

ORIGINAL ARTICLE

'Atypical' Pancreatic Endoscopic Ultrasound-Guided Fine Needle Aspiration Cytology: A Study of Interobserver Agreement

Li Yan Khor, Simin Zeng, Lee Hong Song, Issam Al Jajeh, Kiat Hon Lim, Angela Takano, Syed Salahuddin Ahmed, Javed Iqbal, Wei Keat Wan, Rafay Azhar, Jacqueline Siok Gek Hwang, Sangeeta Mantoo

Department of Anatomical Pathology, Singapore General Hospital, Singapore

ABSTRACT

Objectives The Papanicolaou Society of Cytopathology's terminology scheme for endoscopic ultrasound-guided fine needle aspiration cytology includes an 'atypical' interpretation category. We conducted a retrospective study to determine the frequency of its use within our institution and the interobserver agreement among pathologists with variable experience in interpretation. **Methods** Following IRB approval, diagnoses of endoscopic ultrasound-guided fine needle aspiration procedures over a 6-year period, imaging studies and follow-up biopsy/resection results were collated. Eight pathologists blindly reviewed 18 'atypical', 5 'benign' and 5 'malignant' cases using standard reporting terms. The free-marginal kappa (K_{free}) was calculated to determine interobserver agreement. The 'atypical' reporting rate for each pathologist was also calculated. **Results** Of 598 pancreatic endoscopic ultrasound-guided fine needle aspiration, 29 (4.89%) were reported as 'atypical'; In blinded review, K_{free} showed slight agreement with the 'atypical' category (0.07) and fair agreement with the 'benign' and 'malignant' categories (0.34 and 0.22, respectively). Combining 'suspicious' and 'malignant' diagnoses increased the K_{free} to 0.50 (moderate agreement). The pathologists had an acceptable mean 'atypical' reporting rate of 5.92% (standard deviation: 5, range: 12.2-0.7%). **Conclusions** The inter-observer agreement level for 'atypical' cases was lowest of all groups. Tracking atypical rates may be useful to measure the quality of the endoscopic ultrasound-guided fine needle aspiration interpretation service and as a measure of quality assurance in routine practice.

INTRODUCTION

The role of pancreatic endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) cytology in diagnosis and management of pancreatic lesions is well-established. The Papanicolaou Society of Cytopathology recently proposed a standardized terminology scheme for pancreatobiliary specimens that correlate cytological diagnosis with biological behavior and increasingly conservative patient management of surveillance only [1]. The proposed terminology scheme recommends a six-tiered system which includes an 'atypical' interpretation category. 'Atypical' diagnoses are rendered in cases where qualitative cytologic features fall short of malignancy and overlap with those seen in benign processes. Low cellularity and premalignant changes (dysplasia) may also be included within this category. Additionally, in many instances, gastrointestinal tract contamination is

not easily distinguished from a mucinous neoplasm of the pancreas. The presence of atypical cells in a background of benign pancreatic tissue, where a neoplasm or malignancy cannot be confidently excluded, is also well-placed within this interpretation category [2]. The risk of malignancy of 'atypical' diagnoses has been shown to range from 25% to 100% [1, 3, 4, 5]. Alston *et al.* reported the risk of a pancreatic neoplasm ranges from 6% to 93%, a reflection of the range of benign to malignant/neoplastic lesions represented by this diagnosis [6].

The existing literature reports a mean 'atypical' reporting rate for EUS-FNA ranging from 1% to 14% (mean 5.3%) [4]. However, these studies do not comment on the experience of the reporting pathologists in interpreting these challenging cytologic specimens. We conducted a retrospective study to determine the frequency of use of 'atypical' in pancreatic EUS-FNA within our institution and to determine the interobserver agreement on a uniform test set of EUS-FNA smear samples in a group of pathologists with variable experience in pancreatic cytopathology smear interpretation.

