Pancreatoblastoma: Cytologic and Histologic Analysis of 12 Adult Cases Reveals Helpful Criteria in Their Diagnosis and Distinction From Common Mimics

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BACKGROUND: Pancreatoblastoma (PBL) is a rare malignant pancreatic tumor seen predominantly in childhood, and its cytologic diagnosis remains challenging. **METHODS:** Twelve fine-needle-aspirations from 11 adults were analyzed. **RESULTS:** In total, 6 men and 5 women (median age, 45 years; age range, 32-60 years) had tumors measuring a median 5.6 cm (range, 2.5-12 cm) located in the pancreatic head (n = 7) or tail (n = 4), including 3 with familial adenomatous polyposis (FAP)/FAP-related syndromes and 4 with metastasis at diagnosis. The median follow-up was 39.8 months (range, 0.8-348 months), and 5 patients died of disease. The original cytology diagnoses were: PBL (n = 2), neuroendocrine neoplasm (n = 2), poorly differentiated neuroendocrine carcinoma (n = 2), well differentiated neuroendocrine tumor (n = 1), poorly differentiated carcinoma (n = 2), "positive for malignancy" (n = 1), acinar cell carcinoma (n = 1), and epithelioid neoplasm with endocrine and acinar differentiation versus PBL (n = 1). Universal cytopathologic findings included hypercellularity; 3-dimensional clusters; and single, monotonous, blast-like cells that were from 1.5 to 2.0 times the size of red blood cells with high nuclear-to-cytoplasmic ratio, fine chromatin, small, distinct nucleoli, and a resemblance to well differentiated neuroendocrine tumor and poorly differentiated neuroendocrine carcinoma. Branching pseudopapillae (n = 7) and grooved nuclei (n = 3) raised the differential diagnosis of solid-pseudopapillary neoplasm, but with more atypia. Uncommon features included pleomorphism (n = 4) and numerous mitoses (n = 1). Squamoid morules were seen on smears (n = 5) or cell blocks (n = 6) in 70% of patients and were characterized by epithelioid cells with elongated, streaming nuclei, fine chromatin, absent nucleoli, and positive nuclear β-catenin (n = 6 of 8). The median Ki-67 index was 21% (range, 2%-70%), and neuroendocrine marker expression was common (100%), but acinar markers were variable (63%). **CONCLUSIONS:** A combination of cytologic findings in PBL, including a predominant population of primitive blast-like cells, subtle squamoid morules, frequent neuroendocrine and variable acinar phenotype, should facilitate accurate cytologic diagnosis and distinction from common mimics. ***Cancer Cytopathol* 2019;127:708-719**. © *2019 American Cancer Society*.

KEY WORDS: cytology; fine-needle aspiration (FNA); pancreas; pancreatoblastoma.

INTRODUCTION

Although pancreatic ductal adenocarcinoma (PDAC) accounts for the vast majority of primary pancreatic neoplasms, other less common solid pancreatic tumors are also in the differential, particularly when tumors are large and circumscribed. Such differentials include neuroendocrine neoplasms (NENs) and pure or mixed

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acinar cell carcinomas (ACCs), tumors that characteristically are seen in adults. An additional consideration is pancreatoblastoma (PBL) an extremely rare malignant primary pancreatic epithelial neoplasm that accounts for fewer than 1% of pancreatic tumors in adults.

PBLs have historically been described under many names, including infantile pancreatic carcinoma and pancreatic carcinoma in childhood, mainly because of their predominance in children, in which they account for up to 25% of pancreatic tumors in the first decade.[1-3] Although they originally were considered pediatric tumors, one-third of PBLs occur in adults.[4-7] These tumors were first defined by Becker in 1957,[8] but the term pancreatoblastoma was not proposed until 1975 by Kissane[9] and again by Horie et al in 1977 because of the tumor's morphologic resemblance to 7-week-old fetal pancreatic tissue.[10] Since then, there have been several reports describing its clinicopathologic and molecular characteristics.[1,5,11-13] In 1995, Klimstra et al established the histomorphologic features in an analysis of 14 cases—the largest series in the English literature.

PBL is typically a large (mean size, 10.6 cm), circumscribed, and highly aggressive tumor and shows multilineage differentiation, which is frequently acinar but may also be neuroendocrine, ductal, or even mesenchymal. In addition, it can be distinguished from its acinar, neuroendocrine, and ductal counterparts by the presence of trademark squamoid morules, which may or may not be keratinized.[1,5,13] Because of its rarity and polyphenotypic nature, the diagnosis of PBL can be morphologically and immunohistochemically challenging. Although its histologic profile (including the entity-defining squamoid morules) is fairly well known, its cytologic features remain poorly characterized, with only isolated reports in the literature.[14-23] Herein, we describe the cytologic findings in 12 PBLs diagnosed in 11 adults and review their clinicopathologic characteristics, cytologic features, and histologic features. To the best of our knowledge, this represents the largest series in the cytology literature to date and the second largest in the English literature overall.

MATERIALS AND METHODS

After approval by the institutional review boards of the participating institutions (all primarily focused on adult pathology), a search of the archival pathology files was conducted, which yielded 12 fine-needle aspiration biopsies (FNABs) from 11 patients. Rapid on-site evaluation was performed on all cases that were deemed adequate at the time of evaluation. The cytology material, including smears, ThinPrep slides (Hologic Inc), and cell blocks, as well as immunostains, where available, were reviewed for the presence or absence of cytologic characteristics of PBL that were previously described in the literature, including hypercellularity, clusters, papillae, nuclear grooves, nuclear molding, pleomorphism, mitotic figures, necrosis, squamoid morules, stromal fragments, plasmacytoid tumor cells, tumor cells with high nuclear-to-cytoplasmic ratio, fine chromatin, and small size (similar to that of red blood cells [RBCs]). For the purpose of this study, these small tumor cells with high nuclear-to-cytoplasmic ratios and fine chromatin were characterized as blast-like pancreatoblastoma cells. The presence of cytoplasmic microvesicles and red granules was also noted.

In addition, 10 patients had histologic material (resection (n = 10) plus biopsy (n = 1)) which were also reviewed with confirmation of the diagnosis. The patients' clinicopathologic information was also collated.

