A Practical Approach to the Classification of WHO Grade 3 (G3) Well-differentiated Neuroendocrine Tumor (WD-NET) and Poorly Differentiated Neuroendocrine Carcinoma (PD-NEC) of the Pancreas

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Abstract: High-grade neuroendocrine neoplasms (World Health Organization [WHO] G3) of the pancreas include both well-differentiated neuroendocrine tumor (WD-NET) and poorly differentiated neuroendocrine carcinoma (PD-NEC). According to the WHO classification scheme, the diagnosis of this group of tumors is based on both the histopathology of the tumor and the assessment of proliferation fraction. However, the former can be challenging due to the lack of well-defined histologic criteria, and the latter alone (ie, >20 mitoses/10 high-power fields or Ki67 > 20%) may not sufficiently distinguish WD-NETs from PD-NECs. Given the considerable differences in treatment strategies and clinical outcome, additional practical modalities are required to facilitate the accurate diagnosis of high-grade pancreatic neuroendocrine neoplasms. We examined 33 cases of WHO G3 neuroendocrine neoplasms of the pancreas and attempted to classify them into WD-NET, small cell PD-NEC (PD-NEC-SCC), and large cell PD-NEC (PD-NEC-LCC) or to designate them as "ambiguous" when an uncertain diagnosis was rendered by any of the observers or there was any disagreement in classification among the 3 observers. To simplify the interpretation, both PD-NEC-SCC and PD-NEC-LCC were considered together as PD-NECs in the final analysis. The initial approach was to assess microscopically a single morphologically challenging hematoxylin and eosin section from each case without the knowledge of Ki67 values, performed independently by 3 pathologists to assess the degree of diagnostic concordance, and then evaluate immunohistochemical staining for surrogate biomarkers of known genotypes of WD-NET and PD-NEC, respectively, and, lastly, complete a clinicopathologic review to establish a final definitive classification. Loss of DAXX or ATRX protein expression defined WD-NET, and abnormal p53, Rb, SMAD4 expression signified PD-NEC. When the chosen section displayed an element of WD histopathology, or other tumor sections contained WHO G1/G2 components, or there had been a prior established diagnosis of a primary WD-NET, the final diagnosis was rendered as a WD-NET with high-grade (G3) progression. If a component of conventional adenocarcinoma was present (in slides not seen in the initial review), the diagnosis was established as a combined adenocarcinoma and PD-NEC. All 3 pathologists agreed on the morphologic classification of 33% of the cases (6 WD-NET, 3 PD-NEC-SCC, and 2 PD-NEC-LCC), were conflicted on 2 cases between PD-NEC-SCC and PD-NEC-LCC, and disagreed or were uncertain on the classification for the remaining 20 cases (61%), which were therefore categorized as ambiguous. In the group of cases in which all pathologists agreed on the classification, the 6 WD-NET cases had either loss of DAXX or ATRX or had evidence of a WD-NET based on additional or prior pathology slides. The 7 PD-NEC cases had abnormal expression of p53, Rb, and/or SMAD4 or a coexisting adenocarcinoma. In the ambiguous group (n = 20), 14 cases were established as WD-NETs, based upon loss of DAXX or ATRX in 7 cases and additional pathology evidence of high-grade progression from WD-NET in the other 7 cases; 5 cases were established as PD-NEC based upon abnormal expression of p53, Rb, and/or SMAD4; 1 case remained undetermined with normal expression of all markers and no evidence of entity-defining histologic findings in other slides. On the basis of the final pathologic classifications, the disease-specific survival was 75 and 11 months for the WD-NET and PD-NEC groups, respectively. Thus, we conclude that morphologic diagnosis of high-grade pancreatic neuroendocrine neoplasms is challenging, especially when limited pathologic materials are available, and necessitates better defined criteria. The analysis of both additional sections and prior material, along with an immunohistochemical evaluation, can facilitate accurate diagnosis in the majority of cases and guide the appropriate clinical management and prognosis.

Key Words: pancreas, neuroendocrine tumor, neuroendocrine carcinoma, WHO G3, high grade

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Recent investigations have indicated that there exist uncommon pancreatic well-differentiated neuroendocrine tumors (NETs) that can exhibit characteristic morphologic features of a low-grade or intermediate-grade neoplasm but a proliferative rate that breaches the threshold for the WHO classification of a high-grade (G3)

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neuroendocrine neoplasm.[1] Some cases may be morphologically homogenous and appear well differentiated throughout, with the high-grade nature only revealed by assessment of the mitotic rate or, more commonly, the Ki67 index. Other cases have components of a low-grade or intermediate-grade NET, with a low proliferative rate, either admixed with the high-grade neuroendocrine neoplasm or in a different focus or prior sample from the patient; such cases have been interpreted as high-grade progression of a WD-NET. In both of these scenarios of WD-NETs with a G3 proliferative rate, the tumors do not possess the clinical, pathologic, and genotypical features of a true PD-NEC.[2–6] Mutations in *TP53*, *RB1*, and *SMAD4*, found in PD-NECs, are absent, and loss of *DAXX* or *ATRX* can occur, as in other WD-NETs of the pancreas. Thus, these neoplasms are increasingly being classified as high-grade (G3) WD-NETs, rather than PD-NECs. Although this phenomenon is generally rare in WD-NETs, the prevalence is higher in pancreatic primaries.[3] In the absence of pertinent clinical information (such as symptoms at the initial presentation, results of radiographic assessment, and blood biomarkers) and without evidence of a lower-grade counterpart (WHO G1/G2), the distinction between a high-grade WD-NET and PD-NEC may be challenging, particularly in a common scenario of suboptimal biopsy material or limited tumor sections. The difficulty is enhanced when the morphologic features are not those of classic small cell carcinoma, as pancreatic WD-NETs can particularly resemble large cell PD-NECs. In addition to applying classic but rather inconsistent morphologic criteria, some pathologists may use a combination of their intuition from personal experience and available clinical information to distinguish WD-NET from PD-NEC; others simply use a rigid mitotic count or Ki67 index cutoff point to assign the classification. Given the significant difference in treatment strategies and outcome for WD-NET and PD-NEC, better defined morphologic criteria, ancillary studies, and clinical information are crucial to facilitate the accurate interpretation of these 2 distinct neoplasms.[7,8] The present study was conducted to determine the utility of a selected panel of immunohistochemical (IHC) stains to improve the classification of G3 pancreatic neuroendocrine neoplasms.