MATERIALS AND METHODS

Following Institutional Review Board approval, cases of EUS-FNA procedures performed from January 2007 to December 2012 were extracted from the pathology database. Other recorded clinical data included: age, sex,

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Correspondence Sangeeta Mantoo

Department of Anatomical Pathology

Singapore General Hospital

20 College Road,

Academia, Level 10, Diagnostics Tower

Singapore 169856

Tel +65 81251835

E-mail sangeeta.mantoo@singhealth.com.sg

initial and follow-up radiological findings (either from CT scans, MRI scans or ERCP findings), EUS-FNA diagnoses and follow-up biopsy/resection information, where available. Fluid chemistry levels (for example, CEA and amylase) were not available at the time of the study.

EUS-FNA procedures were performed under conscious sedation using 22 gauge needles with a cytotechnologist present for on-site evaluation of adequacy. For on-site adequacy, only DiffQuik (Baxter, McGraw Park, IL) staining was used. Final pathology diagnosis was performed with additional Papanicolaou-stained slides as well as hematoxylin and eosin stained cell blocks where available.

Five hundred and ninety-eight EUS-FNA cases were reported during this time period as 'benign', 'malignant' or 'atypical'. 'Benign' cases included those with normal tissue like pancreatic acinar (grape-like) and ductal (monolayered sheet with well-spaced nuclei), inflammation and reactive processes with no significant cytologic atypia. 'Malignant' cases included specimens with unequivocal diagnostic features of malignancy including crowded sheets and clusters of cells with significant cytoarchitectural and nuclear atypia, discohesive single abnormal cells, mitoses and necrosis. 'Atypical' cases displayed features as described earlier (**Figure 1**). From the 598 EUS-FNA cases, twenty-eight were selected for review within this study, including 18 cases with 'atypical', 5 cases with 'benign' and 5 cases with 'malignant' diagnoses. Cases reported as 'non-diagnostic' or 'suspicious' were excluded from the initial

selection. Eight participating pathologists with variable experience in evaluating cytology specimens (3 to 20 years) blindly reviewed these cases using standard terms in reporting categories used within our institution: 'non-diagnostic' (unsatisfactory/inadequate), 'benign', 'atypical', 'suspicious' (suspicious for neoplasm) and 'malignant' (positive for neoplasm). The free-marginal kappa (K_{free}) [7] was calculated to determine the interobserver agreement. The ranges of agreement are as follows: -1.0: perfect agreement, 0.0: agreement equal to chance, 0.01-0.2: slight agreement, 0.21-0.4: fair agreement, 0.41-0.6 moderate agreement, 0.61-0.8: substantial agreement and 0.81-1.0: almost perfect agreement.

The 'atypical' reporting rate for each pathologist over the 6 year time period was also calculated by dividing the number of 'atypical' diagnoses made by the individual pathologist over the total number of cases reported for the year.

RESULTS

Five hundred and ninety-eight pancreatic EUS-FNA cases were screened during the study period of 6 years (2007 to 2012), of which 29 (4.9%) were reported as atypical. Twenty-eight cases were selected for blinded review. These patients included 15 males and 13 females with a mean age of 62 years (range: 35-84). Radiological information was available for twenty cases: 16 with a head of pancreas mass, 1 with an ampulla of Vater mass and 3

