Novel Cytological Diagnostic Scoring System for Pancreatic Specimens Obtained by Endoscopic Ultrasound-Guided Fine Needle Aspiration

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Abstract

Background: Cytological diagnosis of pancreatic specimens obtained by endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) is often challenging because of the small amount of sample or well-differentiated adenocarcinoma with weak cytological atypia. Therefore, the sensitivity and specificity of cytological diagnosis for pancreatic cancer should be improved. Hence, we aimed to evaluate the indices, which are used to distinguish malignant from benign lesions for the cytological diagnosis of pancreatic EUS-FNA specimens.

Methods: Seven reviewers, including 3 cytotechnologists and 4 medical doctors, evaluated 20 morphological indices in pancreatic specimens obtained by EUS-FNA (malignant, n=111; benign, n=31). Statistical analyses were performed using Fisher's exact test, logistic regression analysis, area under the receiver operating characteristic curve, and Youden Index.

Results: Among the 20 indices, there was a high incidence rate (>40%) of the following 13 indices in malignant cases: structural atypia, hyperchromatic nucleus, irregular cell polarity, unclear cell boundary, nuclear membrane thickening, anisonucleosis, overlapping, irregular nuclei, high nuclear/cytoplasmic ratio, binding decline, simultaneous appearance of malignant and benign cells, enlarged nucleoli, and background necrosis. When we diagnosed pancreatic specimens using these 13 cytological indices, the cutoff value of 8/9 showed the highest Youden index (0.950) as well as high sensitivity and specificity in distinguishing malignant from benign specimens (98% and 97%, respectively).

Conclusion: Thirteen cytological indices showed high sensitivity and specificity in differentiating malignant and benign lesions using pancreatic EUS-FNA samples. Further validation or prospective studies are necessary to establish criteria for the cytological diagnosis of pancreatic cancer.

Introduction

The rates of mortality and morbidity associated with pancreatic cancer have been increasing worldwide.[1] The prognosis of pancreatic cancer remains poor, with an overall 5-year survival rate of less than 10% due to its aggressive growth and high rate of metastasis. Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) allows for repeated, minimally invasive sampling from the pancreas,[2] and has 85%–91% sensitivity for pathological diagnosis of pancreatic cancer.[3] Previous studies reported that atypical cytological findings by pancreatic EUS-FNA highly indicated pancreatic neoplasms (55%) including cancer (36%).[4] Cytological diagnosis was also highly specific for detection of pancreatic cystic tumors such as intraductal papillary mucinous neoplasm[5] and mucinous cyst neoplasm.[6,7] Thus, cytological diagnosis using pancreatic EUS-FNA provides important information on the indications for surgery.[5,8,9] The Japanese Pancreas Society recommends EUS-FNA as the first choice for pathological diagnosis of pancreatic cancer.[10] Although most pathological diagnoses using EUS-FNA samples are straightforward, some inconclusive pathological findings were reported, especially in small and/or degraded samples, and when differentiating atypical epithelium and well-differentiated cancer with weak

cytological atypia, as well as pancreatic mucinous neoplasms from the contaminants of gastric mucosa.[11] Furthermore, current cytological criteria have several problems such as; 1) until recently there was no widely accepted categorization criteria for pancreatic cytology obtained by EUS-FNA, 2) cytological criteria and standard definition of pancreatobiliary cytology have not been well established, 3) important cytological indices to distinguish malignant from benign have not been well clarified.

Several studies have attempted to identify the definite cytologic criteria, which can predict malignancy in bile duct and pancreatic duct brushings.[12-17] Avadhani et al. reported that 11 cytological indices could be helpful in identifying malignancy in cytological specimens obtained by bile duct brushings.[18] Robins et al. proved that nuclear overlapping, nuclear contour irregularity, and irregular chromatin distribution were the best predictors of malignancy in pancreatic CT-guided FNA specimens.[19] Chi et al. found that the combination of at least two of four indices, including three-dimensional cluster, isolated malignant cells, irregular nuclear contour/nuclear groves/notches, and marked nuclear size variation can help diagnose pancreatic adenocarcinoma in specimens obtained by EUS-FNA.[20]

The cytological morphology of EUS-FNA samples are different from that obtained by other procedures; however, it has not been well clarified. Cytological criteria for pancreatic EUS-FNA samples might be necessary because EUS-FNA has now become a common diagnostic procedure for pancreatic tumors. In the present study, we aimed to investigate specific cytologic criteria, which are useful for both cytotechnologists and medical doctors, for the accurate pathological diagnosis of malignancy in pancreatic EUS-FNA specimens.

Materials And Methods

Specimen selection

Pancreatic EUS-FNA smear specimens (n=142) were obtained from 119 patients admitted to the Tokyo Metropolitan Geriatric Hospital between 2013 and 2018 (men, n=56; women, n=63; median age, 76 years; range, 39–92 years). Cytologic specimens were routinely stained with Papanicolaou stain.[32,33] We selected 111 malignant and 31 benign samples for the present study according to the following criteria: (1) malignant cases had a definitive histological diagnosis of adenocarcinoma, either by biopsy (n=110) or both biopsy and resection (n=9). Of the 111 cases, 110 were diagnosed as malignant by biopsy. In one case, biopsy revealed no pancreatic epithelium; however, cytology and resected specimens were malignant. Nine of the 111 cases were diagnosed as malignant with both biopsy and resected specimens; (2) benign cases had a definitive histological diagnosis of non-malignancy by biopsy (n=31) and a clinical uneventful follow-up of 2-70 months (median value, 35 months). In our institute, we initially diagnose the specimens with FNA using cytology specimens, and then separately diagnose using biopsy specimens (H&E staining and immunostaining). Benign cases included patients with pancreatitis, simple pancreatic cysts, and low-grade dysplasia. Malignant cases included patients with invasive adenocarcinoma and high-grade dysplasia, as it is impossible to distinguish carcinoma *in situ* from invasive carcinomas using EUS-FNA samples. All methods were performed in accordance with the

guidelines and regulations of the Research Ethics Committee of the Tokyo Metropolitan Geriatric Hospital. **Informed consent for study participation**was obtained. The present study was approved by the Research Ethics Committee of the Tokyo Metropolitan Geriatric Hospital (approval number: R17-58).

