Original Article

Whole slide image with image analysis of atypical bile duct brushing: Quantitative features predictive of malignancy

Brian T. Collins¹, R. Cody Weimholt¹

¹Department of Pathology and Immunology, Washington University in St. Louis School of Medicine, St. Louis, Missouri, USA

E-mail: *Dr. Brian T. Collins - bcollins@path.wustl.edu
*Corresponding author

Received: 14 May 15 Accepted: 06 July 15 Published: 31 August 15

Abstract

Background: Whole slide images (WSIs) involve digitally capturing glass slides for microscopic computer-based viewing and these are amenable to quantitative image analysis. Bile duct (BD) brushing can show morphologic features that are categorized as indeterminate for malignancy. The study aims to evaluate quantitative morphologic features of atypical categories of BD brushing by WSI analysis for the identification of criteria predictive of malignancy. Materials and Methods: Over a 3-year period, BD brush specimens with indeterminate diagnostic categorization (atypical to suspicious) were subjected to WSI analysis. Ten well-visualized groups with morphologic atypical features were selected per case and had the quantitative analysis performed for group area, individual nuclear area, the number of nuclei per group, N: C ratio and nuclear size differential. Results: There were 28 cases identified with 17 atypical and 11 suspicious. The average nuclear area was 63.7 µm² for atypical and 80.1 µm² for suspicious (+difference 16.4 µm²; P = 0.002). The nuclear size differential was 69.7 µm² for atypical and 88.4 µm² for suspicious (+difference 18.8 µm²; P = 0.009). An average nuclear area >70 µm² had a 3.2 risk ratio for suspicious categorization. Conclusion: The quantitative criteria findings as measured by image analysis on WSI showed that cases categorized as suspicious had more nuclear size pleomorphism (+18.8 µm²) and larger nuclei (+16.4 µm²) than those categorized as atypical. WSI with morphologic image analysis can demonstrate quantitative statistically significant differences between atypical and suspicious BD brushings and provide objective criteria that support the diagnosis of carcinoma.

Key words: Adenocarcinoma, bile duct, cytology image analysis, whole slide image

INTRODUCTION

Whole slide images (WSIs) provide a method for digitally capturing pathologic slides and the platform offers a variety of options and uses to extend and expand the use and analysis of the traditional morphologic pathologic material. In addition to educational, archival and remote viewing, WSIs can be subject to image analysis. Instead of select static images, an entire slide...
or groups of slides can be studied by the methodology. A variety of vendors offer robust software solutions, which measure defined morphologic features in an automated or semi-automated manner. These can measure traditional staining morphology (H & E staining, Papanicolaou staining, etc.) and quantitative and qualitative features of immunohistochemistry stains (human epidermal growth factor receptor 2 [HER-2/neu]).

In cytopathology, morphologic specimen interpretation serves as the bedrock for specimen interpretation and diagnosis. This interpretation is informed by observational experience, knowledge of expected and observed features and subjective individual judgment. Image analysis offers an objective tool by which measurement of morphologic features can be performed.

In cytopathology, bile duct (BD) brushing is commonly utilized to evaluate pancreatobiliary strictures. Patients who present with biliary stricture can have a variety of possible etiologies including benign inflammatory conditions, intrabiliary lithiasis (stones) or carcinoma (cholangiocarcinoma/adenocarcinoma). The clinical features are not always determinative, and a BD brush cytology can help to stratify patients in the context of other clinical and radiographic findings. BD brush specimens are challenging to interpret since they often demonstrate an inflammatory reactive background due to the local effects of stricture and are often low to intermediate cellularity. As a result, atypical and suspicious categories are commonly reported. Indeterminate diagnostic categories are not as helpful as benign and malignant diagnostic diagnoses for clinical management.

The aim of the study was to evaluate biopsy proven carcinoma with indeterminate BD brush cytology specimens (atypical and suspicious categories) with WSI analysis in order to identify objective morphologic criteria that might further stratify risk of malignancy.