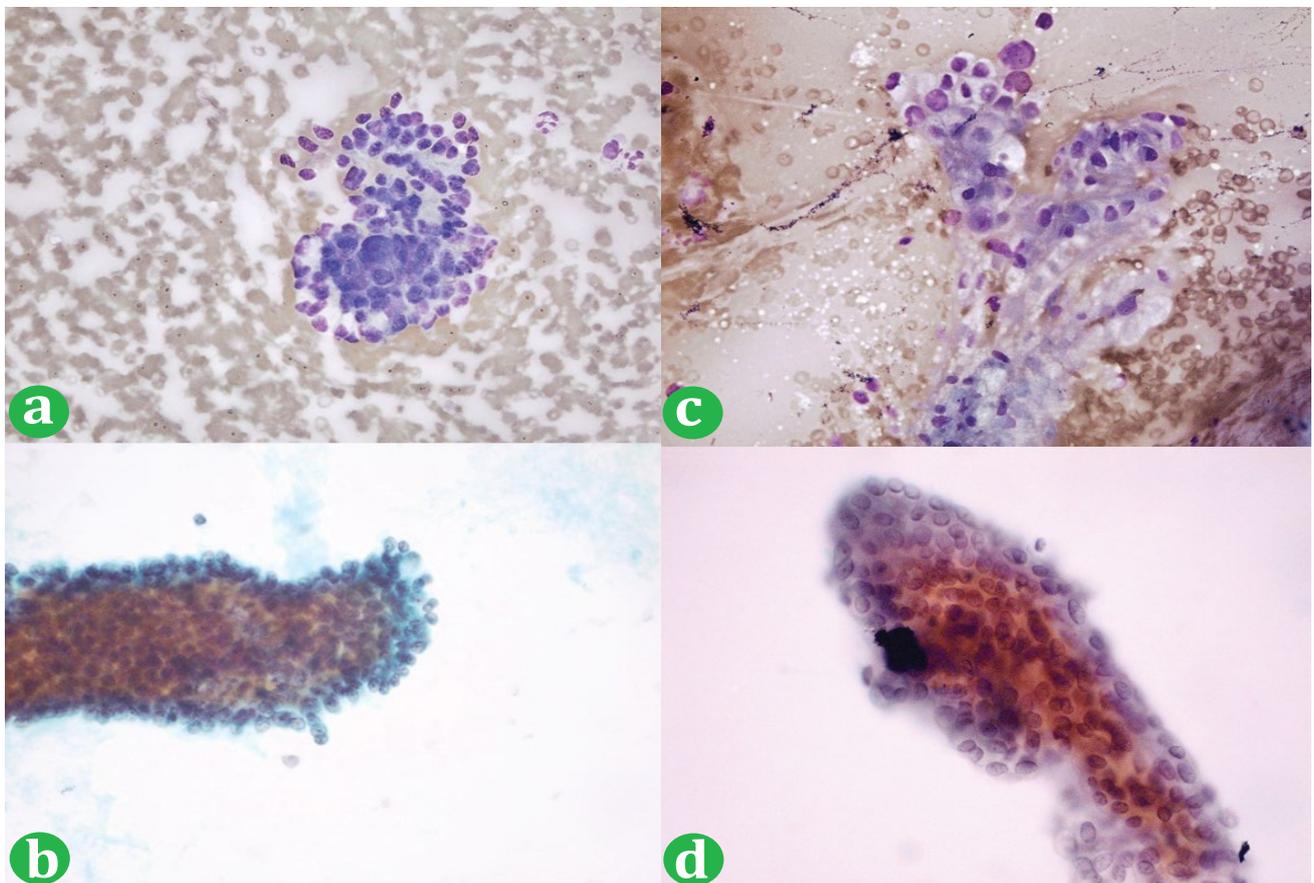


Figure 1. (a, b). 'Atypical' cells on cytology, and inflammation on final histology. (DiffQuik, magnification 20x; Papanicolaou stain, magnification 10x). (c, d). 'Atypical' cells on cytology and adenocarcinoma with mucinous and papillary features on final histology (DiffQuik, magnification 40x; Papanicolaou stain, magnification 20x).

pancreas body/tail masses by imaging studies. All, except one, were described as solid hypodense masses (**Table 1**). Of the 28 cases reviewed, 18 were originally diagnosed as 'atypical' on cytology. Thirteen (72%) of these cases were malignant on histologic follow-up, 1 was benign and 4 did not have follow-up tissue diagnoses. Following blinded review by 8 pathologists with variable experience in interpreting such smears, the free-marginal kappa (K_{free}) was calculated for the 'overall' 28 cases, 18 'atypical', 5 'benign' and 5 'malignant' cases. Slight agreement (K_{free} 0.15 and 0.07, respectively) was observed with the 'overall' and 'atypical' categories and fair agreement (K_{free} 0.34 and 0.22, respectively) was observed with the 'benign' and 'malignant' categories (**Table 2**). As 'suspicious' and 'malignant' diagnoses were used almost interchangeably among the

pathologists, the two interpretations were combined and the K_{free} calculated comparing the following diagnostic categories: 'non-diagnostic', 'benign', 'atypical' and 'suspicious/malignant'. The K_{free} increased to 0.50 (moderate agreement) for a 'suspicious/malignant' diagnosis (**Table 3**).

