Fig. 4.22 Somatostatinoma. **A** Tumour with glandular architecture and psammoma bodies producing somatostatin as shown by immunophenotyping. **B** Psammoma body at transmission electron microscopy level. The psammoma body shows a coral-like configuration. There is an electron-dense central portion surrounded by a paler peripheral area with needle-like crystal structures arranged radially.

Tumour spread and staging

Because somatostatinomas are generally large by the time they are detected, nearly two-thirds have already metastasized to the regional lymph nodes or the liver {105,2045}.

Histopathology

Somatostatinomas share histologic features with other pancreatic endocrine tumours. In contrast to duodenal somatostatin producing tumours, they often lack the prominent gland formation and psammoma bodies.

Somatostatinomas usually show fairly uniform histochemical reactions. The tumour cells are uniformly non-argentaffin and show argyrophilia only with the Hellerstrom-Hellman technique that is selective for somatostatin-producing cells. Some tumours may show focal Grimelius-positive argyrophilia in a small number of cells.

Immunohistochemistry

Synaptophysin is strongly and diffusely positive in almost all tumour cells, while chromogranins are less consistently expressed. Stains for somatostatin show an intense immunoreactivity in a domi-nant population of the tumour cells. Cytoplasmic staining for such products as ACTH, calcitonin, insulin, glucagon, *etc.* may be found in a variable, but small proportion of the tumour cells and may correlate with the focal Grimelius-positive argyrophilia seen in those tumours {292, 464,776}.

Electron microscopy

The tumour cells contain large numbers of intracytoplasmic membrane-bound neurosecretory granules in which two distinct populations can be identified. The majority of the secretory granules are large (range of diameter 250-450 nm), round and moderately electron-dense. The second subset consists of smaller granules (range of diameter 150-300 nm), with somewhat denser cores.

Precursor lesions

Somatostatinomas have not been associated with any precursor lesion.

Somatic genetics

Very little information is available on somatic genetic alterations in these rare tumours. One tumour listed as somatostatinoma, analyzed by CGH showed a loss of chromosome X and gains of chromosomes 7, 11, 14 and 18pter-q1 {2154} and a second exhibited allele loss together with a somatic mutation of *MEN1* {750}.

Genetic susceptibility

Pancreatic somatostatinomas have occasionally been reported in patients with MEN1 {1878}, Von Hippel-Lindau syndrome {1389} and in a small subset of NF1 patients {2217}.

Prognosis and predictive factors

As somatostatinomas are usually large tumours, most are considered endocrine tumours of uncertain behaviour or well differentiated endocrine carcinomas. The largest experience with these uncommon endocrine tumours estimates a 75.2% 5-year survival overall, with a 59.9% in patients with and 100% of the patients without metastases {2091}. However in retrospect not all of these cases may have been functioning tumours.

Gastrinoma

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Definition

A gastrinoma is a functionally active and usually malignant endocrine tumour with clinical symptoms due to inappropriate secretion of gastrin (Zollinger Ellison syndrome; ZES) {2094}. Tumours with immunohistochemical expression of gastrin but without evidence of ZES should not be designated gastrinomas. Two types of gastrinomas can be distinguished, sporadic, non-familial gastrinoma with ZES (≈80% of cases), and familial gastrinoma with ZES in the setting of MEN 1 (≈20%).

ICD-O code

Gastrin cell tumour   8153/1
Gastrin cell tumour, malignant
                               8153/3

Synonyms and historical annotation

Ulcerogenic tumours. The syndrome was named after R.M. Zollinger and E.H. Ellison who first described the association between "islet cell tumours" and ulcerogenic disease in 1955 {2498}. The peptide hormone gastrin was isolated by R. A. Gregory et al. in 1960 {771} and the pathogenesis of ZES was elucidated in 1964 {556}.

Epidemiology

The majority of patients with ZES suffer from a gastrinoma of the pancreas or duodenum. Gastrinomas are relatively common functionally active endocrine tumour of the pancreas accounting for about 20% of cases, second in frequency only to insulinomas {858}. In some series they are approximately half as common as insulinomas {255,858,1111}, whereas in other series gastrinomas and insulinomas have a similar incidence {577}. In the United States, approximately 0.1% of patients with duodenal ulcers have evidence of ZES {577}.

The reported incidence of gastrinomas is between 0.5-4 per million population per year {255,463,987,2094}. ZES is more common in males than in females with a ratio of 3:2. The mean age at the onset of symptoms is 38 years (range 7-83 years) in some series {556,2398} and 50 years (range 45-51 years) in others {220,281, 1001,1802,2114}.

Etiology

The etiology and pathogenesis of sporadic gastrinomas are unknown. Approximately 20% of gastrinomas are part of MEN 1. No other risk factors are known.

Localization

Substantial variation has occurred in the distribution of pancreatic versus non-pancreatic gastrinomas with the passing of time, possibly related to the refinement of clinical and pathological (immunohistochemical) diagnostic procedures. Indeed, the collection of 800 cases in the Zollinger-Ellison Tumour Registry up to 1973 demonstrtated localization of gastrinomas to the pancreas in 53% of patients and to the duodenum and jejunum in 13% {644}. In contrast, the recent experience of a large reference center in the United States revealed an overall incidence of pancreatic localization of 24% with a drop to 14% in sporadic cases whereas a duodenal localization was found in 49% of cases (47% in sporadic ones) {1619}.

Pancreatic gastrinomas more frequently occur in the head of the gland {515,2109}. More than 90% of duodenal gastrinomas are located in the first and second parts of the duodenum and limited to the submucosa in 54% of patients {2227}. The anatomical area comprising the head of the pancreas, the superior and descending portion of the duodenum and the relevant lymph nodes has been called the "gastrinoma triangle" since it harbors the vast majority of these tumours {926,1002,1617,1618,2109}.

Other primary sites of gastrinomas are being identified increasingly {1619}, including stomach, jejunum, biliary tract, liver, kidney, mesentery and heart {62, 374,709,1237,1416,1616,2109,2236}. Some peripancreatic and periduodenal lymph node gastrinomas are thought to represent primary tumours rather than metastases from an occult primary in the duodenum {184,477,2409} and some patients have been cured after resection of the tumourous lymph nodes {51,1617, 1618}. This hypothesis of primary lymph node gastrinomas has been challenged {515,2235}. In two recent studies, however, neuroendocrine and gastrin expressing cells were identified in peripancreatic and duodenal lymph nodes of patients without neuroendocrine tumours, provid-
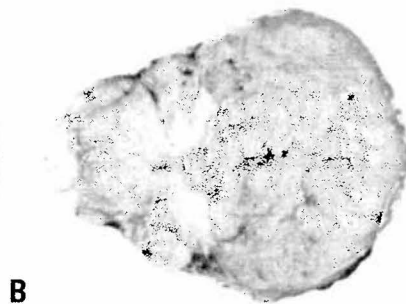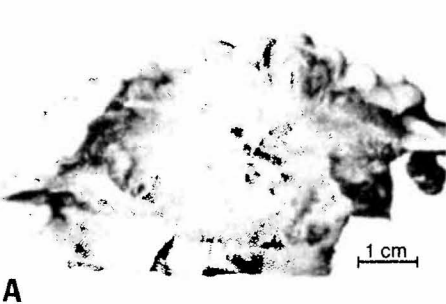

Fig. 4.23 Malignant gastrinoma. **A** Ill-defined gastrinoma in the head of the pancreas invading the papilla Vater and the duodenal wall. **B** Large, malignant gastrinoma with areas of necrosis and fibrosis.

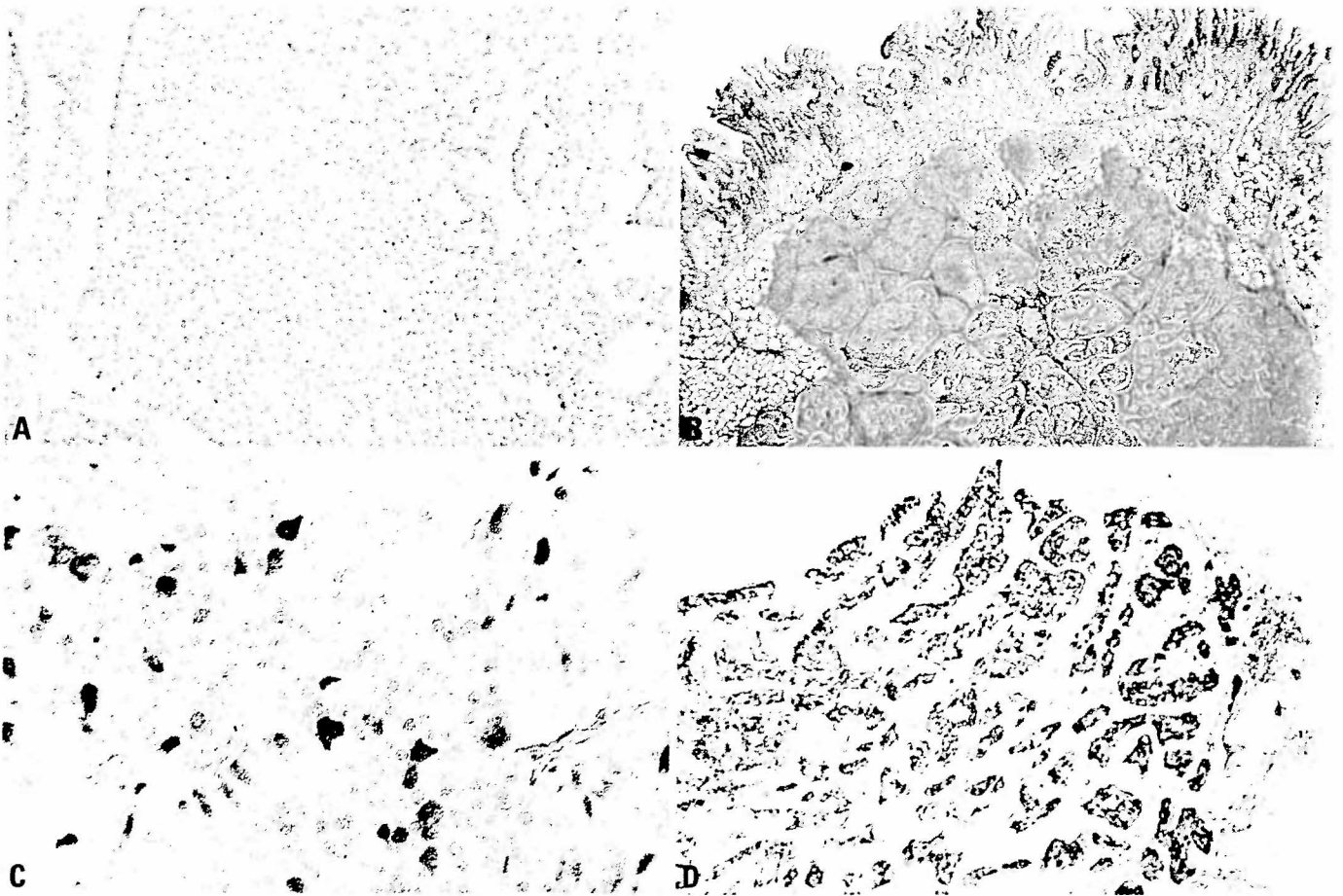**Fig. 4.24** Liver metastasis of a gastrinoma. High proliferative index (Ki-67 labeling).