**MATERIALS AND METHODS**

Over a 3-year consecutive period, the pathology database was searched for BD brush specimens with indeterminate diagnostic categorization. These included “atypical” or “suspicious” in the cytopathologic report during the course of the routine patient evaluation. Cases reported as benign, and adenocarcinoma/malignant/positive were excluded. For this indeterminate group, the corresponding pathology database was cross-referenced, and specimens with a corresponding histologic confirmation of carcinoma were selected. Only cases with an indeterminate cytopathologic diagnosis (atypical/suspicious) and histologic diagnosis of carcinoma were included in the study group. Pathology reports were collected and patient demographics and data were reviewed. BD brush specimens are processed from a liquid fixative (PreservCyt™, Hologic Corporation) and a single non-Gyn Filter ThinPrep™ slide (Hologic Corporation) is made. It is stained by a traditional Papanicolaou staining method and interpreted by a pathologist. The individual slides from each individual case were placed in an Aperio® Scanscope XT 120 (Leica Biosystems Inc., Buffalo Grove, Illinois, USA). The entire WSI was available for review. Using the scroll and zoom in/out capability, the entire slide was systematically reviewed by a single operator without knowledge of original categorization or patient outcome. Postprocessing image adjustment built into the software (brightness/contrast) was adjusted to improve visualization of three-dimensional groups and better identify individual nuclear outlines within a group. The operator was instructed to identify the most cytologically abnormal groups that could be well-visualized and the 10 best were manually selected. These were identified and the outer limit of each individual group was delineated using the “pen tool” which is available in the ImageScope™ software. The operator identified the outer limits of the group cytoplasm and completely encircled the entire group. Groups with benign features such as small orderly monomorphic nuclei and flat sheets were not selected. Instances where the groups were difficult to visualize, or other characteristics were not clearly apparent were not selected; the operator used their best judgment based on the individual group’s morphology. Exclusion criteria included single epithelial cells, groups of indeterminate origin (secondary to crush, poor preservation, or staining), and nonepithelial cells (neutrophils, singly, and in clusters). Ten groups were selected and then a manual process was used to delineate the outside margins of the group and individual nuclei [Figure 1]. Within the 10 selected individual groups, the individual nuclei were manually selected.

Within the Aperio® Spectrum™ software, an individual case was opened in the ScanScope/ImageScope™ software (v10.2.2.2352 Leica Biosystems Inc., Buffalo Grove, Illinois, USA). The entire WSI was available for review. Using the scroll and zoom in/out capability, the entire slide was systematically reviewed by a single operator without knowledge of original categorization or patient outcome. Postprocessing image adjustment built into the software (brightness/contrast) was adjusted to improve visualization of three-dimensional groups and better identify individual nuclear outlines within a group. The operator was instructed to identify the most cytologically abnormal groups that could be well-visualized and the 10 best were manually selected. These were identified and the outer limit of each individual group was delineated using the “pen tool” which is available in the ImageScope™ software. The operator identified the outer limits of the group cytoplasm and completely encircled the entire group. Groups with benign features such as small orderly monomorphic nuclei and flat sheets were not selected. Instances where the groups were difficult to visualize, or other characteristics were not clearly apparent were not selected; the operator used their best judgment based on the individual group’s morphology. Exclusion criteria included single epithelial cells, groups of indeterminate origin (secondary to crush, poor preservation, or staining), and nonepithelial cells (neutrophils, singly, and in clusters). Ten groups were selected and then a manual process was used to delineate the outside margins of the group and individual nuclei [Figure 1]. Within the 10 selected individual groups, the individual nuclei were manually selected.
and delineated by the “pen tool” [Figure 2]. These qualified and delineated “pen tool” groups were recorded within the ImageScope™ software in the “annotations” dialog box. Within the “annotations” dialog box, each individual group is given a “region” designation under a single “layer” category [Figure 3]. Ten of these were recorded for each analyzed group in individual cases. At the completion of the process, the individual region designation allowed the operator to review each group by clicking on the “region” which then takes the view screen to a high power encompassing view of the specific group. The entire manual process of group and nuclei selection took an average of 15–20 min/WSI. After completing the process of delineating groups of interest for the WSI, the objective quantitative image analysis process was performed. The manual process of delineating the groups of interest was performed by a single operator (RCW).

