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

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Abstract

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: 6 males and 5 females, median age 45-years (range 32–60), median size 5.6cm (range 2.5-12cm), located in head (n=7), tail (n=4), 3 with familial adenomatous polyposis (FAP)/FAP-related syndromes and 4 with metastasis at diagnosis. Median follow-up was 39.8 months (range 0.8-348) and 5 died of disease. Original cytology diagnoses were: PBL (n=2), neuroendocrine neoplasm (n=2), poorly-differentiated neuroendocrine carcinoma (PDNEC)(n=2), well-differentiated neuroendocrine tumor(WDNET) (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 vs PBL (n=1). Universal cytopathologic findings included hypercellularity, 3-dimensional clusters, and single monotonous blast-like cells 1.5-2x RBCs, with high nuclear-to-cytoplasmic ratio, fine chromatin, small distinct nucleoli and resemblance to WDNET and PDNEC. 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 (SMs) were seen on smears(n=5) or

cell blocks(n=6) in 70% and were characterized by epithelioid cells with elongated streaming nuclei, fine chromatin, absent nucleoli, and positive nuclear β-catenin(n=6/8). 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 SMs, frequent neuroendocrine and variable acinar phenotype, should facilitate accurate cytologic diagnosis and distinction from common mimics.

Key words: pancreatoblastoma, pancreas, cytology, FNA

The results of this study were presented as a platform presentation at the annual United States and Canadian Academy of Pathology meeting in March 2019.

Introduction

While 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 acinar cell carcinomas (ACCs), tumors which are characteristically 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, where they account for up to 25% of pancreatic tumors in the first decade.[1-3] Although originally considered pediatric tumors, a third of PBLs occur in adults.[4-7] These tumors were first defined by Becker *et al* 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. Additionally, 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. While 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] We herein describe the cytologic findings in 12 PBLs diagnosed in 11 adults and review their clinicopathologic characteristics, cytologic and histologic features. To the best of our knowledge, this represents the largest series in the cytology literature 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 (FNAB) from 11 patients. Rapid on-site evaluation was performed on all cases which were deemed adequate at the time of evaluation. The cytology material, including smears, ThinPrep®, 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). 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.

Additionally, 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 mediastinal lymph node (n=1).

Clinical Characteristics

Patients included 6 males and 5 females (M:F, 1.2: 1) ranging in age from 32 - 60 years (median 45 years); 6 patients were < 50 years at diagnosis and 5 were younger than 40 at diagnosis. No childhood-associated tumors were identified in this study possibly because of the adult-focused hospitals in which the contributors practice. Mean tumor size was 5.6 cm (range 2.5-12cm), 7 (64%) were located in the head and 4 (36%) in the body/tail. One patient (patient # 7) had Gardner syndrome and two (patient #5 and #6) had familial adenomatous polyposis (FAP). Both FAP patients also had a previous history of successfully treated cancers (patient # 5 was a 50-year-old female with colonic adenocarcinoma diagnosed 14 years earlier and patient #6 was a 43-year-old female with small bowel adenocarcinoma diagnosed one year earlier and was recurrent at the time of PBL FNAB). Additionally, we believe that patient #5 had undiagnosed Gardner syndrome since, in addition to FAP, she also 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 (patient #3, 4 and 7) and four 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 seven of 11 patients. On imaging, three tumors were partially cystic, two involved the tail and one was in the head. The biliary tree (including extra and intrahepatic bile ducts as well as common bile duct) was dilated in 3 patients , all of them located in the pancreatic head. 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 (2/7, 29%), neoplastic (2/7, 29%) and malignant (3/7, 43%) differentials. Benign differentials included (1) autoimmune versus chronic pancreatitis in a 33-year-old male and (2) pseudocyst in a 34-year-old. 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 < 35-years-old which likely influenced the benign differential diagnoses. PBL was not raised as a differential in any of the patients. Radiologic diagnoses are also summarized in Table 1.

Cytologic Findings

The FNAB smears were uniformly hypercellular (100%) with numerous 3-dimensional clusters (100%) and singly dispersed cells (Figure 1). Seven (58%) cases showed branching pseudopapillae (Figure 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 (75%) cases.

