Neuroendocrine Neoplasms of the Pancreas

Michael Markow and Barbara Ann Centeno

Introduction

Neuroendocrine tumors of the pancreas are composed of cells with evidence of neuroendocrine differentiation based on immunophenotypic and ultrastructural findings. These historically were called islet cell tumors, based on the assumption that they were derived from the islets of Langerhans. The first reported description came from Nicholls in 1902. He reported his discovery at autopsy of a simple adenoma of the pancreas arising from the island of Langerhans [1]. Fabozzi described a malignant counterpart in 1903 [2]. It has since been recognized that these neoplasms arise from neuroendocrine cells residing in the pancreatic ductal epithelium [3]. The WHO 2004 classification system referred to these as pancreatic endocrine neoplasms [4]. In the WHO 2010 classification, the terminology was modified to neuroendocrine tumor [5]. This chapter will cover the clinical, pathological, and molecular features of functional and nonfunctional pancreatic neuroendocrine tumors and adult nesidioblastosis.

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Neuroendocrine Tumors

Clinical

PanNETs are the second most common pancreatic neoplasm but account for 5 % or less of all pancreatic malignancies, with an annual incidence of 1 in 100,000 [6]. Many are detected incidentally during imaging for another process. They are more common in adults, with an age distribution of 30–60 years (mean of 50 years). Those occurring in children or adolescents are usually associated with a genetic syndrome [6]. There is a slight male predominance.

The tumors occur throughout the pancreas without a predilection for any site. PanNETs that occur in the head of the pancreas are only rarely associated with jaundice.

PanNETs may arise in association with familial syndromes including von Hippel-Lindau (VHL), tuberous sclerosis complex (TSC), neurofibromatosis type 1, and multiple neuroendocrine neoplasia type 1 (MEN 1) (see Table 1). Neoplasms arising in association with MEN 1 and VHL are frequently multifocal and occur in a younger age group [7, 8]. MEN 1 syndrome patients develop multiple microadenomas (<0.5 cm), and dysplastic islets, but can also develop macrotumors (>0.5 cm). The PanNETs that develop in VHL patients are nonfunctional, and up to 60 % show clear cell cytoplasm due to the presence of intracytoplasmic lipids [8, 9]. This morphology is not pathognomonic of VHL as originally presumed, since it is seen in sporadic, nonfunctional tumors and tumors associated with MEN 1 [10].

PanNETs have traditionally been classified as functioning or nonfunctioning. The majority of PanNETs are sporadic and nonfunctioning [11]. They are found during screening of patients with a syndrome that predisposes them to PanNET, the work-up of patients presenting with nonspecific symptoms, or the work-up of patients presenting with widespread metastatic disease. Functioning PanNETs are less common. They often present secondarily to patient symptoms related to the peptide produced by the PanNET. See Table 2 for a list of the syndromes and associated symptoms.

Table 1 Genetic syndromes associated with PanNET

Syndrome	Gene involved	Clinical features	Frequency of PanNET
Multiple endocrine neoplasia, type 1	*MEN 1*	Hyperplasia/neoplasms in multiple endocrine organs	Almost 100 %
von Hippel-Lindau	*VHL*	A variety of neoplasms, including pheochromocytoma, paraganglioma, hemangioblastoma, renal cell carcinoma, PanNET, serous cystadenoma, etc.	11–17 %
Neurofibromatosis, type 1	*NF-1*	Café au lait, neurofibromas, freckling, bony dysplasia	Rare
Tuberous sclerosis	*TSC1* *TSC2*	Hamartomas in many organs	2 %

Table 2 Summary of Clinical Presentations in Nonfunctional and Functional PanNET

Type	Incidence	Hormone	Clinical presentation
Nonfunctioning	1–3	CgA, NSE, PP	Incidental or nonspecific (related to tumor mass)
Insulinoma	0.1–0.3	Insulin	Whipple's triad
Gastrinoma	0.5–1.5	Gastrin	Zollinger-Ellison syndrome
Glucagonoma	0.01–0.1	Glucagon	Glucagonoma syndrome
VIPoma	0.05–0.2	VIP	Verner-Morrison syndrome
Somatostatinoma	<0.1	Somatostatin	SSoma syndrome (rare, <10 %), incidental, nonspecific symptoms related to tumor mass
PPoma	Rare	PP	Nonspecific, tumor mass related
Polyfunctional	Rare	Multiple	Depends on the hormones being secreted

Abbreviations: *CgA* chromogranin A, *PP*pancreatic polypeptide, *VIPoma* VIP-secreting tumor, *VIP* vasoactive intestinal polypeptide

Imaging

The imaging findings of PanNETs apply to both functional and nonfunctional tumors. Typical PanNETs present as solid, circumscribed enhancing masses on computed tomography (CT) imaging studies. They may appear somewhat lobulated and rarely appear infiltrative. Nonfunctioning tumors often are larger and have more irregular borders. Tumors in the head of the pancreas do not commonly obstruct the bile duct, in contrast to ductal adenocarcinomas, which frequently do. By endoscopic ultrasound (EUS), lesions are usually round and circumscribed, homogenous, and hypoechoic. Multiple tumors may be seen, particularly in patients with familial syndromes.

Cystic degeneration is a relatively common finding, identified in up to 10–20 % of PanNETs by CT scan and EUS. A key imaging feature of a cystic PanNET is a thick cyst wall, which is present in most cases.

Octreotide scintigraphy utilizes octreotide, a radiolabeled somatostatin analog, which binds to somatostatin receptors, to identify PanNETs. The Octreoscan, which uses ^{111}In-pentetreotide, was one of the first such functional imaging methods. Octreoscan is used to localize primary tumors and metastases and to plan treatments and monitor therapy response. Unfortunately, it has low sensitivity for the detection of tumors measuring less than 1 cm.

An emerging technology is the use of somatostatin analogs labeled with gallium. The somatostatin analogs are linked to ^{68}Ga with the use of a chelate, most commonly 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA). There are three ^{68}Ga-DOTA-labeled somatostatin analogs currently in use: DOTA-NOC, DOTA-TATE, and DOTA-TOC. Advantages over the traditional Octreoscan are the following: faster image acquisition, improved spatial resolution, higher affinity for somatostatin receptors, and the ability to quantify activity. It appears that a combination of imaging techniques including EUS, MDCT, and ^{68}Ga-DOTA-TOC provides the most optimal diagnosis [12]. ^{68}Ga-DOTA PET/CT imaging techniques can be used to localize functional as well as nonfunctional tumors [13].

Laboratory Testing

Plasma chromogranin A (CgA) is the most useful marker for diagnosing and monitoring patients with neuroendocrine tumors, including pancreatic neuroendocrine tumors. Limitations of the use of CgA as a biomarker include treatment with proton pump inhibitors, chronic atrophic gastritis, impaired renal function, and inflammatory bowel disease. Serum pancreatic polypeptide levels are used less frequently due to lower sensitivity, but when used in combination with CgA can improve sensitivity to 93 %.
Elevated levels of specific hormones are identified with functional syndromes and will be described in those specific sections.