RESULTS

Eleven patients had 12 FNABs of primary pancreatic masses (n = 7) and metastatic PBLs involving the liver (n = 4) and a mediastinal lymph node (n = 1).

Clinical Characteristics

The patients included 6 men and 5 women (male-to-female ratio, 1.2: 1) ranging in age from 32 to 60 years (median age, 45 years); 6 patients were aged <50 years at diagnosis, and 5 were aged <40 years at diagnosis. No childhood-associated tumors were identified in this study, possibly because of the adult-focused hospitals in which the contributors practice. The mean tumor size was 5.6 cm (range, 2.5-12 cm), 7 tumors (64%) were located in the pancreatic head, and 4 (36%) were located in the pancreatic body/tail. One patient (patient 7) had Gardner syndrome, and 2 (patient 5 and 6) had familial adenomatous polyposis (FAP). Both patients who had FAP also had a previous history of successfully treated cancers (patient 5 was a woman aged 50 years with colonic adenocarcinoma diagnosed 14 years earlier, and patient 6 was a woman aged 43 years with small bowel adenocarcinoma who was diagnosed 1 year earlier and was recurrent at the time of PBL FNAB). In addition, we believe that patient 5 had undiagnosed Gardner syndrome because, in addition to FAP, she also

TABLE 1. Clinicopathologic Characteristics of 11 Patients With Pancreatoblastoma

Case	Age, y	Sex	Associated Syndrome	Location	Size, cm	Radiologic Dx	Metastasis at Dx	Follow-Up, mo	Late Mets/ Recurrence	Outcome
1	33	Man		Head	2.8	Pancreatitis	NA	72.2	No	DOD
2	60	Man		Head	2.5	NA	NA	17.9	Recurrence	Alive
3	57	Man		Tail	5.0	Neoplasm	Yes	3.6	No	DOD
4	59	Man		Head	8.7	NA	Yes	85	No	DOD
5	50	Woman	Gardner	Head	7.5	NA	No	143.7	No	DOD
6	43	Woman	FAP	Tail	3.4	NA	No	13.6	No	Alive
7	34	Man	Gardner	Tail	2.5	Pseudocyst	Yes	0.8	No	DOD
8	57	Woman		Head	10.5	NET vs ACC	No	6.5	No	Alive
9	34	Woman		Head	12.0	SCA vs SCAd	No	348	Mets	Alive
10	40	Man		Head	4.0	NA	No	88	Mets	Alive
11	32	Woman		Tail	2.5	NA	No	91	Both	Alive

Abbreviations: ACC, acinar cell carcinoma; DOD, died of disease; Dx, diagnosis; FAP, familial adenomatous polyposis; Mets, metastasis; NET, well differentiated neuroendocrine tumor; SCA, serous cystadenoma; SCAd, serous cystadenocarcinoma; NA, not available.

had a history of resected maxillary osteoma, colonic tubular adenomas, and multiple benign subcutaneous tumors, known features of Gardner syndrome. Three PBLs were metastatic at diagnosis (patients 3, 4, and 7), and 4 metastasized years after initial diagnosis. Two patients developed recurrent pancreatic disease 8 years and 1.5 years after initial resection. Clinical characteristics are summarized in Table 1.

Radiologic Findings

Imaging results were available for 7 of 11 patients. On imaging, 3 tumors were partially cystic, 2 involved the tail, and 1 was in the head. The biliary tree (including extrahepatic and intrahepatic bile ducts as well as the common bile duct) was dilated in 3 patients, all of them located in the pancreatic head. The median tumor size on imaging was 4.8 cm (range, 2.0-13.5 cm). Three showed evidence of metastasis to liver and lungs. Radiologic diagnoses included benign (2 of 7 patients; 29%), neoplastic (2 of 7 patients; 29%), and malignant (3 of 7 patients; 43%) differentials. Benign differentials included 1) autoimmune versus chronic pancreatitis in a man aged 33 years and 2) a pseudocyst in a patient aged 34 years. Malignant differentials included 1) sarcoma/ACC versus neuroendocrine tumor (NET) (n = 2) and 2) serous cystadenocarcinoma versus serous cystadenoma (n = 1). Two cases were called "pancreatic neoplasm." Patients with benign differentials (pseudocyst, pancreatitis, and serous cystadenoma) were aged <35 years, which likely influenced the benign differential diagnoses. PBL was not raised as a differential in any of the patients. Radiologic diagnoses also are summarized in Table 1.

Cytologic Findings

The FNAB smears were uniformly hypercellular (100%) with numerous 3-dimensional clusters (100%) and singly dispersed cells (Fig. 1). Seven cases (58%) showed branching pseudopapillae (Fig. 1) with delicate vascular cores akin to those seen in solid-pseudopapillary neoplasm (SPN), but nuclear grooves were only seen in 3 (25%) of them. Stromal fragments were seen in 9 cases (75%).

After reviewing all cytology samples together, a single-cell population with distinctive cytologic features was identified in all samples. In all cases, these distinctive cells were mostly monotonous, round, blast-like, and small (1.5-2.0 times the size of an RBC), with high nuclear-to-cytoplasmic ratio, scant cytoplasm, and infrequent (n = 4) anisonucleosis (Figs. 1–3). This blast-like cell population could be distinguished from other primary pancreatic tumor cells that would be considered in the main differential diagnosis by virtue of having the following cytologic features: although similar to neuroendocrine cells of well differentiated NETs (WDNETs), "blast-like" cells had more cytologic atypia, higher nuclear-to-cytoplasmic ratio, and more delicate chromatin, and they lacked the coarse salt-and-pepper chromatin of WDNETs (Fig. 1). Although, in areas, some tumor cells had occasional nuclear grooves resembling SPN (25%), the cells were more atypical and had larger nucleoli than SPN cells. In addition, blast-like cells shared some cytologic similarity with ACC tumor cells (Figs. 1 and 2) but lacked the macronucleoli, pleomorphism, and brisk mitotic activity of ACC. The blast-like cells had dark-blue cytoplasm on Diff-Quik stain, and some had cytoplasmic red

