published protocols and recommendations[23,24,277,279]. However, the most important issue should be to reach international consensus on a single recommendation, and to implement the use of standardised protocols worldwide. We have focused particularly on the importance of tumour involvement of the retroperitoneal resection margin, a margin often not evaluated in reports on pancreatoduodenectomy specimens, and on determination of the precise tumour origin (pancreatic, biliary, ampullary, or duodenal). The retroperitoneal

margin was the margin that was most often involved in incomplete resections. This has also been shown previously[128,288], and confirmed in later studies[24,129,133,134,215]. We found that retroperitoneal margin involvement was an independent adverse prognostic factor after curative-intent pancreatoduodenectomy for adenocarcinoma. However, the benefit of a margin-free resection was significantly more pronounced for patients with ampullary tumours compared to patients with pancreatic tumours.

In paper II, we reviewed histopathologic reports from all 25 Norwegian institutions that performed pancreatoduodenectomies in the period 1998-2004. We found that standardised histopathologic evaluation was more important than surgical volume for completeness of histopathologic reporting. Other studies have shown that standardised reporting may improve detection of poor prognostic factors in various cancers (e.g., for estimating the true frequency of lymph node involvement in pancreatic adenocarcinomas[296], as described in the "**Statistical considerations**" section). However, no previous study has to our knowledge investigated whether standardisation actually increases accuracy of prognostic estimates using all available information from the pathology reports. Furthermore, we found that lymph node involvement was the factor that was most dependent on standardised reporting to discriminate between favourable and poor prognostic subgroups. Lymph node evaluation has many times previously been recognised as an important histopathologic prognostic factor, particularly when more than one positive lymph node is detected[132,298-304]. The total number of evaluated lymph nodes has been suggested as an indicator of the quality of histopathologic reporting[296,302,305-309]. However, the total number of lymph nodes extracted during pancreatoduodenectomy (and evaluable for the pathologist) may vary considerably even for a single surgeon.

In paper III, we compared classification of pancreatoduodenectomy specimens by tumour origin (pancreatic, biliary, ampullary, or duodenal) with classification according to the histologic type of differentiation (pancreatobiliary versus intestinal). As detailed in the introduction of this thesis, failure to reach a correct diagnosis of the cancer origin (which may be impossible to determine in some instances[31,70,153,159,161]) may lead to false assumptions regarding long-term survival[25,30-32]. Misclassification of the tumour origin has been reported in up to 39% of long-term survivors[62]. Comparing the original histopathologic reports on pancreatoduodenectomy specimens with an independent reevaluation of the microscopic slides, we found that interobserver agreement in classification of the cancer origin was only fair to moderate (*kappa*=0.37; 95% CI=[0.25, 0.49]), whereas agreement was significantly better (*kappa*=0.68; 95% CI=[0.57, 0.79]) when the reevaluation also

included review of the operative and macroscopic reports. Determination of the probable anatomic tumour origin thus requires careful consideration of clinical, surgical, and macroscopic characteristics, as well as appropriate histopathologic specimen dissection and adequate preparation of microscopic slides (papers I and III). In particular, a whole mount section parallel to the biliary tract, ampulla of Vater, and main pancreatic duct, and also including the peri-Vaterian duodenum, should be obtained in order to demonstrate the tumour's relation to each of these structures of potential origin (paper I).

Staging according to the TNM criteria relies on a correct diagnosis of the cancer origin, since staging criteria are different for the separate origins[33,34]. The World Health Organization's recommendations for classification of these tumours[35] also includes classification according to a morphologic diagnosis, i.e. the histologic type. In particular, ampullary tumours may easily be misclassified as duodenal or pancreatic/biliary tumours. Even in cases when the ampullary tumour is easy to determine, eg., small infiltrating carcinomas with an associated intraampullary component and prominent dysplasia of the ampullary epithelium adjacent to the neoplastic tissue, ampullary tumours may have either intestinal-type or pancreatobiliary-type differentiation. Thus, we hypothesised that classification by the histologic type of differentiation for all four periampullary origins could be more relevant with respect to estimating long-term survival after curative-intent pancreatoduodenectomy for adenocarcinomas. This has not been evaluated previously. There have been some previous reports on intestinal-type pancreatic and biliary tract carcinomas, although not in a context of consecutive reporting for a pancreatoduodenectomy series. This study confirms the previous assumption that intestinal-type pancreatic (and biliary tract) carcinomas are rare, although Albores-Saavedra and colleagues[148] reported that intestinal-type adenocarcinomas might be more frequent than previously believed – according to them, this entity may represent the second-most frequent histologic subtype of ductal pancreatic carcinomas (the typical pancreatobiliary-type carcinomas being the most frequent subtype). However, larger studies must establish the true incidence of these tumours in pancreatic and distal bile duct carcinomas. Importantly, the finding that evaluation of the histologic type of differentiation, pancreatobiliary versus intestinal, was a better predictor of survival than the anatomic tumour origin after pancreatoduodenectomy raises the question whether we really need a separate classification for ampullary carcinomas[162]. In accordance with our findings, van Roest and colleagues[310] recently found that the primary site of origin for the periampullary carcinomas was not significantly associated with survival ($p=0.095$) when adjusting for perineural growth,

resection margin involvement, lymph node status, and angioinvasion. In our study (paper III), we reported that there were significant associations between histologic type of differentiation and the prognostic determinants reported by van Roest and colleagues, and this probably explains why tumour origin rendered insignificant when adjusting for the other factors in the study by Roest and colleagues.

Paper IV evaluated the usefulness of molecular markers in classification and prognosis of pancreatic head adenocarcinomas. Although immunostaining is often used to confirm the finding of a specific histologic type, previous reports on differentiation markers in specimens from pancreatic head and periampullary resections have low sensitivity and/or specificity[148,156,188,191,192,200,202,223,311-315], even when evaluating combined marker expression[156,192,223,313-315]. We demonstrated that immunohistochemical analysis is not necessary to determine the histologic type of differentiation, although specific immunohistochemical phenotypes may identify patients with poor versus favourable prognosis among those who have pancreatobiliary-type tumours or intestinal-type tumours, respectively.

# Conclusions

Referring to the aims stated on page 29, the following conclusions can be drawn.

1. Non-curative (R1) resection is most often due to tumour involvement of the retroperitoneal resection margin (in 32 of 40 R1 resections, 80%; paper I). Resection margin involvement, and retroperitoneal margin involvement in particular, independently predicts a poor prognosis after curative-intent (R0 and R1) pancreatoduodenectomy. Obtaining a curative (R0) resection was found to be significantly more important for patients who have ampullary tumours than for patients who have pancreatic tumours.

2. Standardised histopathologic reporting was more important than institutional volume for completeness of histopathologic reporting and accuracy of survival estimates (paper II), particularly with respect to lymph node staging.

3. Interobserver variation in classification of the cancer origin is not only dependent on differences between independent reviewers in judgement of histopathologic slides. It is also dependent on the methods and extent of evaluation of clinical, surgical, and macroscopic characteristics, as well as the appropriateness of histopathologic specimen dissection and the adequacy in preparation of microscopic slides (papers I and III). The histologic type of differentiation is easier to determine and may also be a better prognostic indicator than the assumed anatomic tumour origin (paper III).

4. Immunohistochemical evaluation of molecular markers was not found necessary to determine the histologic type of differentiation (paper IV). However, immunostaining for the mucin proteins MUC1 and MUC4 may be used to identify patients with particularly poor prognosis after pancreatoduodenectomy for adenocarcinoma.

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# I

BMC Cancer

Research article



# Resectable adenocarcinomas in the pancreatic head: the retroperitoneal resection margin is an independent prognostic factor

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Published: 14 January 2008

*BMC Cancer* 2008, **8**:5 doi:10.1186/1471-2407-8-5

Received: 24 April 2007
Accepted: 14 January 2008

This article is available from: http://www.biomedcentral.com/1471-2407/8/5



## Abstract

**Background:** The retroperitoneal margin is frequently microscopically tumour positive in non-curative periampullary adenocarcinoma resections. This margin should be evaluated by serial perpendicular sectioning. The aim of the study was to determine whether retroperitoneal margin involvement independently predicts survival after pancreaticoduodenectomy within a framework of standardized assessment of the resected specimens.

**Methods:** 114 consecutive macroscopically margin-free periampullary adenocarcinomas were examined according to a prospective standardized protocol for histopathologic evaluation. The retroperitoneal margin was assessed by serial perpendicular sectioning. The periampullary cancer origin (pancreas, ampulla, distal bile duct or duodenum) was registered prospectively and reevaluated retrospectively. Associations between histopathologic factors were evaluated by Chi-square test, Fisher's exact test, Kruskal-Wallis test, and Mann-Whitney test, as appropriate. Survival curves were calculated by the Kaplan-Meier method and compared using the log-rank test. Associations between histopathologic factors and survival were also evaluated by unadjusted and adjusted Cox regression analysis, including stepwise variable selection, in order to identify factors that independently predict a poor prognosis after periampullary adenocarcinoma resections.

**Results:** Microscopic resection margin involvement (R1 resection) was present in 40 tumours, of which 32 involved the retroperitoneal margin. Involvement of the retroperitoneal margin independently predicted a poor prognosis ($p = 0.010$; HR 1.89; CI 1.16–3.08) after presumed curative (R0 and R1) resection. In microscopically curative (R0) resections ($n = 74$), pancreatic tumour origin was the only factor that independently predicted a poor prognosis ($p < 0.001$; HR 4.71 for pancreatic versus ampullary; CI 2.13–10.4).

**Conclusion:** Serial perpendicular sectioning of the retroperitoneal resection margin demonstrates that tumour involvement of this margin independently predicts survival after pancreaticoduodenectomy for adenocarcinoma. Periampullary tumour origin is the only histopathologic factor that independently predicts survival in microscopically curative (R0) resections.

## Background

Resectable primary adenocarcinomas located in the pancreatic head may derive from the pancreatic tissue, the hepatopancreatic ampulla, the distal bile duct or the duodenum, and collectively these cancers may be referred to as periampullary adenocarcinomas [1]. The precise tumour origin is often impossible to determine prior to surgery, and pancreaticoduodenectomy is thus performed for all four types irrespective of tumour origin. Complete tumour removal is one of the most important factors influencing long-term survival after resection [2-6]. However, even after margin-free resection (R0 resection) the recurrence rate is high and the majority of patients succumb to the disease within 5 years [2-6].

The reported proportion of patients having tumour involved resection margins (R1 resection) after pancreaticoduodenectomy varies considerably, in the range 31–85% for pancreatic tumours and 2–27% for ampullary tumours [1,2,7-10]. The large variation may partly be explained by underreporting of R1 resections due to non-standardized protocols for microscopic evaluation of the resection margins [9,11]. Furthermore, little is known about the relative importance of the different resection margins in R1 resections as determinants for survival [5,9,12]. The techniques employed for examination of the resected specimens clearly influence the reported rates of R0/R1 resections. Several groups have suggested guidelines for standardization of histopathologic assessment [13-19]. However, the retroperitoneal resection margin, which is most often involved in non-curative resections [5,13,20,21], is often not systematically evaluated in studies reporting histopathologic prognostic factors after pancreaticoduodenectomy [22-25].

The considerable variations in reported percentages of R1 resections for pancreatic and ampullary tumours may also be explained by difficulties in determining the cancer origin. Even after systematic histopathologic evaluation, the precise origin may be impossible to determine due to tumour destruction of normal periampullary anatomy [13,26-29]. There is also considerable normal variation of periampullary ductal structures, adding to the difficulties [26]. The common practice of reporting data on only a single periampullary subtype makes comparison of studies difficult due to the expected variations in inclusion and exclusion criteria for periampullary subtypes. For example, survival after resection of ductal pancreatic adenocarcinoma may be overestimated if ampullary cases are not adequately excluded [30]. Adjusted Cox regression analysis [31] including tumour origin as a covariate adjusts for some of the uncertainties regarding periampullary subtype classification, and also eliminates redundant or duplicate information resulting from associations between tumour origin and other covariates. Thus, we propose that survival analysis of all periampullary adenocarcinomas should include the tumour origin as a covariate rather than only presenting the results from separate subgroups.