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 times a red blood cell), with high nuclear to cytoplasmic ratio, scant cytoplasm and infrequent (n=4) anisonucleosis (Figure 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 neuroendocrine tumors (WDNETs), "blast-like"cells had more cytologic atypia, higher nuclear to cytoplasmic ratio, more delicate chromatin, and lacked the coarse salt-and-pepper chromatin of WDNET (Figure 1). Although in areas some tumor cells had occasional nuclear grooves resembling SPN (25%), the cells were more atypical than SPN cells and had larger nucleoli than SPN. Additionally, blast-like cells shared some cytologic similarity with ACC tumor cells (Figure 1 – 2), but lacked the macronucleoli, pleomorphism and brisk mitotic activity of ACC. The blast-like cells had dark blue cytoplasm on Diff Quik stain, some with cytoplasmic red granules (50%) and microvesicles (33%) (Figure 1). On Papanicolaou stain, these cells consistently had high nuclear to cytoplasmic ratio, smooth nuclei, delicate immature blast-like powdery chromatin, and distinct nucleoli (Figure 1 - 2). Focal nuclear molding and crushing resembling small cell carcinoma was seen in all cases (Figure 1 – 3). Abnormal mitotic figures were occasionally seen in 5 cases but were striking (19/10HPFs) in one (case #7), a 34-year-old male with metastatic PBL who died 8 days after diagnosis. This case also showed more pleomorphism (Figure 3).

Squamoid morules were seen in 9 (75%) samples, both on smear (n=5) and cell block (n=7) (Figures 2 – 5). These were more readily identifiable on cell block 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

(Figures 2, 4 - 5). Morules were magenta-colored on Diff Quik (Figure 5), greenish bluish on Papanicolaou and pale pink on Hematoxylin and Eosin (H&E) stain (Figure 2, 4 - 5). Keratinization was not seen on alcoholic fixed or cell block sections. One case had only ThinPrep® slides and a cell block for review (Figure 5A-C) and while the blast-like cells and squamoid morules were readily identifiable on both specimens (Figure 5B), the morules were not seen on ThinPrep® (Figure 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, NOS (n=2), poorly-differentiated neuroendocrine carcinoma (PDNEC, n=2) and WDNET (n=1). Two were diagnosed as poorly-differentiated carcinoma, one as "positive for malignancy" and one as ACC. One patient (case #6) whose tumor coexpressed neuroendocrine and acinar immunocytochemical markers, was initially diagnosed as a mixed neuroendocrine and ACC versus PBL. Only 2 (17%) (case #7 and #8) samples were initially diagnosed as PBL and squamoid morules were seen in both (on smear and/or cell block) and were highlighted by positive nuclear β-catenin immunostain done during initial work-up (Figure 3C). Two cases (case #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 immunoprofile were re-classified as PBL. Cytologic findings are summarized in Table 2.

Immunocytochemical Findings on Cytologis Samples

Immunocytochemistry was performed on a limited number of cytology samples. Six cases stained with pancytokeratin (AE1/AE3) were positive. Neuroendocrine markers synaptophysin (n=10/10), chromogranin (n=6/10), CD56 (n=5/6) and NSE (n=2/4) were also expressed by some. Trypsin was positive in 5/8 (63%) and chymotrypsin was positive in 1/2 (50%). Nuclear β-catenin was positive in 6/8 (75%) tested cases and highlighted even subtle squamoid morules (Figure 3 – 5). β-catenin was

performed during initial FNAB work-up (n=3/8, specimens # 6 - 8) and during re-evaluation for this study (n=5/8). Median Ki-67 proliferative index in six tested cases was 21% (range 2 – 70%). See Table 3 for results of other immunostains.

Electron Microscopy Findings

Electron microscopy was performed on 1 tumor (patient #9) which was originally diagnosed as a poorly differentiated carcinoma. Electron microscopy revealed cytoplasmic zymogen granules, a marker of acinar differentiation, abundant mitrochondria 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 – 12.0 cm (median, 5.6 cm). Histologic diagnoses on the pancreatectomy specimens were PBL (n=8) and PDNEC (n=2, patient 10# and 11). All showed small round to spindled blast-like tumor cells with back-to-back arrangement, focal rosettes and acini (Figure 4 - 5). The 8/10 that were originally called PBL also showed heterogeneously distributed well demarcated eosinophilic squamoid morules, which facilitated the PBL diagnosis (Figure 5). While some were more classical in appearance others were absent or ill-defined geographic zones, best seen after (nuclear) β-catenin staining. In one case diagnosed as PDNEC (patient #11) the morules were pale and surrounded by rosette-like structures lined by columnar cells with blast-like nuclei (Figure 4). The other misdiagnosed case showed sheets of small round blue cells in hypercellular stroma and had no definite morules. The absence of, and vague appearance of the morules in these two cases likely led to PBL not being considered during initial work-up and both were also diffusely positive for AE1/AE3, synaptophsin and chromogranin. The PBL diagnosis was made after metastases developed (8 years-post first diagnosis) and the re-reviewed pancreatectomies showed vague morules, prompting a β-catenin stain which showed nuclear labeling, far more than expected based on H&E stain (Figure 4, 5C).