Review

The seven reviewers included two certified pathologists who are licensed medical specialists from the Japanese Society of Pathology and Japanese Society of Clinical Cytology, one senior trainee pathologist, and four cytotechnologists who are licensed specialists from the Japanese Society of Clinical Cytology. All reviewers were blinded to all clinical or radiologic information, histologic diagnoses, and other reviewers' diagnoses. For each case, the seven reviewers evaluated the presence or absence of 20 indices using two microscope glass slides with Papanicolaou stain.

Statistical analysis

A universal standard was developed to clarify the correlation between years of experience and qualifications of 20 indices. In accordance with this universal standard, the presence or absence of these 20 indices was determined. The agreement significantly associated with malignant cases was examined using Fisher's exact test. Logistic regression analysis was used to calculate the odds ratios for individual indices and for malignancy. The number of malignant indices present was calculated for each sample, and the sensitivity, specificity, and accuracy were evaluated for each cutoff point using the Youden Index. *P*-values of < 0.05 (two sided) were considered statistically significant. Then, we compared various cutoffs for high agreement. All analyses were conducted using SPSS version 20 (IBM, Armonk, NY, USA).

Results

Evaluation of 20 indices in malignant and benign cases

The presence or absence of the following 20 cytological indices was evaluated by 7 reviewers based on the results of previous studies:[18,21] (1) necrotic background (Fig. 1A), (2) mucus background (Fig. 1B), (3) hypercellularity (Fig. 1C), (4) two-cell pattern (Fig. 1D), not including contamination of digestive epithelial cells, (5) irregular structure (Fig. 2A), (6) irregular cell polarity (Fig. 2B), (7) overlapping (Fig. 2C), (8) decreased cell adhesion (Fig. 2D), (9) irregular nuclei (Fig. 3A), (10) hyperchromasia (Fig. 3B), (11) nuclear membrane thickening (Fig. 3C), (12) anisonucleosis (Fig. 3D), which is the presence of a mixture of various cell sizes, (13) prominent nucleoli (Fig. 3E), (14) increased mitosis (Fig. 3F), which is indicated by the presence of an increased number of mitotic cells, (15) unclear cell boundary (Fig. 4A), which is indicated by the presence of overlapping cells and unclear cellular boundaries, (16) high nuclear/cytoplasm (N/C) ratio (Fig. 4B), (17) pink intracellular mucus (Fig. 4C), (18) orange-yellow intracellular mucus (Fig. 4D), (19) cannibalism (Fig. 4E), and (20) keratinization (Fig. 4F), which is an important cytologic feature to predict adenosquamous carcinoma. Most of these are typical cytological indices of malignant cells in various organs. The presence of a two-cell pattern, which is the coexistence of malignant and benign cells (Fig. 1D), aids in identifying malignant cells with relatively lower atypia.[18]

The indices related to mucins are specific to malignant cells of the pancreas based on our previous study.[21]

Usefulness of 20 indices to distinguish malignant and benign cases

Seven reviewers, including 4 cytotechnologists and 3 medical doctors, evaluated 20 cytological indices using pancreatic specimens obtained by EUS-FNA (malignant, n=111; benign, n=31). Figure 5 shows the percentages of the presence of these 20 indices among the included specimens. This was used to distinguish malignant from benign tumors. Thirteen indices had an incidence rate of more than 40%, when the specimens were evaluated by cytotechnologists (Fig. 5A), medical doctors (Fig. 5B), and all reviewers combined (Fig. 5C). Furthermore, these 13 indices showed statistically significant differences between malignant and benign cases, when evaluated by cytotechnologists (Fig. 5A), medical doctors (Fig. 5B), and all reviewers combined (Fig. 5C), (*P<0.05). Hypercellularity showed both a high presence rate and statistically significant difference between malignant and benign cases, when evaluated by all reviewers and cytotechnologists, but not when evaluated by medical doctors (Fig. 5).

The 13 indices (gray boxes in Table 1) had higher odds ratios for malignancy than benignity. Several odds ratios could not be determined (N.D.) because the agreement was 0 or 100.

The useful indices to distinguish malignant and benign cases should have the following properties: (1) high incidence rate in malignant cases, (2) statistically significant difference ($P < 0.05$) between malignant and benign cases, and (3) reproducibility in various groups with different backgrounds and skills. Therefore, we determined that the following 13 cytological indices were useful to distinguish malignant and benign cases: irregular structure, hyperchromasia, irregular cell polarity, unclear cell boundary, nuclear membrane thickening, anisonucleosis, overlapping, irregular nuclei, high N/C ratio, decreased cell adhesion, two-cell pattern, prominent nucleoli, and necrotic background.

Cutoff values of the 13 indices indicating tumor malignancy

Each of the 13 indices was given a score of 1 point, based on the evaluation of 4 out of 7 reviewers (Table 2). Malignant cases had higher scores than benign cases (Table 2). To determine the cutoff values of the scores of all 13 malignant indices, we calculated the Youden index.[22] A score of 8 points compared with a score of > 9 points showed the highest Youden index (0.950, Table 3). The cutoff value 8/9 showed high sensitivity (98%, Table 3), specificity (97%), accuracy (98%), and high area under the receiver operating characteristic curve value (0.996).