Starting from 1998, we have employed a standardized protocol for evaluation of pancreaticoduodenectomy specimens, including serial perpendicular sectioning of the retroperitoneal resection margin and prospective evaluation of the cancer origin. The aim of this study was to investigate whether tumour involvement of the retroperitoneal margin is an independent prognostic factor for survival after resection of periampullary adenocarcinoma. Tumour origin was included as a covariate both in the overall adjusted analysis of all presumed curative (R1 and R0) periampullary resections and in a separate subgroup analysis of R0 resections.

## Methods

### *Patient cohort*

The study was approved by the National Committees for Research Ethics in Norway, project number S-05081, and was in compliance with the Helsinki Declaration. From 1998 to 2004, 161 consecutive patients underwent pancreaticoduodenectomy at the Department of Surgery, Rikshospitalet University Hospital, a third-level referral hospital. Of these, 114 patients (55 women and 59 men; median age 68 years; range 41–82) had primary adenocarcinoma with macroscopically free margins (R0 or R1 resections). Seventy six of the 114 included patients died before the end of the study, and the remaining 38 patients were followed up for a median of 4.8 years (range 1.6–8.4). None of the patients received preoperative chemo- or radiotherapy. During the study period, national guidelines did not recommend postoperative chemo- or radiotherapy. All patients underwent a standard Whipple's procedure including a distal gastrectomy. An effort was made to skeletonize the superior mesenteric and portal veins and the superior mesenteric artery in all cases, without performing extended lymphadenectomy. There were three cases with vascular resection (of which one tumour originated in the peripapillary duodenum and two were pancreatic). Intra-operative frozen sections from the bile duct and pancreatic neck resection margins were performed upon macroscopic suspicion of tumour involvement. Perioperative death (in-hospital death or death within 30 days of operation) was 3.5% (4/114). Cases with perioperative death were included in the survival analysis.

### *Standardized protocol for examination of resection specimens*

In this study, we defined the retroperitoneal margin as the area of sharp dissection in the peripancreatic fatty tissue behind the pancreatic head and lateral to the mesenteric

vessels (Figure 1). After fixation of the pancreaticoduodenectomy specimen in formalin, one block from the pancreatic neck and distal bile duct resection margins, respectively, was secured. These sections were taken parallel to the resection margins (shave sections). One block from the stomach and small bowel resection margins, respectively, was also secured. The retroperitoneal margin was identified and inked, and a section parallel to the resection margin (5–10 mm thick slice) was made, from which serial perpendicular sectioning into 5 mm thick slices was performed (Figure 2) [11,13]. The pancreatic duct and the distal bile duct, and their orifice(s) at the duodenal surface were identified, and probes were inserted in order to locate any obstruction within these ducts. A section parallel to the ductal structures, including duodenum, ampulla, distal bile duct and pancreatic parenchyma on a single slide, was made in order to demonstrate the tumour's relation to each of these potential sites of origin (Figure 3) [1]. Cross sections into the tumour were then made to evaluate tumour size and potential infiltration into adjacent structures. Lymph nodes were sampled from the duodenal knee and large and lesser curvatures of the stomach.

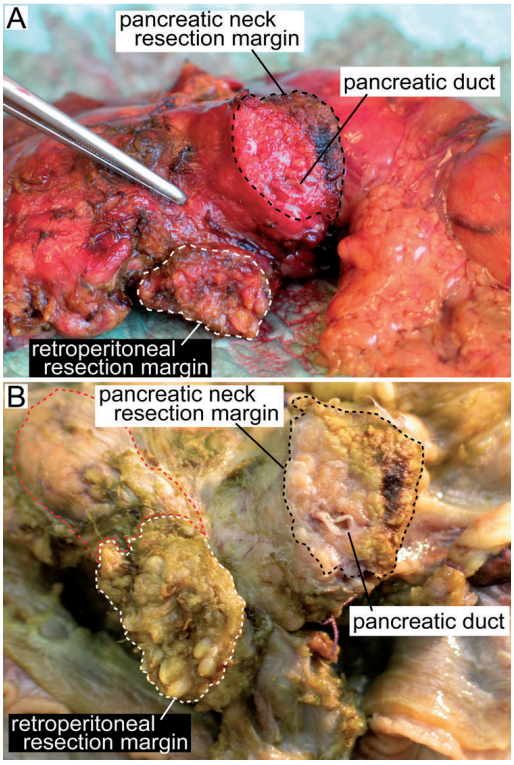


**Figure 1**
Close-up of the posterior aspect of a pancreaticoduodenectomy specimen from a patient with adenocarcinoma in the pancreatic head without tumour infiltration into the retroperitoneal margin, before (A) and after (B) fixation in formalin. The retroperitoneal resection margin was defined as the area of sharp dissection (white stapled area) in the peripancreatic fatty tissue behind the pancreatic head and lateral to the mesenteric vessels. In this case this area was relatively small. In cases with tumour infiltration or inflammation involving the posterior aspect of the pancreatic head, the size of this sharply dissected area may extend into the superior mesenteric vein groove (A, indicated by the forceps) or to a larger part of the posterior pancreatic surface (B, red stapled area). The black stapled area (A, B) indicates the pancreatic neck transection margin.

### *Histopathologic evaluation of specimens*

The following histopathologic factors were prospectively registered by routine examination: Tumour origin, maximum tumour diameter, degree of differentiation, perineural infiltration, vascular infiltration, dysplasia or other tumour associated pathologic changes, lymph node status, and resection margin status (pancreatic, bile duct, stomach, jejunal and retroperitoneal margins evaluated independently). All registrations were later reevaluated by an experienced pathologist. Finally, tumour origin was independently assessed by a second experienced pathologist. The cancer origin was determined by assessing tumour location relative to ductal anatomy and duodenal and pancreatic parenchyma, and by noting any associated epithelial dysplasia or in situ neoplasia. Upon disagreement, consensus was reached by discussion. All tumours were assigned to one of the four types using this approach. The final allocation of tumour origin corresponded with the initial prospective evaluation in 89 of 114 specimens (78%).

An R0 resection was defined as both macro- and microscopically free margins. An R1 resection was defined as tumour within 1 mm of a resection margin upon microscopic examination of haematoxylin and eosin stained sections. An R2 resection was defined as macroscopic residual tumour at the operative site, as described in the surgeon's operative report. Degree of differentiation was classified according to a two-score system as proposed by Lüttges et al. [32], distinguishing high-grade from low-grade carcinomas by presence or no presence, respectively, of areas with poorly differentiated tumour.

### *Statistical analysis*

Survival data were obtained from the National Registry of Norway. The Kaplan-Meier method was used to calculate curves for overall survival and to estimate median survival. Survival curves were compared using the log-rank

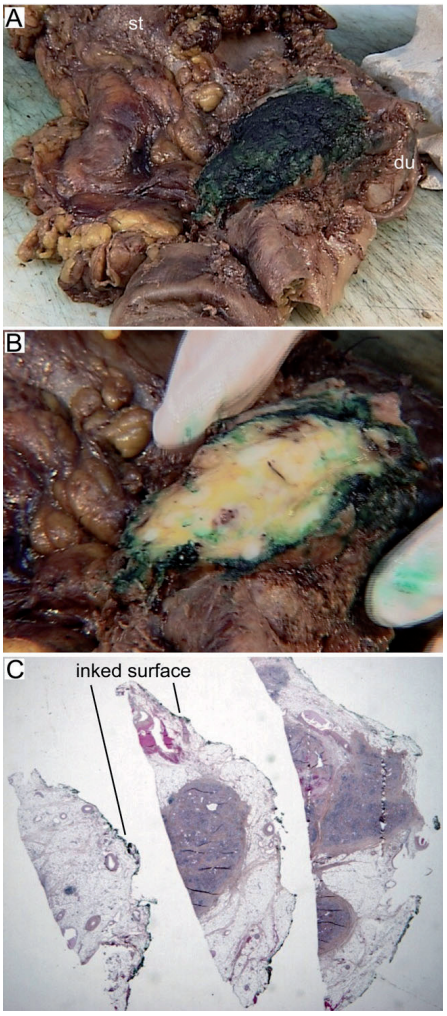


**Figure 2**
(A) Posterior view of a pancreaticoduodenectomy specimen with periampullary adenocarcinoma infiltrating the retroperitoneal resection margin. The specimen includes the pyloric part of the stomach (st), the duodenum (du), adipose tissue which is part of the greater omentum, and the head and uncinate process of pancreas. The retroperitoneal resection margin was identified and marked with ink. (B) A section parallel to this resection margin was made. (C) Perpendicular sections of the retroperitoneal resection margin demonstrate pancreatic parenchyma and connective tissue, including fat, vessels and nerves, with infiltration of tumour cells < 1 mm from the inked margin (visible on higher magnification), thus revealing a non-curative (R1) resection.

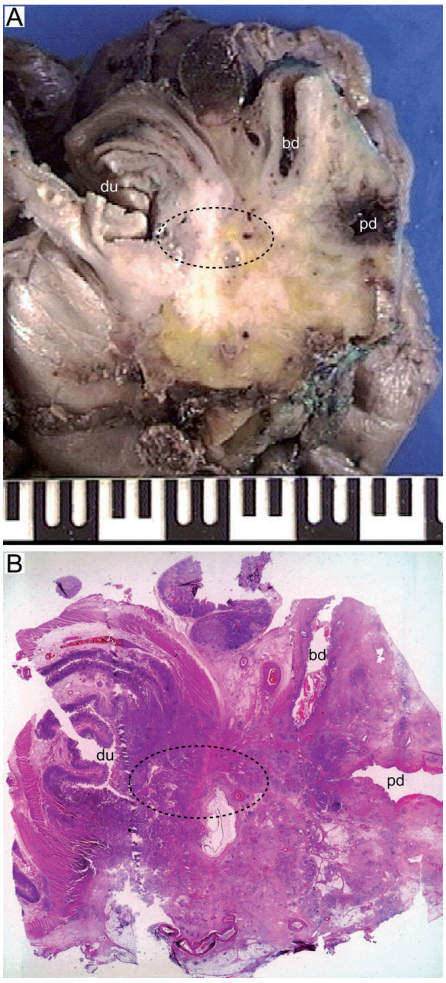


**Figure 3**
Whole-mount section parallel to the ampulla, distal bile duct and pancreatic duct, demonstrating periampullary tumour growth relative to ductal anatomy, for determination of the site of cancer origin. (A) White areas on the macroscopic photograph indicate possible tumour growth. (B) On microscopic examination, this tumour was found to originate from the peri-papillary duodenum (du), although it also involved the entire ampullary region (stapled area), and the distal portions of the distal bile duct (bd) and pancreatic duct (pd). In such cases, and in particular if epithelial dysplasia or in situ neoplasia also affects more than one periampullary sublocation, determination of the cancer origin may be difficult, and virtually impossible without systematic histopathologic examination.

test. Associations between categorical variables were examined using Chi-square test and Fisher's exact test. Mann-Whitney test and Kruskal-Wallis test were performed to compare tumour diameter (measured as a continuous variable) between groups of independent samples. The factors evaluated were: tumour origin, maximum tumour diameter, degree of differentiation, perineural infiltration, vascular infiltration, lymph node status, and resection margin status (pancreatic, bile duct, and retroperitoneal margins; no tumour infiltrated the stomach or jejunal resection margins).