Each individual group was quantitatively analyzed for the number of total nuclei, individual nuclear area (μm²), and total group area (μm²). Based on the known magnification of the images by the software program, these are calculated directly. From this directly measured data, the average number of nuclei per group, average nuclear to cytoplasmic (N/C) ratio per group and range of nuclear area (nuclear size differential) per group were calculated.

After the analysis, the cumulative WSI “layer” region result is exported by the ImageScope™ software to a Microsoft Excel™ spreadsheet file which has an individual line for each delineated and measured region. A large amount of data is recorded, only some of which is pertinent and utilized. The directly measured data fields used are: Group area (μm²), total nuclei per group (numeric), and individual nuclear size (μm²) [Figure 4]. From the Excel™ spreadsheet, the measured group size is known, and the total nuclear area calculated (total nuclei area added per group), and then the (N/C) ratio is calculated by dividing the measured total nuclear area by the measured group area (nuclear area/group area). The overall calculated average N/C ratio for the individual WSI is obtained by taking the added total of each N/C ratio and dividing it by the total group number (total of all N/C ratio/total group number). The range or difference in nuclear size per each individual group was measured, and then the average nuclear size differential per WSI was calculated.

Once the calculations were completed independently and in a blinded fashion, the individual cases were correlated with the pathologic reports. The BD brush WSI cases
were categorized into “atypical” and “suspicious” groups based on the original pathologic classification. Data analysis on the Microsoft® Excel file was performed by the Excel™ software program on individual cases and for analysis of group categories and associated measured outcomes. The P values were calculated using standard calculation formulas and independent two sample t-test calculations (IBM Corporation, SPSS Statistics, version 21 Armonk, New York, USA). A result was considered statistically significant if \( P < 0.05 \). A relative risk ratio was calculated, and it was intended to provide information about the image analysis cut-off values. It is not a standard risk ratio of malignancy. The risk ratio is calculated utilizing the cases that were equal to or greater than the cut-off values as a true positive result. This is intended to help stratify the image analysis features within the context of these indeterminate cytopathology brush classifications where patients were determined to have carcinoma. The study had the approval of the Institutional Review Board.

RESULTS

Twenty-eight patients were identified. All patients were originally categorized as either “atypical” or “suspicious” by the pathologist. BD brush cases were interpreted by a mixture of pathologists during the course of routine clinical service. No attempts were made to further subtype or reclassify the initial categorization. Histologic and surgical excision for all cases confirmed the presence of carcinoma. This was performed from a variety of methods that included forceps biopsy at the time of BD brush specimen collection and subsequent staging and Whipple procedures.

The image analysis results are presented in Table 1. As per the image analysis method described in the materials and methods section, a variety of measured and calculated endpoints were collected. Based on the original cytopathology report, the individual WSI cases were stratified into atypical and suspicious end-point groups. WSI of the BD brushing of the atypical cohort class (17) showed a range of results for each individual case including average total nuclei per group of 12.97 (range: 5.2–29), average area of nucleus 63.59 \( \mu m^2 \) (range: 35.6–86.9 \( \mu m^2 \)), average nuclear area differential 69.7 \( \mu m^2 \) (range: 36.3–110.7 \( \mu m^2 \)), and an average N/C ratio range of 0.52 (range: 0.38–0.66). WSI of the BD brushing of the suspicious cohort class (11) showed a range of results for each individual case including average total nuclei per group of 12.28 (range: 7.3–18), average area of nucleus 80.05 \( \mu m^2 \) (range: 70.4–109.6 \( \mu m^2 \)), average nuclear area differential 88.4 \( \mu m^2 \) (range: 67.7–107.6 \( \mu m^2 \)), and an average N/C ratio range of 0.53 (range: 0.47–0.66).