Discrepancy between score and pathological diagnosis

At a cutoff value of 8/9, 3 cases showed a discrepancy between cytological score and pathological diagnosis (Table 2A, B and C and Supplementary Table 1). Cytological specimens of case A lacked malignant features with contamination of many red blood cells (Fig. 6A). The cytological specimens of case B contained only a small number of cells (Fig. 6B). The cytological specimen of case C showed

epithelial cells with enlargement of the nucleus, nuclear groove, and yellow mucus (Fig. 6C), and case C was diagnosed as mild atypical epithelium but not malignancy.

Discussion

The present study revealed that (1) among the 20 cytological indices, 13 indices showed statistically significant differences between malignant and benign cases, and had high incidence rates in malignant cases; (2) our new scoring system showed high sensitivity and specificity to distinguish malignant and benign lesions in pancreatic EUS-FNA samples.

Previous reports on cytological diagnosis of pancreatic cancers have also shown the usefulness of several cytological features such as high N/C ratio,[23] nuclear membrane thickening,[14,24] anisonucleosis,[20,25] and irregular cell polarity[26,27]. In the present study, these 4 cytological features showed high odds ratios (> 100) and incidence rates (over 90%), suggesting that they are critical for pancreatic cancer diagnosis. Furthermore, we established a new scoring system for pancreatic cancer diagnosis using a combination of cytological indices.

We had previously reported that atypical pancreatobiliary glands with pink mucus (probably sulfomucin) suggested malignancy, and yellow-orange mucin (probably neutral mucin) suggested benignity.[21] In the present study, pink intracellular mucus was detected only in malignant cases and not in benign cases, but its presence in malignant cases was low (12%–44%). These results indicate that pink intracellular mucus possesses high specificity and low sensitivity to distinguish malignant and benign tumors.

The difference in hypercellularity was statistically significant between malignant and benign tumors, when evaluated by cytotechnologists, but not by medical doctors. This might be due to the fact that, in Japan, cytotechnologists routinely evaluate hypercellularity but medical doctors do not.

Among the 20 indices evaluated in the present study, mucus background, yellow-orange mucin in cytoplasm, cannibalism enclosed image, and keratinization did not have statistically significant differences between malignant and benign cases, when evaluated by all reviewers, suggesting that they were not useful to distinguish between malignant and benign tumors.

The sensitivity and specificity of our new scoring system using the 13 cytological indices were higher than those of Avadhani who reported similar diagnostic criteria for the cytological diagnosis of bile duct samples.[18] Pancreatic EUS-FNA can provide fresh samples with few degenerative changes, while the bile duct sample showed degeneration based on the results of bile juice examination. The results showed that the sensitivity and specificity of our scoring system were high, and all 13 indices were useful for diagnosing malignancy by cytology of pancreas obtained by EUS-FNA.

The present study has several limitations. We focused mainly on indices of malignant morphology and not those of benign morphology here. This was a retrospective study using malignant and benign samples obtained by pancreatic EUS-FNA. Hence, prospective and multicenter studies are needed to

determine the usefulness of the diagnostic criteria. Furthermore, we need to evaluate the usefulness of the diagnostic criteria using various samples, including pancreatic juice and liquid-based cytology (LBC).[29-31] This was a single-institution retrospective study, so the result needs to be evaluated in other hospitals.

In conclusion, we introduced a new scoring system for cytologic diagnosis using pancreatic EUS-FNA samples. We proved that all the 13 indices were important findings for diagnosing malignancy in the pancreatic cytology smear of EUS-FNA. A further validation study is warranted.

Declarations

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Author contributions

Y.M., T.I., and T.A. designed the study. Y.K. and Y.M. wrote the initial draft of the manuscript. Y.K., Y.M., S.E., Y.H., H.S., M.K., and T.A. evaluated the cytological specimens. Y.F. and M.M. selected the cases based on the information obtained from the medical records. T.I. contributed to the analysis and interpretation of data and assisted in the preparation of the manuscript. All authors approved the final version of the manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

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Conflict of interest

The authors declare that they have no conflict of interests.

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Tables

Table 1. Odds ratio and 95% confidence interval of 20 indices in malignant cases compared with benign cases

	OR	95% CI of OR	*P*-value
Irregular structure	N.D.	N.D.	N.D.
Hyperchromasia	N.D.	N.D.	N.D.
Irregular cell polarity	316.3	37.70–2652.89	< 0.0001
Unclear cell boundary	458.3	52.80–3978.58	< 0.0001
Nuclear membrane thickening	458.3	52.80–3978.58	< 0.0001
Anisonucleosis	572	64.07–5106.65	< 0.0001
Overlapping	1026.7	102.84–10249.21	< 0.0001
Irregular nuclei	99.1	20.41–481.21	< 0.0001
High N/C ratio	186.9	36.52–956.01	< 0.0001
Hypercellularity	14.6	5.70–37.20	< 0.0001
Decreased cell adhesion	18.4	6.97–48.60	< 0.0001
Two-cell pattern	26.4	7.46–93.39	< 0.0001
Prominent nucleoli	70.9	9.28–541.81	< 0.0001
Necrotic background	30.2	6.83–133.63	< 0.0001
Pink intracellular mucus	N.D.	N.D.	N.D.
Mucinous background	0.6	0.23–1.34	0.1883
Orange-yellow intracellular mucus	0.5	0.21–1.06	0.0705
Increased mitosis	N.D.	N.D.	N.D.
Cannibalism enclosed image	N.D.	N.D.	N.D.
Keratinization	N.D.	N.D.	N.D.