Cox regression models were fitted in order to estimate unadjusted and adjusted survival after presumed curative (R0 and R1) resection, together with the hazard ratios with their 95% confidence intervals. For categorical variables, the group with the best prognosis in unadjusted analysis was set as reference. Hazards were proportional for all covariates, allowing inclusion of covariates in the adjusted analysis without need for stratification. All the examined histopathologic factors were significant in the unadjusted analysis and were thus included in the adjusted models. Two separate models were fitted for adjusted analysis of all histopathologic factors, considering the resection margins collectively (R1 versus R0) and individually (retroperitoneal margin free versus involved, and pancreatic margin free versus involved; omitting the stomach and duodenal resection margins, that had no cases with tumour involvement, and the distal bile duct margin, that had only two cases with tumour involvement). Factors were evaluated using forward stepwise variable selection, thus avoiding inclusion of variables with redundant prognostic information. In order to estimate the relative importance of the individual resection margins, the adjusted analysis was repeated after exclusion of the seven patients that had multiple margin tumour involvement. This analysis gave very similar results, with the same covariates in the final adjusted models as in the analysis including all 114 patients. These seven patients were thus not excluded from the analysis. A separate adjusted Cox regression subgroup analysis was performed for R0 resected patients in order to determine the factors that were independently associated with survival in curative resections. The proportional hazards assumption was evaluated by examination of log minus log plots (see Additional file 1: Verification of the proportional hazards assumption). Likelihood ratio test was computed to examine possible interactions between covariates (see Additional file 2: Evaluation of possible interaction between tumour origin and resection margin status).

All statistical analyses were performed with SPSS 15.0 for Windows software (SPSS Inc., Chicago, Illinois, USA). A two-sided $p < 0.05$ was considered statistically significant.

## Results

In this study of 114 macroscopically margin-free pancreaticoduodenectomies, 65% and 35% were R0 and R1 resections, respectively (Table 1). The retroperitoneal margin was involved by tumour infiltration in 80% of the R1 resections (32 of 40). Seven of the thirty-two tumours that infiltrated the retroperitoneal margin also infiltrated the pancreatic neck transection margin (of which two also infiltrated the distal bile duct margin). Resection margin involvement was significantly associated with each of the other prognostically poor histopathologic factors (regional lymph node involvement, $p < 0.001$; vessel infiltration, $p = 0.001$; perineural infiltration, $p < 0.001$; presence of areas with poor differentiation, $p = 0.005$; large tumour, $p = 0.013$). Resection margin involvement was most frequent when the tumour originated from the distal bile duct or pancreas ($p = 0.009$).

### *Unadjusted overall survival*

In the unadjusted Cox regression analysis of 114 periampullary adenocarcinomas, tumour involvement of the resection margins predicted a poor prognosis compared to margin-free resections (Figure 4; see also Additional file 3: Unadjusted analysis of histopathologic prognostic factors), both when the resection margins were modelled collectively (R1 versus R0 resections, $p < 0.001$) and separately (retroperitoneal margin involved versus free, $p < 0.001$; pancreatic neck transection margin involved versus free, $p = 0.003$; bile duct resection margin involved versus free, $p = 0.005$). As expected, patients with cancer originating from the pancreas had the worst prognosis, with a median postoperative survival of 1.2 years (95% CI: 1.0–1.4), compared to 4.9 years (95% CI: 2.4–7.4) for ampullary tumours ($p < 0.001$). However, although the prognosis for R0 resected patients was significantly associated with tumour origin ($p < 0.001$), the prognosis after non-complete (R1) resections did not depend on tumour origin ($p = 0.45$). Comparing resections of pancreatic and ampullary tumours (Figure 5), resection status was found to be a more powerful predictor for survival for patients with ampullary tumour ($p < 0.001$, Figure 5B) than for patients with pancreatic tumour ($p = 0.30$, Figure 5A). Patients with ductal pancreatic adenocarcinoma had an estimated, statistically non-significant survival benefit of only five months for curative versus non-curative resection ($p = 0.30$, Figure 5A), while most of the patients with R0 resected ampullary tumours were still alive by the end of the study (19 of 31; median survival not reached, >5 years) and all patients with R1 resected ampullary tumours were dead by the end of the study (10 of 10) ($p < 0.001$, Figure 5B). The interaction between resection margin status and tumour origin for these two groups was statistically significant ($p = 0.009$).

**Table 1: Origin of tumour versus margin involvement and other histopathologic characteristics in 114 periampullary adenocarcinomas**

| | Origin of tumour | | | | Total (n = 114) | p-value[a] |
|---|---|---|---|---|---|---|
| | Ampulla (n = 41) | Duodenum (n = 16) | Distal bile duct (n = 17) | Pancreas (n = 40) | | |
| **Margin involvement** | | | | | | 0.009[b] |
| Any margin (R1 resections) | 10 | 2 | 10 | 18 | 40 | |
| Retroperitoneal | 9 | 1 | 8 | 14 | 32 | |
| Pancreatic neck | 2 | 1 | 5 | 7 | 15 | |
| Distal bile duct | 0 | 0 | 0 | 2 | 2 | |
| No margin (R0 resections) | 31 | 14 | 7 | 22 | 74 | |
| **Other histopathologic characteristics** | | | | | | |
| Nodal status | | | | | | 0.005 |
| N1 | 17 | 10 | 7 | 31 | 65 | |
| N0 | 24 | 6 | 10 | 9 | 49 | |
| Degree of differentiation | | | | | | 0.009 |
| Poor | 8 | 5 | 9 | 21 | 43 | |
| High or moderate | 33 | 11 | 8 | 19 | 71 | |
| Vessel involvement | | | | | | 0.013 |
| yes | 9 | 4 | 9 | 21 | 43 | |
| no | 32 | 12 | 8 | 19 | 71 | |
| Perineural infiltration | | | | | | <0.001 |
| yes | 13 | 6 | 11 | 32 | 62 | |
| no | 28 | 10 | 6 | 8 | 52 | |
| Tumour size | | | | | | <0.001[c] |
| large (diameter ≥ 2.6 cm) | 8 | 11 | 6 | 23 | 48 | |
| small (diameter ≤ 2.5 cm) | 33 | 5 | 11 | 17 | 66 | |

[a]Chi-square test, when not otherwise specified
[b]R1 vs R0 resection
[c]Measured as a continuous variable, Kruskal-Wallis test and Mann-Whitney test (pancreatic vs non-pancreatic)

### *Adjusted analysis of presumed curative resections*

In order to establish whether the retroperitoneal resection margin was an independent prognostic factor for clinically resectable periampullary adenocarcinomas (R0 and R1 resections), we performed adjusted Cox regression analysis including in a forward variable selection process all the variables that were significant in the unadjusted analysis. This resulted in the adjusted models (Table 2), in which tumour involvement of one or more resection margins (Table 2A), and the retroperitoneal margin in particular (Table 2B), independently predicted a poor prognosis after pancreaticoduodenectomy for periampullary adenocarcinoma ($p = 0.010$), adjusting for lymph node status and perineural infiltration ($p < 0.01$ for each, in both adjusted models).

### *Survival after microscopically margin-free resections*

Finally, we performed a subgroup analysis of all patients that underwent a curative (R0) resection (n = 74). Pancreatic tumour origin was significantly associated with each of the other prognostically poor histopathologic factors (regional lymph node involvement, $p = 0.008$; vessel infiltration, $p = 0.004$; perineural infiltration, $p < 0.001$; presence of areas with poor differentiation, $p = 0.001$; large tumour, $p < 0.001$). Although these factors were significantly associated with survival in unadjusted analysis, adjusted analysis with stepwise forward variable selection resulted in a final model that included only tumour origin, which was thus the only independent predictor of survival after curative pancreaticoduodenectomy in the present study. The hazard ratio for R0 resected ductal pancreatic versus ampullary adenocarcinoma was 4.71 (95% CI: 2.13–10.4, $p < 0.001$). Median survival for patients with R0 resected pancreatic cancer was 1.3 years (95% CI: 1.0–1.6) while patients with R0 resected non-pancreatic cancer survived median more than 5 years (median survival not reached for ampullary and duodenal cases; $p < 0.001$).

## Discussion

Standardized protocols for evaluation of the resection margins should be mandatory in studies reporting prognostic data on periampullary adenocarcinomas [1,9,13,19,33]. The retroperitoneal margin should be evaluated by serial perpendicular sectioning [13]. Insufficient examination of the retroperitoneal margin might lead to underreporting of R1 resections[9,11]. Although most investigators report overall resection margin involvement to be an independent prognostic factor [2,3,7,8,34], some investigators have concluded other

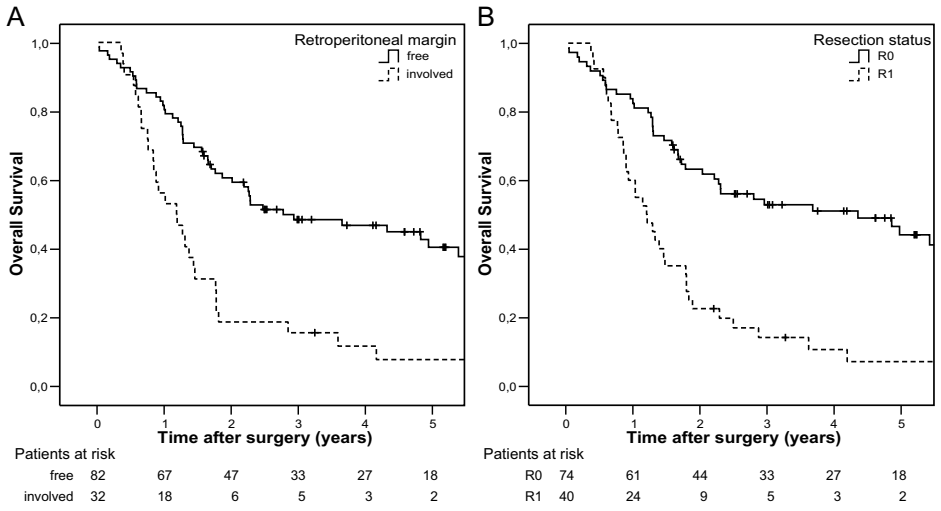


**Figure 4**
Overall survival after pancreaticoduodenectomy for periampullary adenocarcinoma (n = 114) with (A) free versus involved retroperitoneal resection margin ($p < 0.001$) and (B) R0 versus R1 resection ($p < 0.001$).

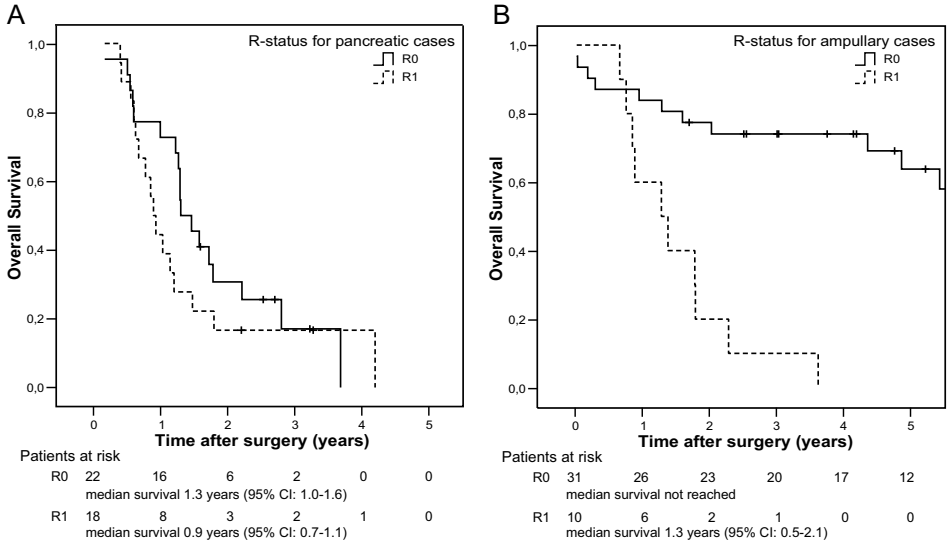


**Figure 5**
Overall survival for R0 versus R1 resection of tumours originating in (A) the pancreas (n = 40; $p = 0.30$) and (B) the ampulla (n = 41; $p < 0.001$).