The image analysis comparison data is presented in Table 2. The average total nuclei per group showed a 0.69 differential \( (P = 0.387) \) (12.97 for atypical and 12.28 for suspicious). The average nuclear area showed a 16.46 \( \mu m^2 \) differential \( (P = 0.002) \) (63.59 \( \mu m^2 \) for atypical and 80.05 \( \mu m^2 \) for suspicious). The average area of nucleus differential size was 18.7 \( \mu m^2 \) \( (P = 0.009) \) (69.7 \( \mu m^2 \) for atypical and 88.4 \( \mu m^2 \) for suspicious). The average N/C ratio differential was 0.008 \( (P = 0.387) \) (0.523 for atypical and 0.531 for suspicious). A \( P \leq 0.05 \) was considered statistically significant.

Data points and thresholds were analyzed and stratified within the cohorts [Table 3]. When categorizing the cases with an average nuclear area >70 \( \mu m^2 \), these included all of the suspicious cases (11/11) and 5 of the

---

**Table 1: Atypical bile duct brush WSI: Image analysis results**

| Image analysis data | Atypical (17) | Suspicious (11) |
|---------------------|--------------|----------------|
| Total nuclei per group, range | 5.2 to 29 | 7.3 to 18 |
| Total nuclei per group, average | 12.97 | 12.28 |
| Area of nucleus, average, range | 35.6 to 86.9 \( \mu m^2 \) | 70.4 to 109.6 \( \mu m^2 \) |
| Area of nucleus, average | 63.59 \( \mu m^2 \) | 80.05 \( \mu m^2 \) |
| Area of nucleus: differential, range | 36.3 to 110.7 \( \mu m^2 \) | 67.7 to 107.6 \( \mu m^2 \) |
| Area of nucleus: differential, average | 69.7 \( \mu m^2 \) | 88.4 \( \mu m^2 \) |
| N/C ratio, range | 0.38 to 0.66 | 0.47 to 0.66 |
| N/C ratio, average | 0.52 | 0.53 |

N/C: Nucleus to cytoplasm

---

**Table 2: Atypical bile duct brush WSI: Comparison data**

| WSI image analysis data points | Atypical | Suspicious | Differential | P value |
|-----------------------------|---------|-----------|-------------|--------|
| Total nuclei per group, average | 12.97 | 12.28 | 0.69 | 0.387 |
| Area of nucleus, average | 63.59 \( \mu m^2 \) | 80.05 \( \mu m^2 \) | −16.46 \( \mu m^2 \) | 0.002 |
| Area of nucleus: differential, average | 69.7 \( \mu m^2 \) | 88.4 \( \mu m^2 \) | −18.7 \( \mu m^2 \) | 0.009 |
| N/C ratio, average | 0.523 | 0.531 | 0.008 | 0.387 |

N/C: Nucleus to cytoplasm; P value ≤0.05 statistically significant
Table 3: Atypical bile duct brush WSI: Risk ratio

| Data points | Atypical | Suspicious | Risk ratio | P value |
|-------------|----------|------------|------------|---------|
| Nuclear area greater than 70 µm² | 5/17 | 11/11 | 3.2 | 0.0015 |
| Nuclear differential greater than 75 µm² | 6/17 | 9/11 | 2.6 | 0.0094 |

a: Nuclear area average per individual WSI case, b: Area of nucleus differential averaged per individual WSI case, P value ≤0.05 statistically significant

DISCUSSION

WSI is becoming more widely available, and the technology offers a method to digitize traditionally prepared pathology slides and smears. There are many practical and theoretical advantages to utilizing a digital pathology specimen. WSI are gaining wide use in educational activities, specimen archival applications, remote viewing uses and second opinion/consultations. [3-4] One advantage to WSI of pathology slides includes the ability to assess and evaluate the digitized information by image analysis. This includes currently Food and Drug Administration approved algorithm for image analysis of HER-2/neu immunohistochemistry. [1,2] The method is applicable for histologic and cytopathologic preparations. For cytopathology, WSI technology can digitize the variety of specimen slides prepared for evaluation including cytospin-concentration techniques, aspirate smears, liquid-based slides, brushing specimens, and cell blocks; each of which include a range of staining methods including Papanicolaou, modified Giemsa, and H & E. Once digitized, the image analysis toolbox is available to objectively analyze and evaluate the cellular elements present in cytopathology specimens. WSI and image analysis technologies provide the opportunity to examine an entire slide or group of slides. As a platform, these can work together as either an automated or semi-automated workflow process.