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Fig. 4.25 A Gastrinoma. Solid growth pattern. **B** Duodenal gastrinoma. Tumour with a partly solid partly trabecular growth pattern invading duodenal glands and the muscularis mucosae. **C** Gastrinoma. Production of gastrin by numerous tumour cells shown by immunophaenotyping. **D** Massive gastrin production by the majority of tumour cells is shown by immunophenotyping.

ing evidence that these embryonic rests might be precursors of nodal gastrinomas {875,1726}. Still, gastrinomas arising from lymph nodes are extremely uncommon. The diagnosis can be made only after exclusion of a primary gastrinoma at another localization. Rarely, ovarian or pancreatic mucinous cystadenomas or -carcinomas may secrete enough gastrin to cause ZES {211,1406}.

Clinical features
Signs and symptoms
Most patients with gastrinomas suffer from typical duodenal ulcer at presentation, but about 20% have no ulcer. In almost every patient the initial symptoms are caused by gastric acid hypersecretion. Abdominal pain from either peptic ulcer disease or gastroesophageal reflux disease remains the most common symptom occurring in more than 75% of patients. Diarrhoea initially may be the only symptom in 10-15% and occurs with abdominal pain in about 50% of patients.

Diarrhoea is caused by high gastric acid output in the duodenum, thereby neutralizing the pancreatic enzymes necessary for digestion. This will cause malabsorption. In late stages of the disease symptoms may be caused by the tumour itself, e.g. right upper quadrant abdominal pain and weight loss.

In early studies more than 90% of patients with gastrinoma and the Zollinger-Ellison syndrome at presentation had a peptic ulcer and in more than 25% the ulcers were multiple or in unusual locations. Past patients frequently presented with complications or severe peptic ulcer disease (e.g. bleeding, perforation, oesophageal strictures) and although this is less common today it still occurs.

Imaging
Endoscopic ultrasonography is the most sensitive method to demonstrate small gastrinomas of the pancreas and duodenum. Extrapancreatic and metastatic lesions can be detected by ultrasonography, CT scan, MRI. Most recently, somatostatin receptor scintigraphy and PET-scan have proven to be the most sensitive methods to demonstrate gastrinomas. The sensitivity is about 70% and specificity 85%. It is even higher for metastatic liver disease, with a sensitivity of 92% and a specificity of 90-100%.

Diagnostic procedures
If the gastric pH is below 2.5 and the serum gastrin concentration above 1,000 picogram per ml (normal <100 picogram per ml) the diagnosis of ZES is confirmed and no other diagnostic studies are actually needed. Unfortunately, the majority (40–50%) of patients present serum gastrin concentrations between 100 and 500 picogram per ml. In these patients a secretin test should be performed in addition to a determination of basal acid and pentagastrin stimulated acid output. Most patients with ZES have a basal acid output above 15

mEq per hour if they have not undergone previous acid-reductive surgery. The secretin test is considered positive when an increase in serum gastrin over the pretreatment value is more than 200 picogram per ml.

Macroscopy

Most sporadic gastrinomas are solitary tumours. In the pancreas tumours are usually well-circumscribed, non-encapsulated and their diameter generally exceeds 2 cm. Their texture varies from soft to firm depending on the amount of fibrous stroma. Multiple pancreatic tumours are more common in patients with MEN 1 associated ZES {1748}. However, most of these MEN associated tumours exhibit immunoreactivity for peptides other than gastrin and, thus, are not causative of ZES {229,1748}.
In the duodenum most gastrinomas are less than 1 cm in diameter. Microgastrinomas (<0.5 cm) are easily overlooked. The size of the tumours is not related to the severity of hormonally induced symptoms. Duodenal gastrinomas appear as well circumscribed, soft, grey to yellow, often polypoid tumours with or without ulcerated overlying mucosa. In older series 13% of tumours were multiple {266}. Multiplicity of tumours, however, is indicative for an associated MEN 1 syndrome {1128, 1129}.

Tumour spread and staging

Duodenal gastrinomas can metastasize while still very small, and give rise to paraduodenal lymph node metastases, which may be larger than the primary. It has, therefore, been suggested that the so-called lymph node gastrinomas are metastases of occult duodenal microgastrinomas {515,2235}. The exact percentage of malignant gastrinomas is unclear. In early studies 60-90% of gastrinoma patients had metastatic disease at the time of diagnosis but in recent studies the percentage has dropped to 34% (range 13-52%) probably due to earlier diagnosis {1002}. Metastases to the liver generally occur late and are only seen in a small percentage of patients at the time of surgery, mostly in patients with with pancreatic tumours {477,515, 2110}.
So far there is no staging system that specifically applies to gastrinomas (see Pancreatic endocrine tumours).
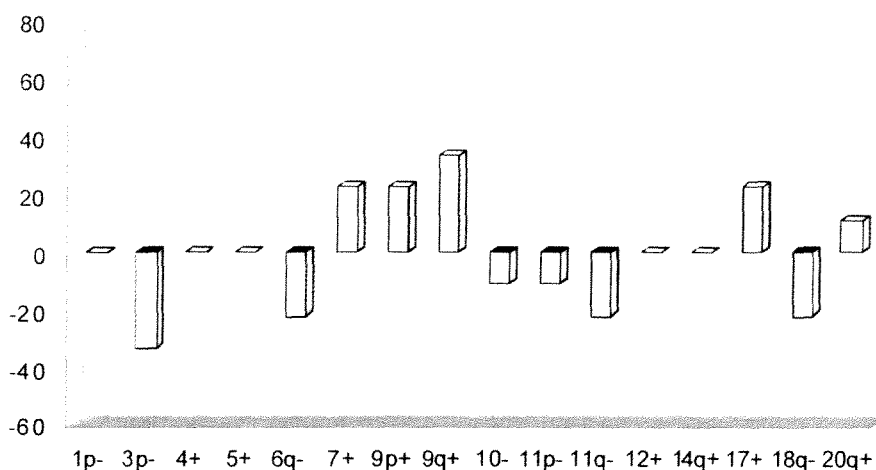

Fig. 4.26 Analysis of chromosomal alterations of gastrinomas, using CGH. Chromosomal gains are prominent on chromosomes 7, 9p, 9q and 17, while chromosomal losses are shown in 3p, 6q and 11q.

Histopathology

The histopathological findings of gastrinomas, either pancreatic or extrapancreatic, almost invariably correspond to those of well differentiated endocrine tumours according to the WHO classification {2097}. As with other pancreatic endocrine tumours malignancy cannot be predicted histologically in most instances, except in the rare tumours in which angioinvasion or infiltration of peripancreatic tissues can be documented {2094}. The growth fraction of gastrinomas was found to be similar to that of other functioning but lower than that of non-functioning pancreatic endocrine tumours whereas a MIB-1 index >10% was invariably associated with development of metastases {391}.
Due to their substantial differences pancreatic and extrapancreatic gastrinomas are described separately as are the histopathological features of extratumoural pancreatic tissue.

Pancreatic gastrinomas

The histological arrangement of pancreatic gastrinomas has no distinctive features with respect to other functioning or non-functioning differentiated endocrine tumours of the pancreas. The early observation of an abundance of glandular like structures {773} was not confirmed by further studies. Actually, pancreatic gastrinomas are predominantly arranged in a mixed trabecular and solid pattern with varying amount of intervening stroma. Tumours with pure, gyriform/ribbon pattern, especially if multi-

ple, are likely to be accompanying neoplasms that are shown by immunohistochemistry to produce nongastrin hormones, mostly glucagon and/or pancreatic polypeptide {229,1748}. Necrosis is uncommon in gastrinomas. Tumour cells tend to show regular round or ovoid nuclei with minimal atypia and well-represented cytoplasm with faint eosiniphilic granularity. Mitoses are infrequent.
Immunohistochemistry. Although gastrinoma may react with Grimelius silver stain or with a number of general neuroendocrine markers, the conclusive evidence for the diagnosis is provided by gastrin immunohistochemistry, which may react diffusely in most tumour cells or in discrete cells or cell clusters. In cases in which the tumours are mostly or entirely unreactive to gastrin antiserum, the use of a panel of antibodies directed against specific sequences of the gastrin molecule may be necessary. Detection of gastrin mRNA by in situ hybridization is recommended in tumours in which constitutive secretion of the hormone results in undetectable or absent gastrin storage in tumour cells.
Electron microscopy. Showing varying proportions of cells either devoid of granules or with nondiagnostic granules {431}, EM has virtually no diagnostic relevance.

Duodenal gastrinomas

These tumours are mostly confined to the mucosa and submucosa and their predominant patterns include broad trabeculae and glandular-like structures.

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Immunohistochemistry. Gastrin immuno-staining is usually intense and diffuse whereas electron microscopy shows more abundant and typical G cell granules {2094}.

Non-tumour pancreatic tissue.

Islet cell hyperplasia and nesidioblasto-sis (i.e., budding off of islet cell clusters from ductal pancreatic epithelium) is frequently encountered in the pancreas of gastrinoma patients {431,2094}. Gastrin has never been convincingly demonstrated in these lesions that may possibly depend on the trophic effect of tumour dependent hypergastrinaemia. A morphometric study of the PP-rich pancreatic region of ventral embryological origin in patients with sporadic gastrinomas showed pronounced PP cell hyperplasia, possibly as a manifestation of a more diffuse disorder of PP cells in this condition {1414}.

Histogenesis

Gastrin gene expression in endocrine pancreas of mammals is restricted to fetal life {240}, and gastrin-containing G-cells are normally not encountered in the postnatal human pancreas. Therefore pancreatic gastrinomas are considered "ectopic" tumours, whereas gastrinomas arising from the duodenum, jejunum or stomach, where G-cells are normally found, are considered "eutopic" tumours. Similar to all pancreatic endocrine tumours the histogenesis of gastrinoma is uncertain.

Somatic genetics

The number of gastrinomas investigated by molecular methods is relatively small and the majority of these studies have focused on the mutational state and allelic loss of the *MEN1* gene on 11q13. Furthermore, many studies did not clearly separate between pancreatic and duodenal tumours and, thus, the results might not be fully representative. Compiled results of CGH studies on 9 pancreatic gastrinomas revealed that chromosomal alterations are less often present than in other types of pancreatic endocrine tumours (including insulinomas), with the exception of losses of 3p and 18q21 which occur more frequently {2105,2107,2154,2490}. The rates of allelic losses at 11q13 and mutations of the *MEN1* gene are the highest of all pancreatic endocrine tumours and have

been found in 90% (28/31) and 37% (19/51) of tumours, respectively {750, 879,2350,2469,2495}. Somatic *MEN1* mutations in primary pancreatic gastrinomas are mostly located in exon 2 and in duodenal tumours they are mainly found in other exons of the gene {734}. Mutations of other genes have not been described.

Genetic susceptibility

Approx. 20-25% of patients suffering from ZES have MEN 1 {1002,2094}. The prevalence of MEN 1 in patients with gastrin producing tumours of the duodenum and upper jejunum has been reported to be 5.3% {2093}. Pancreatic endocrine tumours in the setting of VHL disease consistently lack gastrin immunoreactivity {1357}.