**Table 2: Adjusted Cox regression analysis of histopathologic prognostic factors (n = 114)**

| | | HR | 95% CI | p-value |
|---|---|---|---|---|
| **A. Model 1** | | | | |
| Resection margin status | R1 (vs R0) | 1.90 | 1.17–3.10 | 0.010 |
| Lymph nodes | N1 (vs N0) | 2.24 | 1.28–3.91 | 0.005 |
| Perineural infiltration | yes (vs no) | 2.22 | 1.31–3.75 | 0.003 |
| **B. Model 2** | | | | |
| Retroperitoneal margin | involved (vs free) | 1.89 | 1.16–3.08 | 0.010 |
| Lymph nodes | N1 (vs N0) | 2.29 | 1.32–3.99 | 0.003 |
| Perineural infiltration | yes (vs no) | 2.32 | 1.38–3.92 | 0.002 |

HR, hazard ratio. HR > 1 indicates increased probability of death compared to the reference group

wise [6,22]. Specific data on the retroperitoneal margin were not included in these reports. In the present study we have used a standardized systematic protocol for histopathologic assessment of resection margin involvement, with special attention to the retroperitoneal margin. Our main finding was that resection margin involvement, and retroperitoneal margin involvement in particular, independently predicts a poor prognosis in curative-intent (R0 and R1) resections for periampullary adenocarcinoma. In addition, we found that the anatomic tumour origin was the only independent prognostic factor in macro- and microscopic margin-free (R0) resections.

A problem when considering standardization of histopathologic reporting of pancreaticoduodenectomy specimens is that the definition of the retroperitoneal resection margin varies considerably. Some investigators define this margin simply as "the peripancreatic fat tissue behind the head of the pancreas [13,15]." Others include only the tissue directly adjacent to the proximal 3–4 cm of the superior mesenteric artery [16,19], sometimes with a clear distinction between the "retroperitoneal" and the "posterior pancreatic" resection margins [16]. The retroperitoneal margin is also often synonymously referred to as the "posterior," "mesenteric" or "uncinate" margin [14,17]. Some have advocated examination of the whole peripancreatic fatty tissue resection margin [20,21,35]. Verbeke et al. [9] recently evaluated a standardized protocol for examination of the circumferential resection margin, subdividing this margin into the anterior, posterior and superior mesenteric vein groove circumferential resection margins. In cases with inflammation and tumour invasion it may be difficult to distinguish between such distinct resection margins. Most important for evaluation of tumour margin infiltration is the area of sharp dissection, the extent of which varies depending on the degree of inflammation and tumour invasion. In our study, we thus widened the strictest definition of the retroperitoneal resection margin, but omitted separate analysis of each aspect of the circumferential peripancreatic margin in order to avoid extensive sampling.

The use of non-standardized protocols for histopathologic assessment may not only cause inconsistencies in the reporting of R0 versus R1 rates, but could also lead to differences with respect to classification of the anatomic site of tumour origin [1,13]. In the present study, tumour origin did not independently predict survival in presumed curative (R0 and R1) resections, although this factor was borderline significant when evaluated in a base model adjusting for all other histopathologic factors. There are probably two reasons for this. First, patients with pancreatic tumours (with the poorest prognosis in unadjusted analysis) frequently had resection margin involvement (45%). Thus, adjusting for resection margin status in the adjusted analysis renders tumour origin statistically non-significant. Second, in the unadjusted analysis, tumour origin was significantly associated with survival only in R0, not R1, resections. Consequently, in the adjusted analysis for R0 resected patients, tumour origin was the only histopathologic factor that independently predicted long-term survival after pancreaticoduodenectomy.

Interestingly, patients with ampullary adenocarcinoma had a considerable survival benefit of a retroperitoneal margin-free resection, while a free margin at this site was only non-significantly associated with survival for patients who had adenocarcinoma originating in the pancreas. Even when considering the resection margins collectively, we found only a non-significant tendency towards some five months benefit of having a margin-free resection in the pancreatic group. This is in line with previous reports, since the difference between median survival of patients with margin-free versus margin-involved resections from ductal pancreatic adenocarcinoma has typically been reported to be about half a year [5,8,9,36-38]. In a large, multicenter, prospective study of resected pancreatic cancer, Neoptolemos et al. [36] found that resection margin status was not an independent predictor of survival in ductal pancreatic adenocarcinoma. The retroperitoneal resection margin was however not systematically evaluated, and the R0 rate was exceptionally high (81%), possibly underestimating the rate of R1 resections [9]. In a study primarily comparing pancreaticoduodenectomy with or without vascular resection, Tseng et al. [37] reported that retroperitoneal margin involvement was not an independent prognostic factor in patients with pancreatic adenocarcinoma. However, stepwise variable selection was not performed, and the definition of the retroperitoneal margin was restricted to the area directly adjacent to the superior mesenteric artery. Evaluating individual resection margins in 160 resected pancreatic adenocarcinomas, Kuhlmann et al. [5] found that R0 resection independently predicted a favourable prognosis,

but did not report the independent prognostic importance for survival of the retroperitoneal margin in particular. Thus, to establish whether or not involvement of the retroperitoneal resection margin independently predicts the prognosis also in ductal pancreatic adenocarcinoma, larger studies using standardized evaluation of both tumour origin and the individual resection margins should be performed.

## Conclusion

Systematic histopathologic evaluation confirms that resection margin involvement, and retroperitoneal margin involvement in particular, independently predicts a poor prognosis in curative-intent (R0 and R1) resections of periampullary adenocarcinoma. Involvement of the retroperitoneal margin is frequent in pancreatic, distal bile duct and ampullary tumours, and serial perpendicular sectioning of the retroperitoneal margin should thus be performed in all pancreatic head adenocarcinomas to avoid underestimation of R1 resections.

## Competing interests

The author(s) declare that they have no competing interests.

## Authors' contributions

AW participated in design of the study, registration and ethical approval, patient inclusion, review of clinical data, and histopathologic analysis. He also designed the database, performed the statistical analysis, and drafted the manuscript. ST participated in patient inclusion and had a major responsibility for histopathologic analysis. INF contributed with establishment of the protocol for systematic histopathologic assessment, participated in design of the study, patient inclusion, and histopathologic analysis. MC contributed substantially with choice of statistical methods and participated in statistical analysis. TJE participated in establishing systematic pathologic review of pancreaticoduodenectomy specimens, in design of the study, and in histopathologic analysis. ØM contributed substantially in the discussion of operative methods and performed many of the pancreaticoduodenectomies. OPC participated in design of the study, had a major responsibility for histopathologic analysis, and contributed substantially with critical review of the manuscript. IPG participated in design, registration and ethical approval of the research project, and in patient inclusion and registration of clinical data. He also performed many of the pancreaticoduodenectomies, contributed substantially in the discussion of statistical methods, and drafted the manuscript. All authors critically reviewed the manuscript and approved the final manuscript.

## Additional material

### Additional file 1

*Verification of the proportional hazards assumption. Comparison of hazard ratios in the overall base model with hazard ratios obtained by stratification by each covariate, graphically illustrated by log minus log plots.*
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[http://www.biomedcentral.com/content/supplementary/1471-2407-8-5-S1.pdf]

### Additional file 2

*Evaluation of possible interaction between tumour origin and resection margin status. A possible interaction between tumour origin and resection status was evaluated by comparing likelihoods calculated for models with and without the interaction term, respectively.*
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[http://www.biomedcentral.com/content/supplementary/1471-2407-8-5-S2.pdf]

### Additional file 3

*Unadjusted analysis of histopathologic prognostic factors. Median survival and hazard ratios from unadjusted Cox survival analysis of histopathologic prognostic factors in all resections and in microscopic curative resections.*
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## Acknowledgements

The authors thank Jo Røislien and Henrik René Cederkvist at Biostatistics, Rikshospitalet University Hospital for statistical advice.

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## Pre-publication history

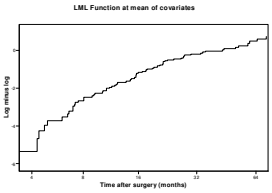
The pre-publication history for this paper can be accessed here:

http://www.biomedcentral.com/1471-2407/8/5/prepub

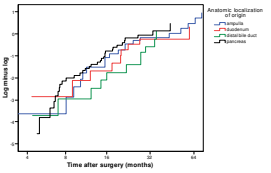
**Verification of the proportional hazards assumption (n=114)**

**Graphical evaluation**
(n=110; excluding perioperative mortality)

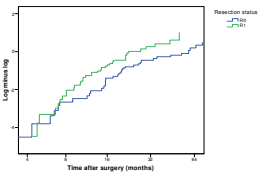
| Covariates | HR | 95.0% CI for HR |
|---|---|---|
| **Base model 1, without stratification** | | |
| Origin | | |
| - duodenal vs. ampullary | 0.57 | 0.21 - 1.52 |
| - distal bile duct vs. ampullary | 0.51 | 0.23 - 1.17 |
| - pancreatic vs. ampullary | 1.19 | 0.62 - 2.31 |
| Resection margin status | 1.82 | 1.09 - 3.06 |
| Lymph node status | 1.55 | 0.84 - 2.86 |
| Poor differentiation | 1.82 | 1.06 - 3.12 |
| Vessel involvement | 1.72 | 1.02 - 2.92 |
| Perineural infiltration | 1.66 | 0.92 - 3.02 |
| Tumour diameter (in cm) | 1.23 | 0.96 - 1.58 |



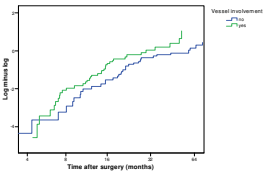

| | | |
|---|---|---|
| **Base model 1, stratified by tumour origin** | | |
| Resection margin status | 1.74 | 1.03 - 2.93 |
| Lymph node status | 1.72 | 0.90 - 3.27 |
| Poor differentiation | 1.83 | 1.06 - 3.15 |
| Vessel involvement | 1.65 | 0.96 - 2.84 |
| Perineural infiltration | 1.59 | 0.86 - 2.93 |
| Tumour diameter (in cm) | 1.21 | 0.94 - 1.57 |



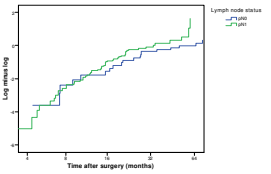

| | | |
|---|---|---|
| **Base model 1, stratified by resection status** | | |
| Origin | | |
| - duodenal vs. ampullary | 0.57 | 0.21 - 1.52 |
| - distal bile duct vs. ampullary | 0.52 | 0.22 - 1.18 |
| - pancreatic vs. ampullary | 1.14 | 0.58 - 2.24 |
| Lymph node status | 1.58 | 0.85 - 2.94 |
| Poor differentiation | 1.91 | 1.11 - 3.28 |
| Vessel involvement | 1.66 | 0.97 - 2.84 |
| Perineural infiltration | 1.62 | 0.90 - 2.95 |
| Tumour diameter (in cm) | 1.22 | 0.95 - 1.56 |



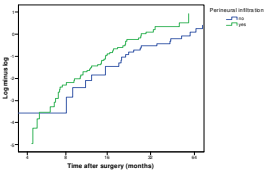

| | | |
|---|---|---|
| **Base model 1, stratified by lymph node status** | | |
| Origin | | |
| - duodenal vs. ampullary | 0.51 | 0.18 - 1.42 |
| - distal bile duct vs. ampullary | 0.50 | 0.22 - 1.14 |
| - pancreatic vs. ampullary | 1.21 | 0.62 - 2.35 |
| Resection margin status | 1.85 | 1.10 - 3.12 |
| Poor differentiation | 1.90 | 1.11 - 3.27 |
| Vessel involvement | 1.77 | 1.04 - 3.00 |
| Perineural infiltration | 1.66 | 0.91 - 3.01 |
| Tumour diameter (in cm) | 1.19 | 0.91 - 1.54 |