MATERIALS AND METHODS

Patient Information

Pancreatic neuroendocrine neoplasms with increased proliferative activity (WHO G3 category, mitosis >20/10 high-power field [HPF], or Ki67 >20%) were identified retrospectively and prospectively using the pathology files at the authors' institution, with IRB approval. These included primary surgical resection specimens, core biopsies, and resections of recurrent or metastatic tumors. Of the 33 cases selected for the study, all patients, except 1, were evaluated clinically at our institution with appropriate radiologic and laboratory studies and surgical or oncologic management. Follow-up information was available for all cases, except 1.

Pathologic Assessment

A single representative hematoxylin and eosin slide was selected from each case to represent the high-grade region of the tumor (in cases in which other material may have displayed lower-grade components). Initially, 3 pathologists specialized in gastrointestinal and hepatopancreatobiliary pathology independently assessed the selected sections from each case. The cases were blinded to the reviewers (L.H.T., O.B., and D.S.K.) by a fourth individual with regard to the patient's identification, the histopathology of additional tumor sections and prior diagnoses, any clinical information, and results of any ancillary studies, including the Ki67 index. Initially, the cases were classified into the following categories: WD-NET, small cell PD-NEC (PD-NEC-SCC), large cell PD-NEC (PD-NEC-LCC), and uncertain, when the subtype could not be definitively assigned on the morphologic findings alone. For purposes of further analysis the PD-NEC-SCC and PD-NEC-LCC groups were considered together as PD-NECs. A consensus diagnosis was achieved when all 3 reviewers agreed. In cases with disagreement among reviewers as to WD-NET versus PD-NEC, or when any individual reviewer considered a diagnosis to be uncertain, the consensus diagnosis was regarded as ambiguous. The secondary evaluation included incorporating the analysis of IHC with surrogate biomarkers of known genotypes for WD-NET and PD-NEC, respectively, and a final complete clinicopathologic review of the cases, including assessment of other slides and prior specimens, for a definitive final classification. The contribution of each type of data to the establishment of the final classification was assessed.

Immunohistochemistry

Standard ABC peroxidase techniques were used for IHC performed on 4-μm-thick sections of formalin-fixed and paraffin-embedded tissue. Antigen retrieval in heated citrate buffer at pH 6.0 was applied for all antibodies. The Ki67 monoclonal antibody (1:100), Rb monoclonal antibody (1:400), p53 monoclonal antibody (1:500), chromogranin-A polyclonal antibody (1:8000), and synaptophysin (1:500) were obtained from Dako (Carpintaria, CA). The SMAD4 monoclonal antibody (1:800) was acquired from Santa Cruz Bio (Santa Cruz, CA). The ATRX polyclonal antibody (1:500) and DAXX (1:100) polyclonal antibody were obtained from Sigma-Aldrich Corporation (St Louis, MO). IHC was performed on BenchMark XT automated equipment (Ventana Medical System Inc., Tucson, AZ). Positive control tissue was stained in parallel with each study case. The Ki67 immunoreactivity was expressed as the percentage of tumor cells with nuclear staining, which was based upon digital counting of >2000 tumor cells in regions with the highest labeling recognizable on scanning magnification. p53 immunoreactivity with strong staining intensity in

>25% tumor cells was regarded as abnormal (positive), and complete loss of SMAD4, DAXX, ATRX, and Rb protein expression (negative), in the presence of positive staining in non-neoplastic cells, was regarded as abnormal.

RESULTS

Patient Information

Of 33 cases chosen for this study, the mean age ± SD was 57 ± 16 years (ranging from 13 to 81 y), with a male to female ratio of 17:16 and a median follow-up of 18.6 ± 35 months (ranging from 1 to 120 mo). Primary pancreatic tumors constituted 20/33 cases, and metastases made up 13/33 cases. Every case had a proliferative index (Ki67 index) >20% with a mean of 60% ± 20% (26% to 93%). The mitotic rate was assessed in all cases, and 15/33 (45%) had a mitotic rate in the G3 range (>20/10 HPF), 17/33 (52%) were in the G2 range (2 to 20/10 HPF), and 1 case was in the G1 range (< 2/10 HPF).

Morphologic Assessment of High-grade Pancreatic Neuroendocrine Neoplasm

Of the 33 slides reviewed independently by 3 pathologists, 8 were core biopsy specimens and 25 were surgical resections or excisional biopsies. The results of the morphologic assessment are shown in Table 1.

Approximately one third (11/33) of the cases, which were all surgical resections, achieved diagnostic consensus by all 3 reviewers, and 61% of the cases were regarded to be ambiguous, because an uncertain diagnosis was rendered by any of the observers or there was disagreement between WD-NET and PD-NEC among the 3 observers. Every biopsy specimen (n = 8) in this cohort failed to achieve consensus among the reviewers. All of the 6 WD-NET cases that achieved consensus revealed some classic histopathologic and cytologic features of WD-NET, which included an organoid, trabecular architecture, a regular intratumoral vascular pattern, abundant granular cytoplasm, and stippled nuclei with inconspicuous nucleoli (Figs. 1A, B). Three cases of PD-NEC that reached consensus demonstrated features of small cell carcinoma, such as geographic tumor necrosis, spindled or fusiform cell morphology, minimal cytoplasm, finely granular, hyperchromatic nuclei with inconspicuous nucleoli, and nuclear molding (Figs. 1C, D). The morphologic features of PD-NEC-LCC appeared to be the least specific and reproducible, as they overlapped with both WD-NET and PD-NEC-SCC. In fact, 1 of the 3 cases classified as PD-NEC-LCC by all 3 reviewers, was reclassified as WD-NET in the final assessment (see below, Table 2). The other 2 PD-NEC-LCC cases had a large expansile growth pattern with subtle peripheral nuclear palisading, rosette/tubule-forming structures within the large nests, irregular large vessels, and tumor necrosis (Figs. 1E, F). The ambiguous cases either shared overlapping morphologic features with WD-NET, PD-NEC-SCC, and PD-NEC-LCC subtypes or were present in suboptimal small biopsies with varying degrees of histologic processing artifact (Figs. 2A–F).

Subclassification of PD-NECs into PD-NEC-SCC and PD-NEC-LCC also revealed poor interobserver concordance, and the 3 observers did not agree or determine the subclassification on 8/13 (62%) cases (Table 1).