Follow-up Information

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

Discussion

The present study is the largest cohort describing the cytologic features of PBL in adults. The absence of pediatric cases is attributable to the fact that the contributors practice in adult-focused instutitions. 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 liver. This underscores the importance of early, accurate diagnosis and distinction from less aggressive mimics like SPN and WDNET.

We found that there were two distinctive cytologic features, i.e. blast-like tumor cells and squamoid morules, which corresponded to the two-cell population crucial for accurate histologic diagnosis, and distinction from related entities with similar immunohistochemical profiles. Blast-like cells were small (1.5-2 x red blood cells) and had fine immature chromatin and small but distinct nucleoli. Squamoid morules were seen in the majority of cases and were present both on 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 stain. 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 two of our cases were definitively diagnosed as PBL on initial FNAB, and two others were only identified when 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 with high nuclear to cytoplasmic ratio, round to oval nuclei, fine or vesicular chromatin and distinct nucleoli.[14-17,20-24] Pleomorphism and mitotic acivity have only rarely been described and were occasionally seen in our cohort.[16,21] A second population of larger squamoid cells has been reported but more frequently on cell block,[14,16,22,24] than smears.[14,15] These may contain biotin-rich optically clear nuclei (BROCN),[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] which we recorded collectively as stromal fragments and saw in 75%. Branching pseudopapillae were also common (60%) in our cohort and in three cases also showed grooved SPN-like nuclei. Pseudopapillae and grooved nuclei have been reported in PBL, albeit infrequently,[14,19] and in one case led to misdiagnosis as SPN.[19]

Squamoid corpuscles (or morules, since they rarely have true squamous characteristics) are the entity-defining hallmark of PBL. They vary in circumscription and number, and range from small 10 – 15-cell aggregates to large ill-defined (1.0 mm) zones, [5] visible on both histology and cytology samples.[5,14,15,23] Morules overexpress estrogen receptor (ER)-β and biotin and show aberrant nuclear/cytoplasmic β-catenin which has been exploited as a diagnostic marker and can highlight even the most subtle morules.[24] The latter is due to 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 "BROCN"/morule-forming tumors like pulmonary blastomas, cribrifom-morular papillary thyroid carcinoma, 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 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]which has led some to propose that PBLs may represent an extracolonic manifestation of FAP.[26] This is supported by the three FAP patients 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 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 fact that two 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 slightly different mechanism than ordinary FAP.

Because of its multilineage differentiation and the broad morphologic spectrum of the squamoid morules that define them, the cyto-histologic diagnosis of PBL can be especially challenging. Squamoid morules can be extremely subtle or absent and were not seen in three of our cases. Based on location and morphology the PBL differential diagnosis must includes WDNET, PDNEC, ACC/mixed endocrine-non-neuroendocrine carcinoma and SPN. Five of our cases were originally diagnosed as NENs which is not surprising since they occured in adults, had neuroendocrine-type morphology and expressed neuroendocrine markers. Additionally, the blast-like cells and scattered plasmacytoid tumor cells seen in some of these cases resemble small cell carcinoma and WDNET, respectively, and none of them were stained with β-

catenin initially. 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 eight cases in our cohort that were interrogated for acinar markers (trypsin, chymotrypsin) 63% showed focal positivity and one 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] Since morules may be either invisible or indistinct zones of pallour 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. Additionally, PBL may even express lymphoid enhancer-binding factor 1 (LEF1), a recently described SPN marker that was also focally positive in one of our cases.[29] Identifying the blast-like cells of PBL can helpful with distinction, as well as a cytokeratin stain which is positive in PBL and negative or only focally positive in SPN.

The PBL diagnosis is not only challenging 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 (< 35-years-old) patients even benign differentials were considered.

Conclusion

The defining cytologic chracteristics of pancreatoblastoma, 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 solid-pseudopapillary neoplasms. Because of the strong association with germline *APC* mutations and FAP the PBL diagnosis show prompt genetic testing in all cases.

Legends to Figures

Figure 1. Cytologic features of pancreatoblastoma. **A.** Hypercellular smear with 3-dimensional clusters of small round cells (Diff Quik stain x 100). **B.** Pseudopapilla with branching fibrovascular core similar to solid-pseudopapillary neoplasm (Diff Quik stain x 100). **C - D.** Singly dispersed monotonous blast-like cells with high nuclear to cytoplasmic ratio, round nuclei, distinct nucleoli, focal plasmacytoid features, cytoplasmic microvesicles (C, Diff Quik stain x 400), fine immature chromatin and prominent nucleoli (D, Papanicolaou stain x 400).