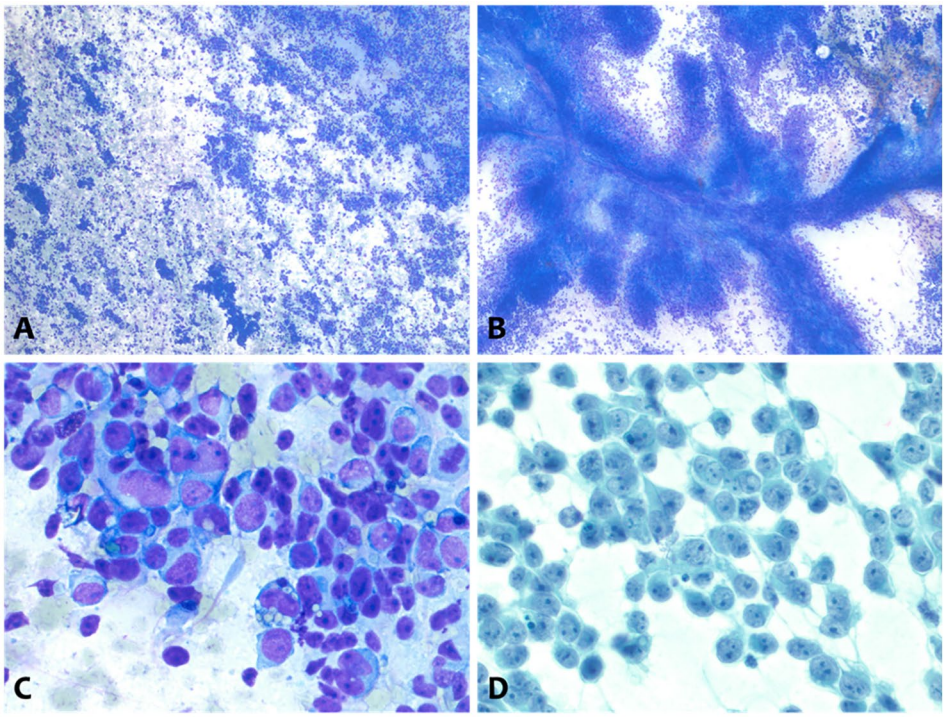

Figure 1. Cytologic features of pancreatoblastoma are shown, including (A) a hypercellular smear with 3-dimensional clusters of small, round cells (Diff-Quik stain, original magnification ×100); (B) pseudopapilla with a branching, fibrovascular core similar to that seen in solid-pseudopapillary neoplasm (Diff-Quik stain, original magnification ×100); (C,D) singly dispersed, monotonous, blast-like cells with (C) high nuclear-to-cytoplasmic ratios, round nuclei, distinct nucleoli, focal plasmacytoid features, and cytoplasmic microvesicles (Diff-Quik stain, original magnification ×400) and (D) fine, immature chromatin and prominent nucleoli (Papanicolaou stain, original magnification ×400).

granules (50%) and microvesicles (33%) (Fig. 1). On Papanicolaou staining, these cells consistently had high nuclear-to-cytoplasmic ratio; smooth nuclei; delicate, immature, blast-like, powdery chromatin; and distinct nucleoli (Figs. 1 and 2). Focal nuclear molding and crushing resembling small-cell carcinoma were seen in all cases (Figs. 1–3). Abnormal mitotic figures were occasionally seen in 5 cases but were striking (19 per 10 high-power fields) in one (case 7)—a man aged 34 years with metastatic PBL who died 8 days after diagnosis. This case also showed more pleomorphism (Fig. 3).

Squamoid morules were seen in 9 samples (75%), on both smears (n = 5) and cell blocks (n = 7) (Figs. 2–5). These were more readily identifiable on cell blocks than on smears. They were composed of whorling or streaming epithelioid cells with abundant, dense, granular cytoplasm; syncytial arrangement; low nuclear-to-cytoplasmic ratio; and elongated nuclei with blunted ends and vesicular chromatin (Figs. 2, 4, and 5). Morules were magenta-colored on Diff-Quik (Fig. 5), greenish/bluish on Papanicolaou, and pale pink on hematoxylin and eosin staining (Figs. 2, 4, and 5). Keratinization was not seen on alcoholic-fixed or cell block sections. One case had only ThinPrep slides and a cell block for review (Fig. 5A-C) and, although the blast-like cells were readily identifiable on both specimens (Fig. 5A-B), the morules were not seen on ThinPrep slides (Fig. 5A).

All cases were categorized as malignant/neoplastic on the original cytologic evaluation. The most common cytologic diagnosis was that of a "neuroendocrine" neoplasm (NEN) (n = 5; 42%), including NEN, not otherwise specified (n = 2), poorly differentiated neuroendocrine carcinoma (PDNEC) (n = 2), and WDNET (n = 1). Two were diagnosed as poorly differentiated carcinoma, 1 as "positive for malignancy" and 1 as ACC. One case (patient 6) in which the tumor coexpressed neuroendocrine and acinar immunocytochemical markers was initially diagnosed as mixed neuroendocrine and ACC versus PBL. Only 2 samples (17%; patients 7 and 8) were initially diagnosed as PBL, and squamoid morules were seen in both (on smear and/or cell block) and were highlighted by positive