Five hundred and ninety-eight EUS-FNA cases were reported between January 2007 and December 2012. The yearly atypical reporting rate fluctuated between 1.6% to 7.0% with an average of 5.1% (standard deviation: 1.83) (**Table 4**). **Figure 2** shows the percentage distribution of pancreatic EUS-FNA cases reported by each of the 8 pathologists involved in the current study, as well as other pathologists within the department who did not report any atypical cases during the study period. The 8 pathologists individually reported between 3 (1%) and 140 (24%) EUS-

Table 1. Clinical data for 28 cases selected for review.

Patient (Age/Race/Sex)	Lesion/mass Site	Lesion/mass Size (cm)	Radiological Features	EUS-FNA Diagnoses	Follow-up Diagnosis	Method	
1	57/C/M	Head	1.4	Cystic	Atypical	Adenoca	f/u bx
2	59/C/F	N/A	N/A	N/A	Atypical	N/A	N/A
3	53/CA/M	N/A	N/A	N/A	Atypical	N/A	N/A
4	71/C/M	N/A	N/A	N/A	Atypical	Adenoca	f/u bx
5	63/C/M	N/A	N/A	N/A	Atypical	Adenoca	f/u bx
6	71/Ma/F	Head	3.2×3.3×1.8	Atrophic Pancreas	Atypical	N/A	N/A
7	75/C/F	Head	1.9×1.8	Poor enhancing mass	Atypical	Met	f/u bx
8	75/C/F	Head	9.7×4.3	Large thick walled cystic lesion	Atypical	Adenoca	f/u bx
9	64/C/M	Head	3.6×1.8	Mass at uncinate process	Atypical	Pancreatic cancer	Rad
10	44/Ma/F	Body	1.7×7.7	Stable mass	Atypical	Adenoca	f/u bx
11	60/C/F	Head	N/A	Dilatation main pancreatic duct	Atypical	Adenoca	f/u bx
12	40/C/M	Head	7.2×5.3	Bilobulated hypodense mass	Atypical	Adenoca	f/u bx
13	65/C/F	N/A	N/A	N/A	Atypical	N/A	N/A
14	73/C/M	Head	3.1×2.5	Cystic mass lesion	Atypical	Carcinoma	f/u bx
15	60/C/M	Body/Tail region	2.9×3.3	Cystic mass lesion	Atypical	Lymphoma	f/u bx
16	62/C/M	Head	N/A	Pancreatic duct dilatation	Atypical	Autoimmune Pancreatitis	rad
17	66/C/M	Head	4.3×3.8×4.9	Lobulated hypoenhancing focus at unicate process	Atypical	Lymphoma	f/u bx
18	35/O/F	Head	2.9×2.6	Ill-defined hypodense mass in unicate process	Atypical	Adenoca	rad
19	59/C/F	N/A	11.6×10.6	Large solid and cystic mass involving pancreas	Malignant	Adenoca	rad
20	71/C/F	Head	N/A	Pancreatic head swelling	Malignant	Carcinoma	rad
21	75/C/F	Ampulla of vater	1.2	Nodule at ampulla of vater	Malignant	Adenoca	f/u bx
22	70/C/M	Body	4.2×2.6	Pancreatic hypodense mass	Malignant	Met	rad
23	84/C/M	N/A	N/A	N/A	Malignant	Adenoca	f/u bx
24	60/C/M	Head	N/A	Suspicion of a low-density mass at the head of pancreas.	Benign	PanIN 1B-II	f/u bx
25	62/C/F	N/A	N/A	N/A	Benign	N/A	N/A
26	64/C/F	Head and Tail	2 and 2.4 respectively	Pancreas mass in the head and tail due to tumour	Benign	N/A	N/A
27	56/C/M	Head	3.2×2.5	Narrowing of main pancreatic duct	Benign	Pancreatitis	rad
28	52/C/M	Head/Tail region	1.6×1.9	irregular hypodense mass	Benign	Adenoca	f/u bx

Adenoca adenocarcinoma; PanIN 1B pancreatic intraepithelial neoplasia 1B; Met metastasis; f/u bx: follow-up biopsy; rad radiological data; C chinese; Ca caucasian; Ma malay; M male; F female N/A Denotes fields where information was unavailable.