Functional Neuroendocrine Tumors

Functional or syndromic PanNETs are named after the functional hormone they secrete, although some may secrete multiple hormones [11]. The diagnostic criteria vary. Based on the clinical definition of function – functional, hormone-producing, clinically symptomatic tumors – approximately 85 % of pancreatic neuroendocrine tumors are nonfunctional. Functional PanNETs are rare. The most common is insulinoma, followed by gastrinomas, glucagonomas, VIPomas, somatostatinomas, PanNET causing carcinoid syndrome, and other rare syndromes [11]. The most common syndromes are summarized in Table 2.

Insulinoma

Insulinomas are insulin-secreting PanNETs. The detection of the insulinoma relies upon the classical symptomatic presentation of Whipple's triad: hypoglycemia with glucose less than 50 mg/dL, neuroglycopenia, and symptomatic relief following administration of glucose. To establish the diagnosis of hyperinsulinism related to insulinoma, the parameters of serum glucose, C-peptide, and proinsulin are measured following a 72-h fast. Together, the criteria of decreased serum glucose, elevated C-peptide, and elevated proinsulin are 99 % sensitive for distinguishing insulinomas from other pancreatic mass lesions [14]. Some authors have reported higher overall sensitivity and specificity rates using the insulin/glucose ratio in conjunction with glucose, C-peptide, and insulin levels [15].

The characterization of a mass lesion is required to distinguish insulinoma from a rare, diffuse pancreatic insulin-secreting lesion known as nesidioblastosis. Conventional multi-detector CT imaging has been reported to have 68.8–94.4 % sensitivity [14, 16] for the detection of mass lesions; cases in which the CT is negative but clinical suspicion remains high can be augmented with MRI, EUS (86.6–92.3 % sensitivity) [14, 17], ^{18}F-FDOPA PET imaging [18],

^{18}F-radiolabeled GLP-1 analog exendin-4 imaging [18], and dual-energy spectral CT [16], among other imaging modalities. For cases in which these imaging modalities are negative, and nesidioblastosis cannot be excluded from the differential diagnosis, intra-arterial [14] calcium stimulation with venous sampling (ASVS) remains an option (see Nesidioblastosis, Adult Type, "Clinical diagnostic criteria") [14].

Glucagonoma

Glucagon targets some of the same pathways as insulin, and therefore has the contrary effect of raising blood glucose, but also has efficacy in pathways that are not directly related to glucose metabolism. The clinical diagnostic triad consists of diabetes mellitus, excess serum glucagon, and a skin rash known as necrolytic migratory erythema (NME) [19]. Diabetes is a simple diagnosis with well-established criteria; however, it is a nonspecific finding. Glucagon elevation is fairly specific in the context of a mass lesion. NME is seen in 70% of cases and has dermatological and histopathological features that are nonspecific. Features of NME are psoriasiform in nature and include papillary dermal hyperplasia with prominent vessels, superficial epidermal necrosis, epidermal hyperplasia, and parakeratosis. Other symptoms of glucagonoma include weight loss, anemia, diarrhea, stomatitis, and psychiatric disorders [20].

When clinical suspicion is aroused enough to impel the use of imaging to search for a mass lesion, a pancreatic mass is readily detected, and the appropriate neuroendocrine tumor work-up can successfully lead to the diagnosis. The imaging studies already described may be employed.

Glucagon may be hypersecreted by enlarged, misshapen pancreatic islets, much in cases where a mass lesion is absent, like insulin is in nesidioblastosis. Some authors consider this phenomenon to be a glucagon-secreting nesidioblastosis [21, 22], while others have called it *glucagon cell adenomatosis*. It presents with the same symptoms of glucagonomas, but without a discrete mass. Instead, the islets are diffusely enlarged with glomeruloid morphology [20, 23]. In contrast to the association of insulinomas with MEN 1, only one case of glucagon cell adenomatosis has been found to have the *MEN 1* (11q13) deletion [23]. The significance of this is unclear.

Gastrinoma

Clinical presentation of gastrinomas includes abdominal pain (with or without gastric ulceration), bleeding, and chronic diarrhea due to unregulated acid production in the stomach. Gastrinomas are famous for their association with Zollinger-Ellison syndrome and are also known to be associated with MEN 1. In both syndromes, gastrinomas tend to be multifocal; nonsyndromic gastrinomas are usually solitary. *ZES* and *MEN 1* mutations can be identified through molecular studies. Care must be

exercised in evaluating functional neuroendocrine tumors biochemically, as gastrinomas can produce separate neuroendocrine hormones (e.g., insulin) in up to 50 % of cases and many may be characterized as polyfunctional pancreatic neuroendocrine tumors if the separate hormone causes distinct clinical symptoms (page 6) [24].

The diagnosis of gastrinoma can be determined using fasting serum gastrinoma (FSG) concentration, endoscopic imaging, conventional CT and MRI for pancreatic masses, and newer imaging and biochemical methods. Any hypochlorhydric state can lead to serum hypergastrinemia, but hypergastrinemia (>2000 pg/mL) in the presence of a mass is highly suggestive of gastrinoma (98 % sensitive) [25]. With chronic functional gastrinomas, the stomach develops characteristically thickened rugal folds in 92 % of cases, grossly reflecting parietal cell hyperplasia due to unopposed gastrin stimulation [24]. Chromogranin A and progastrin have been studied for use in gastrinoma diagnosis, with some initial success of α-aminated gastrin assays. Overall, the biochemical diagnosis of gastrinomas can be complicated by treatment with common proton pump inhibitors and by problems with manufactured reagents that have occurred recently [24]. A stimulation test for gastrinoma can be performed using the glucagon provocation test, which is positive if plasma gastrin levels increase by 200 pg/mL or rise to greater than 35 % above baseline [26]. Secretin, a hormone that stimulates G cells, has been used historically as a clinical gold standard for diagnosis. Due to expense, it is now used primarily for tumor localization in cases of small but functional gastrinomas. The selective arterial secretagogue injection (SASI) test measures plasma gastrin levels after selective injection of secretin in various tributary arteries to the pancreas.

Prognosis is strongly correlated with the presence or absence of metastasis; 10-year survival with no metastasis is 96 %, and 10-year survival with diffuse metastasis is 16 % [24].

Vasoactive Intestinal Peptide-Secreting Tumor (VIPoma)

Pancreatic VIPomas are rare, with only about 1 in 10,000,000 per year [11]. These usually present as larger tumors, greater than 3 cm, and the vast majority occur in the tail of the pancreas (75 %). A majority, 60–80 %, present with metastatic disease. The classical clinical presentation is that of Verner-Morrison syndrome, also known as WDHA [27]. The signs and symptoms include watery diarrhea, with dehydration, hypokalemia, achlorhydria, and metabolic acidosis. Symptoms related to hypokalemia and dehydration include lethargy, nausea, vomiting, muscle weakness, and muscle cramps. Other symptoms include vasodilation with flushing and hypotension, hypercalcemia, and hyperglycemia. Abdominal pain, if present, is mild at most [11].

Somatostatin-Secreting Pancreatic Neuroendocrine Tumor (Somatostatinoma)

Due to the physiologic role of somatostatin as a general inhibitor of neuroendocrine secretion, the clinical presentation is highly variable. The classical presentation of somatostatinoma includes the development of diabetes mellitus, gallbladder disease

(due to cholestasis), diarrhea, weight loss, and steatorrhea [28]. However, preferential inhibition of insulin or other hormones may occur idiosyncratically, leading to primary presentation with hypoglycemia or hypochlorhydria [29]. Pancreatic somatostatinomas are far more likely than duodenal somatostatinomas (2 %) to present with symptoms, presumably due to circumvention of the first-pass effect [28, 30]. Gallbladder disease, the most distinguishing clinical feature of somatostatinomas from other pancreatic neuroendocrine tumors, is seen in 59 % of pancreatic somatostatinomas [30].