Prognosis and predictive factors

Gastrinomas show a high risk of malignant behaviour independent of size and should therefore be classified as tumours with uncertain behaviour or as well-differentiated endocrine carcinomas when gross invasion and/or metastases are present. Gastrinomas in patients with liver metastases seem to behave more aggressively than those with lymph node metastases only {477,2110} and regional lymph node metastases appear to have little influence on the overall survival of patients suffering from ZES {477,2365}. The risk for liver metastases increases with tumour size and pancreatic location of the primary. It is low in MEN 1 patients. Thus, the frequency of liver metastases is 30% in patients with pancreatic gastrinomas and 3% in patients with duodenal tumours {2094}. The growth rate of hepatic metastases as revealed by modern, sensitive imaging studies, also has important predictive relevance. It has been used to separate aggressive from non-aggressive variants of gastrinomas, the former showing a tumour size increase of at least 50% in volume per month {2163} (25% in MEN 1 cases {710}) and the latter no or lower growth. In spite of the occurrence of liver metastases also in the group of indolent gastrinomas, tumour related deaths were almost entirely confined to the aggressive growth group {710,2163}. Metastases to other organs such as lung, pleura, skin, bone and spleen occur very rarely {463}. It has been reported that duodenal gastrinomas are less malignant

(38%) than pancreatic ones (60-70%) {960,2098}. However, recent studies reported a more than 50% malignancy rate of duodenal gastrinomas {1748, 2227}. It may be that the reported less malignant behaviour of duodenal gastrinomas is only due to the earlier detection. The same holds true for the proposed lower malignancy rate of duodenal gastrinomas in MEN 1 patients.
In general, the progression of gastrinomas is relatively slow with a combined 5-year survival rate of 65% (62-75%) and 10-year survival rate of 51% (47-53%) {1002}. Even with metastatic disease a 10-year survival of 46% (lymph node metastases) and 40% (liver metastases) has been reported. Patients with complete tumour resection have excellent 5- and 10-year survival rates (90-100%). Again, patients with pancreatic tumours have a worse prognosis than those with primary tumours in the duodenum (10-year survival 9% versus 59%) {2365}. The rate of tumour related death in aggressive forms of gastrinomas ranges from 38% in MEN 1 cases {710} to 62% in sporadic cases {2163}.
There are no established markers to predict the biological behaviour of gastrinomas. However, some have found that Her2/neu amplification and overexpression of EGF and hepatocyte growth factor (HGF) are associated with aggressive growth {735,1707}.

VIPoma

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Definition

A VIPoma is a functionally active and usually malignant endocrine tumour, arising mostly in the pancreas, which secretes mainly vasoactive intestinal peptide (VIP). VIP-secreting tumours have been associated with the watery diarrhoea syndrome. Additional substances produced by VIPomas, possibly contributing to the clinical syndrome, include peptide histidine methionine (PHM), pancreatic polypeptide (PP), neurotensin, and others.

ICD-O code

VIP-producing tumour
8155/1
VIP-producing carcinoma,
VIPoma, malignant 8155/3

Synonyms

VIPomas have also been referred to as diarrhoeogenic tumours of the pancreas and islet cell tumours of the pancreas with watery diarrhoea {2094}. The syndrome associated with VIPomas, originally known as Verner-Morrison syndrome recognizing its discoverers {2312}, has also been called pancreatic cholera {1437} and WDHA (watery diarrhoea, hypokalemia, achlorhydria) syndrome {1410}.

Epidemiology

Pancreatic VIPomas constitute about 80% of diarrheogenic neoplasms and 3-8% of all endocrine tumours in the pancreas {1111}. Approximately 50% of pancreatic VIPomas are malignant (metastatic) at the time of diagnosis {2090}. Females are affected more often than males and the age of the patients ranges from 19-79 years (mean: 48 years) {294,1539}. By contrast, two thirds of the neurogenic VIPomas are found in paediatric patients {1036,1349}. VIPomas are not familial, except for some tumours associated with MEN 1 {949,1649}.

Etiology

With the exception of those associated with the MEN 1, no etiologic factors are known for VIPomas.

Localization

VIPomas are located in the pancreas in about 80% and outside the pancreas in the remaining 20% {2094}. Pancreatic VIPomas are located more often in the tail (47%) than in the head (23%) or the body (19%) {294,1111,1649}. Extrapancreatic VIP-secreting epithelial tumours are exceedingly rare and include lesions in the small intestine {294}, oesophagus {2360}, and kidney {808}. Neurogenic tumours such as ganglioneuromas, ganglioneuroblastomas, neuroblastomas, and phaeochromocytomas constitute the bulk of the extrapancreatic VIP secreting tumours and are located more commonly in the retroperitoneum (65%) than in the mediastinum (35%) {1126,2094}.

Clinical features

Signs and symptoms

The Verner-Morrison syndrome is characterized by Watery Diarrhoea, Hypokalaemia and Achlorhydria or more often hypochlorhydria (WDHA syndrome) {1538,2313}. The secretory diarrhoea ranges between 0.5-6.0 litres per 24 hours and is usually the most prominent symptom at presentation. It results in severe loss of potassium and bicarbonate, which, in turn, lead to metabolic acidosis and dehydration. Additional features include hypercalcaemia with normal parathormone levels, hyperglycaemia, and occasionally flushing of the face and the chest. Rare instances of tetany have been reported, explained by hypomagnesaemia with normal or elevated calcium levels {949,1163}.

Imaging

As in other endocrine tumours of the pancreas, ultrasound, CT-scan, MRI, octreoscan and PET-scan are the methods currently utilized to localize pancreatic VIPomas and their metastases.

VIP in plasma

Plasma VIP assay should be carried out to confirm the VIPoma diagnosis, a level above 60 pmol/L being virtually diagnostic. Additional corroboration may be afforded by evaluation of plasma PHM levels, as this peptide is more resistant to proteolysis than VIP {202}.

Macroscopy

Pancreatic VIPomas do not present gross features that distinguish them from other endocrine tumours. These neoplasms are generally solitary, except in rare cases associated with MEN 1. They appear as sharply demarcated, but unencapsulated, masses ranging between 2-20 cm in maximum diameter (median diameter: 4.5 cm). The cut surface is variable, generally pink-tan or grey, and may display focal haemorrhage, fibrous septa, cystic change, or even gross calcifications {1552,1649}. Most VIP-producing neurogenic tumours are encapsulated {2094}.
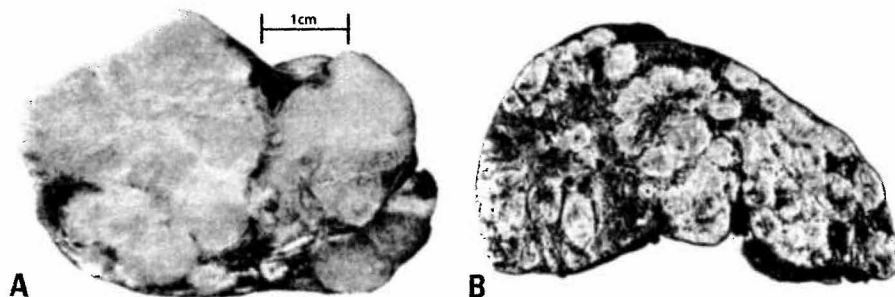

Fig. 4.27 VIPoma. A Large multinodular tumour with partly visible capsule and some necrotic and fibrotic areas. B Multiple liver metastases.

Fig. 4.28 VIPoma. Trabecular and gyriform architecture and partly peripheral palisading of nuclei.

Tumour spread and staging

Metastatic spread of VIPomas is found in about one-half of the pancreatic tumours at the time of diagnosis {1538,2094}. Metastases are more commonly found in the liver (haematogenous spread) than in the regional lymph nodes (lymphatic spread) {294,2090}. Indeed, vascular and perineural permeation is found at the periphery of one-half of the pancreatic VIPomas, generally in association with metastatic spread {2094}. So far there is no staging system that specifically applies to VIPomas (see Pancreatic endocrine tumours).

Histopathology

The microscopic appearance of pancreatic VIPomas does not present distinguishing traits. The tumour cells are rounded or polygonal, with a moderate amount of well-demarcated, faintly granular, eosinophilic cytoplasm. The nuclei may be regular, but often exhibit mild to occasionally moderate pleomorphism: they may be hyperchromatic or display a stippled chromatin pattern, and nucleoli are inconspicuous {294,2094}. Even significant nuclear atypia does not have prognostic significance applicable to a grading system. The mitotic count is usu-

ally low (<2 per 10 HPF), even in malignant tumours, although in about 12% of the tumours it may be relatively high and include the presence of atypical mitoses {2094}. The tumour cells grow in an organoid fashion and may be arranged in solid, trabecular and tubuloacinar patterns. The intervening stroma varies widely, ranging from delicate fibrovascular septa to broad, richly vascularized collagenous bands {294,2094}. Occasional examples of calcium {1552} or amyloid deposits {435} have been reported, the latter being of the islet amyloid polypeptide (IAPP; amylin) variety
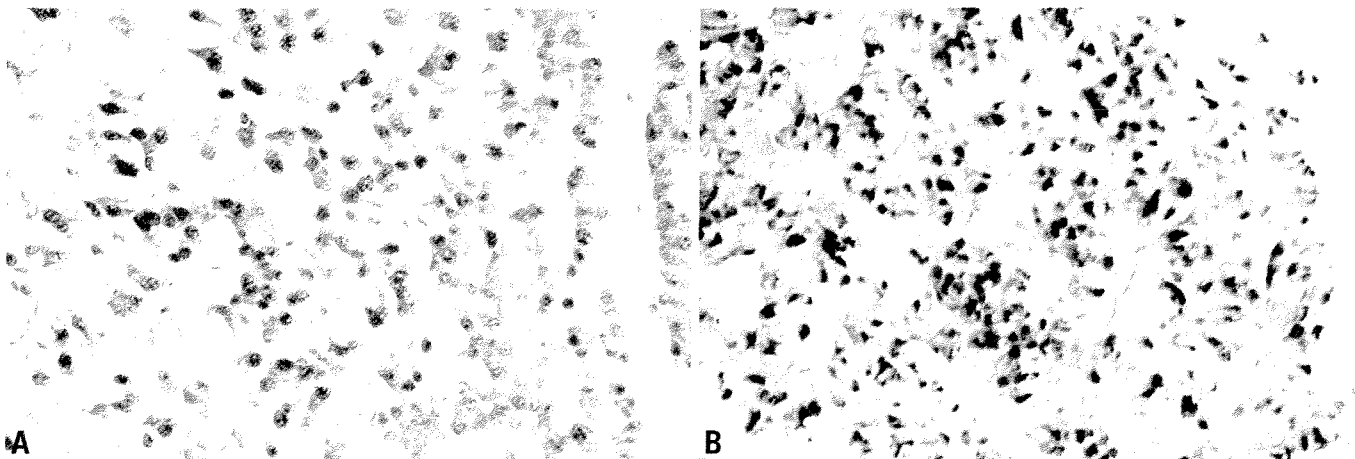

Fig. 4.29 VIPoma. **A** Production of VIP by a large number of tumour cells. **B** Visualization of mRNA for VIP shown by in-situ hybridization.

Immunohistochemistry

Generic endocrine markers, such as synaptophysin, chromogranins and others are positive in more than 90% of the tumours. In multiple studies, VIP has been immunolocalized in 87% of diarrhoeogenic tumours, peptide histidine methionine (PMH), a flanking sequence within the VIP precursor polypeptide, in 57%, PP in 53%, GH-releasing hormone in 50%, and the alpha chain of hCG in 18% {294,1649,2094,2095}. Other pancreatic hormones are expressed in less than 20% of the tumours, and the gastrin-related peptide ghrelin has been found recently in one VIPoma {2328}. All but type 4 somatostatin receptor subtypes have been demonstrated in individual VIPomas {1681,1815}.

Electron microscopy

Whereas the ultrastructure of pancreatic and neurogenic VIPomas has been characterized in numerous publications {1, 294,631,1649}, including the use of immunoelectron microscopy, such techniques are not essential for diagnosis.