Figure 2. Cytologic features of squamous morules in pancreatoblastoma . **A - B.** Smears show 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 x 200). **C.** Two cell population of small blast-like cells on the left and large morular cells on the right (Papanicolaou stain x 400). **D.** Squamoid morule composed of streaming large epithelioid cells with pale cytoplasm, syncytial arrangement and bland elongated, blunted nuclei without nucleoli (Papanicolaou stain x 400).

Figure 3. **A.** Tumor cells focally form acini (upper left, Diff Quik stain x 400) and are more pleomorphic with 5-fold anisonucleolis (lower left, Papanicolaou stain x 400). Magenta colored stroma fragments (right) are also present (Diff Quik stain x 400). **B.** Cell block with sheet of small round blue cells with high nuclear to cytoplasmic ratio (Hematoxylin & Eosin stain x 200). **C.** This squamoid morule was not visible on H&E stain 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.

Figure 4. Case (patient #11, sample #12) misdiagnosed as poorly-differentiated neuroendocrine carcinoma. **A.** Hypercellular smear shows monotonous high nuclear to cytoplasmic ratio cells with powdery chromatin and small nucleoli (Papanocolaou stain x 200). **B.** Cell block from the same case shows a 2-cell population of smaller hyperchromatic blast-like cells with high nuclear to cytoplasmic ratio and a subtle squamoid morule composed of streaming epithelioid cells with abundant eosinophilic cytoplasm and diffuse nuclear positivity for nuclear β-catenin (inset). A peripheral rosette is also present (Hematoxylin & Eosin stain x 200). **C.** Corresponding pancreatic resection which was initially misdiagnosed as a neuroendocrine carcinoma shows central pale pink nuclear β-catenin-positive (inset) squamoid morules surrounded by sheets and glandular groups of elongated spindled blast-like cells (Hematoxylin & Eosin stain x 200).

Figure 5. **A - C.** Lymph node FNA (patient #10, sample #11) originally diagnosed as poorly-differentiated neuroendocrine carcinoma. **A.**ThinPrep® slide with blast-like cells with distinct nucleoli and smaller benign background lymphocytes (Papanicolaou stain x 600). **B.** Corresponding cell block shows a compact squamoid morule highlighted by nuclear β-catenin stain (inset). **C.** 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 (Hematoxylin and Eosin stain x 200). **D.** Case # 1. Pancreatic FNAB from case misdiagnosed as neuroendocrine neoplasm shows spindled to oval small round cells with nuclear molding resembling small cell carcinoma (Papanicolaou stain x 200). **E.** Two-cell population of smaller blast-like cells and a single squamoid cell (arrow) with abundant dense cytoplasm and large nucleus (Diff Quik stain x 200). **F-I.** Squamoid morules ranged from ill-defined (**F**) pale pink clusters (Diff Quik stain x 200) to tight whorled units (**G**) of epithelioid cells with pale cytoplasm and syncytial arrangement (Papanicolaou stain x 200). Corresponding cell block (**H**) and pancreatic resection (**I**) show similar tight eosinophilic squamoid morules surrounded by monotonous blast-like blue cells with intervening cellular stroma (Hematoxylin and Eosin stain x 200).

Table 1. Clinicopathologic Characteristics of 11 Pancreatoblastoma Patients

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

Dx, diagnosis; Mets, metastasis; M, male; F, female; DOD, died of disease; FAP, familial adenomatous polyposis; ACC, acinar cell carcinoma; NET, well-differentiated neuroendocrine tumor; SCA, serous cystadenoma; SCAd, serous cystadenocarcinoma; Mets, metastasis.

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

	1	2	3	4	5	6	7	8	9*	10*	11	12	%
FNA Site	**Pancreas**	**Pancreas**	**Liver**	**Liver**	**Pancreas**	**Pancreas**	**Liver**	**Pancreas**	**Pancreas**	**Liver**	**Node**	**Pancreas**	
Cellular	+	+	+	+	+	+	+	+	+	+	+	+	100
3-D clusters	+	+	+	+	+	+	+	+	+	+	+	+	100
Pseudopapillae			+		+	+	+	+	+			+	58
Grooved nuclei			+						+			+	25
Blast-like cells	+	+	+	+	+	+	+	+	+	+	+	+	100
High N/C	+	+	+	+	+	+	+	+	+	+	+	+	100
Size x RBC	**1.5-2 x**	**1.5-2 x**	**1.5-2 x**	**1.5-2 x**	**1.5-2 x**	**1.5-2 x**	**5-20 x**	**1.5-2 x**	**1.5-2 x**	**1.5-2 x**	**1.5-2 x**	**1.5-2 x**	
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
1st Cytologic Diagnosis	**NEN**	**NEC**	**Pos**	**NEN**	**ACC**	**Mixed NE-Acinar vs PBL**	**PBL**	**PBL**	**PDCA**	**PDCA**	**NEC**	**NET**	
1st Histologic Diagnosis	**PBL**	**PBL**	**PBL**	**PBL**	**PBL**	**PBL**		**PBL**	**PBL**		**NEC**	**NEC**	