Due to vague symptoms and rarity of disease, somatostatinomas present with metastatic lesions 80 % of the time, and overall survival at 5 years is 60 %. In addition to the detection of a mass lesion in the pancreas, somatostatinomas can be identified serologically using immunoassays for somatostatin-like immunoreactivity (SLI) levels. Stimulation of SLI production using tolbutamide or arginine may have clinical utility but remains to be validated in large studies. Reference intervals are difficult to establish due to the paucity of cases; however, elevation of SLI levels to greater than 50 times mean values is accepted by some as diagnostic for somatostatinoma.

Pancreatic Polypeptide-Secreting Tumor (PPoma)

Pancreatic polypeptide-secreting tumors are extremely rare; only 24 pure PPomas have been described in the literature [25, 31]. They more often occur secondarily with other neuroendocrine tumors, often as a separate pauci-functional mass or discrete cell population within a mass that is secreting another major hormone. The physiologic role of pancreatic polypeptide is not fully elucidated, but similarly to somatostatin, it has a role in inhibiting other hormones. Therefore, abdominal pain is the most prominent of symptoms. Other signs and symptoms include diarrhea (including WDHA syndrome: watery diarrhea, hypokalemia, achlorhydria) [26], steatorrhea, and gallbladder contraction [25].

Clinically, PPomas may be detected by performing an assay for fasting serum PP concentrations (>300 pM) or by administering secretin to stimulate PP production (positive cutoff >5000 pM). Of the 24 pure PPomas reported in the literature, eight metastasized (33 %); prognosis is guarded, but it appears that metastatic rate is not dissimilar from nonfunctional pancreatic neuroendocrine tumors (38 %) [23].

Polyfunctional Pancreatic Endocrine Tumor

A polyfunctional PanNET is one that produces more than one hormone type and therefore is associated with more than one clinical syndrome. These are extremely rare and mostly have been documented in case reports [24, 32, 33, 35, 36]. These may respond to surgical debulking and treatment with peptide receptor radiotherapy [35].

Pathology

Gross

PanNETs typically appear as well-circumscribed, solitary masses. The cut surface is tan-brown and fleshy when the stromal component is minimal and sometimes more yellow, firm, and sclerotic when there is a stromal reaction. Some cases have a complete capsule. A percentage of these tumors present as predominantly cystic masses or as masses with cystic degeneration.

Histopathology

PanNET is typically a stroma-poor neoplasm, in contrast to ductal adenocarcinoma. The stroma may contain amyloid. There are no specific features associated with any of the functional types or that differentiate nonfunctional from functional types. Two exceptions exist. PanNET with psammomatous calcifications and gland formations is suggestive of somatostatin-secreting tumors. When dense sclerotic stroma is present, it suggests a serotonin-secreting tumor.
The cells are arranged in anastomosing cords, nests, and trabeculae (Fig. 1). They may form glandular structures, pseudorosettes, or tubuloacinar structures. These structures are surrounded by intervening vascular rich, thin stroma. The individual cells have rounded, monotonous nuclei with salt-and-pepper chromatin. The cytoplasm may be abundant and eccentric, imparting a plasmacytoid appearance.

A number of morphological variants have been described. The lipid-rich (also known as clear cell) variant is characterized by abundant foamy/microvesicular cytoplasm (Fig. 2). Some of these are associated with von Hippel-Lindau syndrome (VHL) [9] or MEN 1 [10]. An oncocytic variant, characterized by abundant, granular, eosinophilic cytoplasm and large prominent nucleoli, is also recognized. This variant has been found to be more aggressive in some studies [37]. Other morphological variants include a rhabdoid variant that may also have signet ring features [38], a small cell variant with an increased nuclear to cytoplasmic ratio, lacking the necrosis and mitotic activity typically seen in small cell carcinomas, and also a peliotic variant (Fig. 3) with pools of blood and dilated blood vessels.

PanNETs may show significant nuclear pleomorphism, called endocrine atypia, similar to that seen in other endocrine organs [39]. These degenerative, symplastic cells can be abundant and atypical, leading to a misdiagnosis of carcinoma.

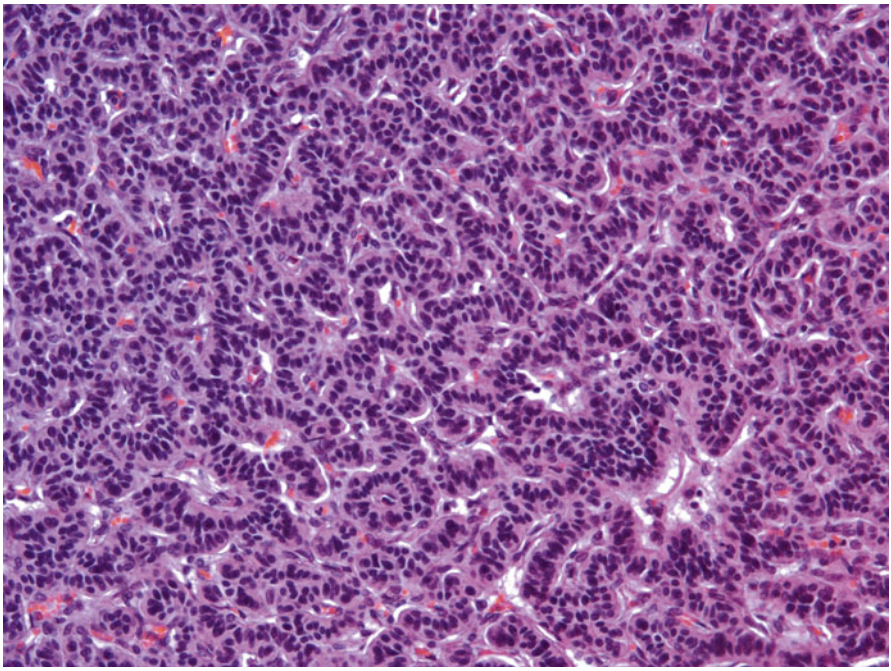

Fig. 1 Pancreatic neuroendocrine tumor growing in cords and trabeculae. The nuclei are round to oval with evenly dispersed chromatin. The cytoplasm is scant (hematoxylin and eosin, 200×)