Precursor lesions

No precursor lesions are known for VIPomas. Whereas early reports attributed some cases of WDHA syndrome to islet cell hyperplasia {2313}, such association has not been reported in the recent literature {2094

Histogenesis

The histogenesis of the pancreatic VIPomas is obscure since, as pointed out above, no normal or neoplastic islet cells appear to produce VIP. A somewhat perplexing finding is that, in spite of the existence of VIP-containing neurons in the normal pancreas, all neurogenic tumours associated with VIP-hypersecretion so far have been extrapancreatic {1238}.
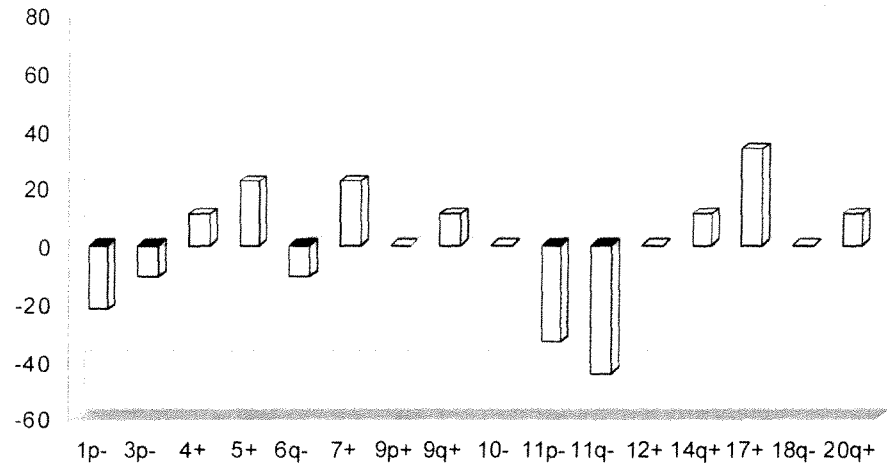

Fig. 4.30 Analysis of chromosomal alterations of VIPomas, using CGH. Chromosomal gains are shown on chromosomes 5, 7 and 17 while losses occur predominantly on 1p, 11p and 11q.

Somatic genetics

The CGH data on nine examined VIPomas exhibited frequent chromosomal gains and losses involving various chromosomes in all but one. The chromosomal loci involved were similar to those of other malignant pancreatic endocrine tumours. However, LOH analysis revealed a higher frequency of corresponding allelic imbalances {2105,2107, 2490}.

MEN1 mutations have been found in 4 of 9 examined sporadic VIPomas {95, 2018}. No mutations could be identified in the genes VHL, PTEN, SDHD and DPC4 {382,1721,1722}.

Genetic susceptibility

VIPomas are very rarely associated with MEN 1 {949,1649}. No association has been described with VHL or NF1.

Prognosis and predictive factors

As outlined in the Introduction, most VIPomas confined to the pancreas are classified as well-differentiated endocrine tumours with uncertain behaviour. Those exhibiting metastatic activity fall into the well differentiated endocrine carcinoma variety. Poorly differentiated, small cell (high grade) endocrine carcinomas have not been reported in association with the VIPoma syndrome.

After treatment, a meta-analysis study reported a 59.6% 5-year survival for patients with metastases and 94.4% for patients without metastases {2090}.

Little is known with respect to the prognosis of VIPomas in particular. It is understood that, like most endocrine tumours of the pancreas, VIPomas tend to have an indolent biological behaviour, even when metastatic {1858}. The massive diarrhoea with ensuing electrolyte imbalance associated with these tumours may initially pose a higher threat to the patient's life than the growth and spread of the tumour itself. No histopathologic criteria for prognosis have been developed so far.

Serotonin-secreting tumour

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Definition
A serotonin-secreting tumour is a usually malignant neoplasm of the pancreas that may become functionally active (syndromic) only after metastasizing to the liver. It produces the clinical symptoms of the carcinoid syndrome.

ICD-O code   8241/3

Synonyms
Carcinoid (obsolete); Carcinoid-islet cell tumour (obsolete)

Epidemiology
The actual incidence of serotonin secreting tumours of the pancreas is not known because evidence of serotonin production iis not routinely sought. Pancreatic tumours causing the carcinoid syndrome are extremely rare {508,1498,1656,1730, 2096,2294,2384}.

Clinical features
When a patient develops liver or retroperitoneal metastases, the carcinoid syndrome may occur with typical flushing, diarrhoea and bronchoconstriction, accompanied by elevated plasma 5-HT levels and urinary 5-HIAA excretion. In the absence of metastases, the tumour remains clinically silent, and the production of serotonin is only demonstrable by immunohistochemistry {614}. The carcinoid syndrome is due to a variety of factors released, including serotonin, kallikreins, substance P, and other tachykinins and prostaglandins.
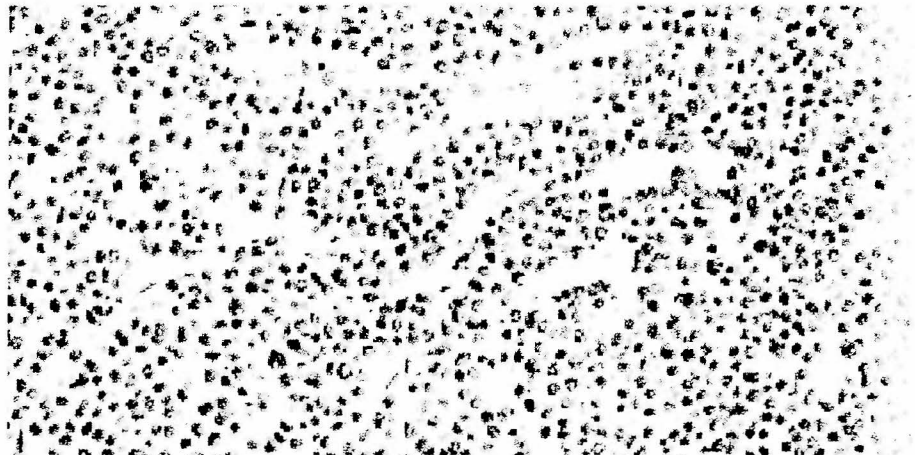

Fig. 4.31 Serotonin-secreting tumour. High-grade small cell carcinoma with solid architecture.

Imaging
Ultrasonography, CT scan, MRI, somatostatin receptor scintigraphy and PET-scan are currently the most sensitive methods for the detection of these tumours and their metastases.

Plasma / urine
The diagnosis is confirmed by documentation of elevated plasma levels of 5-HT and/or high urinary 5-HIAA excretion {615}.

Macroscopy
The tumours are usually large at diagnosis and do not present gross features that distinguish them from other pancreatic endocrine tumours.

Tumour spreading and staging
The tumours are frequently malignant and metastasize to lymph nodes and liver.

Histopathology
Most serotonin secreting tumours share histological features with other well differentiated pancreatic endocrine tumours and do not resemble midgut endocrine tumours (carcinoids). In one report, a poorly differentiated (small cell) endocrine carcinoma secreted serotonin {1701}.

Immunohistochemistry
The tumour cells are immunopositive for serotonin and may express other hormones as well.

Somatic genetics
Somatic genetic alterations in serotonin-producing endocrine tumours of the pancreas have not been reported.

Genetic susceptibility
No association with MEN 1, Von Hippel Lindau or NF1 has been documented {1250,1357,1989}.

Prognosis and predictive factors
All syndromic examples have liver metastases and therefore the ultimate prognosis is poor; however, the disease may progress very slowly.
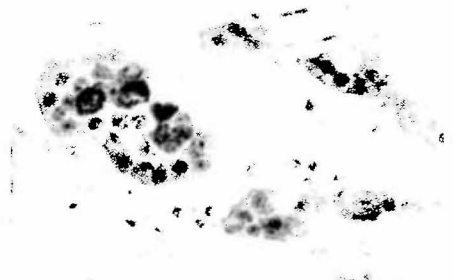

Fig. 4.32 Serotonin secreting tumour in the head of the pancreas.

Fig. 4.33 Serotonin secreting tumour. Serotonin production shown by immunohistochemistry.

ACTH and other ectopic hormone producing tumours

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Definition
Tumours that secrete ectopic hormones (e.g. ACTH, GHRH, PTHrP, calcitonin) are usually malignant neoplasms of the pancreas that produce clinical symptoms related to the specific peptide.

ICD-O code
8150/3

Epidemiology
ACTH-secreting pancreatic endocrine tumours comprise about 10% of ectopic Cushing syndrome cases and occur predominantly in women (64% of cases).

Etiology
The etiology of these tumours is unclear.

Localization
No preferential localization has been reported.

Clinical features
Ectopic secretion of ACTH may lead to Cushing syndrome with some or all of its features. However, the clinical symptoms generally appear within a shorter period of time than those caused by an ACTH-secreting tumour of the pituitary or a cortisol-secreting tumour of the adrenal cortex. In 5% of patients with ZES and 14% of those with Cushing syndrome, the same tumour produces both syndromes {1436,2094}.

The other ectopic hormones which may be secreted by pancreatic endocrine tumours and may produce a syndrome include growth hormone releasing hormone (GHRH) and growth hormone (GH)

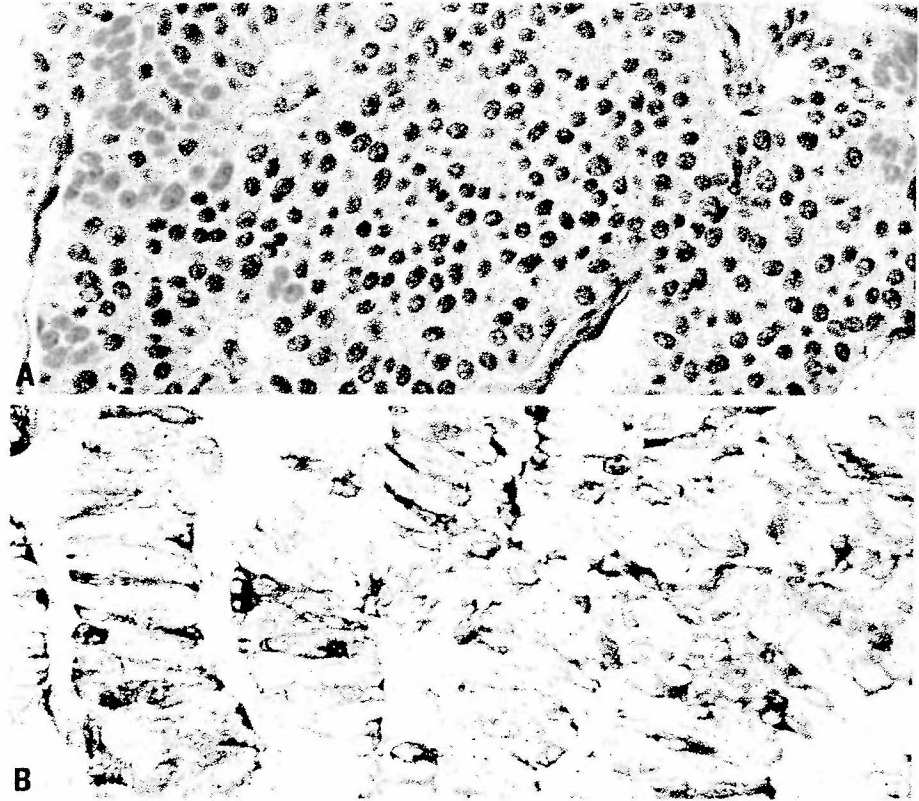**Fig. 4.34** Large, multi-nodular, focally necrotic, ACTH secreting tumour invading the spleen.