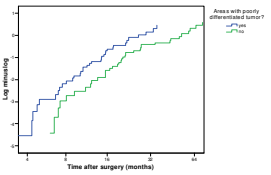

| | | |
|---|---|---|
| **Base model 1, stratified by vessel infiltration** | | |
| Origin | | |
| - duodenal vs. ampullary | 0.56 | 0.21 - 1.47 |
| - distal bile duct vs. ampullary | 0.56 | 0.24 - 1.28 |
| - pancreatic vs. ampullary | 1.17 | 0.60 - 2.28 |
| Resection margin status | 1.74 | 1.03 - 2.94 |
| Lymph node status | 1.53 | 0.83 - 2.82 |
| Poor differentiation | 1.75 | 1.02 - 3.02 |
| Perineural infiltration | 1.71 | 0.94 - 3.13 |
| Tumour diameter (in cm) | 1.23 | 0.96 - 1.58 |




| | | |
|---|---|---|
| **Base model 1, stratified by perineural infiltration** | | |
| Origin | | |
| - duodenal vs. ampullary | 0.57 | 0.21 - 1.54 |
| - distal bile duct vs. ampullary | 0.50 | 0.22 - 1.14 |
| - pancreatic vs. ampullary | 1.18 | 0.61 - 2.30 |
| Resection margin status | 1.84 | 1.10 - 3.09 |
| Lymph node status | 1.50 | 0.82 - 2.75 |
| Poor differentiation | 1.95 | 1.13 - 3.37 |
| Vessel involvement | 1.75 | 1.03 - 2.98 |
| Tumour diameter (in cm) | 1.24 | 0.96 - 1.60 |




| | | |
|---|---|---|
| **Base model 1, stratified by presence of poor differentiation** | | |
| Origin | | |
| - duodenal vs. ampullary | 0.62 | 0.23 - 1.70 |
| - distal bile duct vs. ampullary | 0.54 | 0.24 - 1.24 |
| - pancreatic vs. ampullary | 1.16 | 0.59 - 2.29 |
| Resection margin status | 1.86 | 1.10 - 3.13 |
| Lymph node status | 1.50 | 0.81 - 2.77 |
| Vessel involvement | 1.67 | 0.98 - 2.86 |
| Perineural infiltration | 1.73 | 0.95 - 3.16 |
| Tumour diameter (in cm) | 1.21 | 0.94 - 1.55 |



**Evaluation of possible interaction between tumour origin and resection margin status**

**A. Analysis including all four tumour origins (ampulla, duodenum, distal bile duct, pancreas) and resection margin status (free vs involved) (n=114)**

| | df | p-value | HR | 95.0% CI for HR Lower | Upper |
|---|---|---|---|---|---|
| Covariates (assuming no interaction) | | | | | |
| R-status | 1 | 0.000 | 2.61 | 1.59 | 4.27 |
| Origin | 3 | 0.002 | | | |
| Origin(duodenum vs ampulla) | 1 | 0.699 | 1.17 | 0.54 | 2.54 |
| Origin(distal bile duct vs ampulla) | 1 | 0.652 | 0.84 | 0.40 | 1.78 |
| Origin(pancreas vs ampulla) | 1 | 0.002 | 2.50 | 1.41 | 4.44 |
| -2 Log Likelihood of model coefficients | | | | | |
| 607.31 | 4 | | | | |
| Covariates (assuming interaction) | | | | | |
| R-status | 1 | 0.000 | 6.03 | 2.51 | 14.48 |
| Origin | 3 | 0.000 | | | |
| Origin(duodenum vs ampulla) | 1 | 0.387 | 1.51 | 0.59 | 3.84 |
| Origin(distal bile duct vs ampulla) | 1 | 0.972 | 0.98 | 0.28 | 3.47 |
| Origin(pancreas vs ampulla) | 1 | 0.000 | 4.72 | 2.19 | 10.20 |
| Origin*R-status | 3 | 0.103 | | | |
| Origin(duodenum vs ampulla)*R-status | 1 | 0.493 | 0.54 | 0.09 | 3.19 |
| Origin(distal bile duct vs ampulla)*R-status | 1 | 0.437 | 0.53 | 0.11 | 2.61 |
| Origin(pancreas vs ampulla)*R-status | 1 | 0.014 | 0.25 | 0.08 | 0.75 |
| -2 Log Likelihood of model coefficients | | | | | |
| 601.24 | 7 | | | | |
| Change in Chi-square | | | | | |
| 607.31 - 601.24 = 6.07 | 3 | 0.10<p<0.20* | | | |

**B. Analysis including the two largest groups of tumour origin (ampulla, pancreas) and resection margin status (free vs involved) (n=81)**

| | df | p-value | HR | 95.0% CI for HR Lower | Upper |
|---|---|---|---|---|---|
| R-status | 1 | 0.000 | 7.21 | 2.74 | 18.97 |
| Origin | 1 | 0.000 | 5.81 | 2.43 | 13.89 |
| Origin*R-status | 1 | 0.009† | 0.20 | 0.06 | 0.67 |

* non-significant

† significant

**Unadjusted analysis of histopathologic prognostic factors**

| | | No of patients | Median survival (years) | HR | 95% CI for HR | p-value |
|---|---|---|---|---|---|---|
| **A. R0 and R1 resections (n=114)** | | | | | | |
| R-status | R0 (ref) | 74 | 4.4 | | | |
| | R1 | 40 | 1.2 | 2.82 | 1.78–4.49 | < 0.001 |
| Retroperitoneal margin | free (ref) | 82 | 2.8 | | | |
| | involved | 32 | 1.2 | 2.61 | 1.63–4.18 | < 0.001 |
| Pancreatic neck margin | free (ref) | 99 | 2.2 | | | |
| | involved | 15 | 1.2 | 2.45 | 1.35–4.43 | 0.003 |
| Distal bile duct margin | free (ref) | 112 | 1.9 | | | |
| | involved | 2 | 0.6 | 8.27 | 1.88–36.4 | 0.005 |
| Origin | ampulla (ref) | 41 | 4.9 | | | < 0.001 |
| | duodenum | 16 | 2.3 | 1.09 | 0.50–2.38 | 0.822 |
| | distal bile duct | 17 | 2.5 | 1.20 | 0.58–2.49 | 0.615 |
| | pancreas | 40 | 1.2 | 3.15 | 1.79–5.53 | < 0.001 |
| Lymph nodes | N0 (ref) | 49 | NR | | | |
| | N1 | 65 | 1.3 | 3.30 | 1.97–5.53 | < 0.001 |
| Poor differentiation | no (ref) | 71 | 3.7 | | | |
| | yes | 43 | 1.2 | 2.59 | 1.64–4.11 | < 0.001 |
| Vessel involvement | no (ref) | 71 | 3.7 | | | |
| | yes | 43 | 1.3 | 2.80 | 1.76–4.47 | < 0.001 |
| Perineural infiltration | no (ref) | 52 | 5.4 | | | |
| | yes | 62 | 1.3 | 3.17 | 1.93–5.19 | < 0.001 |
| Tumour size (continuous) | diameter (cm) | 114 | 1.8 | 1.27 | 1.08–1.48 | 0.003 |
| **B. R0 resections (n=74)** | | | | | | |
| Origin | ampulla (ref) | 31 | NR | | | < 0.001 |
| | duodenum | 14 | 5.0 | 1.51 | 0.59–3.84 | 0.389 |
| | distal bile duct | 7 | NR | 1.00 | 0.28–3.55 | 0.998 |
| | pancreas | 22 | 1.3 | 4.71 | 2.13–10.4 | < 0.001 |
| Lymph nodes | N0 (ref) | 41 | NR | | | |
| | N1 | 33 | 1.7 | 2.89 | 1.51–5.55 | 0.001 |
| Poor differentiation | no (ref) | 53 | 5.4 | | | |
| | yes | 21 | 1.3 | 2.94 | 1.54–5.62 | 0.001 |
| Vessel involvement | no (ref) | 54 | 5.4 | | | |
| | yes | 20 | 1.3 | 2.43 | 1.24–4.75 | 0.009 |
| Perineural infiltration | no (ref) | 43 | 6.0 | | | |
| | yes | 31 | 1.7 | 2.38 | 1.27–4.48 | 0.007 |
| Tumour size (continuous) | diameter (cm) | 74 | 4.3 | 1.23 | 1.00–1.50 | 0.045 |

HR, hazard ratio. HR > 1 indicates increased probability of death compared to the reference group

ref, reference for categorical variables

NR, not reached

# II

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III

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# Pancreatobiliary versus intestinal histologic type of differentiation is an independent prognostic factor in resected periampullary adenocarcinoma

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Published: 11 June 2008

*BMC Cancer* 2008, **8**:170 doi:10.1186/1471-2407-8-170

Received: 7 July 2007
Accepted: 11 June 2008

This article is available from: http://www.biomedcentral.com/1471-2407/8/170



## Abstract

**Background:** Resectable adenocarcinomas in the pancreatic head, by definition "periampullary", originate from ampullary, duodenal, biliary, or ductal pancreatic epithelium. Typically, periampullary adenocarcinomas have either intestinal or pancreatobiliary type of differentiation, and the type of differentiation might be prognostically more important than the anatomic site of origin. The aim of the study was to determine whether the histologic type of differentiation is an independent prognostic factor in periampullary adenocarcinoma, and whether tumour origin predicts the prognosis in pancreatobiliary type carcinomas independently of resection margin involvement, tumour size, nodal involvement, perineural and vascular infiltration, and degree of differentiation.

**Methods:** Histopathologic variables in 114 consecutively resected periampullary adenocarcinomas of pancreatobiliary (n = 67) and intestinal (n = 47) type differentiation were evaluated using a standardized, systematic protocol for evaluation of the resected specimen (study group). Histologic type of differentiation and tumour origin were compared as predictors of survival, and the results were validated by comparison with a historical control group consisting of 99 consecutive pancreaticoduodenectomies performed before standardization of histopathologic evaluation. Associations between histopathologic variables were evaluated by Chi-square and Mann-Whitney tests. Survival was estimated by the Kaplan-Meier method, comparing curves using log-rank test, and by univariate and multivariable Cox regression analysis.

**Results:** Both in the study group (n = 114) and in the historical control group (n = 99), the histologic type of differentiation independently predicted survival, while tumour origin predicted survival only in univariate analysis. Independent adverse predictors of survival in the study group were pancreatobiliary type differentiation (p < 0.001; HR 3.1; CI 1.8–5.1), regional lymph node involvement (p < 0.001; HR 2.5; CI 1.5–4.4), vessel involvement (p = 0.012; HR 1.9; CI 1.2–3.1),

and increasing tumour diameter (measured in cm, p = 0.011; HR 1.3; CI 1.1–1.5). For pancreatobiliary differentiated adenocarcinomas (n = 67), lymph node status, vessel involvement, and tumour diameter remained independent prognostic factors, while tumour origin did not independently predict the prognosis due to significant association with tumour size (p < 0.001) and lymph node involvement (p = 0.004).

**Conclusion:** Pancreatobiliary versus intestinal type of differentiation independently predicts poor prognosis after pancreaticoduodenectomy for periampullary adenocarcinoma. Lymph node involvement, vessel infiltration, and increasing tumour diameter are adverse predictors of survival in tumours with pancreatobiliary differentiation.

## Background

Resectable primary adenocarcinomas located in the pancreatic head may derive from the pancreas, the ampulla, the distal bile duct, or the duodenum. Collectively, these tumours may be referred to as "periampullary" adenocarcinomas, of which those originating from the pancreas have the worst prognosis [1]. The histopathologic and biologic features associated with ductal pancreatic adenocarcinoma are different from non-pancreatic periampullary tumours [2], and it has thus been customary to consider these four subtypes of periampullary adenocarcinoma as separate entities.