Secondary Evidence for the Classification of Pancreatic Neuroendocrine Neoplasms

Whereas all cases had a Ki67 index of >20% in this cohort, 35% (7/20) of the confirmed WD-NET case had Ki67 > 55%, and 33% (4/12) of the confirmed PD-NEC case had Ki67 < 55% (Table 2). Thus, both the morphology and the Ki67 could not accurately distinguish these 2 pathologic entities. After the initial morphologic assessment, IHC was performed using surrogate biomarkers of known genotypes for WD-NET (ie, DAXX and ATRX loss)[9] and PD-NEC (ie, p53 overexpression; Rb or SMAD4 loss),[3,10] respectively. Loss of DAXX or ATRX protein expression was mutually exclusive and occurred in WD-NETs in 10/33 cases. Abnormal p53, Rb, or SMAD4 expression characterized PD-NECs and was found in 11/33 cases (Table 2 and Fig. 3). In no cases were there concurrent abnormalities in DAXX/ATRX along with p53, Rb, or SMAD4. Thus, IHC confirmed 3/6 of WD-NET and 6/7 of PD-NEC cases that had reached consensus; and 60% (12/20) cases with no consensus (including 1 with incorrect classification) were defined as WD-NET or PD-NEC based upon the results of IHC. Nonetheless, 8/20 of the ambiguous cases remained unclassified after IHC analysis.

Additional pathologic and clinical information was further acquired to facilitate the classification of this group of high-grade neuroendocrine neoplasms. When a case either contained WHO G1/G2 areas in other tumor sections within the same neoplasm (8/19) or had a prior pathologic diagnosis of a G1/G2 WD-NET (11/19), the final diagnosis of the high-grade neoplasm in the study cohort was rendered as WD-NET, reflecting high-grade progression from G1/G2 to G3; 19 cases fulfilled this criterion (Table 2), including 13 in which the morphologic diagnosis was ambiguous (uncertain diagnosis rendered by any of the observers or disagreement among the 3 observers) or wrong and 10 cases in which IHC failed to demonstrate abnormalities in the markers examined.

Therefore, the combined immunoprofile and clinicopathologic assessment confirmed 20 WD-NETs in the cohort of 33 high-grade cases; 50% (10/20) of the WD-NET cases had loss of DAXX or ATRX expression, and 95% (19/20) had evidence of a concurrent or prior G1/G2 WD-NET (high-grade progression). Twelve of the 33 cases were confirmed as PD-NEC, of which the majority (11/12) had abnormal Rb, p53, or SMAD4 expression, and 4/12 had a component of ductal carcinoma present on other sections of the tumor (Table 2). The distinction between WD-NET and PD-NEC could not be established for 1 case in this cohort: the morphologic assessment of the case was categorized as ambiguous, the clinical information and the prior pathology were not available

TABLE 1. Morphologic Assessment of High-grade Pancreatic Neuroendocrine Neoplasms

Consensus	Reviewer 1	Reviewer 2	Reviewer 3	Specimen Type
WD-NET	WD-NET	WD-NET	WD-NET	Resection
WD-NET	WD-NET	WD-NET	WD-NET	Resection
WD-NET	WD-NET	WD-NET	WD-NET	Resection
WD-NET	WD-NET	WD-NET	WD-NET	Resection
WD-NET	WD-NET	WD-NET	WD-NET	Resection
WD-NET	WD-NET	WD-NET	WD-NET	Resection
Ambiguous	WD-NET	Ambiguous	WD-NET	Biopsy
Ambiguous	WD-NET	WD-NET	Ambiguous	Resection
Ambiguous	Ambiguous	WD-NET	WD-NET	Biopsy
Ambiguous	WD-NET	WD-NET	Ambiguous	Resection
Ambiguous	WD-NET	WD-NET	Ambiguous	Resection
Ambiguous	WD-NET	WD-NET	Ambiguous	Resection
Ambiguous	WD-NET	WD-NET	Ambiguous	Biopsy
Ambiguous	WD-NET	WD-NET	PD-NET-LCC	Resection
Ambiguous	WD-NET	WD-NET	PD-NET-LCC	Biopsy
Ambiguous	Ambiguous	Ambiguous	Ambiguous	Biopsy
Ambiguous	Ambiguous	Ambiguous	PD-NEC-SCC	Resection
Ambiguous	PD-NEC-SCC	Ambiguous	PD-NEC-SCC	Resection
PD-NEC-LCC	PD-NEC-LCC	PD-NEC-LCC	PD-NEC-LCC	Resection
Ambiguous	Ambiguous	Ambiguous	Ambiguous	Biopsy
PD-NEC-LCC	PD-NEC-LCC	PD-NEC-LCC	PD-NEC-LCC	Resection
PD-NEC-LCC	PD-NEC-LCC	PD-NEC-LCC	PD-NEC-LCC	Resection
PD-NEC-SCC	PD-NEC-SCC	PD-NEC-SCC	PD-NEC-SCC	Resection
PD-NEC-SCC	PD-NEC-SCC	PD-NEC-SCC	PD-NEC-SCC	Resection
PD-NEC-SCC	PD-NEC-SCC	PD-NEC-SCC	PD-NEC-SCC	Resection
PD-NEC	PD-NEC-LCC	PD-NEC-SCC	PD-NEC-LCC	Resection
PD-NEC	PD-NEC-SCC	PD-NEC-SCC	PD-NEC-LCC	Resection
Ambiguous	WD-NET	PD-NEC-LCC	PD-NEC-LCC	Resection
Ambiguous	PD-NEC-LCC	PD-NEC-LCC	Ambiguous	Resection
Ambiguous	Ambiguous	Ambiguous	PD-NEC-SCC	Resection
Ambiguous	Ambiguous	PD-NEC-SCC	Ambiguous	Biopsy
Ambiguous	Ambiguous	PD-NEC-LCC	Ambiguous	Biopsy
Ambiguous	Ambiguous	PD-NEC-LCC	PD-NEC-LCC	Resection

because the patient was not seen at our institution, and the selected biomarkers did not demonstrate an abnormal immunoprofile.

Using the final IHC and clinicopathologic classification (and omitting the single without a definitive diagnosis), the mean Ki67 index for the WD-NETs (46% ± 14%, ranging 30% to 80%) was significantly lower than that for the PD-NECs (72% ± 20%, ranging 26% to 93%) ($P = 0.012$). However, there was significant overlap, and 7/20 WD-NETs had a Ki67 > 55%, whereas 8/12 of PD-NECs were < 55%.

In the group confirmed as WD-NETs (n = 20) after every level of assessment, 13 were categorized as ambiguous and 1 as PD-NEC-LCC in the initial morphologic assessment (Table 2); we confirmed the correct classification by the loss of DAXX/ATRX expression (Fig. 3D) in 7/14 cases and with the clinical and pathologic evidence of WD-NET with high-grade progression in the remaining 7 cases. Similarly, in the group of confirmed PD-NECs, all 5 cases initially rendered as ambiguous had abnormal p53, Rb, or SMAD4 protein expression (Figs. 3A–C and Table 2).