Fig. 4.35 ACTH-secreting tumour. **A** Partly solid, partly glandular architecture. **B** Immunophenotyping shows ACTH production by the tumour.

(acromegaly) {592,1928,1929}, corticotropin releasing hormone (CRH) (Cushing syndrome) {1436}, parathyroid hormone (PTH) and PTH-related peptide (PTHrP) (hypercalcemia) and calcitonin (diarrhoea) {78}.

Imaging
Ultrasonography, CT scan, MRI, somatostatin receptor scintigraphy (octreoscan) and PET-scan are currently the most sensitive methods for the detection of these tumours and their metastases.

Diagnostic procedures
The diagnosis is confirmed by documentation of elevated plasma levels of the appropriate hormone.

Macroscopy
Ectopic hormone producing tumours do not present gross features that distin-

guish them from other pancreatic endocrine tumours. They are generally solitary and large. In rare cases they are associated with MEN 1 {507,1219,1905, 2155}.

Tumour spread and staging
Because ectopic hormone producing tumours are large by the time they are detected, the majority have already metastasized to the regional lymph nodes or the liver {507,1905}.

Histopathology
The microscopic appearance of ectopic hormone producing tumours is not distinctive.

Immunohistochemistry
The hormone causing the syndrome can be detected by immunostaining, but the number of immunoreactive cells may
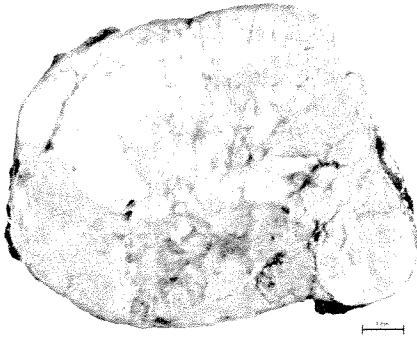
Fig. 4.36 Calcitonin-secreting tumour. Large, sharply defined tumour surrounded by a capsule showing necrotic and small haemorrhagic areas.

vary greatly. In addition to this hormone there may be other peptides identified {87,390,969}.

Somatic genetics

LOH of 3p was found in 3 of 3 PTHrP producing tumours, but in none of the two investigated ACTH-producing tumours {382}.

Genetic susceptibility

No association with MEN 1, VHL or NF1 has been documented.

Prognosis and predictive factors

Ectopic hormone producing tumours tend to be large, malignant and already metastatic to the liver at the time of diagnosis. Their ultimate prognosis is, therefore, poor {390,519}.

Fig. 4.37 Calcitonin-secreting tumour. A Well differentiated tumour displaying a trabecular architecture B Calcitonin immunoreactivity in a large number of tumour cells.

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Non-functioning tumours and microadenomas

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Definition

A non-functioning pancreatic endocrine tumour (NF-PET) is a usually malignant low grade endocrine neoplasm from which the patient suffers no paraneoplastic symptoms referable to the inappropriate secretion of any of the hormones or bioamines produced by functioning pancreatic endocrine tumours. For this reason, the term "nonsyndromic" pancreatic endocrine tumour may more accurately describe this group, but this term is not widely used. Since no specific clinical syndrome has been reported with inappropriate secretion of PP, these tumours are designated "PPoma" based only on their immunohistochemical profile and high levels of circulating PP, and are included with the NF-PETs.

Within the group of NF-PETs, examples measuring less than 0.5 cm are regarded as microadenomas and are clinically benign.

ICD-O code

Non-functioning pancreatic endocrine tumour   8150/1
Non-functioning pancreatic endocrine carcinoma   8150/3
Microadenoma   8150/0

Synonyms

Islet cell tumour, insuloma (obsolete), non-functioning pancreatic endocrine neoplasm.

Epidemiology

The prevalence of NF-PETs differs between incidental microadenomas and clinically relevant NF-PETs. Microadenomas rarely come to clinical attention; their prevalence has been estimated based on autopsy studies to be 0.4–1.5% {779,1086,2094} depending upon the amount of pancreatic tissue examined. Clinically relevant NF-PETs are much less common, with a prevalence of approximately 0.2-2 per million population {2094}. Based on surgical series, NF-PETs constitute 30-40% of all PETs (see Introduction, page 177) {248, 1069,1111,2094}, although their com-

mon incidental detection during diagnostic imaging procedures is expected to increase their frequency in future studies. Clinically significant NF-PETs may occur at any age but are rare in childhood {2048}. The age range is 12-79, with a mean age of 49.7 years {494,543,1 069,1314,2307}. Both sexes are equally affected (male to female ratio is 1:1.15). NF-PETs in patients with MEN 1 occur at a younger age.

Etiology

No etiologic factors are known with the exception of those tumours associated with MEN 1 {580,1114,1741} and VHL {896,938}. There is a suggestion of an association with tuberous sclerosis as well {2311}.

Localization

NF-PETs are more common in the head of the pancreas, with approximately two-thirds of surgically resected tumours occurring there {494,543,1069,1314, 2307}. Because these tumours are hormonally silent, the lesions most likely to cause local symptoms (i.e., those in the head of the gland) are most commonly detected.

Clinical features
Signs and symptoms

These tumours are hormonally silent and are initially asymptomatic. When they grow large, they present with symptoms due to local disease or distant metastases. Lesions in the pancreatic head may induce back pain or jaundice, but much less commonly than pancreatic adenocarcinoma. Often NF-PETs in the body and tail of the pancreas do not present with symptoms but the mass may be palpated on examination. In addition, patients may complain of nausea, vomiting, diarrhoea and lethargy. Approximately 15-20% of the patients are asymptomatic {1069}.
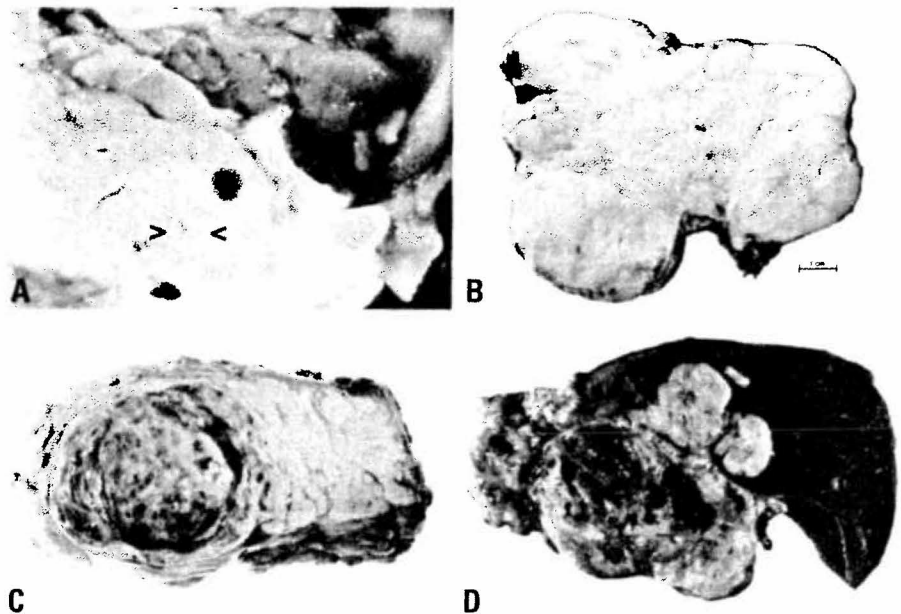

Fig. 4.38 A Circumscribed microadenoma (between arrows) with tan-cut surface (diameter, 0.2 cm). **B** Large, malignant non-functioning tumour with areas of necrosis and fibrosis. **C** Gross appearance of cystic pancreatic endocrine neoplasm (PEN). Note the large central cyst with minimal yellow tumour parenchyma surrounding the cavity. **D** Gross appearance of malignant NF-PET. The tumour is multinodular and invades the spleen.
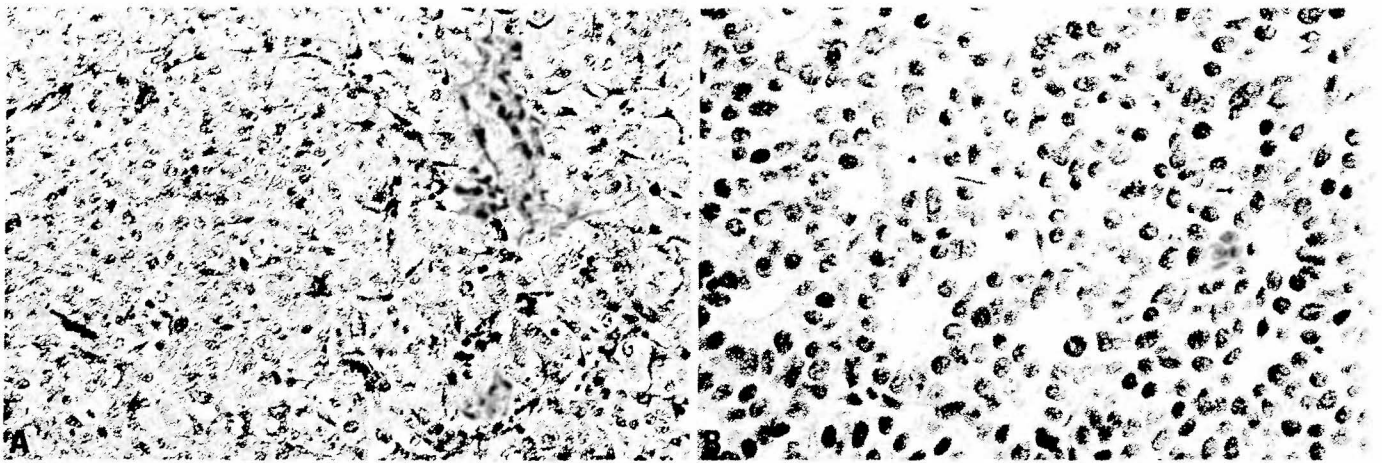
Fig. 4.39 Non-functioning tumour. **A** Liver metastasis displaying solid and trabecular architecture. Pan. **B** Solid and glandular architecture.

Imaging

Ultrasonography, CT scan, MRI, somatostatin receptor scintigraphy (octreoscan) and PET-scan are currently the most sensitive methods for the detection of these tumours and their metastases {1165, 2068}.

Diagnostic procedures

These tumours can be diagnosed by measurement of levels of circulating chromogranins, HCG-alpha-subunit, PP and others. Pancreatic hormone levels may be elevated despite the absence of a functional syndrome.

Macroscopy

Since microadenomas measure by definition less than 0.5 cm, most examples are not grossly detectable. Those that are identified on macroscopic examination of the pancreas appear as circumscribed, but unencapsulated red to tan soft nodules.

Larger NF-PETs may exhibit a wide range of gross appearances. Some tumours are sharply circumscribed and partially or entirely surrounded by a fibrous capsule. The consistency is often soft and fleshy and the tumours vary from red-tan to yellow. Other examples have a more pronounced fibrous consistency, and gross lobulation may occur. Areas of necrosis are uncommon, but degenerative cystic change may be found {1301}.

Tumour spread and staging

NF-PETs grow initially by direct extension into local structures, including peripancreatic soft tissues and spleen for tumours in the tail of the gland and duodenum or common bile duct for tumours in the head. Tumour thrombi within large veins may be found. Metastases occur both to regional lymph nodes as well as to the liver; distant metastases are usually limited to the later stages of the disease {494,543,1069,1314,2307}.