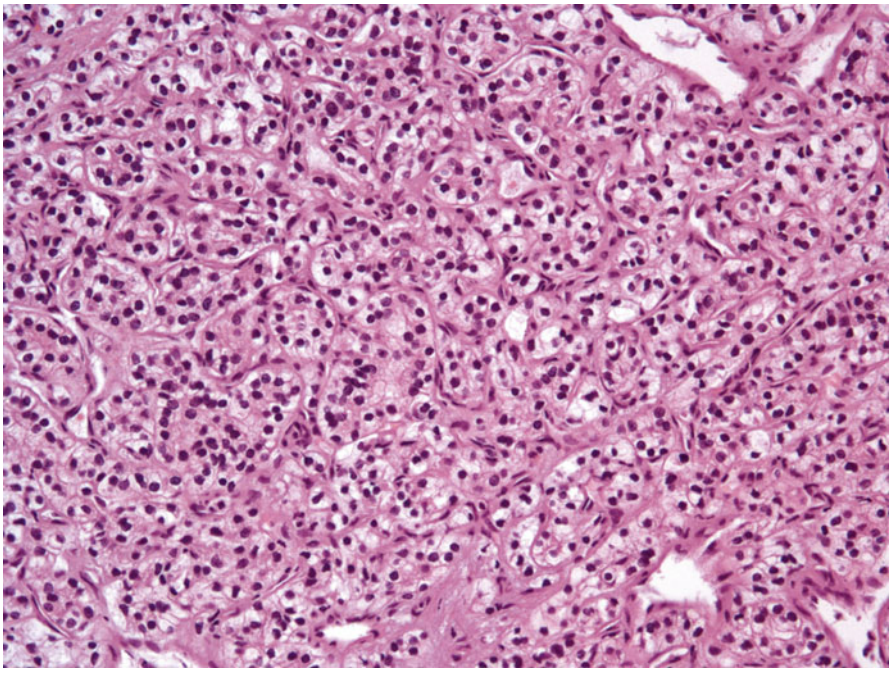

Fig. 2 Pancreatic neuroendocrine tumor with abundant clear cytoplasm (hematoxylin and eosin, 200×)

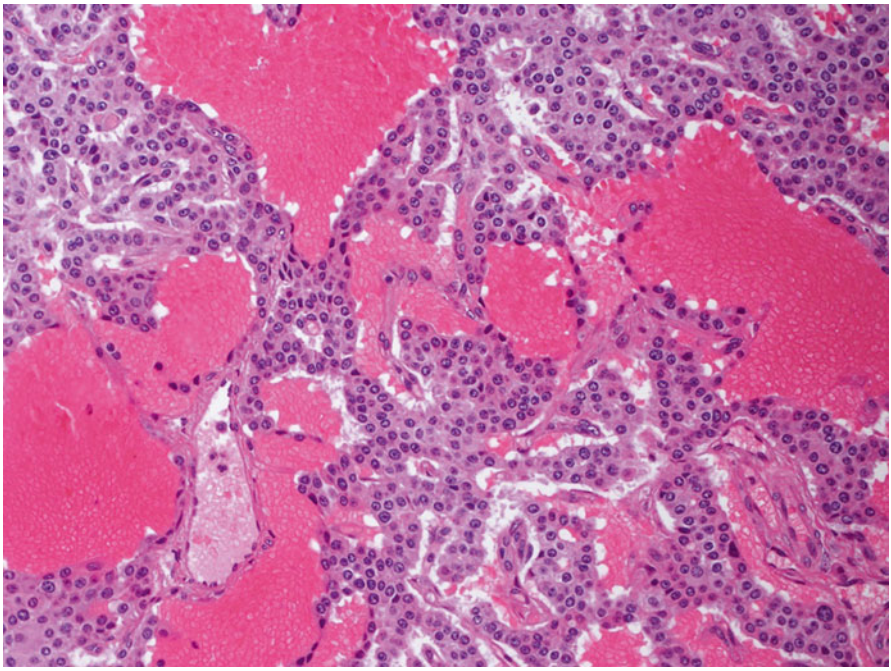

Fig. 3 Pancreatic neuroendocrine tumor showing a peliotic pattern. There are multiple dilated vascular spaces (hematoxylin and eosin, 200×)

Cytopathology

The low-power assessment of PanNET smears typically shows a cellular smear with a monomorphic cellular appearance (Fig. 4). The cells are arranged in loosely cohesive tissue fragments, which may have pseudorosettes, and as single cells. Due to the vascularity of these tumors, fibrovascular cores with loosely attached tumor cells may also be present. The cytoplasm varies from delicate, amphophilic scant cytoplasm to more abundant well-defined, granular cytoplasm with eccentric nuclei, imparting a plasmacytoid appearance [40, 41]. Cytoplasmic variations are seen in the oncocytic, rhabdoid, signet ring, lipid-rich, and clear cell variants [9, 42]. The lipid-rich variant commonly occurs in patients with von Hippel-Lindau syndrome [42]. The nuclei of all neuroendocrine tumor variants are usually round to oval, and the nuclear membranes remain smooth. The chromatin is finely granular with a "salt-and-pepper" appearance. The oncocytic variant retains the nuclear membrane configuration and chromatin pattern but also has prominent nucleoli. The pleomorphic variant of PanNET is the exception and shows anisonucleosis or "endocrine atypia" [42].

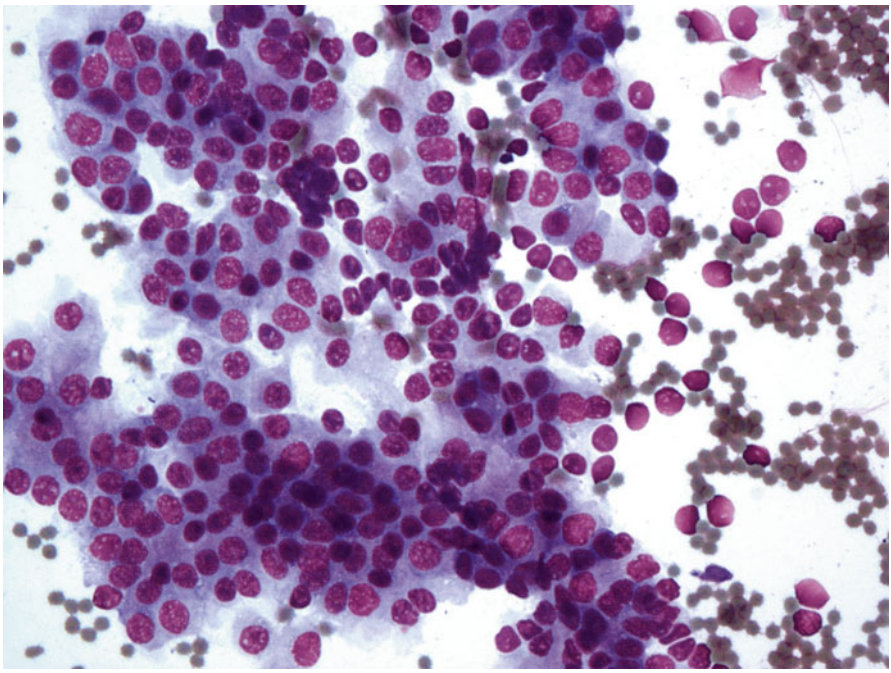

Fig. 4 Fine-needle aspirate smear demonstrating a monomorphic population of cells with round to oval uniform nuclei. The cytoplasm is variable in quantity and fragile. Stripped nuclei are seen in the background (Diff-Quik, 400×)

Ancillary Studies

Ki-67

The cell proliferation marker Ki-67 has gained substantial currency in the use of pancreatic neuroendocrine tumor prognosis. The Ki-67 index was incorporated in the 2010 WHO criteria for determining the histologic grade of pancreatic neuroendocrine tumors. Low grade is <3 %, intermediate is 3–20 %, and high grade is >20 % (Fig. 5a, b) [5]. It is used as an adjunct to mitotic index, which can have poor interobserver variability, and the highest possible grade is assigned according to either the Ki-67 or mitotic index criteria. The Ki-67 index is assessed by counting the areas of greatest proliferation, or so-called hot spots.