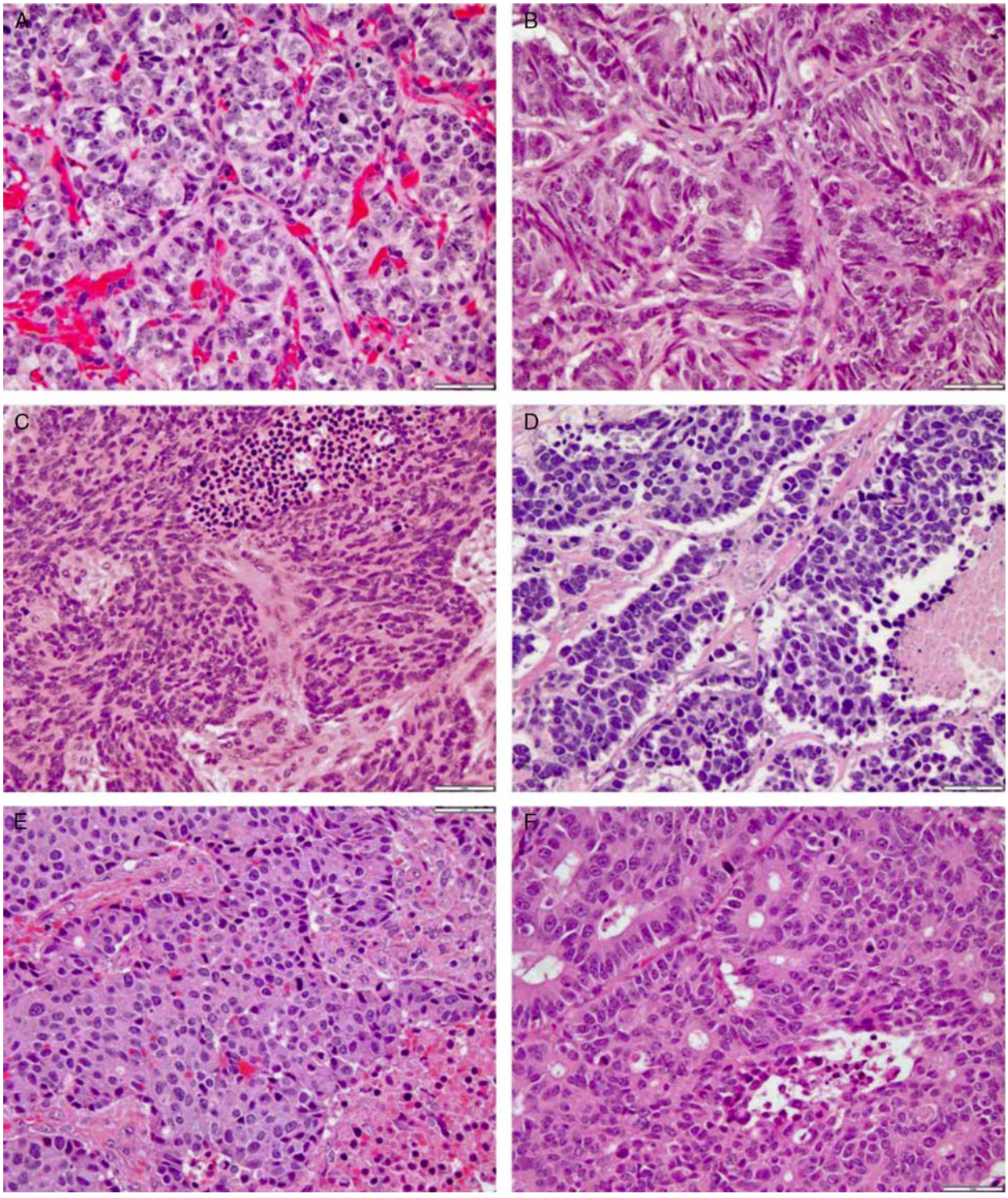

FIGURE 1. Typical morphologic features of pancreatic WD-NET, PD-NEC-SCC, and PD-NEC-LCC. WD-NETs (A and B) revealed nested/organoid and trabecular architecture, a regular intratumoral vascular pattern, abundant granular cytoplasm, and stippled nuclei with inconspicuous nucleoli. PD-NEC-SCC (C and D) demonstrated stromal desmoplasia (C), tumor necrosis (C), fusiform (oat cell) nuclei lacking nucleoli, and nuclear molding. PD-NEC-LCC (E and F) displayed tumor necrosis, expansile and irregular nests with peripheral palisading, and rosettes/tubular structures within the large nests.

TABLE 2. Classification of High-grade Pancreatic Neuroendocrine Neoplasms by Secondary Evidence

Initial Consensus	IHC Abnormalities	Ki67%	Other Histologic Components	Confirmed Classification
WD-NET		50	G1/G2 WD-NET	WD-NET
WD-NET	DAXX	70	G1/G2 WD-NET	WD-NET
WD-NET	ATRX	50	G1/G2 WD-NET	WD-NET
WD-NET		40	G1/G2 WD-NET	WD-NET
WD-NET	DAXX	35	G1/G2 WD-NET	WD-NET
WD-NET		32	G1/G2 WD-NET	WD-NET
Ambiguous		35	G1/G2 WD-NET	WD-NET
Ambiguous		65	G1/G2 WD-NET	WD-NET
Ambiguous	DAXX	50	G1/G2 WD-NET	WD-NET
Ambiguous	ATRX	35	G1/G2 WD-NET	WD-NET
Ambiguous	DAXX	30	G1/G2 WD-NET	WD-NET
Ambiguous		60	G1/G2 WD-NET	WD-NET
Ambiguous	ATRX	40		WD-NET
Ambiguous	DAXX	80	G1/G2 WD-NET	WD-NET
Ambiguous	DAXX	49	G1/G2 WD-NET	WD-NET
Ambiguous		38	G1/G2 WD-NET	WD-NET
Ambiguous		60	G1/G2 WD-NET	WD-NET
Ambiguous		50	G1/G2 WD-NET	WD-NET
Ambiguous		70	G1/G2 WD-NET	WD-NET
Ambiguous	p53/Rb	88		PD-NEC
Ambiguous	p53/SMAD4	38	Ductal adenocarcinoma	PD-NEC
Ambiguous	p53/Rb	70		PD-NEC
Ambiguous	p53/Rb	85		PD-NEC
Ambiguous	p53	60		PD-NEC
Ambiguous		70		Undetermined
PD-NEC-LCC	DAXX	66	G1/G2 WD-NET	WD-NET
PD-NEC-LCC	Rb	44		PD-NEC
PD-NEC-LCC		26	Ductal adenocarcinoma	PD-NEC
PD-NEC-SCC	p53	80	Ductal adenocarcinoma	PD-NEC
PD-NEC-SCC	Rb	90		PD-NEC
PD-NEC-SCC	p53/Rb	94	Ductal adenocarcinoma	PD-NEC
PD-NEC	Rb	84		PD-NEC
PD-NEC	p53	88		PD-NEC