So far there is no staging system that specifically applies to NF-PETs (see Pancreatic endocrine tumours).

Histopathology

NF-PETs have organoid growth patterns typical of differentiated endocrine tumours and are not significantly different from most types of functioning PETs {439}. A nesting growth pattern is most common, but trabecular and gyriform patterns may also occur. Intratumoural heterogeneity is common, particularly in larger examples {1111,1112}. The stromal component varies considerably, from simple fine capillary-sized vessels between tumour cell nests to broad areas of dense, hyalinized collagen. Some tumours exhibit amyloid-like stroma, and stromal calcifications (exceptionally including psammoma bodies) may be found. Most cells contain moderate amounts of amphophilic to slightly basophilic, finely granular cytoplasm. The nuclei are generally centrally located without apparent polarization with respect to the fibrovascular stroma, although eccentric nuclei may be found, imparting a rhabdoid configuration to the cells {1717}. Nuclei are generally round to oval with minimal atypia and coarsely clumped ("salt-and-pepper") chromatin. Some examples demonstrate more nuclear abnormalities, including enlarged nuclei and irregular nuclear membranes {2485}; prominent nucleoli

may also be found. Most NF-PETs have very few mitotic figures. In many examples, they are essentially undetectable, but as many as 10 mitoses per 10 HPF are allowable in a NF-PET (more than 10 mitoses per 10 HPF qualifies the tumour as a well-differentiated endocrine carcinoma). Necrosis is highly variable; many NF-PETs have none, but punctate foci or larger, infarct-like areas may be found. In some NF-PETs, small ductules may be found between nests of endocrine cells. These ductular cells have more abundant cytoplasm and round nuclei without endocrine cytologic features. Although there may be close juxtaposition of these ductules to the neoplastic endocrine cells, the two cell types remain immunohistochemically distinct, and the ductules are likely entrapped and non-neoplastic. True glandular differentiation may also occur in NF-PETs, with luminal spaces being formed within nests of endocrine cells. In contrast to entrapped ductules, these lumina are lined by cells cytologically indistinguishable from the surrounding tumour cells.

Immunohistochemistry

NF-PETs express general markers of endocrine differentiation including synaptophysin, chromogranins, CD56 and CD57 {858,1336,1551}. Although NF-PETs are not associated with clinical syndromes due to inappropriate hormone secretion, it is common for immunohistochemical staining to demonstrate peptide hormones or serotonin in highly variable proportions of the tumour cells. For example, PPomas have expression of PP in the majority of the tumour cells {1239,2253,2255}. Many

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tumours with predominant expression of somatostatin (D-cell tumours) fall into the non-functioning category as well. In all NF-PETs, there is often expression of more than one peptide, including insulin, glucagon, somatostatin, pancreatic polypeptide, vasoactive intestinal polypeptide, gastrin, adrenal corticotrophic hormone, or others. {899,1235, 2094}. In only rare cases will the entire tumour fail to stain for any of these peptides, although occasionally only scattered cells are positive. Microadenomas are more likely to show diffuse expression of a single peptide, most often glucagon or PP {2094}.

Microadenomas may be difficult to distinguish from enlarged but non-neoplastic islets. Microadenomas normally exceed 500 μm in maximum diameter; in addition, immunohistochemical staining for islet peptides is helpful, since they lose the normal proportion and distribution of peptide cell types that are retained in enlarged non-neoplastic islets.

Many NF-PETs also express glycoproteins including CEA and CA19.9 {899, 1030}. Tumours demonstrating gland formation are particularly likely to stain for glycoproteins. Focal acinar differentiation may also be detected, generally in single widely scattered cells (less than one third) that stain for trypsin (-ogen) or chymotrypsin (-ogen) {1030,2448}.

Immunohistochemical staining for MIB-1 may be used to demonstrate the proliferative rate of NF-PETs. Most tumours show a low proliferative rate, with a labeling index of 1-5%, but staining of up to 10% of the tumour cell nuclei is acceptable {2094}. A greater percentage of immunoreactivity for MIB-1 suggests a diagnosis of high grade endocrine carcinoma.

Electron microscopy
Electron microscopy is generally not necessary for diagnostic purposes. If ultrastructural examination is performed, the tumour cells contain relatively abundant dense core neurosecretory granules that vary in size and shape from one tumour to another. Most have a nonspecific morphology, with a dense granule core separated by a halo from the limiting membrane. The granules are randomly distributed in the cytoplasm, and usually measure only 100-350 nm, easily separating them from larger, apically polarized zymogen granules of acinar cell carcinomas {578,772}. Characteristic secre-
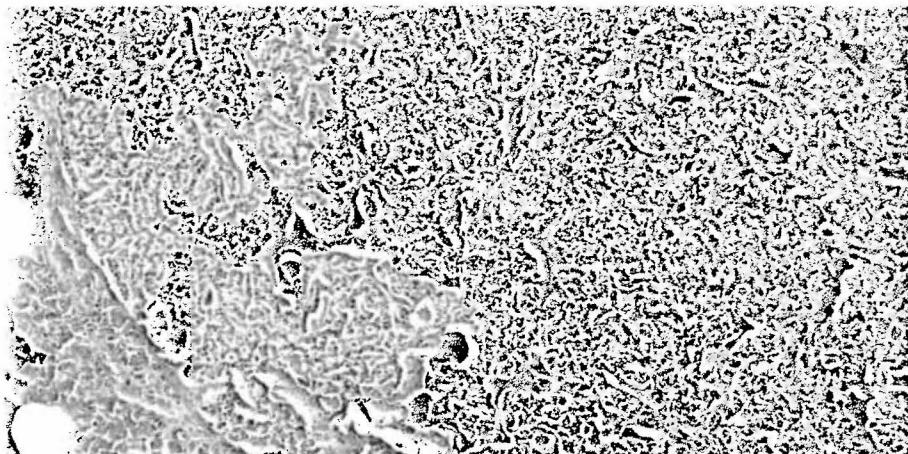

Fig. 4.40 Microadenoma. The tumour lacks a capsule and shows an uniform trabecular architecture.
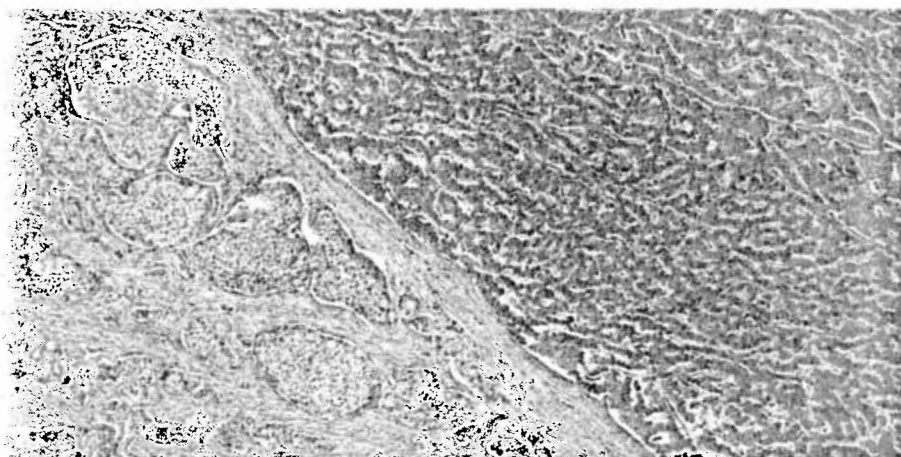

Fig. 4.41 Pancreatic endocrine tumour. Heterogeneity of architecture: nested vs. trabecular pattern.

tory granules of α cells or β cells are usually absent in NF-PETs.

Morphologic variants
A number of morphologic variants of NF-PETs have been described. Oncocytic PETs have cells with abundant granular eosinophilic cytoplasm in the majority of the tumour {320,753,1786}. These cells are filled with mitochondria. In many oncocytic PETs, the nuclei are enlarged

and moderately atypical, frequently with a prominent nucleolus. Some PETs demonstrate marked nuclear pleomorphism, with very large irregular and anaplastic forms {2485}. These tumours have been designated pleomorphic PETs and are frequently confused with higher grade lesions such as anaplastic carcinomas or ductal adenocarcinomas. Although the nuclei are markedly atypical, the cells generally also have abun-
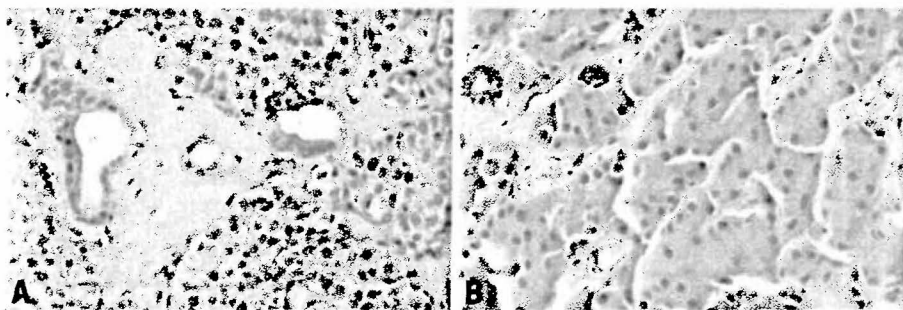

Fig. 4.42 A Non-functioning tumour showing entrapped non-neoplastic ductules. **B** Non-functioning tumour showing oncocytes with abundant granular eosinophilic cytoplasm.

dant cytoplasm, and the mitotic rate is not increased relative to other NF-PETs (distinguishing them from high grade endocrine carcinomas). This type of endocrine pleomorphism has not been shown to have adverse prognostic significance {2485}. Clear cell change may also occur in NF-PETs. In many of the reported examples, the cytoplasm contains innumerable clear vacuoles, sometimes scalloping the nucleus. Clear cell PETs have been reported more frequently in patients with VHL and may be confused with metastatic renal cell carcinoma {896}. None of these variants appear to have any prognostic significance.

Precursor lesions

There is no generally accepted precursor lesion for sporadic NF-PETs. Patients with MEN 1 have been reported to demonstrate islets with altered morphology, possibly representing precursors of NF-PETs.

Somatic genetics

CGH data from 28 tumours indicate that NF-PETs harbour the highest number of different chromosomal gains and losses compared to the other tumour types. These genetic aberrations involve chromosomal loci that are generally enriched in clinically malignant tumours {2490}. Gains of chromosome 4 and losses of 6q appear to be early events as they are already detectable in 40% and 50% of tumours with a diameter of less than 2 cm, respectively {2107}. Results of LOH and CGH studies correspond well and a variety of allelic imbalances have been described {120,121} indicating a high degree of genomic imbalance in these tumours.

Rarely identified somatic mutations in non-functioning PETs include genes such as *MEN1* (2/26) {750,880,1530, 2350}, *PTEN* (1/6) {1723}, *K-RAS* (1/30) {1531} and *TP53* (1/30) {1531}. No mutations could be found in *VHL* {382,1530}, *CDKN2A/p16* {1531}, *SMAD3* {2025} and *DPC4* {1722}.