This new use of Ki-67 is controversial, as the scheme was incorporated into the WHO 2010 with very limited data to support it. Some advocate the alternative of using the Memorial Sloan-Kettering criteria (based on mitotic indices) or using a different proliferation marker like PHH3. Large head-to-head comparisons of repro-

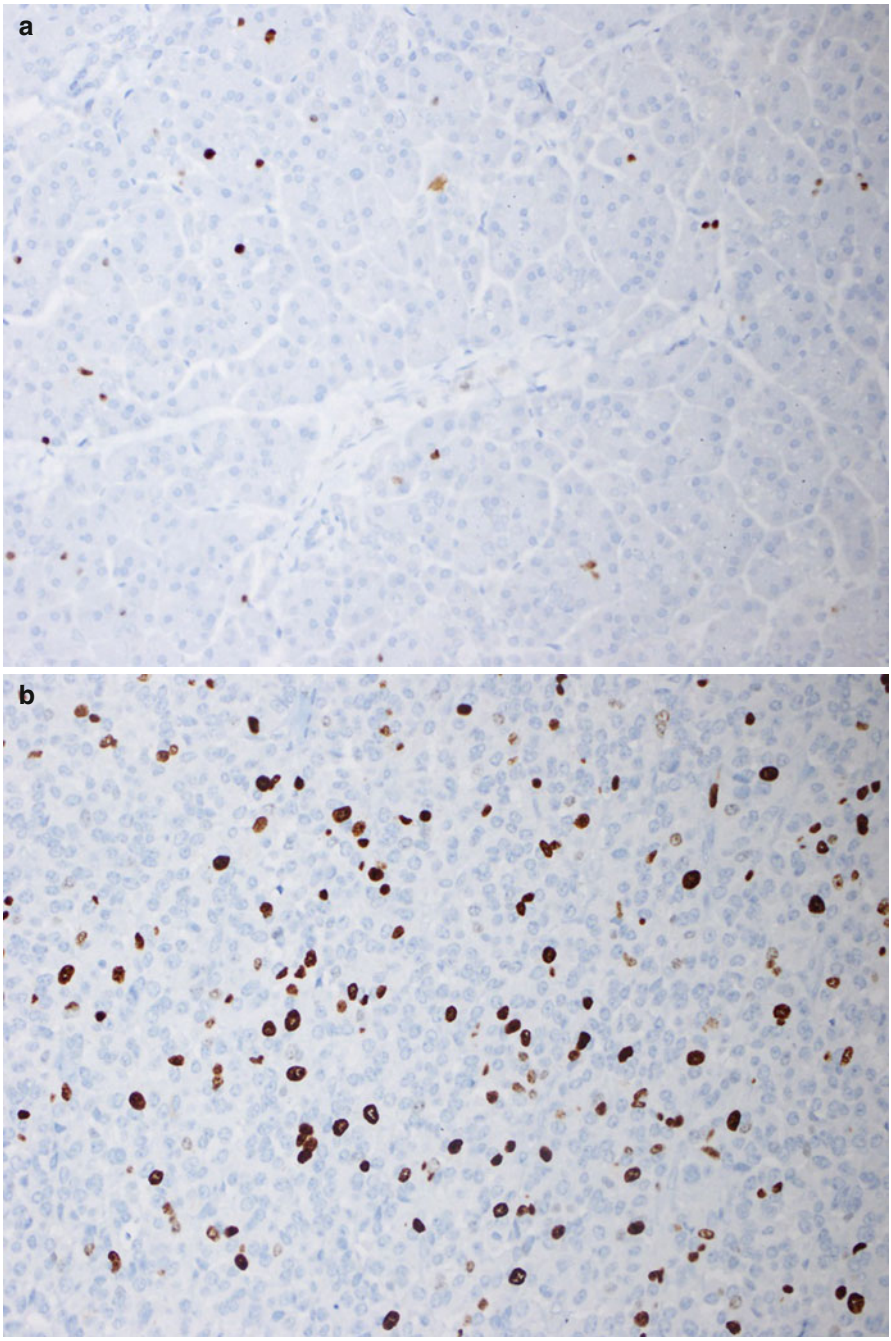

Fig. 5 Images of Ki-67 labeling (**a**) Pancreatic neuroendocrine tumor, grade 1, with a Ki-67 labeling index of 2 % (peroxidase, antiperoxidase, 200×). (**b**) Pancreatic neuroendocrine tumor, grade 2, with a Ki-67 labeling index of 15 % (peroxidase, antiperoxidase, 200×)

ducibility of these prognostic methods against Ki-67, using surgical resection specimens and under practical circumstances of hand counting, have not been performed. However, many studies have attempted to demonstrate the utility of Ki-67 and PHH3 in cell blocks from fine-needle aspirates, usually in conjunction with an automated cytometric counting system [43, 44].

Since the WHO 2010 reclassification, some data has come out to support the utility of Ki-67 in prognostication of pancreatic neuroendocrine tumors. It has use in upgrading grade 1 low-mitotic-count pancreatic neuroendocrine tumors to grade 2 based on an elevated Ki-67 index. PanNETs upgraded in this manner have been found to better correlate to tumor size and metastatic potential [45]. Still other studies have suggested making minor modifications to the WHO 2010 score, like altering the low-grade to intermediate-grade Ki-67 cutoff from 3 to 5 % [46]. A study comparing WHO 2010 Ki-67 index criteria to the WHO 2000/2004 showed that the Ki-67 index criteria are easier to use and more reproducible but that with this technique, there were no benign tumors since even G1 tumors metastasized. The WHO 2000/2004 was better at prognostication, most likely because this system included more parameters [47]. This study shows that the Ki-67 proliferation index alone is not sufficient to predict prognosis and needs to be combined with staging information at the time of resection.

Ultimately, Ki-67 index studies are hamstrung by the problem of site localization. It is widely recognized that different tissues have different mitotic or proliferation indices for assessing malignant potential. This becomes altogether more complicated in the GI tract, where the presence of a single or multiple sub-centimeter neoplasms prior to metastasis obscures discovery of the tissue of origin. Immunohistochemical markers for specific hormones are not helpful, as hormone-producing neuroendocrine tumors can arise in areas away from their native tissue, practically anywhere in the GI tract. Until it is possible to localize sites using immunohistochemical, molecular, or other methods, it will remain difficult to develop robust, site-specific criteria using the Ki-67 index. This is especially true in the pancreas, as its adjacency to multiple other GI organs can confuse radiographic localization of solitary neoplasms [48]. Another confounding problem with the use of the Ki-67 index is heterogeneity of Ki-67 expression within different parts of the same tumor.

PHH3

PHH3, or phosphohistone 3, is a marker of active mitosis (M phase). By contrast, Ki-67 is expressed during S, G2, and M phases. Theoretically, PHH3 should be a superior surrogate marker of mitotic activity, although this remains to be validated in large studies. PHH3 has been compared to the use of mitotic counts in pancreatic neuroendocrine tumors and found to have superior interobserver agreement (Fleiss' κ of 0.69 vs. 0.46) [49].