Table 2. Inter-observer agreement amongst eight pathologists following blinded review.

	K_{free}	Interpretation of agreement level
Overall 28 cases	0.15	Slight agreement
5 Benign cases	0.34	Fair agreement
5 Malignant cases	0.22	Fair agreement
18 Atypical cases	0.07	Slight agreement

Table 3. Inter-observer agreement amongst eight pathologists after combining the ‘suspicious’ and ‘malignant’ categories.

	K_{free}	Interpretation of agreement level
Overall 28 cases	0.23	Fair agreement
5 Malignant cases	0.50	Moderate agreement
18 Atypical cases	0.13	Slight agreement

Table 4. Atypical reporting rate for pancreatic EUS-FNA smears (2007 to 2012).

Year	Total Number of Pancreas EUS-FNA	Atypical cases (n)	Atypical rate (%)
2012	138	8	5.8
2011	127	2	1.6
2010	86	5	5.8
2009	86	6	7.0
2008	75	4	5.3
2007	77	4	5.2
Total	598	29	

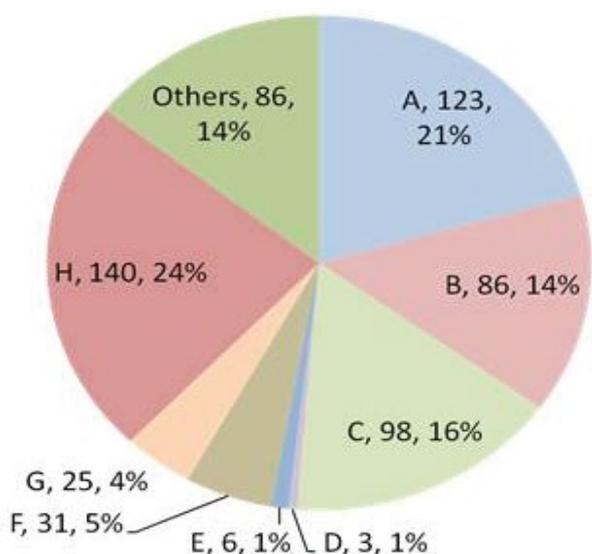


Figure 2. Percentage distribution of pancreatic EUS-FNA cases reported by each pathologist, ‘A’ to ‘H’ (‘Others’ refer to pathologists that did not report any atypical cases from 2007 to 2012).

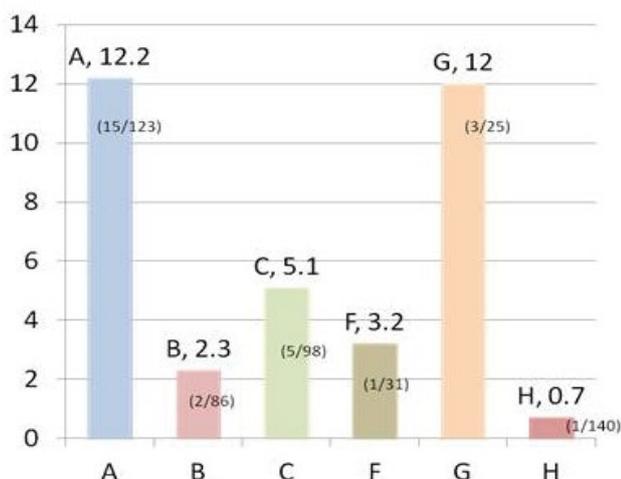


Figure 3. Atypical reporting rate of eight pathologists, ‘A’ to ‘H’ (pathologists ‘D’ and ‘E’ were excluded as they reported less than 20 pancreatic EUS-FNA cases from 2007-2012).

FNA cases within the study period. The mean ‘atypical’ reporting rate was 5.9% (standard deviation: 5, range: 12.2-0.7%); two pathologists who reported less than 20 EUS-FNA cases were excluded (**Figure 3**).