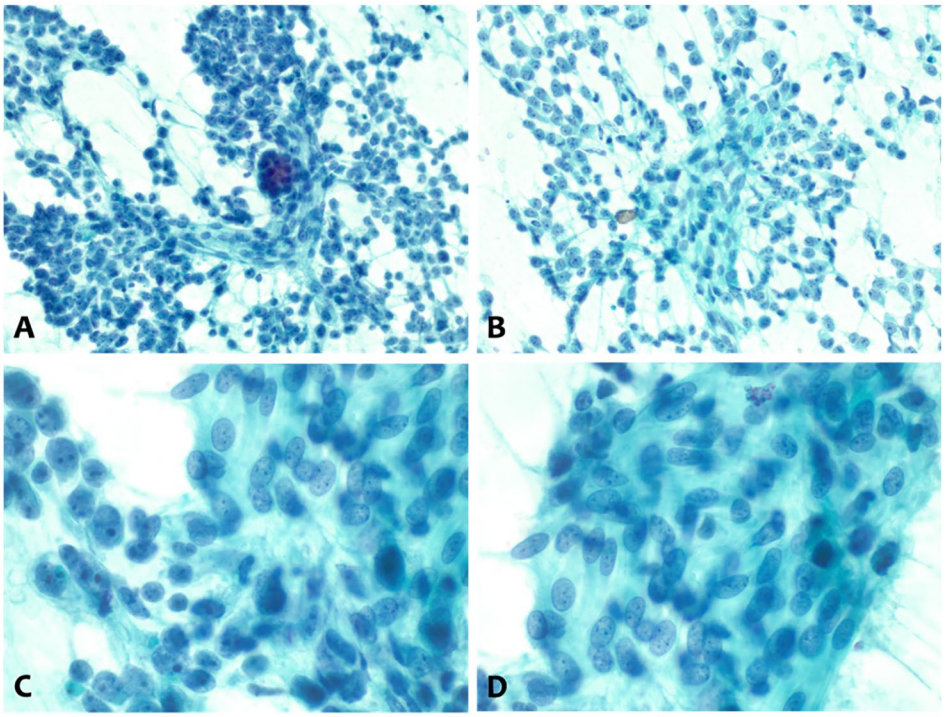

Figure 2. Cytologic features of squamous morules in pancreatoblastoma are shown. (A,B) Smears reveal a 2-cell population of smaller, peripheral, single blast-like cells focally dissected by a squamoid morule composed of larger streaming epithelioid cells with pale cytoplasm, syncytial arrangement, and bland, elongated, and blunted nuclei without nucleoli (Papanicolaou stain, original magnification ×200). (C) A 2-cell population of small blast-like cells is present on the left, and large morular cells are seen on the right (Papanicolaou stain, original magnification ×400). (D) A squamoid morule composed of streaming, large epithelioid cells with pale cytoplasm, syncytial arrangement, and bland, elongated, blunted nuclei without nucleoli is shown (Papanicolaou stain, original magnification ×400).

nuclear β-catenin immunostaining done during initial workup (Fig. 3C). Two cases (patients 8 and 10) that were initially diagnosed as neuroendocrine neoplasms at outside institutions were submitted to the authors as consults and, based on cytomorphology and supporting immunoprofiling, were reclassified as PBL. The cytologic findings are summarized in Table 2.

Immunocytochemical Findings on Cytologic Samples

Immunocytochemistry was performed on a limited number of cytology samples. Six cases stained with pancytokeratin (AE1/AE3) were positive. Neuroendocrine markers synaptophysin (n = 10 of 10), chromogranin (n = 6 of 10), CD56 (n = 5 of 6) and neuron-specific enolase (n = 2 of 4) also were expressed by some. Trypsin was positive in 5 of 8 cases (63%), and chymotrypsin was positive in 1 of 2 cases (50%). Nuclear β-catenin was positive in 6 of 8 (75%) tested cases and highlighted even subtle squamoid morules (Figs. 3–5). β-Catenin immunostaining was performed during initial FNAB workup (n = 3 of 8 specimens; patients 6-8) and during re-evaluation for this study (n = 5 of 8 specimens). The median Ki-67 proliferative index in 6 tested cases was 21% (range, 2%-70%). For results of other immunostains, see Table 3.

Electron Microscopy Findings

Electron microscopy was performed on 1 tumor (patient 9) that originally was diagnosed as a poorly differentiated carcinoma. Electron microscopy revealed cytoplasmic zymogen granules (a marker of acinar differentiation), abundant mitochondria, and prominent desmosomes, supporting the diagnosis of carcinoma.

Histologic Findings

Ten of 11 pancreatic tumors and 1 liver metastasis (patient 4) were resected. Tumors ranged in size from 2.5 to 12.0 cm (median, 5.6 cm). The original histologic diagnoses on the pancreatectomy specimens were PBL (n = 8) and PDNEC (n = 2; patients 10 and 11). All showed

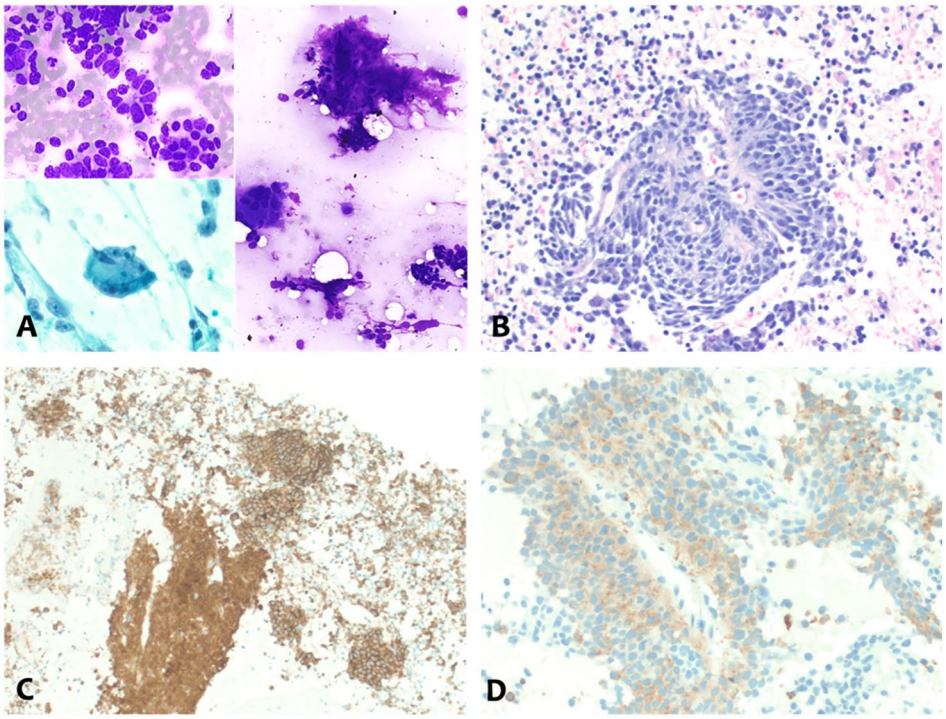

Figure 3. (A) Tumor cells focally formed acini (*Top Left*; Diff-Quik stain, original magnification ×400) and were more pleomorphic, with 5-fold anisonucleolis (*Bottom Left*; Papanicolaou stain, original magnification ×400). (*Right*) Magenta-colored stroma fragments are also present (Diff-Quik stain, original magnification ×400). (B) A cell block shows a sheet of small, round, blue cells with high nuclear-to-cytoplasmic ratio (H&E stain, original magnification ×200). (C) This squamoid morule was not visible on H&E staining but was highlighted by nuclear β-catenin staining. Adjacent blast-like cells showed membranous and cytoplasmic β-catenin staining. (D) Synaptophysin showed granular cytoplasmic staining in the same case.