The precise origin of a periampullary adenocarcinoma is often difficult to determine even with standardized histopathologic evaluation, particularly if the tumour is large and involves more than one potential site of origin [3-8]. Tumour destruction of normal periampullary anatomy [9], and presence of epithelial dysplasia in more than a single periampullary compartment, occurs frequently. Data in reports from a single subtype of periampullary adenocarcinoma may be confounded by inadvertent inclusion of tumours from other subtypes [6]. For example, inadequate exclusion of ampullary carcinomas from series of ductal pancreatic adenocarcinoma may lead to overestimation of long-term survival [10].

In addition to the commonly evaluated histopathologic factors, the histologic type of differentiation has been shown to have biologic and prognostic relevance for ampullary adenocarcinoma [6,7,11-14]. Kimura et al [13] were the first to demonstrate that adenocarcinomas originating in the ampulla of Vater may be classified as having either "intestinal" or "pancreatobiliary" type of differentiation, of which patients with the latter type consistently have been shown to have a worse prognosis [6,7,11-14]. This classification scheme is now widely accepted for ampullary adenocarcinoma and has also been suggested for extrahepatic bile duct carcinoma [15] and ductal pancreatic adenocarcinoma [16], but has not, to our knowledge, been applied previously as a basis for analysis of prognostic factors after periampullary adenocarcinoma resections. In the present study, we hypothesized that an evaluation of the histologic type of differentiation could independently predict the prognosis after periampullary resections and possibly give more precise information about patient prognosis than evaluation of tumour origin.

## Methods

### *Patients*

Permission for the study was obtained by the National Committees for Research Ethics in Norway. The patients included in the study comprised all patients (n = 213) with primary periampullary adenocarcinoma who underwent a pancreaticoduodenectomy with curative intent between 1980 and 2004 at Rikshospitalet University Hospital, a third-level referral hospital. In January 1998, the procedure for histopathologic reporting changed from a non-standardized procedure to a standardized procedure, in particular with respect to assessment of resection margins and tumour origin. Patients resected before and after the first of January 1998 were therefore assigned to a historical control group and a study group, respectively.

From 1998 to 2004 (study group), a total of 161 patients underwent pancreaticoduodenectomy, of which 114 patients had primary adenocarcinoma with macroscopically free margins (R0 or R1 resections). Excluded cases comprised patients with benign lesions (n = 22), neuroendocrine tumours (n = 9), invasive IPMN (n = 4), secondary carcinoma (n = 6), acinar cell carcinoma (n = 1), adenosquamous carcinoma (n = 1), and non-curative resection (i.e. macroscopic residual tumour, R2 resection; n = 4). Histopathologic features were analyzed in order to determine (1) whether the histologic type of differentiation is an independent prognostic factor in periampullary adenocarcinoma, and (2) to evaluate predictors of poor prognosis in the subgroup of patients that had a pancreatobiliary differentiated periampullary tumour.

Among the 114 patients in the study group, 82 were dead by the end of the study and the remaining 32 were followed for a median of 5.8 years (range 2.4–9.3). In the subgroup of patients with pancreatobiliary type adenocarcinoma, 58 (of 67) were dead by the end of the study, and the remaining 9 patients were followed median 6.1 years

(range 3.5–8.7). The relatively many deaths and long follow-up time for the censored cases thus permitted a subgroup analysis of pancreatobiliary cases. Perioperative death (in-hospital death or death within 30 days of operation) was 3.5% (4/114) in the study group, among which three patients had ampullary tumour (3/41) and one had tumour originating in ductal pancreatic tissue.

The patients resected between 1980 and 1997 (historical control group, n = 99) provided a separate dataset in which to validate the main conclusions from the study group analysis. Tumour origin and differentiation type was compared in the two datasets obtained by standardized (study group) and non-standardized (historical control group) histopathologic evaluation, respectively. In the historical control group, 89 (of 99) patients were dead by the end of the study. The remaining 10 patients were followed median 12.4 years (range 9.4–20.8). In the subgroup of patients with pancreatobiliary type adenocarcinoma, 69 (of 73) were dead by the end of the study, and the remaining 4 patients were followed median 13.5 years (range 10.4–20.8). Perioperative death was 4.0% (4/99) in the historical control group. Cases with perioperative death were included in the survival analyses. No patients were lost to follow-up. Data from this series has been reported previously [12].

### *Histopathologic assessment of specimens*

In the study group, histopathologic factors were prospectively registered by routine examination according to a standardized, systematic protocol, and reevaluated retrospectively. The evaluated histopathologic factors were tumour origin, histopathologic type of differentiation, pT stage, maximum tumour diameter, resection margin involvement (with special attention to the retroperitoneal margin), perineural and vascular infiltration, regional lymph node involvement, and degree of differentiation.

Approximately 15 tissue samples were taken from each specimen including whole-mount blocks for most cases. A section parallel to the ampulla, distal bile duct, pancreatic duct, and parallel to the longitudinal duodenal axis was made in order to demonstrate the tumour's relation to each of these sites of potential tumour origin. The cancer origin was determined by tumour location relative to ductal anatomy and duodenal and pancreatic parenchyma, and by associated epithelial dysplasia or in situ neoplasia. Macroscopic pictures were also taken in selected cases.

The histologic type of differentiation was classified according to the criteria first suggested by Kimura et al [13], later revised by Albores-Saavedra et al [7] (figure 1). In brief, pancreatobiliary tumours typically have simple or branching glands and small solid nests of cells surrounded by a desmoplastic stroma, have cuboideal to low columnar epithelium arranged in a single layer without nuclear pseudostratification, and the nuclei are rounded but with marked variation in size and shape from one cell to the next. Intestinal tumours typically resemble colon cancer, may consist of solid nests with cribriform areas,

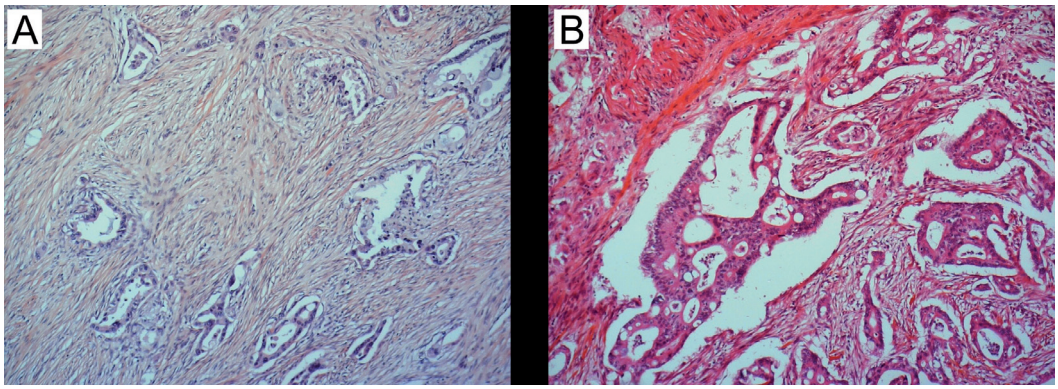



**Figure 1**

**The two dominant types of histologic differentiation in periampullary adenocarcinomas:** (A) The pancreatobiliary type typically has simple or branching glands and small solid nests of cells surrounded by a desmoplastic stroma, and cuboideal to low columnar epithelium arranged in a single layer without nuclear pseudostratification, the nuclei rounded but with marked variation in size and shape from one cell to the next. (B) The intestinal type typically resembles colon cancer, may consist of solid nests with cribriform areas, has tall and often pseudostratified columnar epithelium with oval nuclei located in the more basal aspects of the cytoplasm, and there may also often be presence of mucin.

have tall and often pseudostratified columnar epithelium with oval nuclei located in the more basal aspects of the cytoplasm, and there may also often be presence of mucin. Cases with mixed type differentiation were classified according to the dominant pattern, without performing cut-off optimalization prior to classification [17]. All tumours were assigned to one of these two histologic types of differentiation using this approach. In the historical control group (n = 99), cases with mixed type differentiation were identified (n = 13; 7 predominantly pancreatobiliary and 6 predominantly intestinal), and survival was compared between cases with mixed and single type of histologic differentiation in each group with respect to the predominant histologic pattern of differentiation. Patients with mixed type of differentiation had the same prognosis as patients with only the predominant type, eg. with only pancreatobiliary ($p = 0.35$) or only intestinal ($p = 0.21$) type of differentiation, respectively, thus indicating that classification based on the predominant pattern is applicable.

For both tumour origin and histologic type of differentiation, the prospective registrations were reevaluated independently by two experienced pathologists, a routine pathologist (ST) and a senior pathologist (OPFC). The routine pathologist reviewed only the microscopic slides and was blinded towards clinical and macroscopic data, while the senior pathologist also considered this information when determining the anatomic site of tumour origin. Upon disagreement, final determination of tumour origin and histologic type of differentiation was reached by a second reevaluation of the slides and by reevaluation of the histopathologic and operative reports.

In the historical control group (non-standardized protocol), all histopathologic reports and microscopic slides were reevaluated by a single pathologist (OPFC). The histopathologic factors registered in this group were tumour origin, histopathologic type of differentiation, maximum tumour diameter, resection margin involvement, perineural infiltration, regional lymph node involvement, and degree of differentiation. Only pancreatobiliary or intestinal adenocarcinomas were included in the analysis of histopathologic factors. R2 cases were not excluded from the analysis in the historical control group due to non-standardized reporting for this cohort.

### *Statistical analysis*

Survival data was obtained from the National Registry of Norway, updated May 30, 2007. The Kaplan-Meier method was used to calculate curves for overall survival and to estimate median survival. Survival curves were compared using the log-rank test. Associations between categorical variables were examined using Chi-square test. Mann-Whitney test was performed to compare maximum tumour diameter (measured as a continuous variable) between groups of independent samples. Interobserver agreement was estimated by Cohen's kappa and categorized as poor (kappa < 0.20), fair (0.21 < kappa < 0.40), moderate (0.41 < kappa < 0.60), substantial (0.61 < kappa < 0.80), or almost perfect (kappa > 0.80). Cox regression models were fitted in order to estimate univariate and multivariable survival, together with the hazard ratios and their 95% confidence intervals. For categorical variables, the group with the best prognosis in univariate analysis was set as reference. The multivariable survival model included all histopathologic factors, and the factors were further evaluated using stepwise variable selection. The model obtained from multivariable analysis in periampullary adenocarcinomas was tested in the ampullary subgroup in order to evaluate how well multivariable analysis with the same set of covariates could predict the prognosis in this group for which classification by histologic type of differentiation is already established. A separate multivariable Cox regression subgroup analysis was performed for patients with pancreatobiliary differentiated periampullary adenocarcinoma in order to determine the factors that were independently associated with survival in this subgroup. Finally, the main findings obtained from the study group analysis were validated by analysis of histopathologic factors in a historical control group.

Statistical analyses were performed in SPSS 15.0 for Windows software (SPSS Inc., Chicago, Illinois, USA). R version 2.3.1 (open source statistical software [18]) was used for testing goodness-of-fit based on martingale residual processes. For all tests, a two-sided $p < 0.05$ was considered statistically significant.

## Results

In the study group comprising 114 periampullary adenocarcinomas, there were 67 with pancreatobiliary and 47 with intestinal histologic type of differentiation. These consisted of 40 pancreatic, 41 ampullary, 17 common bile duct, and 16 duodenal adenocarcinomas. Interobserver agreement between the senior and routine pathologist was almost perfect (kappa 0.90; 95% CI 0.82–0.99) for determination of histologic type of differentiation, while it was only fair (kappa 0.37; 95% CI 0.25–0.49) in classification of tumour origin. However, while the routine pathologist who reevaluated the microscopic slides was blinded towards clinical and macroscopic data, the senior pathologist not only reevaluated the microscopic slides but also considered information from the operative and macroscopic reports. This type of information may be more important for accurate tumour origin classification than for histologic type classification. Thus, comparing final consensus with the original reports, interobserver agreement was substantial for both tumour origin classification (25 reclassified cases; kappa 0.68; 95% CI 0.57–

0.79) and histologic type classification (13 reclassified cases; kappa 0.74; 95% CI 0.61–0.87).