OR, odds ratio; CI, confidence interval; N.D., not detected. *P*-value was determined by logistic regression between malignant and benign cases. Gray boxes indicate 13 indices to distinguish malignant from benign.

Table 2. Number of cases with the 13 cytological scores

Score	Malignant, N (%)	Benign, N (%)
0	0 (0)	8 (26)
1	0 (0)	9 (29)
2	0 (0)	3 (10)
3	0 (0)	1 (3)
4	1[A] (1)	1 (3)
5	0 (0)	1 (3)
6	0 (0)	3 (10)
7	0 (0)	2 (6)
8	1[B] (1)	2 (6)
9	5 (5)	1[C] (3)
10	1 (1)	0 (0)
11	25 (23)	0 (0)
12	35 (32)	0 (0)
13	43 (39)	0 (0)

The gray boxes indicate the malignant scores at a cutoff value of 8/9 based on the results of Table 3. "A, B, C" indicate cases with discrepancy between scoring and pathological diagnosis (details in Supplementary Table 1).

Table 3. Sensitivity, specificity, and accuracy of 13 malignant scores at various cutoff points

Score cut point	Sensitivity	Specificity	Accuracy	Positive predictive value	Negative predictive value	Youden Index
0 compared with > 1	100	26	84	83	100	0.258
1 compared with > 2	100	55	90	89	100	0.548
2 compared with > 3	100	65	92	91	100	0.645
3 compared with > 4	100	68	93	92	100	0.677
4 compared with > 5	99	71	93	92	96	0.701
5 compared with > 6	99	74	94	93	96	0.733
6 compared with > 7	99	84	96	96	96	0.830
7 compared with > 8	99	90	97	97	97	0.894
8 compared with > 9	98	97	98	99	94	0.950
9 compared with > 10	94	100	95	100	82	0.937
10 compared with >11	93	100	94	100	79	0.928
11 compared with >12	70	100	77	100	48	0.703
12 compared with >13	39	100	52	100	31	0.387

Figures

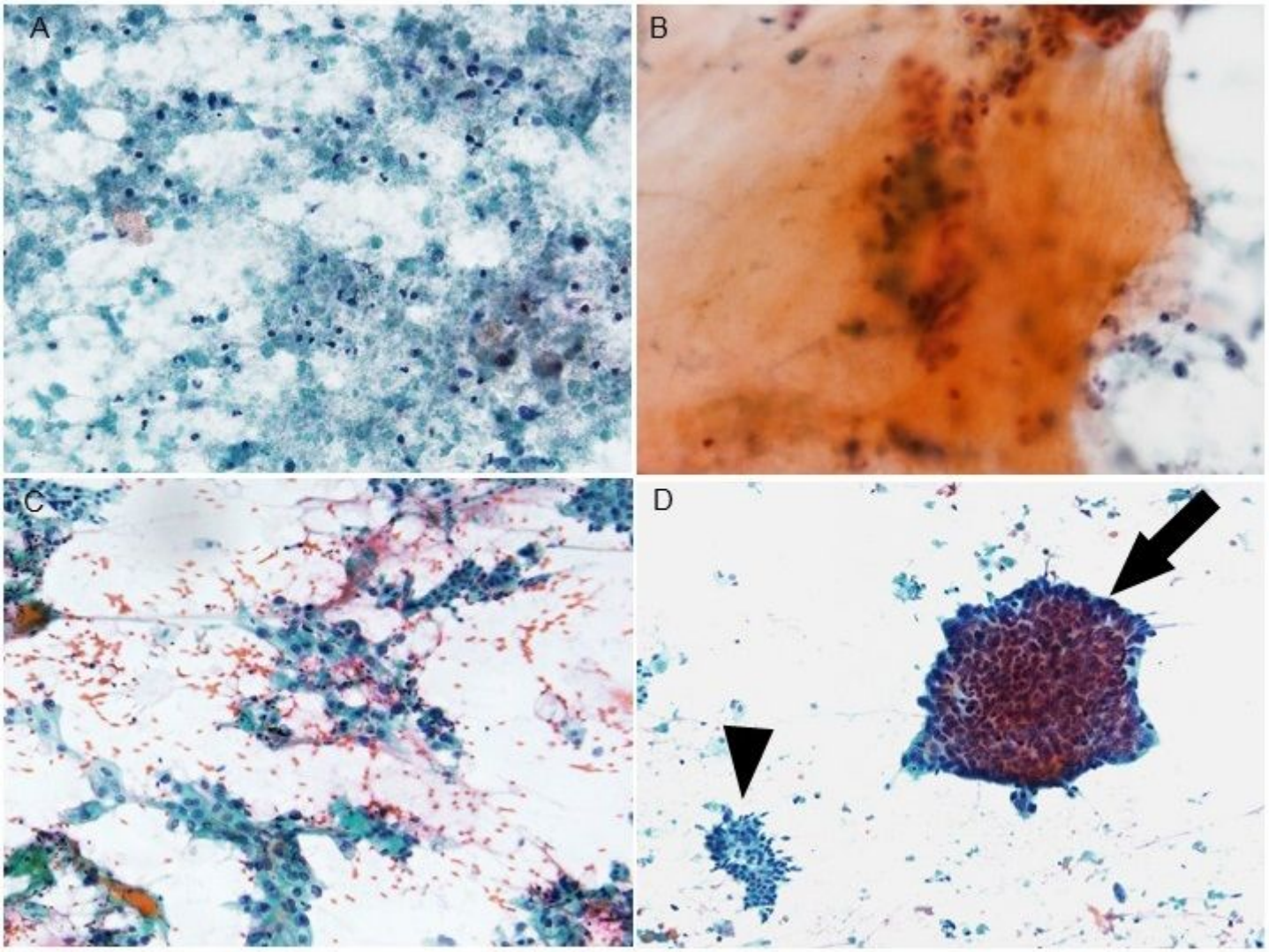

Figure 1

Abnormal findings in the background and cellularity of malignant pancreatic samples obtained by EUS-FNA. (A) Necrotic background. (B) Mucinous background. (C) Hypercellularity. (D) Two-cell pattern. Benign cell cluster (arrowhead) and malignant cell cluster (arrow) exist simultaneously. Original magnification, ×400 in A, B; ×100 in C, D. Papanicolaou stain; EUS-FNA: Endoscopic ultrasound-guided fine needle aspiration