The median disease-specific survival for the entire cohort based upon the final classification of WD-NET (n = 20) and PD-NEC (n = 12) was 75 and 11 months, respectively ($P < 0.0001$) (Fig. 4A). Similarly, the median disease-specific survival for the morphologically ambiguous cases upon the final classification of WD-NET (n = 14) and PD-NEC (n = 5) was 120 and 11 months, respectively ($P < 0.001$) (Fig. 4B).

DISCUSSION

In this study, we simulated a practical scenario and challenged our morphologic intuition for diagnosing difficult cases of high-grade WD-NET and PD-NEC. As demonstrated by the results, pathologists with extensive experience in neuroendocrine neoplasms could not reach consensus on approximately two thirds of these highly selected cases in the absence of an ancillary workup and the pertinent clinicopathologic information on the patients, particularly those with suboptimal or limited tumor tissue in biopsies.

Certain morphologic features, although not entirely specific, might have helped to distinguish between WD-NETs and PD-NECs. A geographic pattern of necrosis, although more commonly seen in PD-NECs, can be present in WD-NETs with high-grade transformation.[3] Despite having high-grade transformation, WD-NETs may retain certain organoid histologic patterns, such as nested, trabecular, or loosely cohesive architecture with a relatively organized vascular network and a hyalinized type of intratumoral fibrosis (Figs. 1A, B, 2A, B, E). In contrast, PD-NECs have expansile large and irregular nests, an infiltrative growth pattern with randomly oriented large vascular structures, and desmoplastic-type fibrosis (Figs. 1C–F). Cytologically, WD-NETs, particularly pancreatic primaries, usually have abundant granular cytoplasm, which results in a low nuclear to cytoplasmic ratio, and stippled chromatin (Figs. 1A, B, 2B, E); conversely, PD-NECs have less granular cytoplasm and a higher nuclear to cytoplasmic ratio with either open chromatin with conspicuous nucleoli (large cell NEC) or hyperchromatic and molded nuclei lacking nucleoli (small cell carcinoma) (Figs. 1C–F, 2C, D). Nevertheless, the classic description of small and large cell neuroendocrine carcinoma in the pulmonary system[11,12] does not perfectly translate to the gastrointestinal tract and the pancreatobiliary system.[12,13] Thus, there are overlapping features between both WD-NET and PD-NEC and between PD-NEC-SCC and PD-NEC-LCC; in fact, the overlap between PD-NEC-SCC and PD-NEC-LCC was such that these 2 entities were combined as simply "PD-NEC" for purposes of the analysis.

The marked differences in proliferative rate between WD-NETs and PD-NECs suggest that this feature may be sufficient for their distinction. Rereview of the cases for