### *Histopathologic prognostic factors in periampullary adenocarcinoma*

As expected patients with pancreatic tumours had the poorest prognosis among all periampullary adenocarcinomas in univariate analysis ($p < 0.001$, figure 2). Pancreatobiliary type of differentiation was an adverse predictor of survival both in the whole cohort of periampullary adenocarcinomas ($p < 0.001$, figure 3A) and in the ampullary subgroup ($p < 0.022$, figure 3B).

Table 1 describes associations between histologic type of differentiation and the other histopathologic factors. Compared to intestinal type adenocarcinomas, pancreatobiliary type adenocarcinomas significantly more often showed presence of histopathologic features associated with a poor prognosis, in particular resection margin involvement, perineural infiltration, areas with poor differentiation, advanced pT stage, and pancreatic tumour origin ($p < 0.001$ for each).

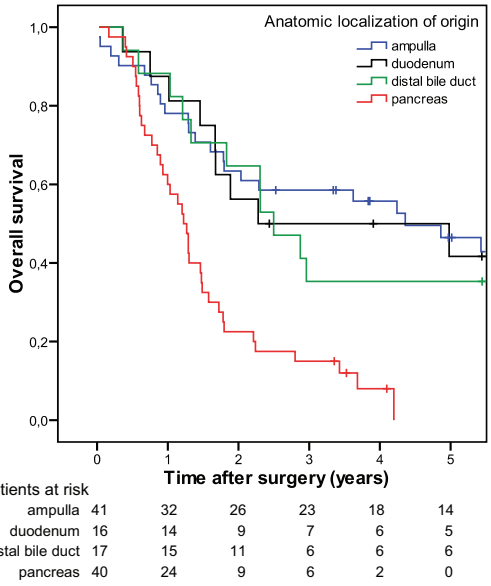



**Figure 2**
**Overall survival after pancreaticoduodenectomy for periampullary adenocarcinoma (n = 114) originating in duodenum (n = 16), ampulla (n = 41), distal bile duct (n = 17), and pancreas (n = 40) ($p < 0.001$).**

In multivariable analysis adjusting for tumour origin, pT stage, maximum tumour diameter, degree of differentiation, regional lymph node metastasis, resection margin involvement, vessel involvement, and perineural infiltration, the histologic type of differentiation was found to be an independent predictor of survival ($p = 0.032$; HR 2.8; 95% CI 1.1–7.1), while tumour origin was only borderline significant ($p = 0.054$). Stepwise backward variable selection resulted in a final model that included the histologic type of differentiation, which in fact was the strongest predictor of survival (table 2). The validity of the final model was tested in the ampullary subgroup, confirming that pancreatobiliary versus intestinal type of differentiation was an independent adverse predictor of survival also among these patients ($p < 0.002$; HR 4.0; 95% CI 1.6–9.6).

### *Prognostic factors in pancreatobiliary differentiated periampullary adenocarcinomas*

A separate analysis of histopathologic factors among the patients who had pancreatobiliary adenocarcinoma (n = 67) was performed in order to identify prognostic factors in this subgroup, and in particular, to evaluate whether tumour origin could independently predict the prognosis in pancreatobiliary type adenocarcinoma. In univariate survival analysis, pancreatic tumour origin was significantly associated with a poorer prognosis compared to non-pancreatic tumour origin (table 3, figure 4A). Even when adjusting for pT stage, the difference in survival between patients who had pancreatic and non-pancreatic tumour origin was statistically significant ($p = 0.003$; HR = 2.9; 95% CI 1.4–6.0). However, adjusting for tumour diameter instead of pT stage demonstrated that there was in fact no survival difference between patients who had pancreatic and non-pancreatic tumours ($p = 0.25$). The pT staging for periampullary adenocarcinomas is based on the assumption that clinical outcome depends more on tumour extension beyond organ of origin than of tumour size. An ampullary pT3 tumour slightly invading the pancreas may thus be as small as 1 cm, while pancreatic pT3 tumours are normally much larger. In the present subgroup analysis of pancreatobiliary differentiated tumours, pancreatic pT3 tumours were significantly larger than non-pancreatic pT3 tumours (median diameter 3.5 versus 2.6 cm; $p = 0.033$).

Among all 67 pancreatobiliary differentiated adenocarcinomas, pancreatic tumours significantly more often than non-pancreatic tumours had regional lymph node metastasis (29/38 for pancreatic versus 12/29 for non-pancreatic tumours, $p = 0.004$) and were larger (median diameter 3.1 cm for pancreatic versus 2.0 cm for non-pancreatic tumours, $p < 0.001$) (figure 4B). The differences in survival seen among all cases (figure 4A) are thus related to the differences in tumour diameter (figure 4B) between

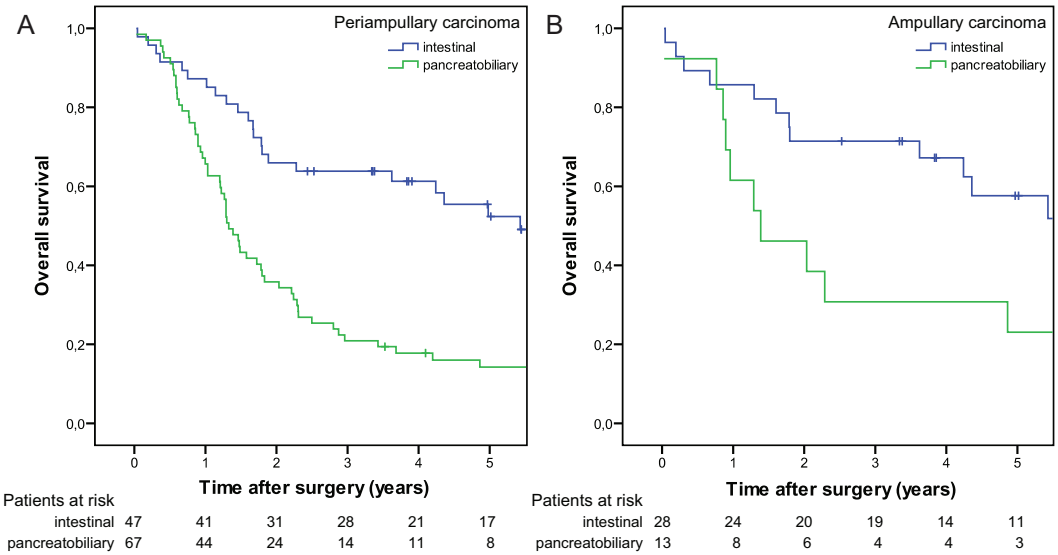



**Figure 3**

**Overall survival after pancreaticoduodenectomy:** (A) Periampullary adenocarcinoma (n = 114) with intestinal (n = 47) and pancreatobiliary (n = 67) type of histologic differentiation (p < 0.001). (B) Ampullary adenocarcinoma (n = 41) with intestinal (n = 28) and pancreatobiliary (n = 13) type of histologic differentiation (p = 0.02).

**Table 1: Associations between histologic type of differentiation and other histopathologic factors in 114 periampullary adenocarcinomas**

| | | Differentiation of adenocarcinoma | | |
|---|---|---|---|---|
| | | intestinal | pancreatobiliary | p-value[a] |
| Tumour origin | ampulla | 28 | 13 | < 0.001 |
| | duodenum | 16 | 0 | |
| | distal bile duct | 1 | 16 | |
| | pancreas | 2 | 38 | |
| pT stage | pT1 | 10 | 2 | < 0.001 |
| | pT2 | 20 | 9 | |
| | pT3 | 8 | 46 | |
| | pT4 | 9 | 10 | |
| Lymph node status | N0 | 23 | 26 | 0.282 |
| | N1 | 24 | 41 | |
| Resection margin status | R0 | 40 | 34 | < 0.001 |
| | R1 | 7 | 33 | |
| Vessel involvement | free | 35 | 36 | 0.025 |
| | involved | 12 | 31 | |
| Perineural infiltration | no | 35 | 17 | < 0.001 |
| | yes | 12 | 50 | |
| Areas with poorly differentiated tumour | no | 39 | 32 | < 0.001 |
| | yes | 8 | 35 | |
| Tumour size (maximum tumour diameter) | small (≤ 2.5 cm) | 31 | 35 | 0.108[b] |
| | large (> 2.5 cm) | 16 | 32 | |

[a]Chi-square test, when not otherwise specified
[b]Mann-Whitney test (histologic type of differentiation vs tumour size measured as a continuous variable)

**Table 2: Multivariable Cox regression analysis of histopathologic prognostic factors in periampullary adenocarcinomas (n = 114)**

| | | HR | 95% CI | p-value |
|---|---|---|---|---|
| Histologic type | pancreatobiliary (vs intestinal) | 3.1 | 1.8–5.1 | < 0.001 |
| Lymph node involvement | N1 (vs N0) | 2.5 | 1.5–4.4 | < 0.001 |
| Vessel involvement | involved (vs not involved) | 1.9 | 1.2–3.1 | 0.012 |
| Tumour size | diameter (measured in cm) | 1.3 | 1.1–1.5 | 0.011 |

HR, hazard ratio. HR > 1 indicates increased probability of death compared to the reference group (for categorical variables) or compared to each increase of one cm in tumour size (continuous variable)
CI, confidence interval

non-pancreatic and pancreatic tumours. Selecting tumours of comparable size (range 2.0–3.0 cm, n = 30) demonstrated no difference in survival between pancreatic (n = 18) and non-pancreatic cases (n = 12) (p = 0.851, figure 4C). These groups were comparable with respect to tumour diameter (median 2.5 and mean 2.4 cm for both groups), and the equal survival was not due to less frequent lymph node metastasis among pancreatic cases (positive lymph nodes in 15/18 pancreatic compared to 6/12 non-pancreatic cases).

Starting with all the histopathologic factors in the base model for multivariable analysis, backward variable selection thus resulted in a final model that did not include tumour origin (table 3). Only lymph node status, vessel involvement and tumour diameter independently predicted the prognosis after resection of pancreatobiliary type periampullary adenocarcinoma. The final model obtained from stepwise backward analysis was confirmed by repeating variable selection with forward stepwise analysis. Although perineural infiltration also seemed to be an important prognostic factor in univariate analysis, this factor did not independently predict survival, due to a strong association with lymph node metastasis (p = 0.002), vessel involvement (p < 0.001), and tumour diameter (p = 0.024).

***Validation of main conclusions in an independent dataset***

We finally validated our main findings by performing a separate analysis of histopathologic prognostic factors in the historical control group consisting of patients operated in our institution before standardization of histopathologic assessment. Among these patients, 73 and 26 were upon reevaluation of the histologic slides found to have pancreatobiliary and intestinal differentiation, respectively. Tumour origin for all 99 cases was classified as ampullary (n = 23), duodenal (n = 14), distal bile duct (n = 10), and pancreatic (n = 52).