DISCUSSION

In their review of EUS-FNA samples reported within their institution, Saieg *et al.* applied the newly proposed standardized terminology scheme to 155 cases reported over a 12-month period. Twenty-nine (18.7%) ‘atypical’ diagnoses were re-interpreted as ‘neoplastic-other’ as these cases were of cystic neoplasms with no features of high grade dysplasia [8]. This reiterates the importance of knowledge of radiological findings when interpreting such specimens. Cyst fluid CEA and amylase levels are also helpful to the cytopathologist in rendering an accurate diagnosis [9, 10]. This additional information would no doubt have improved the interobserver agreement in this study. However, the clinico-radiological findings were not made available to the reviewing pathologists in this study in order to assess interpretation differences based solely on the cytomorphologic findings. In the case of cystic lesions, fluid CEA and amylase levels were not recorded as those tests were not available within our institution at the time of the study. Additionally, the interpretation categories were kept at 5 tiers as they are the standard reporting terms the pathologists used in our institution before the introduction of the fairly new 6-tier system. For the purposes of our study, which was to assess differences in the ‘atypical’ interpretation, the exclusion of a ‘Neoplastic-benign/other’ category was deemed acceptable.

The inter-observer agreement level for benign and malignant control cases was ‘fair’ (0.34 and 0.22, respectively). This was lower than expected and could be attributed to the wide range of interpretive experience (3 to 20 years) and confidence level among the participating pathologists. The ‘atypical’ reporting rates recorded in the literature range from 1% to 14% (mean, 5.3%) [4]. The mean ‘atypical’ reporting rate of 5.9% within our institution for the 6 year study period falls well within this range. The differences in individual ‘atypical’ reporting rates amongst cytopathologists were remarkably different, although the number of cases reported by each pathologist also varied widely (**Figure 3**), reiterating the fact that likely contributing factors for these differences include frequency of interpreting such cases, the varying years

of practice experience, confidence level and expertise in pancreatic cytopathology smear interpretation, excluding the limitations due to the inherent nature of the lesion or material itself [11]. This was similarly shown by Schneider *et al.* who reported improved rates of sensitivity and negative predictive value of EUS-FNA performed at a specialized cytology institute compared to a standard pathology service [12].

In our study, the overall inter-observer agreement for the 28 pancreas EUS-FNA smears after review was poor, especially for the 18 atypical smears. It improved after combining the 'suspicious' and 'malignant' categories together, especially for the 5 malignant cases, which suggests that, as expected, the pathologists had stronger threshold levels for a 'malignant' diagnosis. The inter-observer agreement level for the 18 atypical cases was the lowest compared to the other diagnostic groups reviewed (5 benign and 5 malignant cases). There are many well-documented reasons why cytological smears are interpreted as atypical, including low specimen cellularity, gastro-intestinal tract contaminants, poor cell preservation and under-diagnosis [9]. The variability in both the atypical reporting rates and overall poor inter-observer agreement amongst reviewing pathologists has implications for practice as well as education. In low-volume centers, cytopathologists and cytopathology trainees who have limited exposure to pancreatic EUS-FNA's or a limited range of benign to malignant/neoplastic lesions which are sampled need to be cautious in interpretation until adequate experience is gained, and with the hindsight of histologic correlation. Where available, the addition of cell blocks can be useful to provide additional information in rendering a diagnosis. However, these are ever dependent on adequate sampling. We performed a cell block utility study using paired smears and cell blocks resulting in 63 (83%), 9 (11%) and 2 (3%) of 73 cases confidently diagnosed as malignant, benign and non-diagnostic, respectively, with the addition of cell block material and only 2 (3%) cases remained as 'atypical' (data not shown).

This is a single center study that has evaluated the difficult area of the 'atypical' diagnostic category in pancreatic EUS-FNA. We have established our rates and compared them against the published literature and studied interobserver variations in the reporting of this category. Additionally, quality criteria and thresholds in non-gynecological cytology are not well-established in such specimens. Our results may provide the basis for developing atypical rates as a measure of quality both as a service as a whole as well as for tracking in routine practice as an additional measure of quality assurance.