small, round-to-spindled, blast-like tumor cells with back-to-back arrangement, focal rosettes, and acini (Figs. 4, 5). The 8 of 10 tumors that originally were called PBL also showed heterogeneously distributed, well demarcated, eosinophilic squamoid morules, which facilitated the PBL diagnosis (Fig. 5). Whereas some morules were more classical in appearance, others were absent or ill-defined geographic zones, best seen after (nuclear) β-catenin staining. In 1 case diagnosed as PDNEC (patient 11), the morules were pale and surrounded by rosette-like structures lined by columnar cells with blast-like nuclei (Fig. 4). The other misdiagnosed case (patient 10) showed sheets of small, round, blue cells in hypercellular stroma and had no definite morules (Fig. 5C). The absence and vague appearance of the morules in these 2 cases likely led to PBL not being considered during initial workup, and both also were diffusely positive for AE1/AE3, synaptophysin, and chromogranin. The PBL diagnosis was made after metastases developed (8 years after the first diagnosis), and the re-reviewed pancreatectomy specimens showed vague morules, prompting a β-catenin stain, which showed nuclear labeling far more than expected based on hematoxylin and eosin staining alone (Figs. 4, 5C).

Follow-Up Information

Follow-up information was available for all cases. The median follow-up was 72.5 months (range, 0.8-348 months). At last follow-up, 6 patients (55%) were alive, including 4 with metastases or recurrence (liver metastases, n = 2; abdominal carcinomatosis, n = 1; pancreatic recurrence, n = 1), and 5 patients died of disease, 2 within 6 months of diagnosis.

DISCUSSION

The current study is the largest cohort to date describing the cytologic features of PBL in adults. The absence of pediatric cases is because the contributors practice in adult-focused institutions. Tumors favored the pancreatic head, were fairly large (median, 5.6 cm), and 45% of patients died of disease. Of those that were alive at last follow-up, the majority had recurrent or metastatic disease, with metastases most frequently to the liver. This

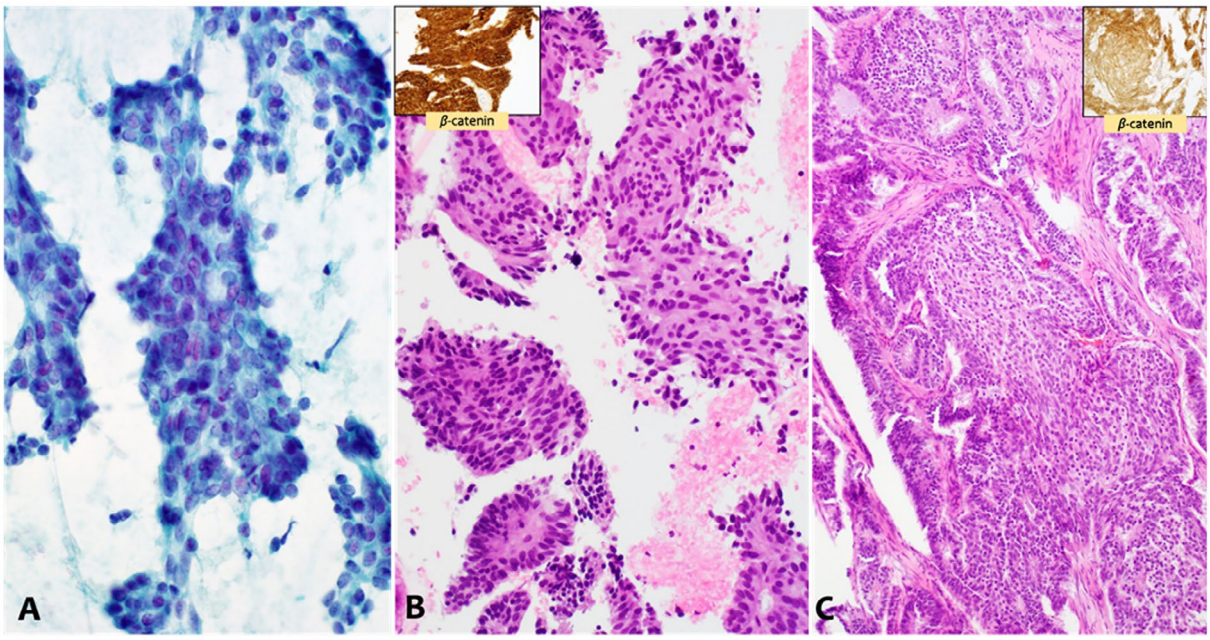

Figure 4. This case (patient 11, sample 12) was misdiagnosed as poorly differentiated neuroendocrine carcinoma. (A) A hypercellular smear shows monotonous cells with a high nuclear-to-cytoplasmic ratio, powdery chromatin, and small nucleoli (Papanicolaou stain, original magnification ×200). (B) A cell block from the same case shows a 2-cell population of smaller, hyperchromatic, blast-like cells with a high nuclear-to-cytoplasmic ratio and (*Inset*) a subtle, squamoid morule composed of streaming epithelioid cells with abundant eosinophilic cytoplasm and diffuse nuclear positivity for nuclear β-catenin. A peripheral rosette is also present at the lower left (H&E stain, original magnification ×200). (C) A corresponding pancreatic resection, which was initially misdiagnosed as a neuroendocrine carcinoma, shows central, pale-pink, nuclear β-catenin–positive squamoid morules (*inset*) surrounded by sheets and gland-like groups of elongated, blast-like cells (H&E stain, original magnification ×200).

underscores the importance of early, accurate diagnosis and distinction from less aggressive mimics like SPN and WDNET.