Genetic susceptibility

NF-PETs occur in MEN 1 patients and multiple microadenomas ("microadenomatosis") are the hallmark of pancreatic involvement in this syndrome {1129}. Approximately 12-17% of VHL patients develop multiple NF-PETs that typically have a clear-cell cytology {811,896}.
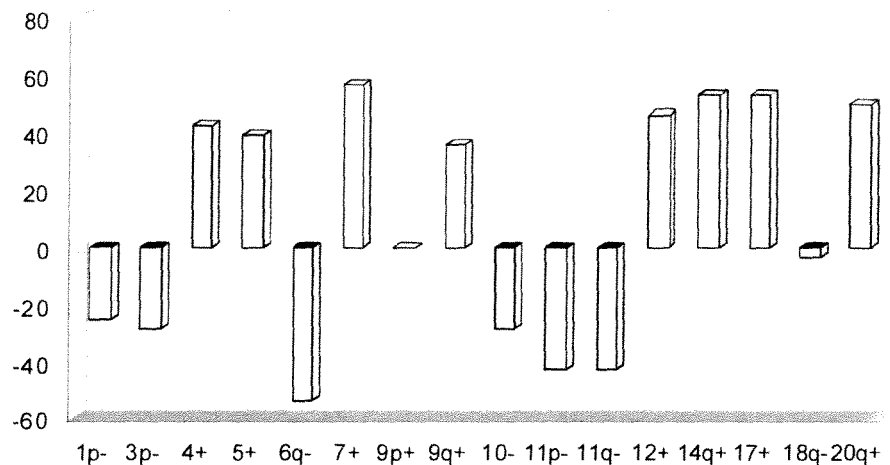

Fig. 4.43 Summary of chromosomal alterations of non-functioning tumours, using CGH. Chromosomal alterations are more numerous than in functioning pancreatic endocrine tumours. Chromosomal gains occur predominantly on chromosomes 4, 5, 7, 9q, 12, 14q, 17 and 20q, while losses are frequent at 1p, 3p, 6q, 10, 11p and 11q.

Prognosis and predictive factors

Pancreatic endocrine microadenomas are benign tumours {2094} and there is no evidence that they progress to clinically relevant malignant PETs.

NF-PETs are relatively aggressive neoplasms. Approximately 65-80% of cases are associated with clear-cut evidence of malignant behaviour (gross invasive growth or metastasis), and tumour recurrence following resection is common {494,543,1069,2094,2307}. With current earlier detection on imaging techniques this figure may decrease. Once distant metastases occur, cure is highly unlikely, although the rate of tumour progression may be slow. Most patients with metastatic NF-PETs survive for several years, and in some instances survival for a decade or more in the presence of hepatic metastases may occur. Following surgical resection, the five year survival for NF-PETs is reportedly 65%, but the ten year survival is only 45% {899}. In addition, a sizeable proportion of patients with NF-PETs present initially with distant metastases, perhaps because there is no paraneoplastic syndrome to draw clinical attention to the tumour early in its course.

In NF-PETs, specific peptide production has no impact on survival {899}. Some studies have demonstrated a correlation between overall nuclear grade and prognosis {899}. Other factors reportedly predictive of more aggressive behaviour include loss of progesterone receptor

expression {1715,2314}, aneuploidy {1068}, increased Ki-67 or PCNA labeling index {391,1715,2330}, increased fractional allelic loss {1830}, upregulated CD44 isoform expression {959}, and immunohistochemical expression of CK19 {491}. Loss of heterozygosity (LOH) at several chromosomal loci has also been reported to correlate adversely with prognosis, including chromosomes 1p {541}, 3p {120,382}, 6q {121}, 17p {788}, 22q {2385}, and X {1749}.

Mixed exocrine-endocrine carcinomas

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C. Bordi

Definition
Mixed exocrine-endocrine carcinomas of the pancreas are malignant epithelial neoplasms in which the exocrine and endocrine cells are intimately admixed in the primary tumour as well as in its metastases and each component comprises at least one third of the tumour tissue. They include mixed ductal-endocrine carcinoma and mixed acinar-endocrine carcinoma. Exocrine tumours in which the endocrine component is represented by scattered individual cells should not be included in this category. Endocrine tumours with entrapped non-neoplastic ducts or other parenchymal elements of the pancreas are also not considered mixed tumours.

ICD-O code   8154/3

Synonyms
Mixed exocrine-endocrine tumour, duct-islet cell tumour, mixed carcinoid-adenocarcinoma

Epidemiology
Mixed ductal-endocrine and mixed acinar-endocrine carcinomas are exceedingly rare in the pancreas {1110}. Mixed ductal-endocrine carcinomas account for approximately 0.5% of all ductal adenocarcinomas {1638} and mixed acinar-endocrine carcinomas for 15% of all acinar cell carcinomas {1106}. These tumours mostly occur in the in the 7th or 8th decades of life {1638}. Mixed acinar-endocrine carcinomas also rarely occur in childhood. Acinar-endocrine carcinomas have a slight female predominance {1106}, while ductal-endocrine carcinomas occur more frequently in males {1638}.

Localization
The majority of mixed ductal-endocrine carcinomas occur in the head of the pancreas {1110,1638}, while acinar-endocrine carcinomas are evenly distributed in the pancreas {1106}.

Clinical features
Symptoms and signs
Presenting symptoms are, in the majority of patients, non-specific and include obstructive jaundice, abdominal pain and weight loss. Only one patient with a mixed ductal-endocrine carcinoma presented with a hormonal syndrome: ZES due to inappropriate gastrin secretion {2212}.

Imaging
Ultrasonography, CT scan, MRI, somatostatin receptor scintigraphy (octreoscan) and PET-scan are currently the most sensitive methods for the detection of these tumours and their metastases.

Diagnostic procedures
There are no specific laboratory tests to diagnose these tumours.

Macroscopy
Mixed ductal-endocrine carcinomas present as solid masses, showing grey-white or yellow colour, with areas of necrosis and measure 3-10 cm in diameter {1110,2212}. Mixed acinar-endocrine carcinomas are tan to yellow, circumscribed with a soft, fleshy cut surface. Their diameter ranges around 3-11 cm, with a mean of 8 cm {1106}.

Tumour spread and staging
Metastases affect the liver and regional lymph nodes, although distant spread to other organs is reported in some patients {1106,2212}. Mixed ductal endocrine carcinomas are staged using the same criteria as ductal adenocarcinoma.

Histopathology
Mixed ductal-endocrine carcinoma
The exocrine component of these tumours is represented by moderately to poorly differentiated ductal or glandular structures or mucinous (colloid) carcinomatous structures. The ductal carcino-
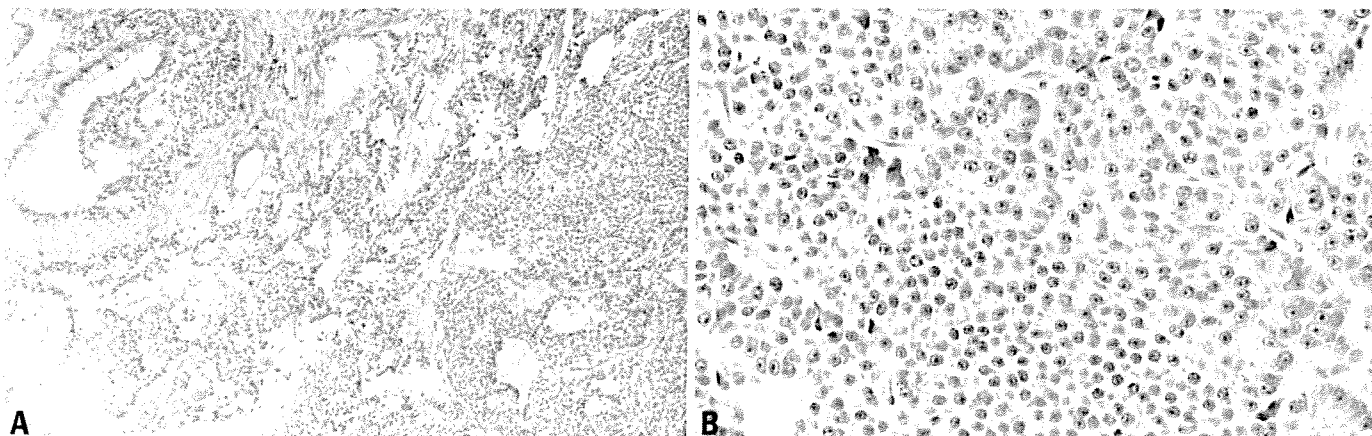
Fig. 4.44 Mixed ductal endocrine carcinoma. **A** Solid architecture with rare glandular formations in the endocrine part of the tumour (right) and ductular architecture (center and upper left corner). Large entrapped pancreatic duct. **B** Mixed acinar-endocrine carcinoma with solid areas composed of small endocrine cells and acinar formation by larger cells of the acinar phaenotype.
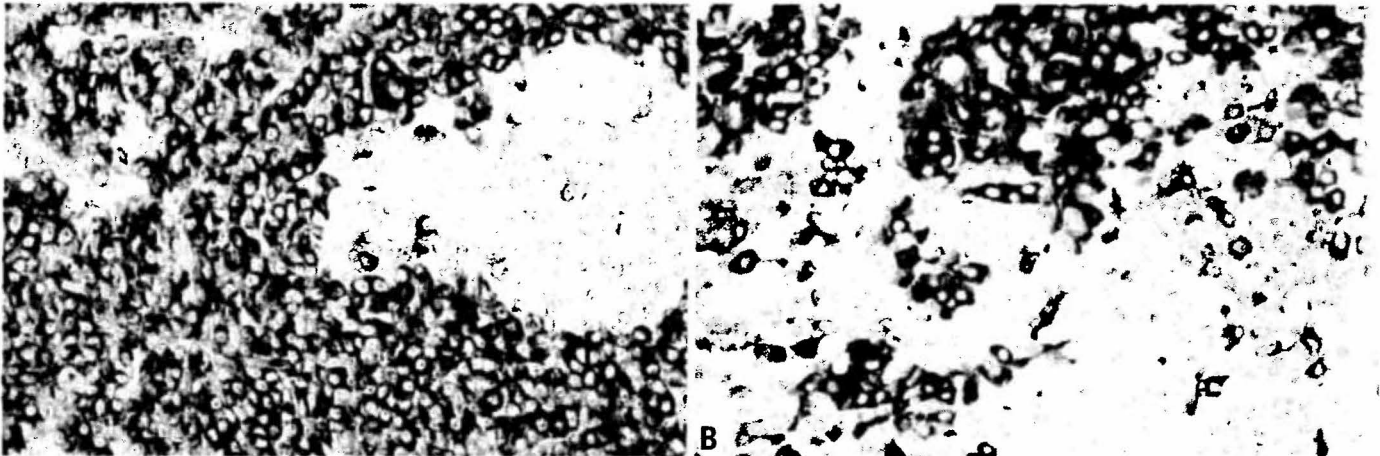
Fig. 4.45 Mixed acinar-endocrine carcinoma. **A** Immunostaining for trypsin labels the acinar component of the tumour. **B** Immunostaining for chromogranin A labels the endocrine component.

matous cells are columnar, with pale cytoplasm, ovoid or round nuclei and distinct nucleoli. In some cases, scattered goblet cells are also present. Histochemically, the ductal cells stain for neutral and acid mucins (positive for periodic acid-Schiff and alcian blue). The endocrine component can be well or poorly differentiated. The well differentiated endocrine component is represented by solid, trabecular or acinar structures formed by small to medium sized cells, with granular eosinophilic or amphophilic cytoplasm and nuclei smaller than those of ductal cells. The endocrine cells are positive for Grimelius' stain, while they are negative for mucin stains. The cell population and growth pattern of the poorly differentiated endocrine component are similar to those of small cell carcinoma of the lung {1638}. Simple lumen formation in a pancreatic endocrine tumour is not sufficient evidence of a neoplastic ductal component.