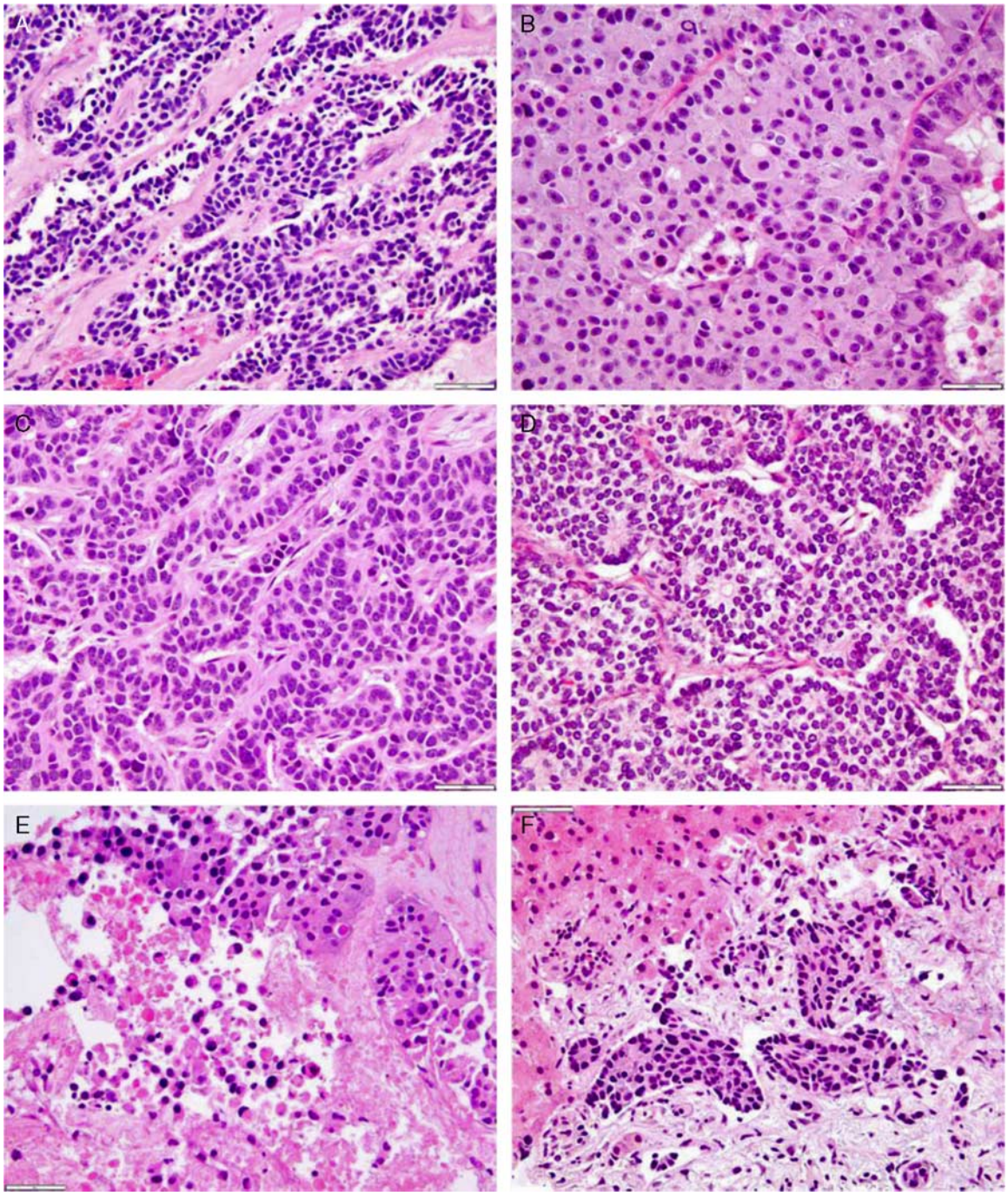

FIGURE 2. Morphologically ambiguous pancreatic neuroendocrine neoplasms. Two cases of WD-NET (A and B) were initially considered as morphologically ambiguous due to an infiltrative growth pattern with irregular architecture, significant intratumoral fibrosis, single cell (A) and punctate (B) tumor necrosis, and brisk mitotic activity. However, upon retrospective review, the tumors appeared to retain some morphologic features of WD-NETs such as a hyalinized type of fibrosis (A), delicate vascular pattern (B), and abundant granular cytoplasm and low nuclear to cytoplasm (N/C) ratio (B). The 2 cases of PD-NEC (C and D) shared some morphologic features of WD-NET such as the vascular patterns and nested or trabecular architecture, although the cytologic features such as large nuclei, high N/C ratio, and minimal cytoplasm might have suggested PD-NEC. The distinction between WD-NET (E) and PD-NEC (F) was especially difficult in small biopsies wherein the architecture of the tumor could not be fully appreciated. Although the cytologic features of the tumor (abundant cytoplasm and low N/C ratio) were suggestive of a WD-NET (E), the presence of extensive tumor necrosis rendered the tumor as ambiguous. Similarly, the small nested structures in a PD-NEC (F) without the context of the global architecture of the tumor could culminate in an incorrect classification of this neuroendocrine neoplasm.

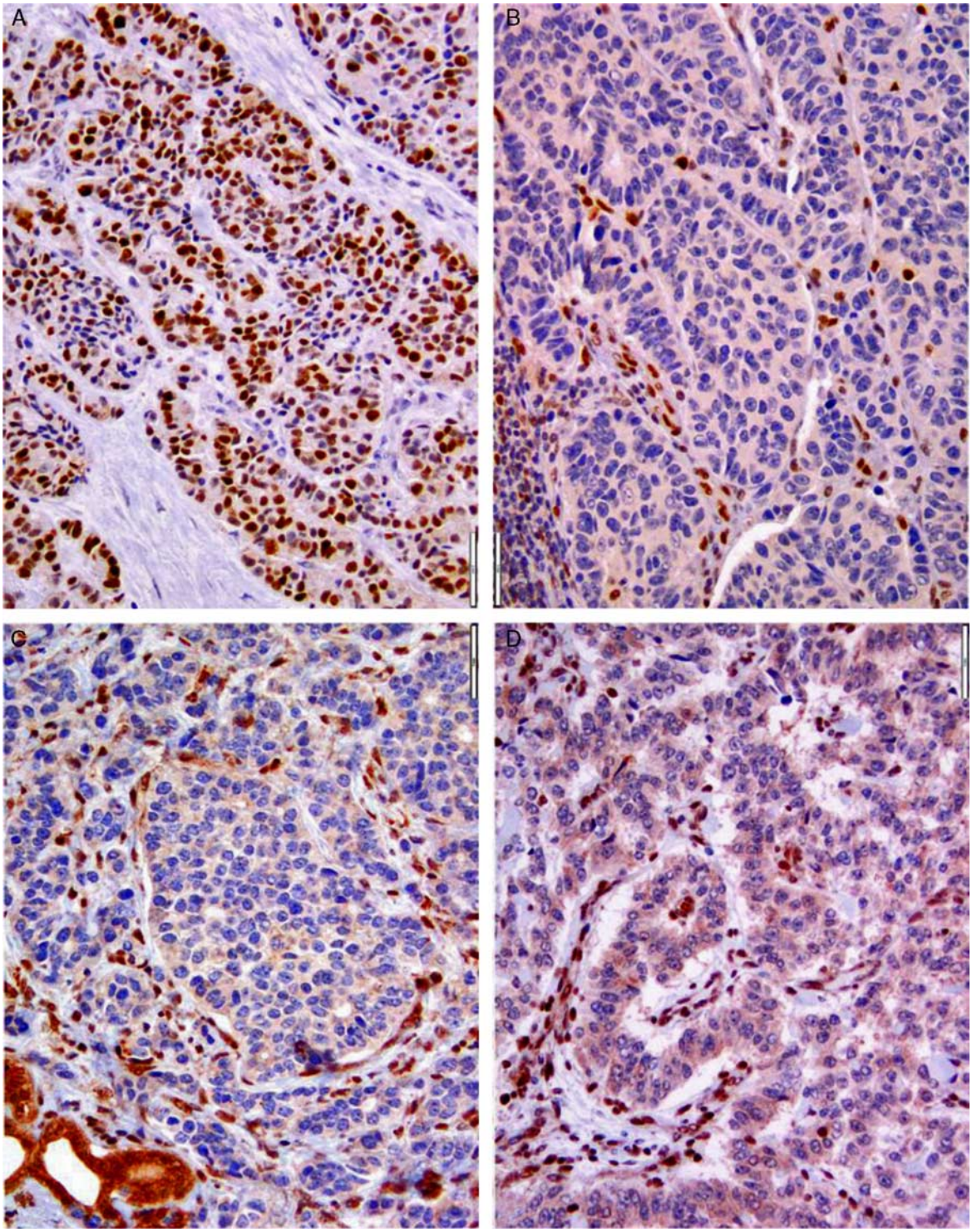

FIGURE 3. Abnormal p53, Rb, SMAD4, and DAXX expression by IHC in high-grade pancreatic WD-NET and PD-NEC. The expression of abnormal p53 served as a surrogate biomarker of *TP53* gene mutation, which was observed in 67% of PD-NECs (A). Similarly, loss of Rb (B) and SMAD4 (C) protein expression were associated with PD-NEC. In contrast, loss of DAXX (D) or ATRX (data not shown) expression was seen in 40% to 50% WD-NET but not in PD-NEC.