We found that there were 2 distinctive cytologic features, ie, blast-like tumor cells and squamoid morules, which corresponded to the 2-cell population crucial for accurate histologic diagnosis and distinction from related entities with similar immunohistochemical profiles. Blast-like cells were small (1.5-2 times the size of RBCs) and had fine, immature chromatin and small but distinct nucleoli. Squamoid morules were seen in the majority of cases and were present on both smears and cell blocks. Morules showed a broad cytomorphologic spectrum, ranging from well defined, tight clusters of epithelioid cells with eosinophilic cytoplasm, low nuclear-to-cytoplasmic ratio, and whorled or streaming cytoplasm, to more ill-defined, loosely cohesive, syncitial groups best seen with β-catenin staining. The 2-cell population of clearly malignant, blast-like cells juxtaposed with bland, streaming epithelioid morules was extremely helpful in correctly identifying these cases as PBL. Despite these features, only 2 of our cases were definitively diagnosed as PBL on initial FNAB, and 2 others were only identified when a review of the first 10 samples highlighted this (2-cell population) characteristic.

The cytologic features of PBL were first described by Silverman et al in 1989.[21] Since then, other brief reports have highlighted its cytomorphology (Table 4).[14-20,22-24] In findings similar to our own, others have shown that PBL aspirates are typically hypercellular, with clusters and single, small cells that have a high nuclear-to-cytoplasmic ratio, round-to-oval nuclei, fine or vesicular chromatin, and distinct nucleoli.[14-17,20-24] Pleomorphism and mitotic activity have only rarely been described and were occasionally seen in our cohort.[16,21] A second population of larger squamoid cells has been reported more frequently on cell blocks[14,16,22,24] than on smears.[14,15] These may contain biotin-rich, optically clear nuclei,[14,15,23] which we looked for but did not see in our cohort. Other reported cytologic findings in PBL include spindled cells,[16,17,21,22] immature mesenchyme,[17,21,22] and stromal fragments,[19,21]

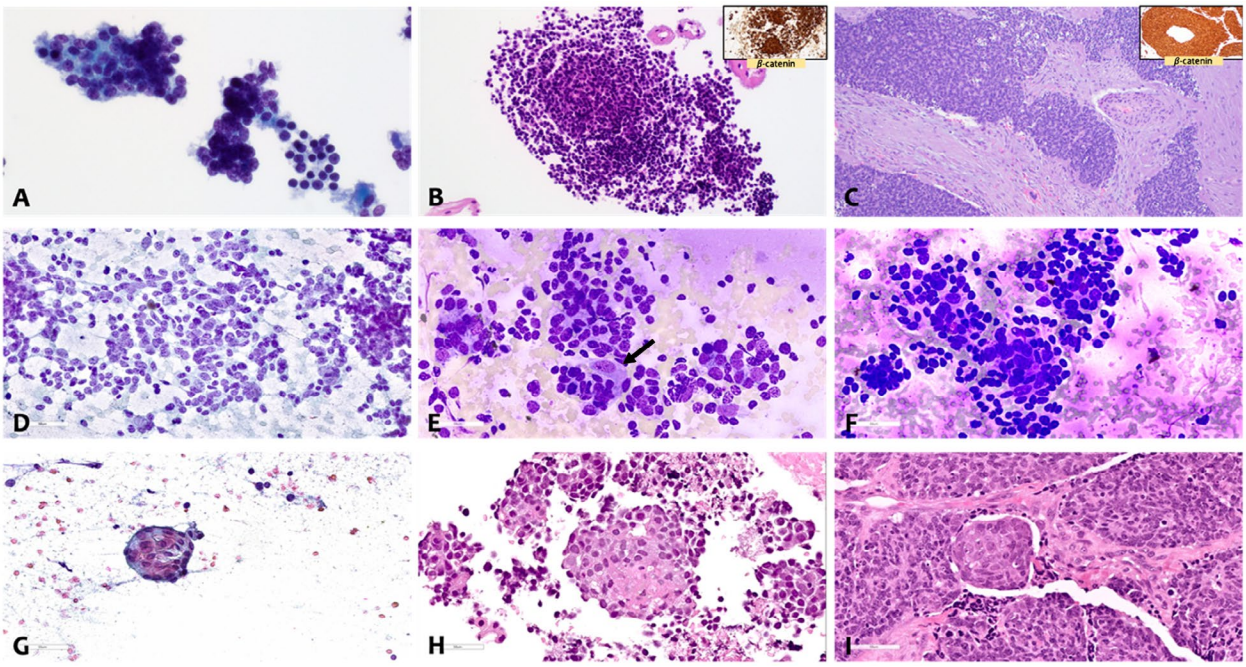

Figure 5. (A-C) This mediastinal lymph node fine-needle aspiration (FNA) (patient 10, sample 11) was originally diagnosed as poorly differentiated neuroendocrine carcinoma. (A) A ThinPrep slide shows blast-like cells with distinct nucleoli and smaller, benign background lymphocytes (Papanicolaou stain, original magnification ×600). (B) The corresponding cell block shows a compact, squamoid morule highlighted by nuclear β-catenin stain (*inset*). (C) A corresponding pancreatic resection shows large sheets of monotonous, small, round-to-elongated blue cells with diffuse nuclear β-catenin staining (*inset*) and immature, hypercellular stroma (H&E stain, original magnification ×200). (D) A pancreatic FNA biopsy from case 1 that was misdiagnosed as neuroendocrine neoplasm shows spindled-to-oval, small, round cells with nuclear molding resembling small-cell carcinoma (Papanicolaou stain, original magnification ×200). (E) A 2-cell population of smaller blast-like cells is shown along with a single squamoid cell (arrow) with abundant, dense cytoplasm and a large nucleus (Diff-Quik stain, original magnification ×200). (F-I) Squamoid morules ranged from (F) ill-defined, pale-pink clusters (Diff-Quik stain, original magnification ×200) to (G) tight, whorled units of epithelioid cells with pale cytoplasm and syncytial arrangement (Papanicolaou stain, original magnification ×200). (H) A corresponding cell block and (I) pancreatic resection show similar tight, eosinophilic, squamoid morules surrounded by monotonous, blast-like blue cells with intervening cellular stroma (H&E stain, original magnification ×200).

which we recorded collectively as stromal fragments and saw in 75% of specimens. Branching pseudopapillae also were common (60%) in our cohort and, in 3 cases, also showed grooved, SPN-like nuclei. Pseudopapillae and grooved nuclei have been reported in PBL, albeit infrequently,[14,19] and in 1 case led to misdiagnosis as SPN.[19]