A comparison between PHH3 and Ki-67 suggests that Ki-67 should continue to remain the standard for now. This is due in part to the phenomenon of mitotic hot spots or aggregates of proliferating cells. The standard practice is to count Ki-67

expression across 500–2000 cells, and the preferred practice is to focus on the fields with the greatest numbers. The PHH3 antibodies used in immunoperoxidase reactions so far have stained less intensely than Ki-67, and PHH3 has therefore been less useful for detecting hot spots and led to underestimation of mitotic indices [43].

No large study has examined the use of PHH3 in the examination of metastatic potential or overall survival in pancreatic neuroendocrine tumors, although its use has shown promising results in other organ systems. Forthcoming validation of PHH3 as a prognostic marker for pancreatic neuroendocrine tumors may show substantial utility.

Molecular Alterations

Information about the genetic basis of this disease has been limited. DAXX/ ATRX, MEN 1, and mTOR pathway genes (*TSC2, PTEN, PIK3CA*) have recently been shown to be altered in sporadic pancreatic neuroendocrine tumors [50]. The genes most commonly affected in pancreatic ductal adenocarcinoma, including *KRAS*, *TGF-β* pathway (*TGFBR1, SMAD 3, SMAD 4*), *CDKN2A*, and *TP53*, were rarely altered, if at all [50]. These suggest an alternate oncogenetic pathway for PanNET.

DAXX/ATRX

Alpha thalassemia/mental retardation syndrome X-linked *(ATRX)* and death domain-associated protein (*DAXX)* are the two subunits of a transcription/remodeling complex. Mutation in one gene is mutually exclusive of mutations in the other. In the study by Jiao et al., 42.6 % of all PanNETs had loss in either ATRX or DAXX. Loss of immunolabeling for *DAXX* and *ATRX* correlated with mutation in the gene [50]. Subsequent studies detected mutations of *ATRX* or *DAXX* within 18 and 23 % of PanNETs by whole exome sequencing or FISH. PanNETs with mutations in *ATRX* or *DAXX* had a worse prognosis than *ATRX*- or *DAXX*-wild type tumors, including increased metastatic rate and decreased progression-free survival [51, 52].

Mutations of *ATRX* and *DAXX* lead to misplacement of histone H3.3 at pericentric and telomeric chromosome regions (at loci of DNA repeat sequencing), leading in turn to aberrant lengthening of telomeres. Failure to deposit H3.3 is an epigenetic phenomenon that leads to permanent somatic chromosome changes, possibly accounting for some of the additive cytogenetic abnormalities identified in sporadic pancreatic neuroendocrine tumors [53].

mTOR Pathway Genes

NF, TSC, and VHL are all associated with loss of suppression of the mTOR pathway. The mTOR pathway is a prominent regulator of cell growth, and its gain of function promotes oncogenesis.

Genes Associated with Familial Syndromes

Familial syndromes with increased pancreatic neuroendocrine tumors include multiple endocrine neoplasia (MEN) type 1, neurofibromatosis (NF) type 1, von Hippel-Lindau (VHL) syndrome, and tuberous sclerosis (TSC). MEN 1 is known to have multiple microcarcinomas of the neuroendocrine type, as well as multiple neuroendocrine tumors [54]. Loss-of-function mutations of the *MEN* gene are seen in 10–27 % of insulinomas and 39–45 % of gastrinomas. The pathways involved in PanNET development patients with a genetic predisposition are shown in Fig. 6. It is believed that genes on 11q distal to the *MEN* locus may harbor additional oncosuppressor exons.

Other Molecular Alterations and Cytogenetic Abnormalities

Sporadic pancreatic neuroendocrine tumors rarely have abnormalities in *TP53* and have various cytogenetic abnormalities, including 1 del, 11q del, 9q gain, 4 gain, 6q del, and 22q loss of heterozygosity (93 % of insulinomas). Deletions of *p16INK4a*

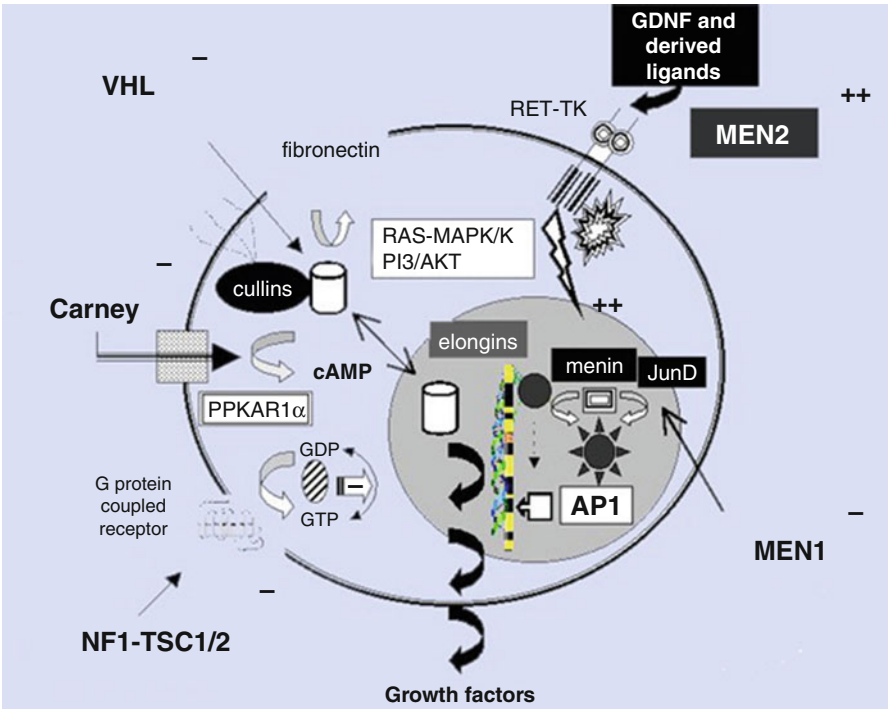

Fig. 6 This is a schematic summary of the pathways involved in genetic predisposition to neuroendocrine tumors (Reprinted with permission from [94])

and *p16/MTS1* are seen in gastrinomas. Cyclin D1 overexpression is seen in 43 % of pancreatic neuroendocrine tumors [55].

Palladin and RUNX1T1

Palladin is a nonproliferative prognostic immunohistochemical marker that is expressed in pancreatic neuroendocrine tumors. Physiologically, it has been demonstrated to play a role in regulating cell motility. It has been shown to have predictive value for liver metastasis. A study of 38 patients showed good immunohistochemical accuracy of palladin for predicting liver metastases. Using an Allred score cutoff of three of nine, palladin had a 100% sensitivity, 63% spcificity and overall positive predictive accuracy of 76% for predicting liver metastases in PanNET [56].

Another nonproliferative prognostic marker is *RUNX1T1* expression, discovered using gene expression profiling on frozen samples of PanNETs. Similar to the palladin protein, a study of RUNX1T1 expression loss in 37 patients using an Allred score of four of nine as the cutoff showed an 85 % sensitivity and 96 % specificity in predicting liver metastasis in tumors with a score of four or less [57]. RUNX1T1 is known to regulate neuronal differentiation in glial tissue; however, its function in the pancreas or pancreatic neuroendocrine cells is not yet elucidated [57].