which we did reach agreement showed that mitotic activity appears to have influenced the classification of G3 WD-NETs (mean mitoses 11.7 ± 10/10 HPF) and PD-NETs (mean mitoses 47/10 ± 19 HPF); however, this does not seem to be the case in the ambiguous group, which had mean mitoses of 13.6 ± 9/10 HPF and 33 ± 2/

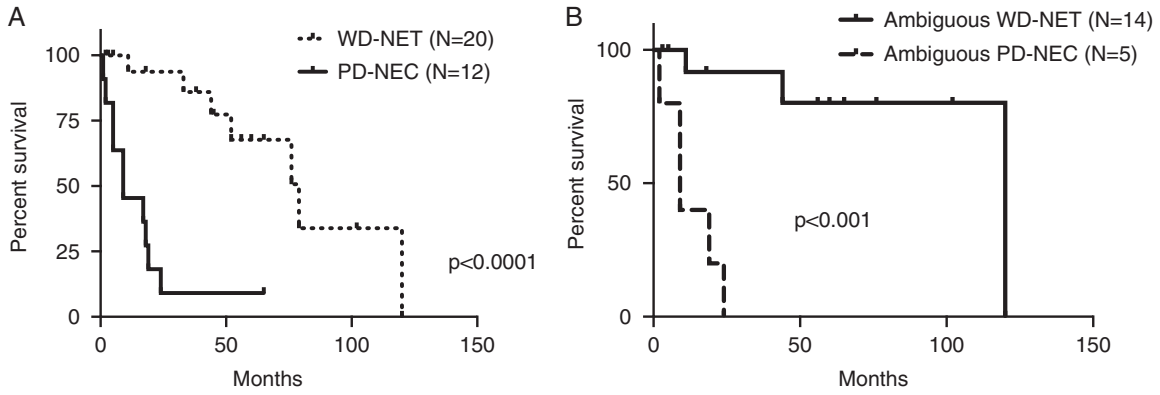

FIGURE 4. Disease-specific survival of high-grade pancreatic neuroendocrine neoplasm. Disease-specific survival of the entire cohort (A) and disease-specific survival of morphologically ambiguous cases (B).

10 HPF in the cases ultimately classified as WD-NET and PD-NEC, respectively. Thus additional morphologic characteristics might have played a role to place them in the ambiguous category. In previous investigations, a cutoff Ki67 index of 55% has been suggested to separate G3 WD-NETs from PD-NECs.[14] Whereas the Ki67 indices were unknown to the reviewers at the initial morphologic assessment, 7/20 (32%) WD-NETs and 8/12 (67%) PD-NECs (on the basis of the final classification) had a Ki67 index >55%. Thus, it is apparent that, although PD-NECs generally have a higher average proliferative index (72% ± 20%, ranging 26% to 93%) than WD-NETs (46% ± 14%, ranging 30% to 80%), there is no absolute cutoff value that can sufficiently distinguish these 2 neoplasms.[15] On the basis of the inadequacy of pure morphologic or proliferation rate criteria to distinguish G3 WD-NET and PD-NEC, it is clear that assessment of additional biomarkers and clinical features is necessary to improve histopathologic diagnosis.

Genomic investigations have discovered recurrent and mutually exclusive *DAXX* and *ATRX* mutations, which culminate in loss of corresponding protein expression in tumor cells, in approximately 44% of pancreatic WD-NETs.[9] This genotype is specific for WD-NET and has not been seen in other pancreatic neoplasms, including PD-NECs.[3,10] In contrast, pancreatic PD-NECs share some of the genotypic alterations of conventional pancreatic ductal adenocarcinoma including frequent gene mutations in *TP53* and, less commonly *KRAS*, *p16*, and *SMAD4*, which have not been identified in pancreatic WD-NETs in a number of investigations.[3,10] Furthermore, *RB1* gene mutations and the associated loss of Rb protein expression are commonly observed in high-grade PD-NECs, with a frequency in the small cell subtype of >91%[16] and in the large cell subtype of 50% to 60%, regardless of the anatomic site of tumor origin.[17,18] *RB1* and *TP53* mutations have not been identified in WD-NETs.[3,10] In the current study, we have demonstrated that these genotypes and corresponding phenotypes for pancreatic WD-NET (DAXX and ATRX) and for PD-NEC (p53, SMAD4, and Rb) as assessed by IHC are indeed very useful to aid in the differential diagnosis. For the WD-NETs in this study, the DAXX/ATRX immunoprofile facilitated the correct interoperation in 50% (7/14) cases that were morphologically ambiguous. Furthermore, the p53/SMAD4/Rb immunophenotype exhibited even better efficacy (particularly p53 and Rb), and abnormal expression of at least 1 of these proteins supported the diagnosis of PD-NEC in all except 1 morphologically ambiguous case. Of note, the loss of SMAD4 expression was only present in 1 case of PD-NEC, which also had a p53 abnormality; thus inclusion of SMAD4 may not provide supplementary value for the diagnosis. Similar results were observed in the consensus cases (3/6 in WD-NETs and 5/6 in PD-NECs). However, in the absence of these mutations (~50% of DAXX/ATRX and ~10% of p53/SMAD4/Rb) the classification of a high-grade neuroendocrine neoplasm with ambiguous morphology cannot be established by IHC.

We have previously demonstrated that despite a high proliferative index and overlapping morphologic features between G3 WD-NET and PD-NEC, there are certain clinical and pathologic characteristics that can assist in distinguishing the 2 neoplasms.[3] In this study, we have further emphasized that when dealing with metastatic high-grade neuroendocrine neoplasms, the consideration of WD-NET with high-grade progression is frequently supported by a coexisting or prior lower-grade WD-NET component in the sample at hand or at another site of disease (eg, the G1/G2 WD-NET primary pancreatic tumor in the face of a high-grade liver metastasis). In fact, every biopsy specimen (n = 6), except for 1, with a metastatic high-grade NE neoplasm, initially rendered as morphologically ambiguous and ultimately confirmed as a WD-NET with high-grade progression, had a previously documented lower-grade pancreatic WD-NET in other specimens. In resection specimens, the lower-grade component may be overt and usually constitutes a significant component (> 50%) of the tumor. The sections chosen for inclusion in the current study were specifically selected to exclude any lower-grade regions known to exist elsewhere within primary WD-NETs. In small biopsies, the heterogenous tumor grades may not be well appreciated.