Squamoid corpuscles (or morules, because they rarely have true squamous characteristics) are the entity-defining hallmark of PBL. They vary in circumscription and number, and range from small 10-cell to 15-cell aggregates to large, ill-defined (1.0 mm) zones,[5] which are visible on both histology and cytology samples.[5,14,15,23] Morules overexpress estrogen receptor-β and biotin and show aberrant nuclear/cytoplasmic β-catenin staining, which has been exploited as a diagnostic marker and can highlight even the most subtle morules.[24] The latter is because of an upregulated Wnt signaling pathway, which normally promotes keratinization and hair folliculogenesis in utero.[12,13,25,26] These findings suggest a shared Wnt-related pathogenesis (confounded by estrogen pathway alteration) in biotin-rich, optically clear nuclei/morule-forming tumors like pulmonary blastomas, cribriform-morular papillary thyroid carcinoma, the complex-pyloric type of intracholecystic papillary neoplasm, and even morule-forming endometrial carcinomas.[27] This interesting angle warrants further investigation. Aberrant Wnt pathway activation manifests as somatic *CTNNB1* mutations (in 90% of cases) and loss of heterozygosity (LOH) of *APC* (in 10%).[11] Other abnormalities include upregulation of the R-spondin/LGR5/RNF43 module, a progenitor-like pancreatic cell expression profile, and LOH of chromosome 11p.[11,26] *APC*/β-catenin pathway alterations are seen in patients with and without FAP,[12,13,26]

TABLE 2. Cytologic Features of Pancreatoblastoma in 12 Fine Needle Aspiration Biopsy Samples

	Sample												
Feature	1	2	3	4	5	6	7	8	9[a]	10[a]	11	12	%
FNA site	Pancreas	Pancreas	Liver	Liver	Pancreas	Pancreas	Liver	Pancreas	Pancreas	Liver	Node	Pancreas	
Cellular	+	+	+	+	+	+	+	+	+	+	+	+	100
3D clusters	+	+	+	+	+	+	+	+	+	+	+	+	100
Pseudopapillae			+		+	+	+	+	+			+	58
Grooved nuclei			+						+			+	25
Blast-like cells	+	+	+	+	+	+	+	+	+	+	+	+	100
High N/C	+	+	+	+	+	+	+	+	+	+	+	+	100
Size x RBC	1.5-2.2	1.5-2.0	1.5-2.0	1.5-2.0	1.5-2.0	1.5-2.0	5-20.0	1.5-2.0	1.5-2.0	1.5-2.0	1.5-2.0	1.5-2.0	
Molding	+	+	+	+	+	+	+	+	+	+	+	+	100
Plasmacytoid							+						8
Pleomorphism	+	+					+	+					33
Mitoses							+						8
Necrosis	+	+					+	+	+	+		+	58
Vesicles	+			+		+	+	+					42
Red granules	+	+	+	+		+						+	50
SM-smear	+	+	+				+					+	42
SM-Cell block	+					+	+	+	+		+	+	58
Stromal fragments	+	+	+				+	+	+	+	+	+	75
First cytologic Dx	NEN	NEC	Pos	NEN	ACC	Mixed NE-acinar vs PBL	PBL	PBL	PDCA	PDCA	NEC	NET	
First histologic Dx	PBL	PBL	PBL	PBL	PBL	PBL		PBL	PBL	NEC	NEC		

Abbreviations: +, positive; ACC, acinar cell carcinoma; Dx, diagnosis; FNA, fine-needle aspiration; N/C, nuclear-to-cytoplasmic ratio; NE, neuroendocrine; NEC, neuroendocrine carcinoma; NEN, neuroendocrine neoplasm; NET, well differentiated neuroendocrine tumor; PBL, pancreatoblastoma; PDCA, poorly differentiated carcinoma; Pos, positive for malignancy; RBC, red blood cell; SM, squamoid morule.
[a]Samples 9 and 10 were from the same patient.

TABLE 3. Immunocytochemical Profile of Pancreatoblastoma in 12 Fine Needle Aspiration Biopsy Samples

	Sample												
	1	2	3	4	5	6	7	8	9[a]	10[a]	11	12	No./Total No.
FNA Site	Pancreas	Pancreas	Liver	Liver	Pancreas	Pancreas	Liver	Pancreas	Pancreas	Liver	Node	Pancreas	
AE1/AE3	+	+	ND	ND	ND	ND	+	ND	+	ND	+	+	6/6
Synaptophysin	+	+	+	+	+	+	+	+	ND	ND	+	+	10/10
Chromogranin	+	–	+	ND	+	+	+	–	–	ND	+	–	6/10
CD56	+	ND	–	ND	ND	+	+	ND	ND	ND	+	+	5/6
NSE	+	+	ND	ND	–	ND	ND	ND	–	ND	ND	ND	2/4
Trypsin	ND	ND	+	ND	+	+	+	+	–	ND	–	–	5/8
Chymotrypsin					–	+							1/2
β-catenin(nuclear)	ND	+[b]	–	ND	–	+	+	+	ND	ND	+[b]	+[b]	6/8
LEF1	ND	ND	ND	ND	ND	ND	–	ND	ND	ND	+	ND	1/2
Ki-67 index, %	ND	50	ND	ND	2	ND	70	11	ND	ND	17	27	
Myo-D	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	–	ND	0/1
Myogenin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	–	ND	0/1
Desmin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	–	ND	0/1
CD99	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	–	ND	0/1
CDX2	ND	ND	ND	+	ND	ND	ND	ND	ND	ND	ND	ND	1/1
CK7	–	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0/1
CK20	–	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0/1
First cytologic Dx	NEN	NEC	Pos	NEN	ACC	Mixed NE-acinar vs PBL	PBL	PBL	PDCA	PDCA	NEC	NET	

Abbreviations: +, positive; –, negative; ACC, acinar cell carcinoma; Dx, diagnosis; FNA, fine-needle aspiration; ND, not done; NE, neuroendocrine; NEC, neuroendocrine carcinoma; NEN, neuroendocrine neoplasm; NET, well differentiated neuroendocrine tumor; NSE, neuron-specific enolase; PBL, pancreatoblastoma; PDCA, poorly differentiated carcinoma; Pos, positive for malignancy; SM, squamoid morule.
[a]Samples 9 and 10 were from the same patient.
[b]β-Catenin staining was performed during specimen re-evaluation for study (samples 2 and 12) or for consultation (sample 11).