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

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

Case #	1	2	3	4	5	6	7	8	9	10	11	12	n/n
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
Trpysin	ND	ND	+	ND	+	+	+	+	-	ND	-	-	5/8
Chymotrypsin					-	+							1/2
β-catenin (nuclear)	ND	+*	-	ND	-	+	+	+	ND	ND	+*	+*	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
1st Cytologic Diagnosis	NEN	NEC	Pos	NEN	ACC	Mixed NE-Acinar vs PBL	PBL	PBL	PDCA	PDCA	NEC	NET	

FNA, fine needle aspiration; ND, not done; +, positive; -, negative; SM, squamoid morule; Dx, Diagnosis; NEN, neuroendocrine neoplasm; NEC, neuroendocrine carcinoma; Pos, positive for malignancy; ACC, acinar cell carcinoma; NE, neuroendocrine; PBL, pancreatoblastoma; PDCA, poorly differentiated carcinoma; NET, well differentiated neuroendocrine tumor. Samples # 9 and 10 are from the same patient; *, β-catenin stain was performed during specimen re-evaluation for study (#2, 12) or consultation (#11).

Table 4. Cytologic Features of Pancreatoblastoma in the Literature

	Silverman *et al*[21]	Henke *et al*[16]	Hasegawa *et al*[15]	Pitman *et al*[17]	Zhu *et al*[22]	Rajpal *et al*[18]	Sahu *et al*[23]	Redelman *et al*[19]	Sigel *et al*[20]	Das *et al*[14]	Nunes *et al*[24]
	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 x RBC	1.5 x	1.5 x	6-8 Microns		1.5 x		1.5 x	1.5 x	<3x lymphoid		1.5 x
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 Diagnosis	**PBL**	**PBL**	**PBL**	**PBL**	**PBL**	**ACC**	**PBL**	**SPN**	**ACC**	**PBL**	**PBL**

N/C, nuclear to cytoplasmic ratio; RBC, red blood cell; +, positive; 1, negative; SM, squamoid morule; IHC, immunohistochemistry; EM, elctron microscopy; NE, neuroendocrine; ACC, acinar cell carcinoma; PBL, pancreatoblastoma; SPN, solid-pseudopaillary neoplasm.

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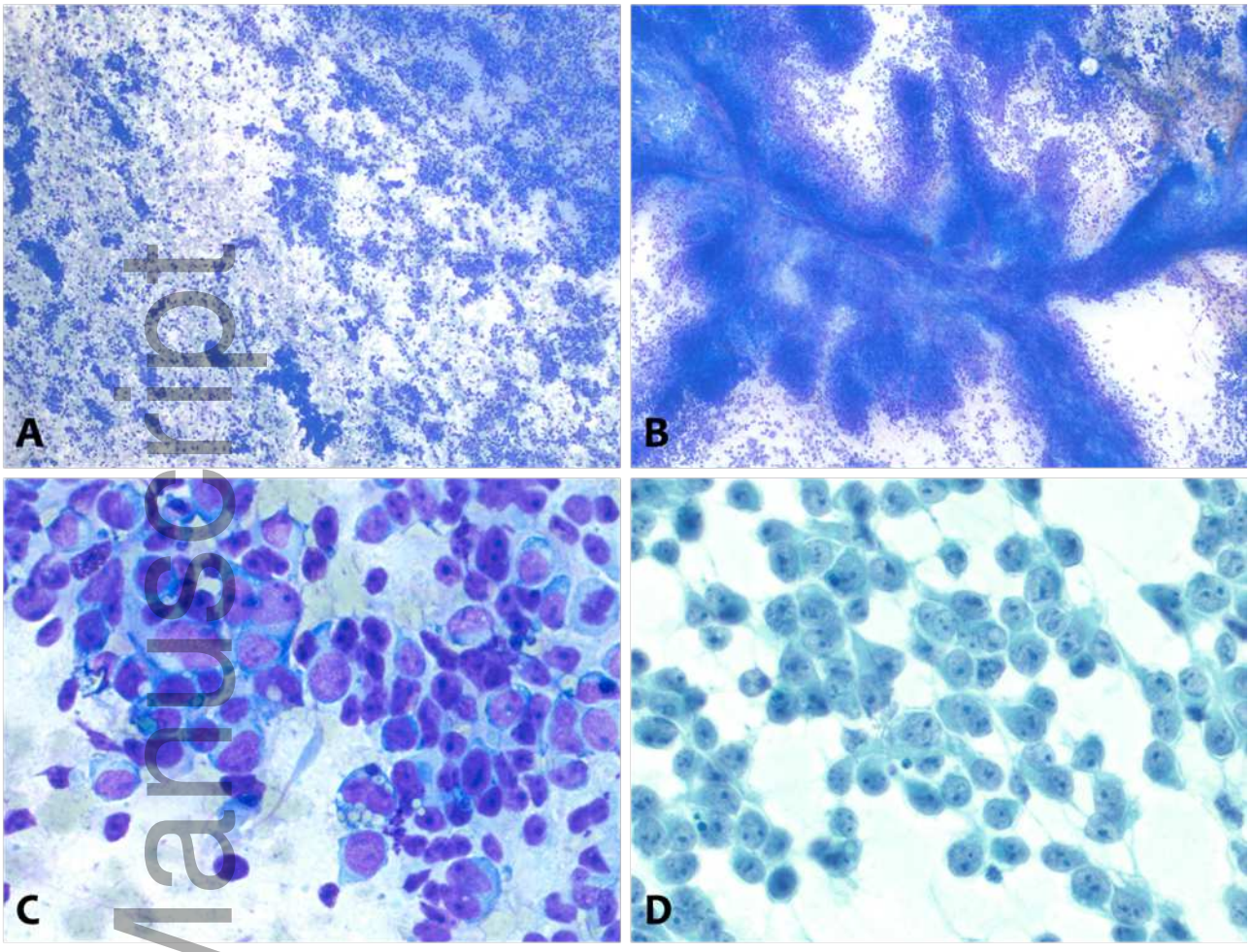