MicroRNA

Alterations of microRNA metabolism have been associated with the development of many neoplasms, including PanNETs. Expression of miR-103 and miR-107, combined with loss of expression of miR-155, is characteristic of PanNETs in one series studied [58]. Expression of miR-204 is seen in insulinomas, and expression of miR-21 is correlated with Ki-67 index and metastatic potential. The specific downstream translational derangements of these microRNA sequences have yet to be elucidated. MicroRNA heat mapping with tumor profiling promises to have greater diagnostic and theragnostic efficacy as greater numbers of pancreatic carcinomas are studied [58].

Other

Immunoperoxidase reagents for immunohistochemical studies are available for all known hormones of clinical significance, including insulin, glucagon, gastrin, somatostatin (SLI), VIP, pancreatic polypeptide, and even motilin [59].

Nesidioblastosis, Adult Type

Background

Nesidioblastosis (also called congenital hyperinsulinism) is a controversial entity that is distinct from the insulinoma but is traditionally defined as proliferations of β cells which do not form discrete masses. Due to the rarity of nesidioblastosis, and the degree of overlap of clinical presentation between nesidioblastosis and insulinoma, no rigorously studied criteria have been developed to differentiate the two entities. The commonly accepted working definition is the exclusion of insulinoma using a combination of radiographic examination, bimanual surgical examination, and gross pathologic examination [60]. Microscopically, there are no absolute size criteria applied to differentiate nesidioblastosis and insulinoma; instead, nesidioblastosis and insulinomas have distinctly different pathological features, described below [61]. Meanwhile, the clinical criteria for the two entities are better defined. Although no specific marker exists to differentiate nesidioblastosis from insulinomas, there are clinical parameters that can be used to strongly support the diagnosis of nesidioblastosis [62]. For the diagnosis of nesidioblastosis, close clinicopathologic correlation is essential.

Nesidioblastosis comes in two main varieties, the diffuse type and focal type [60]. The focal type is characteristically seen only in infants; in fact, only one case has been reported in an adult [63]. Nesidioblastosis, focal type, is distinct from the insulinoma by not forming a discrete mass, and it is distinct from diffuse nesidioblastosis by forming patchy areas of irregular, hyperplasic islets that are increased in cellular density. These areas of islet hyperplasia are only sporadically affected, in contrast to the diffuse form in which islets are nearly equally affected throughout the pancreas [64]. We highlight the diagnostic criteria of nesidioblastosis, focal type, to offer evidence for its distinction as an entity from diffuse nesidioblastosis and insulinoma. Nesidioblastosis, focal type, has additional histopathological features that will not be described here. Further description of the clinical and histopathological features of diffuse nesidioblastosis can be seen below.

Prior to its subtyping, nesidioblastosis was first described as a phenomenon in children in 1938 [65] and first described in adults in 1975 [66]. Nesidioblastosis, diffuse type, is traditionally believed to be a rare entity; only 138 cases have been reported [60–63, 67–98]. Diffuse nesidioblastosis is responsible for 60 % of cases of neonatal nesidioblastosis and essentially 100 % of adult cases [60]. Although nesidioblastosis has been characterized as a β-cell proliferation, it has recently been reported to express glucagon in rare cases, although some believe this should be designated as a separate entity [81, 90]. Nesidioblastosis also can occur concomitantly with insulinomas [22, 76]; and nesidioblastosis, adult type, is associated with prior stomach surgery, especially gastric bypass surgery [69, 90–98]. The commonness of postprandial episodic hypoglycemia with hyperinsulinemia in postoperative

gastric bypass patients, combined with the intrinsic difficulty of diagnosing nesidioblastosis, suggests an underdiagnosis of diffuse nesidioblastosis, adult type [98].

Pathogenesis and Molecular Biology

Nesidioblastosis was originally coined to reflect the phenomenon of endocrine cells appearing to displace ductal epithelial cells of the pancreas (*nesidio-*, islet; *blastos*, germ) [65]. Originally, pathologists believed that its etiology was embryogenic; therefore, some texts utilize the term nesidioblastosis to refer to the embryogenic displacement of epithelial cells by neuroendocrine cells. Others use the term nesidioblastosis only to describe the histological features, without any congenital connotation. Nesidioblastosis was believed to be a diffuse proliferation of neuroendocrine cells within glands (ectopic placement, as in heterotopic pancreas within upper alimentary tract) which was consistent with a congenital, presumed embryological, malformation. The identification of nesidioblastosis exclusively in the pediatric population during the first 37 years after its discovery likely contributed to the conception of nesidioblastosis as an embryologic or congenital disease [65, 66].

However, no convincing studies emerged to demonstrate an embryological pathogenesis for nesidioblastosis, and the recognition of adult-onset diffuse-type nesidioblastosis forced a reconsideration of pathogenesis. The growing evidence that a subset of postoperative gastric bypass patients are developing diffuse-type nesidioblastosis demonstrates that other factors must be at play.

What do we know now about the pathogenesis of nesidioblastosis? While the exact etiology remains unclear, and further studies are required to elucidate a working model to explain both pediatric- and adult-onset nesidioblastosis, we have determined some of the biochemical and molecular factors underlying the disease.

Familial cases of nesidioblastosis have been the underpinning of the challenge against an embryologic model of pathogenesis and include mutations in genes *ABCC8*, *KCNJ11*, *HADH1*, *GCK*, *GLUD1*, *SLC16A1*, *UCP2*, and *HNF4a* [89]. Of these mutations, authors focus on *GLUD1*, *GCK*, and *SLC16A1* as more likely to present in adults.

The *ABCC8* and *KCNJ11* genes, most common of the familial genes, are not typically found in adults with sporadic nesidioblastosis. Homozygous recessive genotypes for either gene, or heterozygosity of both genes, causes defects in ATP-driven K^+ channels on islet cells, leading to hyperplasia of islet cells. Impairment of K^+ receptors leads to constitutively active insulin secretion, of which cell hypertrophy and proliferation are downstream effects [60]. The effects of these two germline mutations can be modified by somatic mutations. Inheritance of paternal *ABCC8* or *KCNJ11* genes with a somatic loss of heterozygosity at the 11p15 region can likewise lead to hypertrophic islet cells and hyperplastic islets. Unsurprisingly, the therapeutic value of diazoxide, an agonist of the ATP-driven K+ channels, is limited in cases of nesidioblastosis with these mutations.

GCK mutations in glucokinase cause hyperinsulinism due to failure of the negative feedback loop in glycolysis. *GLUD1* mutation upregulates glutamate dehydro-

genase, causing increased α-ketoglutarate and efficacy of insulin secretion that is stimulated by amino acids [99]. Together, mutations of *GCK* and *GLUD1* are generally responsive to medical therapy, and so their histopathology has been poorly studied due to absence of resection specimens [60]. Similarly to *GLUD1*, mutation of the gene *HADH1*, which encodes the protein SCHAD, is found in mice knockout studies to increase activity of glutamate dehydrogenase. Increased glutamate dehydrogenase activity is caused by the loss of functional inhibition by wild-type SCHAD glutamate dehydrogenase; the mutant is less capable of downregulating glutamate dehydrogenase. For this reason, both *GLUD1* and *HADH1* are associated with protein-induced hypoglycemia [99].