which has led some to propose that PBLs may represent an extracolonic manifestation of FAP.[26] This is supported by the 3 patients with FAP in our small cohort. Another syndrome seen in childhood PBL is Beckwith-Wiedemann syndrome, which is also associated with chromosome 11p LOH.[28] These syndromic associations

TABLE 4. Cytologic Features of Pancreatoblastoma in the Literature

Feature	Silverman 1990[21]	Henke 2001[16]	Hasegawa 2003[15]	Pitman & Faquin 2004[17]	Zhu 2005[22]	Rajpal 2006[18]	Sahu 2009[23]	Redelman 2014[19]	Sigel & Klimstra 2013[20]	Das 2016[14]	Nunes 2018[24]
Study year	1989	2001	2003	2004	2005	2006	2009	2013	2013	2016	2017
Hypercellularity	+	+	+	+	+	+	+	+	+	+	+
Acinar groups	+	+	+	+	+	+	+		+		
Pseudopapillae								+		+	
Grooved nuclei								+			
High N/C ratio	+	+		+	+		+	+			+
Fine chromatin	+	+	+	+	+			+	–		
Small/indistinct nucleoli	+	+	+	+	+			+	+		
Optically clear nuclei			+				+			+	
Size × RBC	1.5	1.5	6-8 Microns		1.5		1.5	1.5	<3 × Lymphoid		1.5
Molding								+			
Plasmacytoid	+										
Pleomorphism	+	+	No			No	+	+	No	No	
Mitoses		+	No				+			No	
Necrosis	+		No	+						No	+
SM smear			+							+	
SM cell block		+		+	+					+	+
Spindle cells	+	+	+	+	+						
Stromal fragments	+	+						+			
Mesenchyme	+			+					No		
Ancillary studies IHC/EM	Both	+/–	+/– Biotin	+/–	Both	No	No		+/–	No	+/–
Differentiation	NE			NE + ACC	NE				NE + ACC		NE
Cytologic Dx	PBL	PBL	PBL	PBL	PBL	ACC	PBL	SPN	ACC	PBL	PBL

Abbreviations: –, negative; +, positive; +/-, Immunohistochemistry without electron microscopy; ACC, acinar cell carcinoma; EM, electron microscopy; IHC, immunohistochemistry; N/C, nuclear-to-cytoplasmic ratio; NE, neuroendocrine; PBL, pancreatoblastoma; RBC, red blood cell; SM, squamoid morule; SPN, solid-pseudopapillary neoplasm.

emphasize not only the importance of accurate diagnosis of PBL on FNAB but also the need for genetic testing in these patients, with counselling when appropriate. The finding that 2 patients in our study (and others in the literature) had Gardner syndrome rather than ordinary FAP raises the question of whether PBL is more likely to occur in Gardner syndrome and may have a slightly different mechanism than ordinary FAP.

Because of its multilineage differentiation and the broad morphologic spectrum of the squamoid morules that define them, the cytohistologic diagnosis of PBL can be especially challenging. Squamoid morules can be extremely subtle or absent and were not seen in 3 of our cases. On the basis of location and morphology, the PBL differential diagnosis must include WDNET, PDNEC, ACC/mixed endocrine–non-neuroendocrine carcinoma, and SPN. Five of our cases were originally diagnosed as NENs, which is not surprising because they occurred in adults, had neuroendocrine-type morphology, and expressed neuroendocrine markers. In addition, the blast-like cells and scattered plasmacytoid tumor cells seen in some of these cases resembled small-cell carcinoma and WDNET, respectively, and none of them were stained initially with β-catenin. PBL is also morphologically closely related to ACC, as the blast-like cells and occasional acini resemble acinar cells and may express trypsin or chymotrypsin. Of the 8 cases in our cohort that were interrogated for acinar markers (trypsin, chymotrypsin), 63% showed focal positivity, and 1 was misdiagnosed as ACC on FNAB. If the immunocytochemical workup is limited to a neuroendocrine or acinar panel, then cases can be easily misdiagnosed as NEN or ACC. Unless that second population of squamoid cells is identified (by light microscopy or immunocytochemistry), many PBLs will be missed, as happened in several of our cases.[18,20] Because morules may be either invisible or indistinct zones of pallor on low-power examination, a high index of suspicion is required to ensure that a β-catenin stain is performed. The papillary-type structures, pale chromatin, and nuclear grooves seen in some of our cases make SPN another key cytologic differential. Although none of our cases were misdiagnosed as SPN, others in the literature have been.[19] The SPN differential poses an interesting conundrum for pathologists, in that the β-catenin immunostain will not always allow its distinction from PBL, as the stain is positive in both and can also be diffuse in PBL. In addition, PBL may even express lymphoid enhancer-binding factor 1, a recently described SPN marker that was also focally positive in 1 of our cases.[29] Identifying the blast-like cells of PBL can help with distinction as well as cytokeratin staining, which is positive in PBL and negative or only focally positive in SPN.

The PBL diagnosis is challenging not only on cytology but also on imaging. Large tumors often outstrip their blood supply and undergo cystic degeneration. As a result, radiologic differentials include not only solid primary pancreatic malignancies (of neuroendocrine, ductal, and acinar lineage) but also benign and malignant cystic lesions like pseudocyst and serous cystic neoplasms. Interestingly, the PBL differential was not mentioned in any of our cases, and, in the younger patients (aged <35 years), even benign differentials were considered.

Conclusion

The defining cytologic characteristics of PBL, which include small, round blast-like cells and squamoid morules, may be subtle but are helpful in distinguishing it from other solid, cellular, stroma-poor pancreatic neoplasms for which it is often mistaken. Cytologic diagnosis often requires ancillary staining with multiple lineage (neuroendocrine and acinar) markers as well as β-catenin, which highlights even the most subtle squamoid morular areas. Accurate diagnosis is critical because of its prognostic and therapeutic difference from more aggressive neuroendocrine carcinomas and less aggressive tumors like solid-pseudopapillary neoplasm. Because of the strong association with germline *APC* mutations and FAP, a diagnosis of PBL should prompt genetic testing in all cases.

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AUTHOR CONTRIBUTIONS

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