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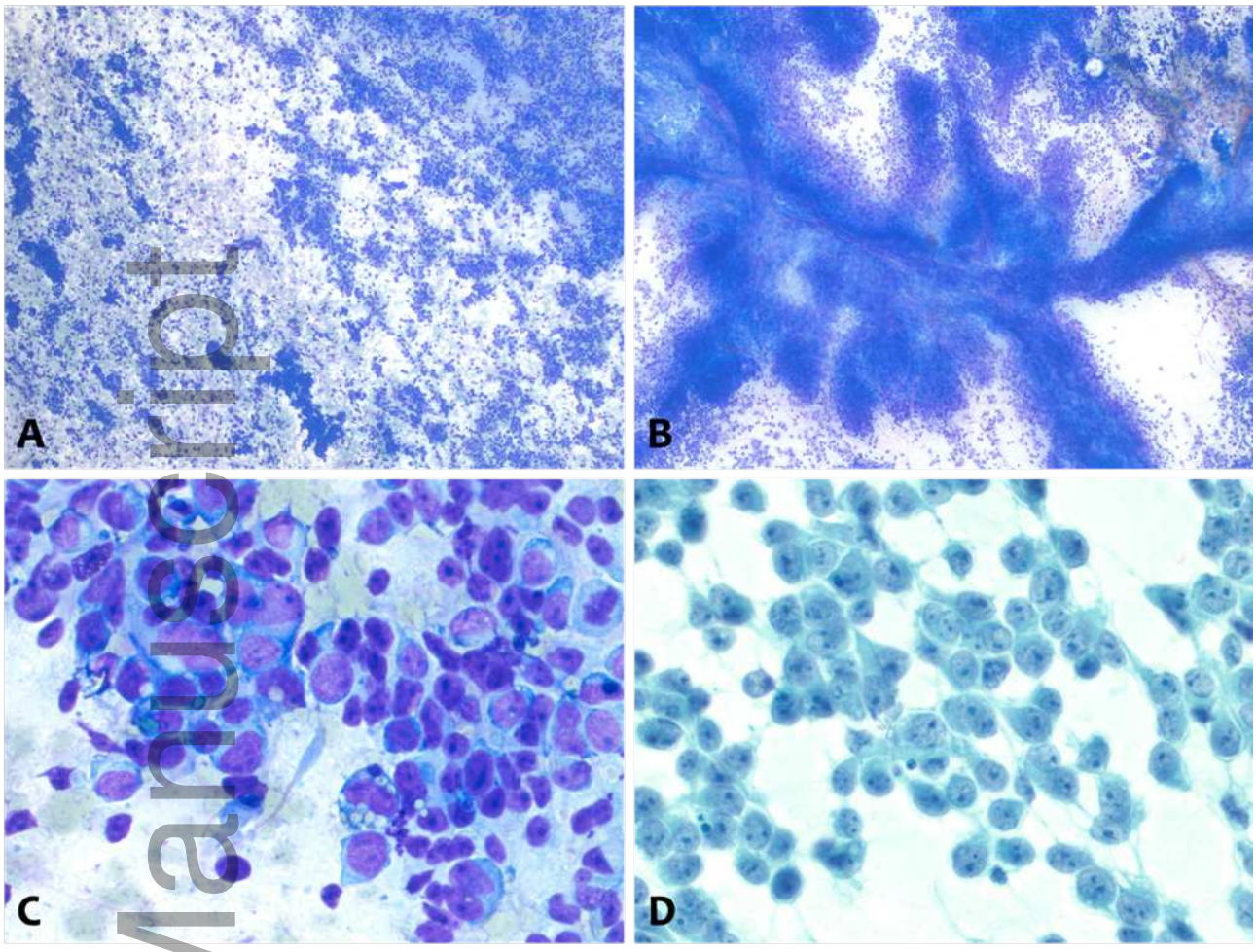