SLC16A1 encodes MCT1, which is a membrane-bound pyruvate transporter. Ostensibly, it removes excess pyruvate, allowing glycolysis to proceed faster. It is associated with anaerobic exercise-induced hypoglycemia. Ultimately, thorough work-up of nesidioblastosis may need to include testing for these genetic aberrances, as treatment efficacy varies demonstrably among nesidioblastosis cases with differing genetic mutations [99, 100].

Studies attempting to implicate the *menin* gene of MEN 1 failed to demonstrate a germline mutation in known cases of nesidioblastosis [61].

In studies surrounding the mechanism of gastric bypass surgery causing nesidioblastosis, glucagon-like protein 1 (GLP-1) has been identified as an inducer of cell proliferation in islets, with subsequent insulin overproduction and postprandial hypoglycemia. GLP-1 targets all islet cell types (including α and β cells) in rat models and also stimulates progenitor cell differentiation [100]. Meanwhile, GLP-1 is demonstrably elevated in gastric bypass patients after glucose intake [93, 101] and acts in a glucose-dependent manner [102]. GLP-1 is a logical candidate for causing β-cell hyperplasia "accidentally" while being endogenously produced to stimulate α-cell production, after bypass surgery, although its full effects remain to be elucidated. However, the GLP-1 hypothesis of nesidioblastosis development does not explain why β cells would predominate in number or function over α cells.

Increased levels of growth factor expression, including IGF1Rα, IGF2, and TGFRβ3, are also seen in nesidioblastosis. These factors stimulate β-cell proliferation and are also elevated in gastric bypass patients. The association is probably causal, but this remains to be demonstrated. The efficacy of growth factors as markers in individual cases of nesidioblastosis appears to be based on underlying subtype (diffuse vs. focal) [103].

Clinical Diagnostic Criteria

Nesidioblastosis is referred to more popularly in clinical circles as congenital hyperinsulinism (CH) due to the historical confusion, etiological uncertainty, and antiquated histological meaning of the term nesidioblastosis. However, the use of the term nesidioblastosis to describe the specific situation of adult-onset CH has gained traction due to the confusion of using "congenital" to describe an adult-onset disease with obvious environmental associations [60]. Given the confusion of

terminology, clarification within the pathology report or during communication with clinicians can help facilitate understanding of the underlying diagnosis. Some also prefer use of the term persistent hyperinsulinemic hypoglycemia (PHH).

Clinically speaking, nesidioblastosis and insulinoma are both under the diagnostic category of organic hyperinsulinism or the overproduction of insulin regardless of blood glucose level. Therefore, in nesidioblastosis, fasting causes hypoglycemia as glucose is depleted over time, and the elevated insulin/glucagon ratio prohibits liberation of new glucose into the blood. The first diagnostic step is a 48- or 72-h fast. Serial measurements of serum insulin, proinsulin, and C-peptide are taken until the patient begins having symptoms of neuroglycopenia. If the above peptides remain elevated, then organic hyperinsulinism is the diagnosis, and the patient is evaluated for insulinoma or an endogenous insulin-producing mass. In the absence of a radiographically identifiable mass, the traditional approach has been to undertake a laparotomy with bimanual examination of the pancreas in an attempt to localize a smaller mass. Depending on clinical parameters, some have recommended stopping the bimanual evaluation in the absence of an identifiable mass and performing a spleen-sparing distal pancreatectomy.

A newer diagnostic modality has been developed, which is helpful for mass localization, and in the absence of mass localization, it is capable of demonstrating nesidioblastosis clinically without biopsy. The procedure is called intra-arterial calcium stimulation with venous sampling (ASVS). It begins with infusion of calcium gluconate serially into each of the arteries feeding the pancreas: pancreatic, hepatic, gastroduodenal, superior mesenteric, and splenic. Throughout, serial measurements of insulin content in the hepatic vein are taken before and after the introduction of calcium gluconate into the tributary arteries. In the event of a localized mass, only one or two of the arteries will have elevated insulin levels. In the event of nesidioblastosis, all of the arteries tested should cause elevations in insulin draining to the hepatic vein. ASVS is comparable to the histopathological gold standard, with 100 % sensitivity in cases studied [80].

Histopathology

The histopathological features of nesidioblastosis are quite distinct from insulinomas and other neuroendocrine neoplasms on histopathological sectioning. Cytopathological examination of the cells cannot distinguish among these entities.

Histologically, nesidioblastosis, diffuse type, appears as diffuse, scattered islets with no mass formation. Anlauf et al. proposed four major criteria as essential for diagnosis and four minor criteria as adjunctive for diagnosis [61].

Major Criteria:

Exclusion of an insulinoma by macroscopic, microscopic, and immunohistochemical examination

Multiple β cells with enlarged, hyperchromatic nuclei and abundant clear cytoplasm
Islets with normal spatial distribution of various cell types
No mitotic proliferative activity of endocrine cells

Minor Criteria:

Irregular shape and occasional enlargement of islets
Increased number of islets
Lobulated islet structure (histological nesidioblastosis)
Macronucleoli in β cells

Using the major criteria as the pathologic standard, the Anlauf et al. group obtained 100 % specificity and 88 % sensitivity [61]. Other authors endorse anisonucleosis of islet cells as the main determining factor for nesidioblastosis, in conjunction with the absence of an identifiable mass and with clinical symptoms. While little data is available to support these criteria, they are becoming more widely used. Least used is the older criteria of calculating β cells as a percentage of pancreatic mass or measuring islet diameters.

It is important to understand that prior to the adoption of the diffuse and focal types of nesidioblastosis, most descriptions (all of the minor criteria) were applied mostly to the focal type. The diffuse type is usually comparatively bland, with regularly shaped islets that have subtle anisonucleosis, subtle islet cell hypertrophy, and subtle islet hyperplasia. The major criterion of low proliferative activity for the diffuse type does not apply to the focal type; the focal type typically has a higher proliferative rate. There is no established cutoff for the Ki-67 index for either type.

Treatment

Treatment of nesidioblastosis can vary based on genetic subtype. Nesidioblastosis may be removed if focal, through either enucleation or distal pancreatectomy. Nesidioblastosis, diffuse type with *ABCC8* or *KCNJ11* mutations, can be treated with octreotide and diazoxide medical therapy, although total or subtotal pancreatectomy remains an option in refractory cases.

Abbreviations

ASVS	Arterial calcium stimulation with venous sampling
CgA	Chromogranin A
CH	Congenital hyperinsulinism
CT	Computed tomography

DOTA	1,4,7,10-tetraacetic acid
EUS	Endoscopic ultrasound
FISH	Fluorescence in situ hybridization
FSG	Fasting serum gastrinoma
GLP-1	Glucagon-like protein 1
MDCT	Multiple detector computed tomography
MEN 1	Multiple neuroendocrine neoplasia, type 1
NF	Neurofibromatosis
NME	Necrolytic migratory erythema
PanNET	Pancreatic neuroendocrine tumor
PET	Positron emission tomography
PHH	Persistent hyperinsulinemic hypoglycemia
PHH3	Phosphohistone 3
PP	Pancreatic polypeptide
PPoma	Pancreatic polypeptide-secreting tumor
SASI	Selective arterial secretagogue injection
SLI	Somatostatin-like immunoreactivity
TSC	Tuberous sclerosis complex
VHL	Von Hippel-Lindau
VIP	Vasoactive intestinal peptide
WHO	World Health Organization
ZES	Zollinger-Ellison syndrome

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