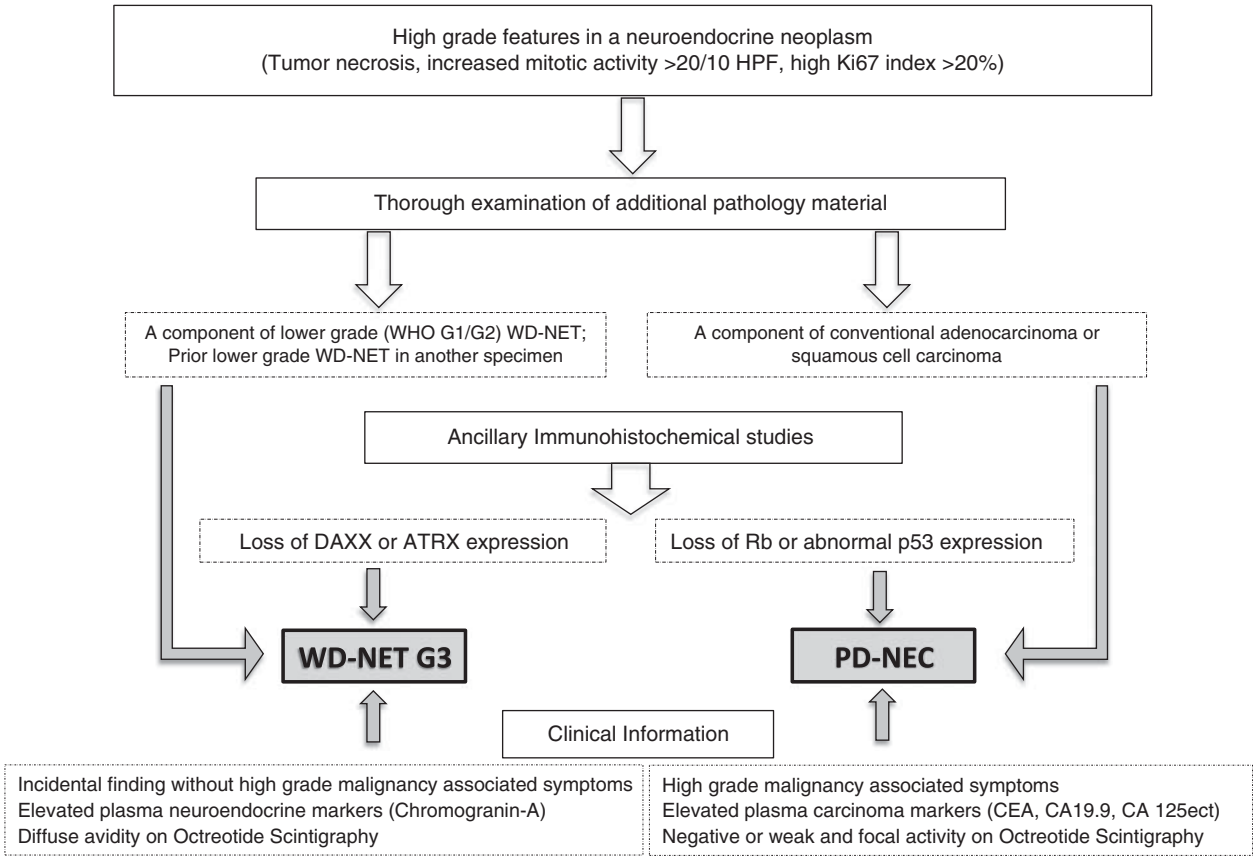

FIGURE 5. Recommended diagnostic algorithm for pancreatic high grade neuroendocrine neoplasms.

Similarly, in the presence of a coexisting conventional carcinoma (ie, squamous cell carcinoma or adenocarcinoma), a high-grade neuroendocrine neoplasm would be considered a PD-NEC, as the combination with a non-neuroendocrine carcinoma component is extraordinarily rare in WD-NETs.

In the absence of a coexisting lower-grade WD-NET or a conventional carcinoma component, additional clinical information (history of the disease, symptoms at the presentation, serum biomarkers, and radiographic assessment) can play an important role in the establishment of the correct classification of a high-grade neuroendocrine neoplasm, particularly when dealing with recurrence or metastasis. Given the relatively protracted clinical course, the primary diagnosis of a WD-NET may have taken place several years earlier (up to 10 y before recurrence).[19,20] In fact, most cases (10/11) of metastatic WD-NETs with high-grade progression in this cohort had a previous history of a lower-grade (WHO G1/G2) tumor; this facilitated the correct classification of morphologically ambiguous cases in the absence of abnormal IHC biomarker expression tested in this study. Therefore, WD-NETs can be heterogenous in grade, and they are unlikely to have an exclusively high-grade component in a resection specimen, and a lower-grade component almost inevitably can be identified in 1 of the tumor sections or in a prior specimen. In contrast, patients with PD-NECs have rapid clinical deterioration,[8] and they are unlikely to have a prior similar malignancy in the extended history. In contrast to WD-NET, PD-NECs are homogenously high grade in any type of specimen, although some tumors may reveal paradoxical reduction of Ki67 after chemotherapy.

Additional clinical data, as discussed in our previous investigation,[3] such as onset age (younger for WD-NET), initial clinical presentations (often asymptomatic in WD-NETs), in vivo biomarkers (ie, chromogranin, CEA, CA19.9 ect), and radiographic studies (ie, octreotide scintigraphy, fluorodeoxyglucose–positron emission tomography–computed tomography) are also helpful in providing supplementary information for classifying these high-grade neoplasms.

We have thus proposed a diagnostic algorithm for high-grade neuroendocrine neoplasms. This algorithm is mostly useful for pancreatic primaries for their known mutated *DAXX* and *ATRX* genotype in WD-NETs (Fig. 5). Although loss of DAXX and ATRX expression are not involved in substantial numbers of extrapancreatic WD-NETs, data exist verifying the restriction of p53 and Rb abnormalities to the PD-NEC category for nonpancreatic primaries.

In summary, due to the lack of easily recognized morphologic criteria, pathologists are challenged when trying to distinguish a high-grade (G3) WD-NET from a PD-NEC, which is critical for clinical treatment decisions.

The combined morphologic features, with knowledge of the histology of prior specimens or other sites of disease, and ancillary IHC can facilitate the accurate diagnosis in the majority of cases and can provide guidance for the appropriate clinical management and prognosis. Although at this time the distinction between PD-NEC-SCC and PD-NEC-LCC may not have a direct clinical impact, future molecular investigations may reveal differences in treatment response and novel diverse therapeutic regimens. The distinction of these 2 subtypes of PD-NEC remains challenging and somewhat subjective, and none of the markers evaluated in the current study appeared to be helpful. Further molecular and genomic investigation may provide insights on these 2 related but phenotypically diverse carcinomas.

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