CONCLUSION

The overall poor agreement level amongst participating pathologists indicates that occasional interpretation of pancreatic cytology should be discouraged. In order to be a reliable and responsible interpreter of pancreatic EUS-FNA cytology samples, regular and frequent exposure to such specimens is essential. The results also emphasize

the limitations of evaluating EUS-FNA samples solely based on cytomorphology. Adjunct imaging, fluid chemistry and clinical information are essential to increase diagnostic accuracy. Proper training guidelines and aspects on workload should be established in the development of a high-quality pancreatic EUS service. In addition, development of quality control based on aggregated and individualized reporting rates for the 'atypical' may serve as a simple tool for improving and monitoring of a routine EUS service.

Ethics Statement

Subjects have given their informed consent. This study protocol has been approved by the institute's committee on human research and thus meets the standards of the Declaration of Helsinki in its revised version of 1975 and its amendments of 1983, 1989, and 1996 [JAMA 1997;277:925-926].

Conflict of Interest

The authors have declared that no competing interests exist.

References

1. Pitman MB, Centeno BA, Ali SZ, Genevay M, Stelow E, Mino-Kenudson M, et al. Standardized terminology and nomenclature for pancreaticobiliary cytology: The Papanicolaou Society of Cytopathology Guidelines. *Cytojournal* 2014; 11:3. [PMID: 25191517]
2. Bergeron JP, Perry KD, Houser PM, Yang J. Endoscopic ultrasound-guided pancreatic fine-needle aspiration: potential pitfalls in one institution's experience of 1212 procedures. *Cancer Cytopathol* 2015; 123:98-107. [PMID: 25410732]
3. Bellizzi AM, Stelow EB. Pancreatic cytopathology: a practical approach and review. *Arch Pathol Lab Med* 2009; 133:388-404. [PMID: 19260745]
4. Abdelgawwad MS, Alston E, Eltoum IA. The frequency and cancer risk associated with the atypical cytologic diagnostic category in endoscopic ultrasound-guided fine-needle aspiration specimens of solid pancreatic lesions: a meta-analysis and argument for a Bethesda System for Reporting Cytopathology of the Pancreas. *Cancer Cytopathol* 2013; 121:620-8. [PMID: 23881871]
5. Layfield LJ, Schmidt RL, Hirschowitz SL, Olson MT, Ali SZ, Dodd LL. Significance of the diagnostic categories "atypical" and "suspicious for malignancy" in the cytologic diagnosis of solid pancreatic masses. *Diagn Cytopathol* 2014; 42:292-6. [PMID: 24578254]
6. Alston E, Bae S, Eltoum IA. Atypical cytologic diagnostic category in EUS-FNA of the pancreas: follow-up, outcomes, and predictive models. *Cancer Cytopathol* 2014; 122:428-34. [PMID: 24436110]
7. Brennan RL, Prediger, DJ. Coefficient Kappa: Some uses, misuses, and alternatives. *Educ Psychol Meas* 1981; 41: 687-699.
8. Saieg MA, Munson V, Colletti S, Nassar A. The impact of the new proposed Papanicolaou Society of Cytopathology terminology for pancreaticobiliary cytology in endoscopic US-FNA: A single-Institutional experience. *Cancer Cytopathol* 2015; 123:488-94. [PMID: 25994860]
9. Opong KW, Dawwas MF, Charnley RM, Wadehra V, Elamin K, White S, et al. EUS and EUS-FNA diagnosis of suspected pancreatic cystic neoplasms: Is the sum of the parts greater than the CEA? *Pancreatology* 2015; 15:531-7. [PMID: 26375415]
10. McKinley M, Newman M. Observations on the application of the Papanicolaou Society of Cytopathology standardised terminology and nomenclature for pancreaticobiliary cytology. *Pathology* 2016; 48:353-6. [PMID: 27114371]

11. Payne M, Staerke G, Gong Y. Indeterminate diagnosis in fine-needle aspiration of the pancreas: reasons and clinical implications. *Diagn Cytopathol* 2009; 37:21-9. [PMID: 18973122]

12. Schneider AR, Nerlich A, Topalidis T, Schepp W. Specialized clinical cytology may improve the results of EUS (endoscopic ultrasound)-guided fine-needle aspiration (FNA) from pancreatic tumors. *Endosc Int Open* 2015; 3:E134-7. [PMID: 26135655]