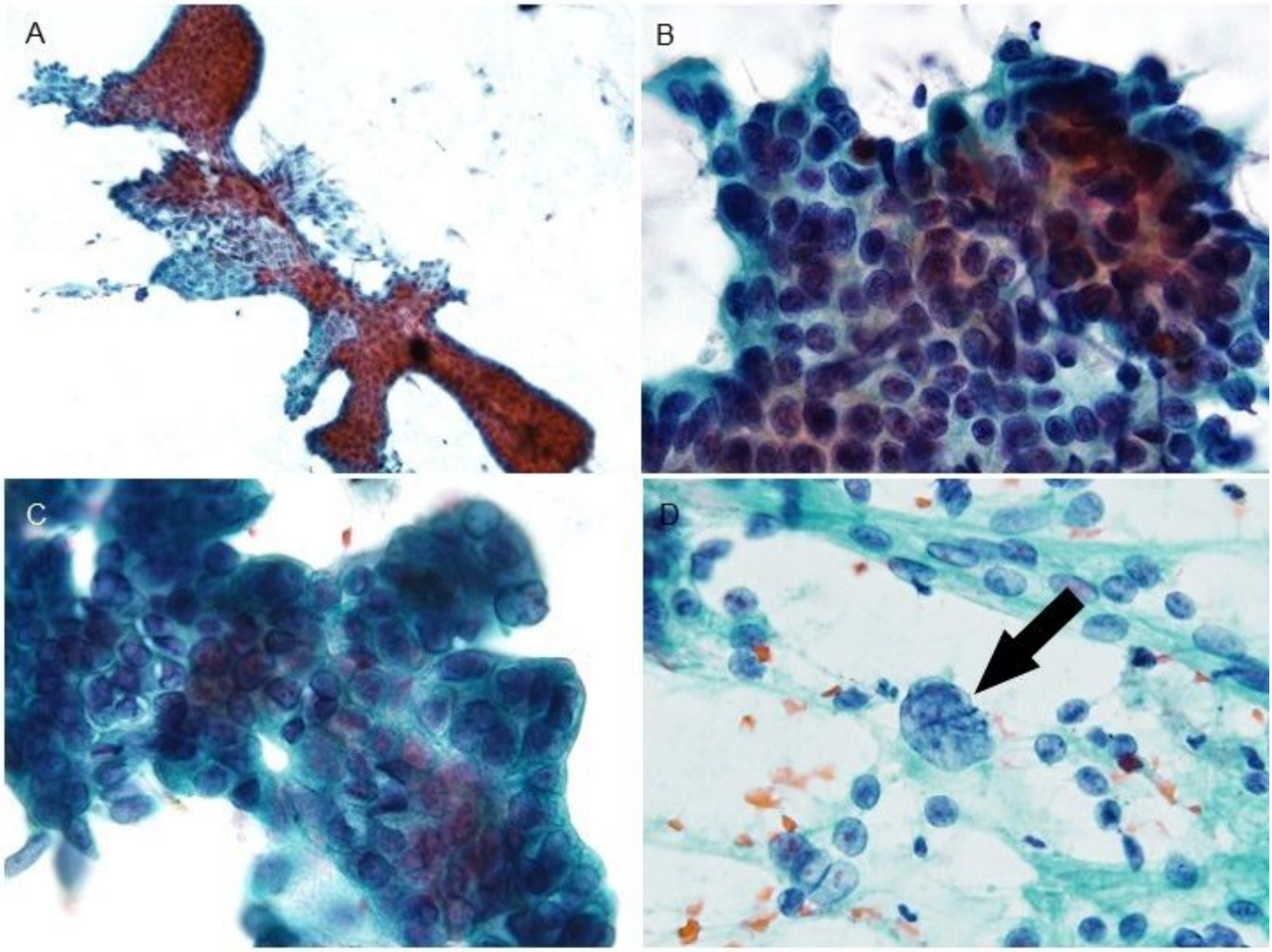

Figure 2

Abnormal structure of malignant pancreatic samples obtained by EUS-FNA. (A) Irregular structure. (B) Irregular cell polarity. (C) Overlapping. (D) Decreased cell adhesion, and the cells were detached from the cluster (arrow). Original magnification, ×100 in A; ×400 in B, C, and D. Papanicolaou stain; EUS-FNA: Endoscopic ultrasound-guided fine needle aspiration