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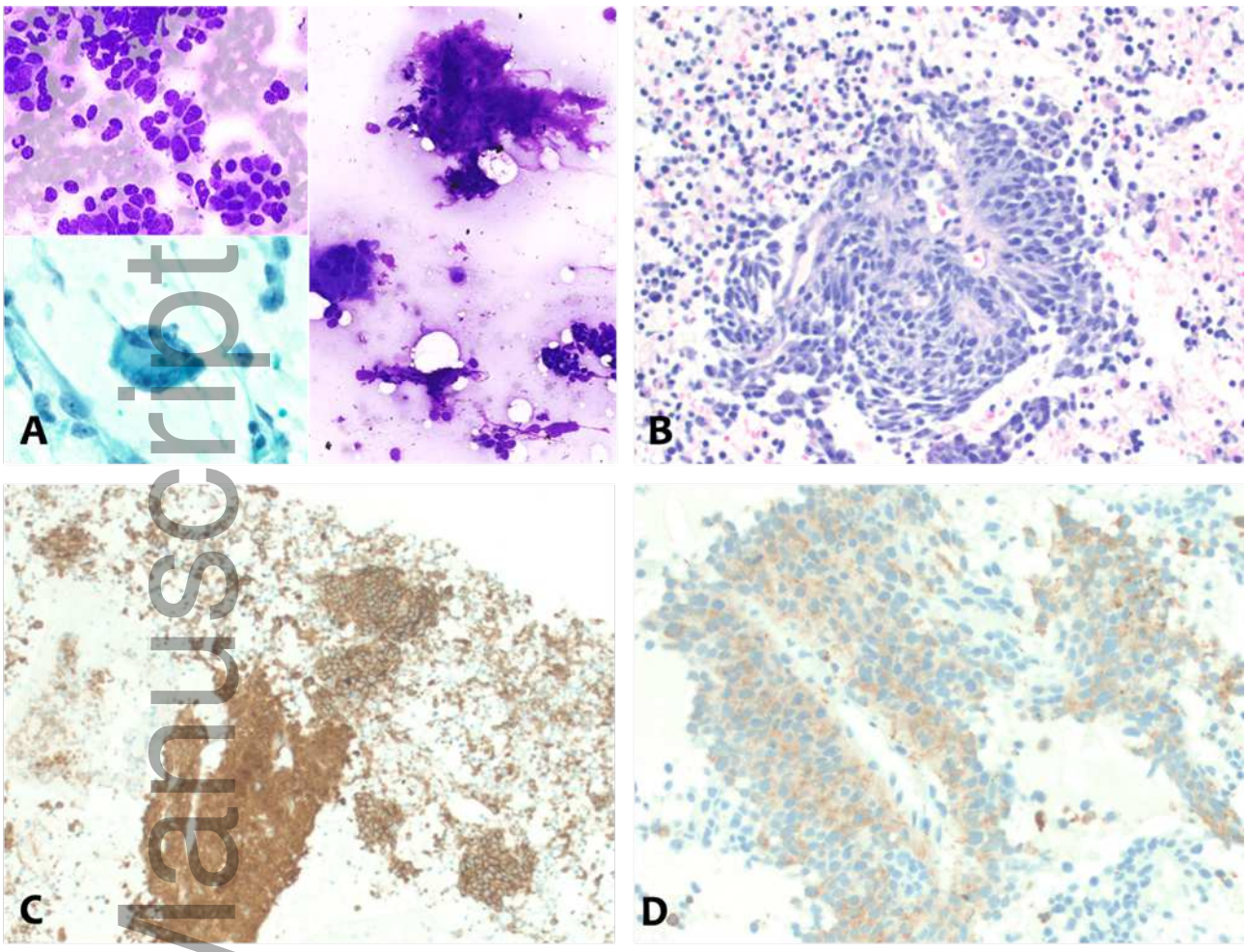