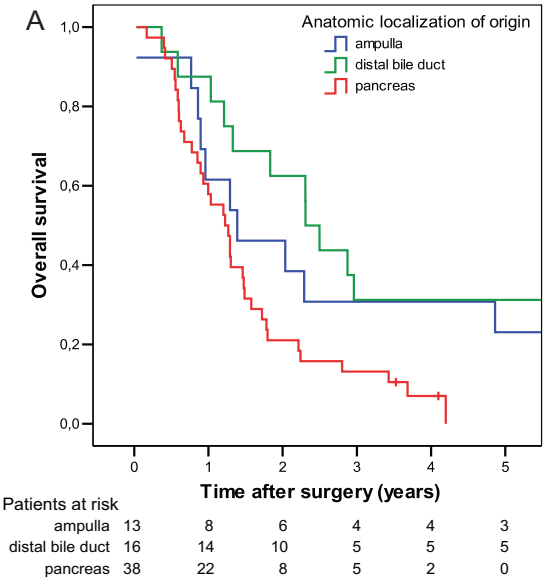
In univariate survival analysis, pancreatobiliary type of differentiation (p < 0.001) and pancreatic tumour origin (pancreatic versus ampullary, p = 0.03) both predicted a poor prognosis. Adjusting for maximum tumour diameter, lymph node and resection margin involvement, degree of differentiation, and whether there was presence of perineural infiltration, the histologic type of differentiation remained highly significant (p < 0.001; HR 2.7; 95% CI 1.5–4.9). In contrast, although approaching significance, tumour origin did not significantly predict the prognosis after adjustment for these other factors (pancreatic versus ampullary, p = 0.10; HR 1.6; 95% CI: 0.9–2.9).

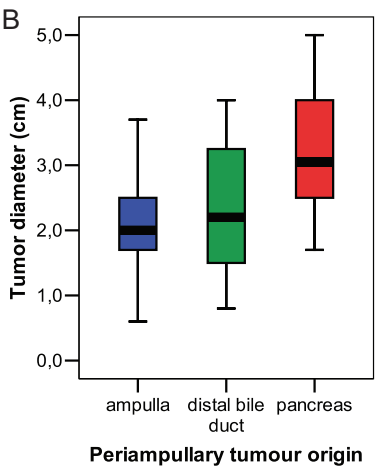
In the subgroup analysis including only pancreatobiliary differentiated tumours (n = 73), stepwise variable selection resulted in a final multivariable model in which

**Table 3: Survival analysis of histopathologic prognostic factors in pancreatobiliary resections (n = 67)**

| | | | p-value | |
|---|---|---|---|---|
| | HR | 95% CI | Univariate | Multivariable |
| Pancreatic tumour origin (pancreatic vs non-pancreatic) | 2.3 | 1.3–4.0 | 0.004 | |
| pT stage (pT3 or pT4 vs pT1 or pT2) | 2.0 | 0.9–4.2 | 0.073 | |
| Resection margin involvement (R1 vs R0) | 1.7 | 1.0–2.8 | 0.052 | |
| Lymph node involvement (N1 vs N0) | 3.0 | 1.6–5.4 | < 0.001 | 0.007 |
| Poor differentiation (yes vs no) | 1.6 | 0.9–2.6 | 0.094 | |
| Vessel involvement (yes vs no) | 3.1 | 1.8–5.3 | < 0.001 | 0.035 |
| Perineural infiltration (yes vs no) | 2.8 | 1.4–5.5 | 0.003 | |
| Tumour size (continuous, measured in cm) | 1.7 | 1.3–2.1 | < 0.001 | < 0.001 |

HR, hazard ratio. HR > 1 indicates increased probability of death
CI, confidence interval



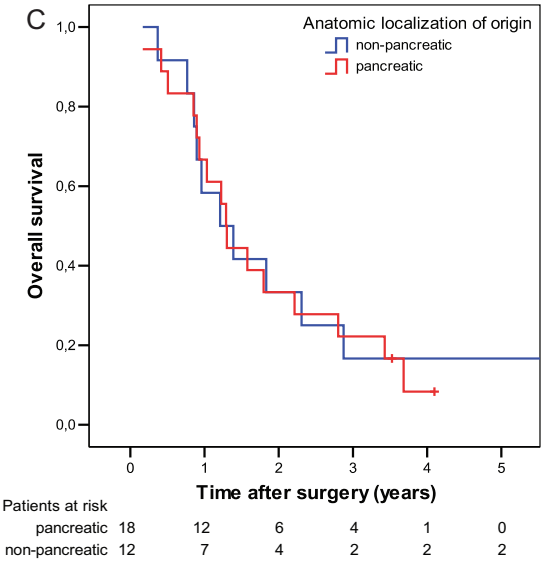




**Figure 4**

**Pancreaticoduodenectomy for pancreatobiliary type periampullary adenocarcinoma:** (A) Univariate survival for patients with pancreatobiliary type periampullary adenocarcinomas (n = 67) originating in ampulla (n = 13), distal bile duct (n = 16), and pancreas (n = 38) (p = 0.009). (B) Boxplot of maximum tumour diameter in the same 67 tumours with pancreatobiliary differentiation (p < 0.001, pancreatic versus non-pancreatic). (C) Survival for patients with non-pancreatic (n = 12, of which 6 ampullary and 6 biliary) and pancreatic (n = 18) tumours of comparable size (2 cm ≤ maximum diameter ≤ 3 cm) (p = 0.851).

lymph node status (N1 versus N0, $p < 0.001$; HR 3.4; 95% CI: 1.9–6.0) was confirmed to independently predict the prognosis, adjusting for grade of differentiation (high/moderate versus low, $p = 0.002$; HR 2.6; 95% CI 1.4–4.8) and resection margin status (R1/2 versus R0, $p = 0.011$; HR 1.9; 95% CI 1.2–3.2). Although significant in univariate analysis ($p = 0.021$), tumour diameter was not confirmed to independently predict survival in this cohort, possibly due to the small size of these tumours (mean diameter 2.1 cm; 95% CI 1.9–2.4) and a significant association between tumour diameter and lymph node status ($p = 0.04$). Finally, after stepwise variable selection, tumour origin and perineural infiltration did not remain in the final multivariable model evaluating histopathologic factors of pancreatobiliary differentiated periampullary adenocarcinomas, in accordance with the results from the study group analysis.

## Discussion

In the present study, we evaluated the prognostic importance of the two main histologic types of differentiation, pancreatobiliary and intestinal types, in presumed curative resections for pancreatic head adenocarcinomas. We found that the pancreatobiliary histologic type of differentiation was independently associated with a poor prognosis, while tumour origin did not significantly predict survival when adjusting for other histopathologic prognostic factors. In pancreatobiliary type adenocarcinomas, survival depended on factors related to the disease stage (tumour size and regional lymph node involvement), but not on the anatomic structure of origin. The main conclusions from this study were confirmed by analysis in an independent dataset.

For multiple reasons, we suggest that determination of the histologic type of differentiation is a useful adjunct to classification of the anatomical site of origin in periampullary tumours. Failure to reach a precise diagnosis of tumour origin may lead to false assumptions regarding long-term survival [2,7,10]. Periampullary anatomy may be distorted by carcinoma or affected by inflammation and fibrosis [9]. The normal ampulla, defined as the junction between the distal bile duct and the main pancreatic duct, has a variable length and is absent in a large proportion of the normal population [9]. Resected ductal pancreatic adenocarcinomas in the pancreatic head typically have a mean diameter of ~3 cm[1], and may involve the entire ampullary region [5]. Determination of tumour origin should therefore be standardized and include a section parallel to (and including) the ductal structures in order to evaluate the tumour's relation to the ductal anatomy. However, even with standardized evaluation, interobserver variability may be considerable unless clinical and macroscopic data is also emphasized.

The present study is the first report on the distribution of pancreatobiliary and intestinal type differentiation in a cohort of resected adenocarcinomas of all periampullary locations. With respect to ampullary tumours, it has been known for more than a decade that these may have either "intestinal" or "pancreatobiliary" histologic type of differentiation [13], and that the intestinal type has a significantly better prognosis [7,11-14]. Intestinal type biliary tract [15] and pancreatic [16] carcinomas have also been reported, but there is sparse data comparing pancreatobiliary type adenocarcinomas of these three different origins [19-21]. Although many studies have compared ductal adenocarcinomas of different periampullary origin [19-22], most studies do not state specifically whether intestinal type ampullary adenocarcinomas were excluded in such comparisons. It should be noted that ductal adenocarcinoma is not synonymous with pancreatobiliary type of histology, since it has recently been shown that ductal pancreatic carcinomas may have features of intestinal differentiation [16]. Pancreatobiliary ampullary carcinomas that involve the pancreas may be indistinguishable from pancreatobiliary pancreatic carcinomas that extend into the ampulla [2,8], and low ratios of ampullary versus periampullary adenocarcinomas in many studies might reflect a tendency towards misclassification of advanced pancreatobiliary type ampullary carcinomas as ductal pancreatic carcinomas.

An explanation for the often reported more favourable prognosis in non-pancreatic versus pancreatic carcinoma could therefore be that comparison has not been strictly limited to pancreatobiliary type adenocarcinomas. The reason why there are prognostic differences between equally advanced non-pancreatic and pancreatic adenocarcinomas may simply be that the latter more often has a pancreatobiliary type of histologic differentiation [7]. Thus, the question whether survival differences between pancreatic and non-pancreatic pancreatobiliary type adenocarcinomas should be attributed to disease stage or biology, or both, has not been definitely answered [2]. The present study suggests that survival differences between periampullary adenocarcinomas of comparable size are more dependent on the histologic type of differentiation than on the anatomic origin.

In the UICC/AJCC classification of pancreatic head malignancies, adenocarcinomas originating from the peri-Vaterian duodenum, the ampulla of Vater, the distal bile duct, and the ductal pancreatic tissue are considered separate entities [23,24], and the TNM staging criteria are different for each origin. However, since adenocarcinomas arising from the peri-Vaterian duodenum and the ampulla of Vater may be indistinguishable [7], and since it may also be difficult to discriminate between pancreatobiliary differentiated ampullary and distal bile duct adenocarcino-

mas [15], pT stage might be classified according to inappropriate anatomic location. Furthermore, pT1 in ductal pancreatic carcinoma includes tumours with maximum diameter up to 2 cm, while pT1 in ampullary carcinomas includes only small tumours not invading either the duodenum (pT2) or the pancreas (pT3). A relatively large pancreatic tumour could therefore still be at pT1 stage compared to a small ampullary tumour extending into the duodenum, thus classified as pT2 (or even extending into the pancreatic tissue, thus classified as pT3). Importantly, pT stage was the factor with the lowest p-value both in univariate and multivariable survival analysis of all periampullary adenocarcinomas as well as in the subgroup of pancreatobiliary differentiated adenocarcinomas. The present study therefore demonstrates that individual factors related to tumour stage are more reliable than pT (or TNM) stage group in multivariable analysis of prognostic factors in pancreatic head carcinomas.

## Conclusion

Pancreatobiliary versus intestinal type of differentiation independently predicts poor prognosis after pancreaticoduodenectomy for periampullary adenocarcinoma. In pancreatobiliary type periampullary adenocarcinoma, lymph node involvement, vessel involvement, and increasing tumour diameter were adverse predictors of survival in the present study.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

AW participated in design of the study, registration and ethical approval, patient inclusion, review of clinical data, and histopathologic analysis. He also designed the database, performed the statistical analysis, and drafted the manuscript. ST participated in patient inclusion and had a major responsibility for histopathologic analysis. INF contributed with establishment of the protocol for systematic histopathologic assessment, participated in design of the study, patient inclusion, and histopathologic analysis. MC contributed substantially with choice of statistical methods and participated in statistical analysis. TJE participated in establishing systematic pathologic review of pancreaticoduodenectomy specimens, in design of the study, and in histopathologic analysis. ØM contributed substantially in the discussion of operative methods and performed many of the pancreaticoduodenectomies. OPFC participated in design of the study, had a major responsibility for histopathologic analysis, and contributed substantially with critical review of the manuscript. IPG participated in design, registration and ethical approval of the research project, and in patient inclusion and registration of clinical data. He also performed many of the pancreaticoduodenectomies, contributed substantially in the discussion of statistical methods, and drafted the manuscript. All authors critically reviewed the manuscript and approved the final manuscript.

## Acknowledgements

The authors thank Anders Bergan who registered clinical and histopathologic data from patients in the historical control group.

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