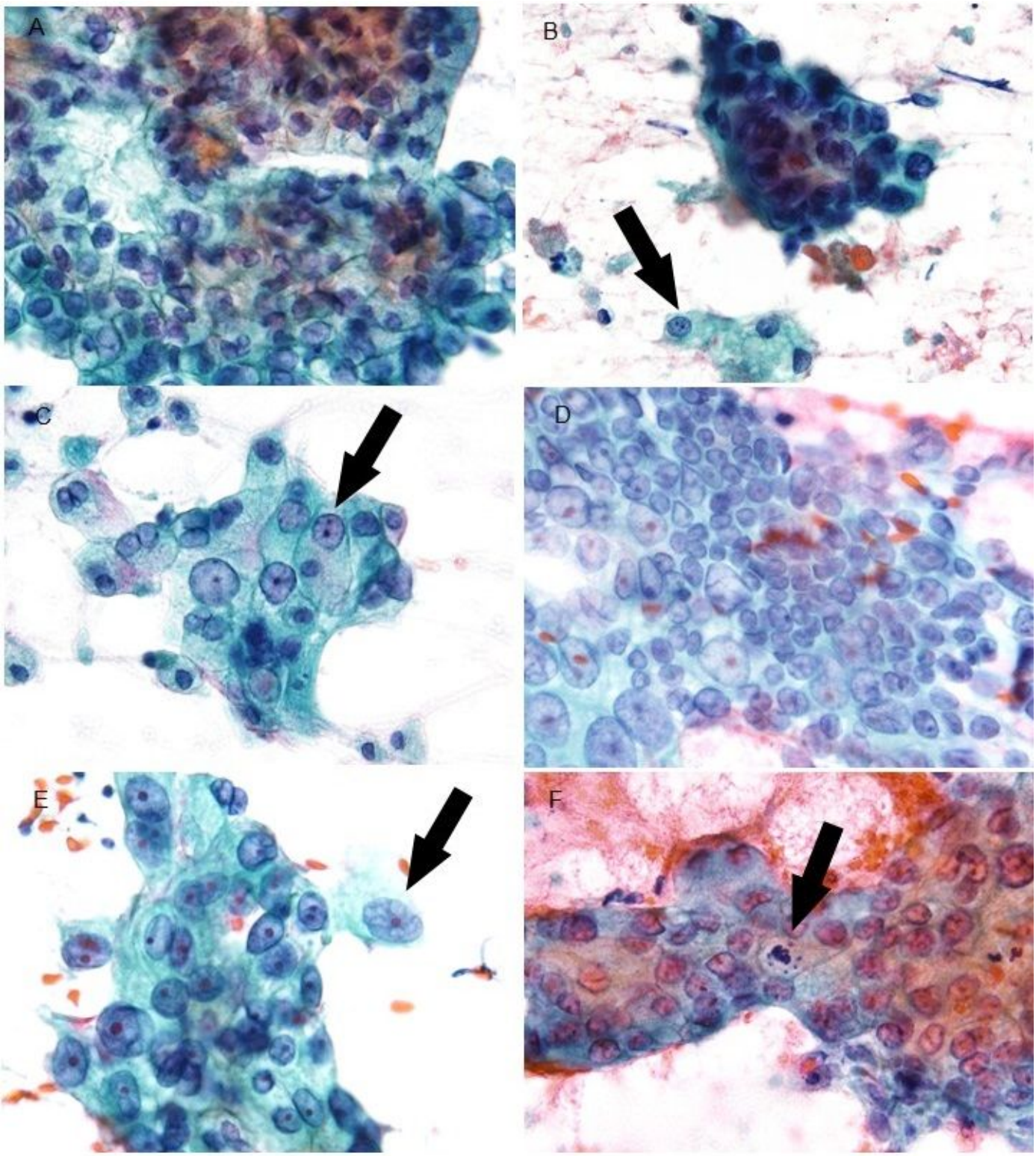

Figure 3

Nuclear findings of malignant pancreatic samples obtained by EUS-FNA. (A) Irregular nuclei. (B) Hyperchromasia. Atypical cells showed hyperchromatin compared with background macrophages (arrow). (C) Nuclear membrane thickening (arrow). (D) Anisonucleosis. (E) Prominent nucleoli (arrow). (F) Increased mitosis (arrow). Original magnification, ×400. Papanicolaou stain; EUS-FNA: Endoscopic ultrasound-guided fine needle aspiration