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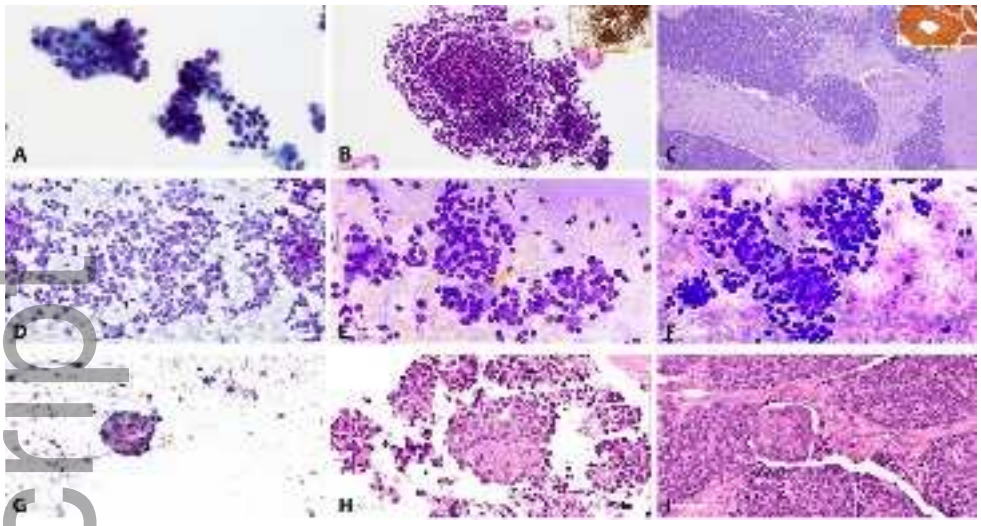

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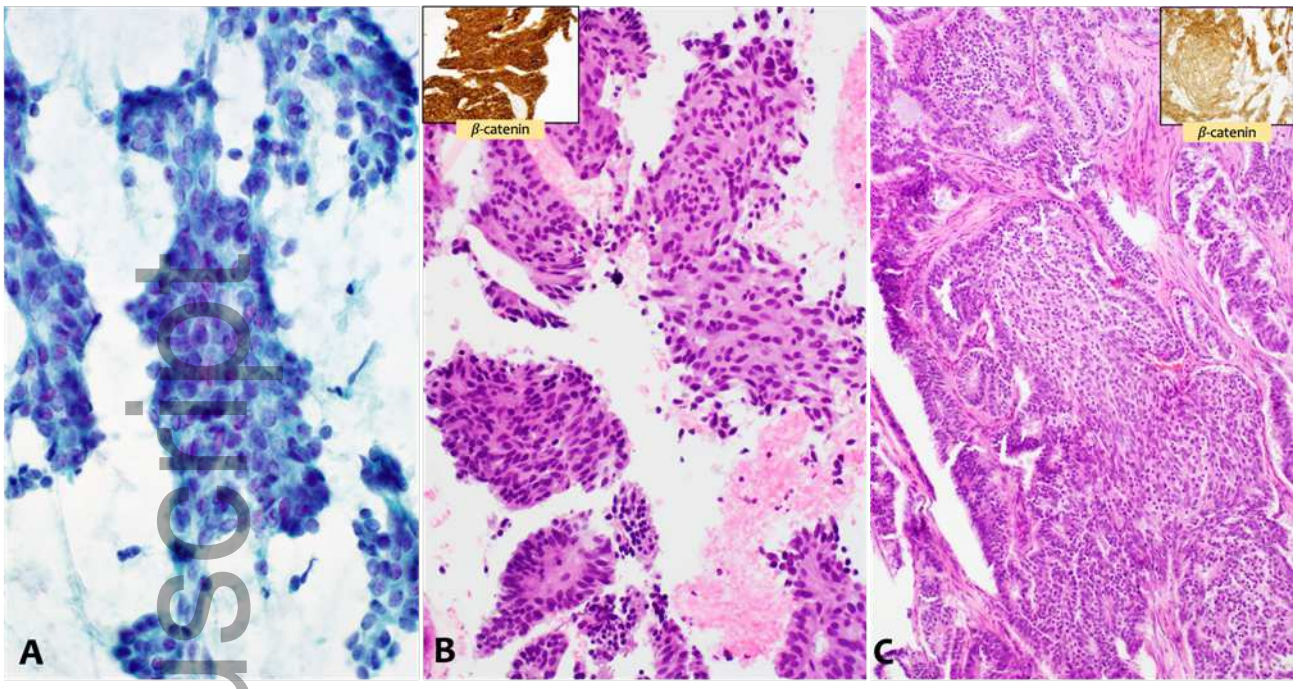

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