Immunohistochemistry. Neoplastic duct cells are strongly positive for cytokeratins 7 and 19, carcinoembryonic antigen and CA 19.9 {1110,1280,1638}. The endocrine cells consistently express synaptophysin and often chromogranins. Among hormones somatostatin is the one most often detected {1110,1280, 1638}, whereas gastrin was detected in the single tumour that presented with a functional syndrome {2212}.

Mixed acinar-endocrine carcinoma
In many mixed acinar-endocrine carcinomas both tumour components can be convincingly identified only with immuno-

histochemical staining. In some cases the acinar component can be recognized in H&E stained sections due to the organization of neoplastic cells in small glandular units with basal nuclei and apical cytoplasmic granularity. The endocrine component in contrast, consists most frequently of solid nests or trabecula of cells with randomly oriented nuclei and amphophilic cytoplasm.

Immunohistochemistry. The acinar differentiation is defined by immunoreactivity for pancreatic enzymes including trypsin, chymotrypsin and lipase {1106}. Chromogranins are expressed in most tumours. A minority of cases also show focal staining for hormones such as glucagon, somatostatin, gastrin and pancreatic polypeptide {1106}. A few cells may coexpress both acinar and endocrine markers ("amphicrine cells"). The only study reporting Ki67 labelling index of mixed ductal-endocrine carcinomas indicated different values varying from very low, in a case of moderately differentiated carcinoma, to 67.1%, in a case of mixed carcinoma with a poorly differentiated (small cell) endocrine component {1638}.

Electron microscopy. Ultrastructurally, ductal cells with mucingranules, acinar cells with characteristic zymogen granules ranging from 250-525 nm, or endocrine cells with small dense core granules measuring from 100-300 nm can be identified. Some cases may show amphicrine cells with an admixture of intracytoplasmic mucin or zymogen granules with small endocrine granules {582,1106}.

Precursor lesions
No precursor lesions are known for these tumours.

Somatic genetics
Detailed genetic studies on mixed exocrine-endocrine carcinomas of the pancreas have not been reported. No somatic or germline mutations in the MEN1 gene were detected in four tumours analysed (Papotti M and Bussolati G unpublished observations).

Genetic susceptibility
A genetic susceptibility for mixed exocrine-endocrine pancreatic carcinomas has not been reported.

Prognosis and predictive factors
The behaviour of mixed exocrine-endocrine carcinomas is dictated by the exocrine component. Mixed ductal-endocrine carcinomas and mixed acinar-endocrine carcinomas, both have the same poor prognosis as ductal adeno carcinoma and acinar cell carcinoma respectively {1106,1110}.

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Poorly differentiated endocrine carcinoma

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Definition
A poorly differentiated endocrine carcinoma (PDEC) of the pancreas is a highly malignant neoplasm composed of small to intermediate-size cells showing endocrine differentiation and a high proliferative rate (>10 mitoses per 10 HPF).

ICD-O code   8041/3

Synonyms
Small cell carcinoma, high grade neuroendocrine carcinoma, neuroendocrine carcinoma, oat cell carcinoma (obsolete).

Epidemiology
PDECs are rare tumours accounting for about 1% of all malignant pancreatic tumours {1536,1629} and no more than 2-3% of pancreatic endocrine neoplasms {2094}. They invariably arise in elderly patients, with a male predominance {1817}. The rate of identification of these neoplasms may, however, be influenced by several factors: (1) the endocrine nature of these tumours may have been unrecognized, with most of them having been misclassified as exocrine tumours in the past; (2) alternatively, some PDECs have been incorrectly diagnosed as pancreatic "carcinoid" tumours; and (3) a metastatic PDEC from an occult primary has been mistaken for a primary pancreatic PDEC {2094}, (4) conversely a primary pancreatic PDEC has been interpreted as a metastasis from an occult pulmonary small cell carcinoma.

Etiology
An association with cigarette smoking has been reported {363} but requires confirmation.

Localization
A predominant location in the pancreatic head has been reported {1817}.

Clinical features
Signs and symptoms
PDECs may present with symptoms similar to those of the exocrine pancreatic tumours {473,1000,1069}. Presentation with widespread metastases may occur. Lesions in the pancreatic head may induce back pain and jaundice due to obstruction of the common bile duct. Rarely these tumours may present with massive haemorrhage as a result of either penetration into the gastrointestinal tract or erosion of vessels in the retroperitoneum. Individual tumours were associated with Cushing syndrome {418}, carcinoid syndrome {747} and another with hypercalcaemia {897}.

Imaging
A majority of these tumours are large and thereby detected by standard procedures such as ultrasonography, CT scan of the abdomen as well as MRI {2005}. Smaller tumours in the head of the pancreas can be detected by endoscopic ultrasonography {53}. Somatostatin receptor scintigraphy (octreoscan) is often negative due to lack of expression of somatostatin receptor types 2 and 5.
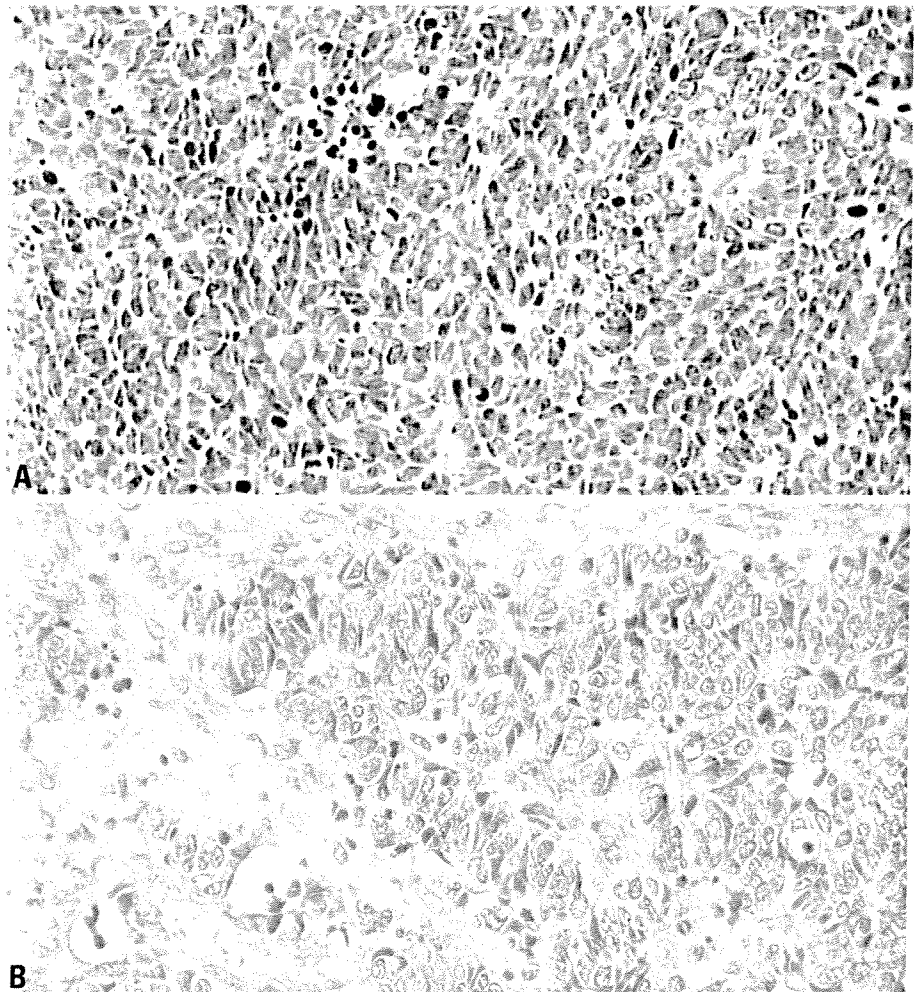
Fig. 4.46 Poorly differentiated endocrine carcinoma. **A** Solid sheets of poorly differentiated endocrine cells with brisk mitotic activity and apoptotic cells. **B** Higher magnification detailing the solid pattern of the poorly differentiated endocrine carcinoma.

Diagnostic procedures

In general serum concentrations of chromogranins A and B are normal but neuron specific enolase (NSE) may be elevated. Other markers such as CA 19-9, CA 50 and 5-HT may also show increased serum levels.

Macroscopy

The tumours have an average diameter of approximately 4 cm {1817}. They appear as firm, white-grey masses with ill-defined borders, often showing areas of necrosis and haemorrhage.

Tumour spread and staging

Invasion of the adjacent duodenum or of other peripancreatic tissues is frequent. Extensive, widespread metastases are the rule, involving regional and distant lymph nodes, as well as intra- and extra-abdominal organs such as liver and lung {1817.2094}.

Histopathology

The tumours commonly consist of tightly packed nests and diffuse irregularly shaped sheets of cells, often, with extensive central necrosis. Tumour cells are of small to intermediate size with hyperchromatic, round to oval nuclei and scanty, poorly defined cytoplasm and closely resemble those of the small cell carcinoma of the lung. {1817.2094}. Mitoses are abundant (>10 per 10 HPF). Some examples also have a more organoid architecture and moderately abundant cytoplasm.

Immunohistochemistry

PDECs stain diffusely for cytosolic/microvesicular markers such as synapto-physin or PGP9.5 but are negative or only focally and weakly positive for granular markers such as chromogranins {812}. As a rule no reactivity for peptide hormones is found. Abnormal nuclear accumulation of TP53, a feature virtually absent in low-grade endocrine carcinomas, is commonly though not invariably found. The Ki67-MIB1 labelling index is consistently above 10% and often exceeds 50%. Tumours with more abundant cytoplasm have more intense immunoreactivity for neuroendocrine granule markers and for peptide hormones {2094}.

Electron microscopy

The diagnostic finding is the presence of sparse small (100-200 nm in diameter), round, membrane-bound, dense-cored secretory granules in the tumour cells. In addition there are free ribosomes, poorly developed rough endoplasmic reticulum and intermediate filaments {812.2094}.

Differential diagnosis

As is usually the case for small cell carcinomas of whatever origin, cytokeratin immunostaining and lack of reactivity for common leucocyte antigen may be crucial in the differential diagnosis with non-Hodgkin lymphoma. PNET can be distinguished by diffuse immunoreactivity for CD99 (O13), although both tumours may strongly express cytokeratins {1371, 1549}.

Prominent cytologic atypia, extensive necrosis, a Ki67 index above 10% and diffuse nuclear expression of TP53 are useful in differentiating PDECs with intermediate sized cells from well differentiated endocrine carcinomas.

Finally, the distinction of a metastasis from an extra-pancreatic PDEC may be difficult by histological and/or immunohistochemical criteria. This situation demands accurate clinical evaluation of the patients.

Somatic genetics

Specific molecular alterations are so far unrecognised in PDECs. The most important differential molecular feature as compared to well-differentiated endocrine tumours and carcinomas, is the presence of alterations of TP53 gene {1838}, leading to nuclear protein accumulation, as in most small cell carcinomas of other sites.

Genetic susceptibility

As opposed to well-differentiated endocrine tumours and carcinomas, PDECs have been very infrequently associated with MEN 1 {1628}. No data are available on their occurrence in the setting of VHL.

Prognosis and predictive factors

The highly aggressive behaviour of PDECs and the usually advanced and unresectable stage at the time of diagnosis make the mortality of these tumours virtually 100%. The survival time ranges from 1 month to one year, despite initial favourable response to chemotherapy {1533,1629}.