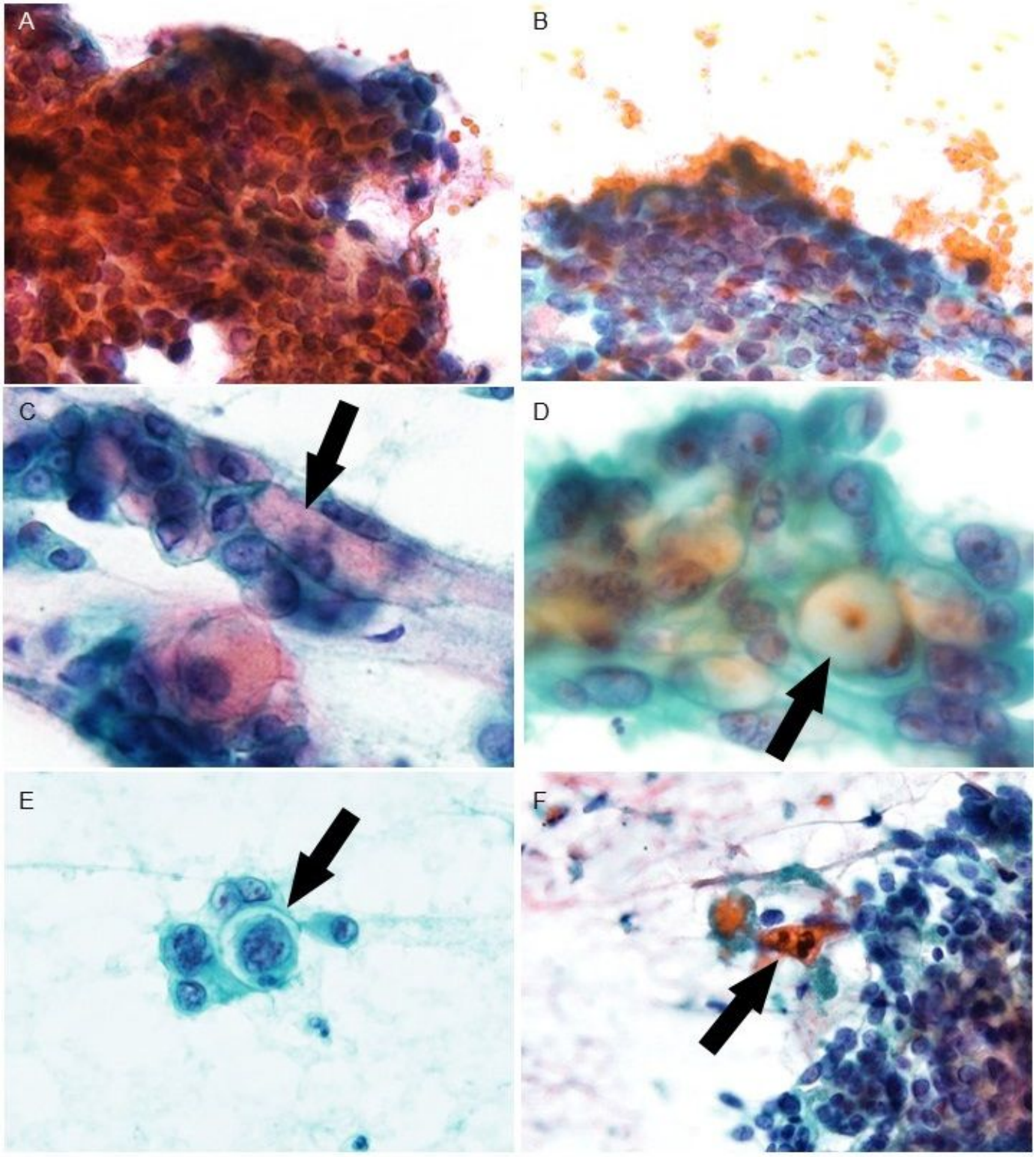

Figure 4

Nuclear and cell findings of malignant pancreatic samples obtained by EUS-FNA. (A) Unclear cell boundary. (B) High N/C ratio. (C) Pink intracellular mucus (arrow). (D) Orange-yellow intracellular mucus (arrow). (E) Cannibalism (arrows). (F) Keratinization (arrow). Original magnification, ×400 in A, B, and F; ×600 in C, D, and E. Papanicolaou stain; EUS-FNA: Endoscopic ultrasound-guided fine needle aspiration; N/C ratio: Nuclear/cytoplasmic ratio

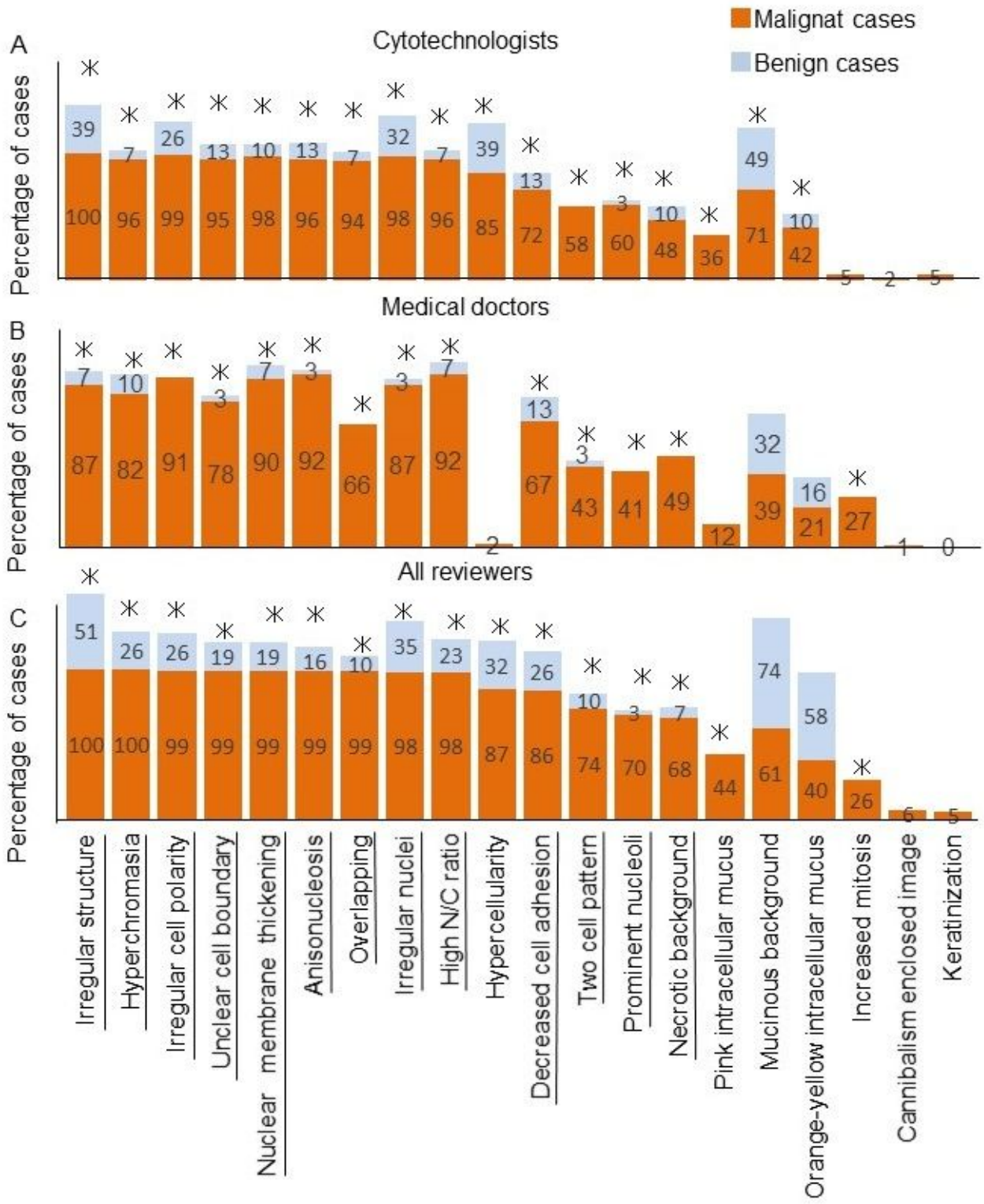

Figure 5

Incidence rate of the 20 indices in malignant and benign cases. Results based on the evaluation of four of seven reviewers. Orange bar, malignant cases (n=111); blue bar, benign cases (n=31); number in boxes, the percentage of the cases; *, P < 0.05 by Fisher's exact test between malignant and benign cases.

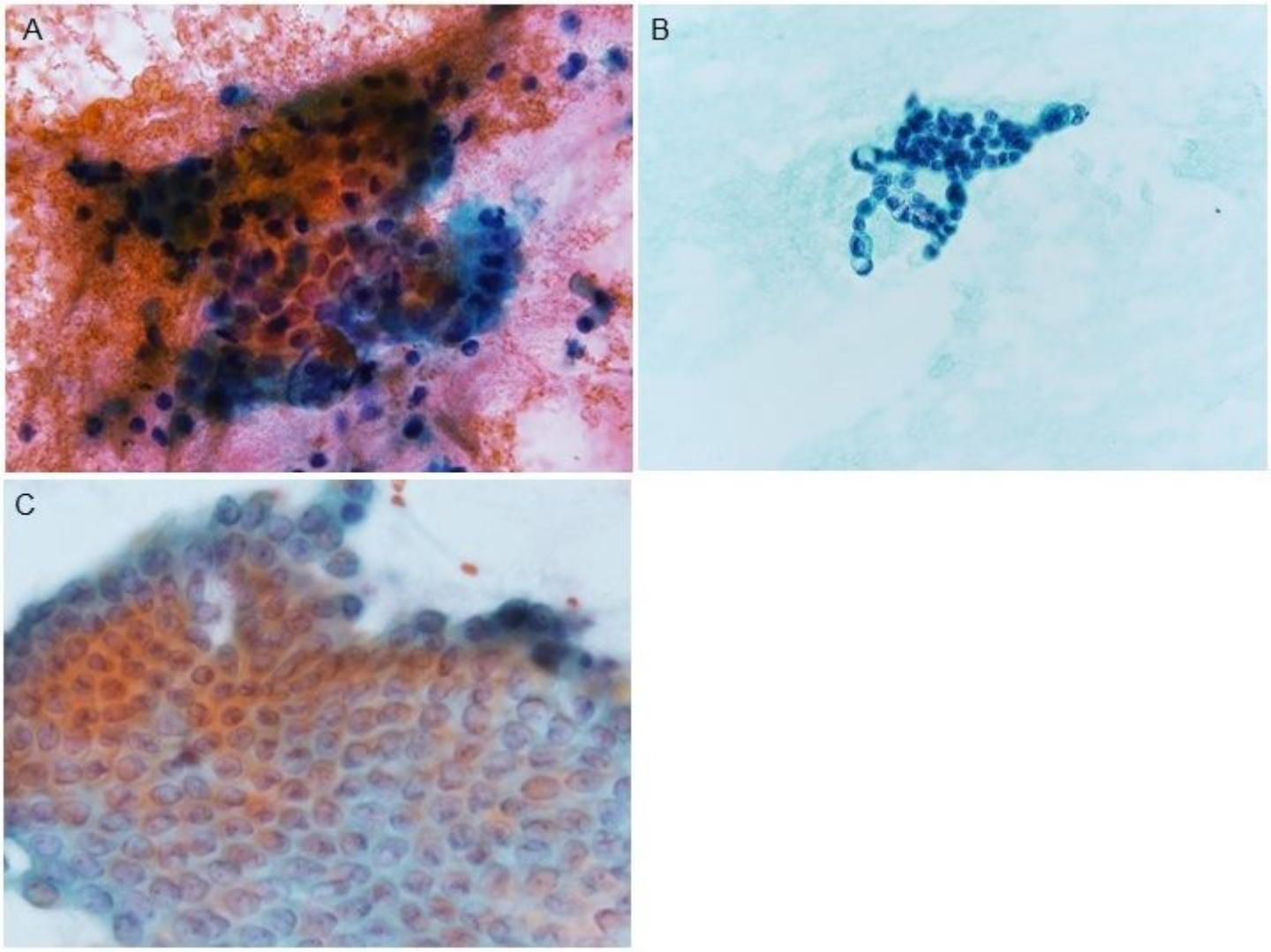

Figure 6

Cytological features of cases showing discrepancy between the criteria and cytological diagnosis. (A) Case A: Weak atypical cells were observed against a background of numerous red blood cells. (B) Case B: A small number of atypical cells were observed against a clean background. (C) Case C: Epithelial cells with nuclear atypia and yellow mucus. Original magnification, ×400 in A, and C; ×100 in B. Papanicolaou stain.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable1112020.docx