Pathology diagnosis is based on morphologic observation of glass slides utilizing long standing traditional tools of light microscopy. For cytopathology, the morphologic judgment classifies specimens with a precise diagnosis. Due to innate features of sampling methods and clinical circumstances, there are cytopathology specimens that have an indeterminate classification, outside the benign or malignant groups. BD brushing specimens are one area where definitive classification is not always possible. The morphologic diagnostic challenges of BD brushing are well described and well-known by those who practice cytopathology and clinicians who rely on the testing to help manage their patients. [7,11] In brief, some of the factors are worth discussing. BD strictures are often in anatomic locations that are difficult to reach by endoscopy. To obtain cellular elements, a BD brushing sample is frequently collected. Traditionally, the brush is introduced into the area of stricture and moved back and forth in the area of stricture to dislodge and collect cells. These areas of stricture are often narrow and have associated reactive glandular epithelial cells and surrounding fibrosis, whether it is a benign inflammatory or neoplastic stricture. The narrowed region and fibrosis can contribute to a sparsely cellular sample. The combination of a sparsely cellular sample and background reactive epithelial cells often contributes to an indeterminate diagnostic classification. These categories tend to include “atypical epithelial cells” and “suspicious for carcinoma.” The criteria utilized for these diagnostic reporting groups are not standardized and rests on the judgment and experience of the interpreting pathologist. In general, a negative categorization and malignant categorization have good reproducibility and both negative predictive value and positive predictive value across different studies. The indeterminate criteria are not as reproducible or have strong statistical certainty related to patient outcome. [9]

Therein lies the value in utilizing an objective measurement method to assist in stratifying and further classifying those indeterminate diagnostic categories which can be present in cytopathology, and specifically in BD brush cytology. Bridging the observational judgment of malignancy assessment by an individual pathologist with an adjunctive objective ancillary testing system has the potential to assist in diagnostic clarification and provide further information for the clinician in managing their patient. For patients with a BD brush diagnosed as either atypical or suspicious for malignancy, can image analysis data point performed on a WSI provide specific metrics, which can assist in further stratifying risk of malignancy. A variety of authors has described utilizing DNA image cytometry on BD cytology brush specimens using a variety of methods and data points. [6-14] Ours is the first to apply an image analysis algorithm to BD brush WSI and the technique has only been infrequently described in cytopathology. [15]

In this study, there were a number of similarities and differences between the atypical and suspicious cohorts. There was no statistical difference between average total nuclei per group (12.97 vs. 12.28). Theoretically, adenocarcinoma in BD brushing has been observed to have more single atypical and malignant cells, which
represent a loss of cellular cohesion in carcinoma in contrast to a reparative epithelial process where the groups are larger, hold together and lack single atypical cells. There was no statistical difference between the average N/C ratio (0.523 vs. 0.531). Adenocarcinoma can be recognized by alteration in the cellular N/C ratio, where benign cells retain a moderate to abundant volume of cytoplasm in contrast to malignant cells that have less differentiation and less cytoplasm imparting a higher N/C ratio.

There were differences observed, which involved the average nuclear area and an average of the nuclear size differential. Adenocarcinoma in BD brush often show nuclear enlargement. The “two cell population” of malignant cells and benign cells intermixed in a BD brush is morphologically observed at a medium power and largely imparted by nuclear size with malignant cells being larger than benign BD nuclei. The average area of the nucleus was statistically different with 63.59 µm² for atypical compared to 80.05 µm² for the suspicious cohort (differential of 16.46 µm²) (P = 0.002). This difference in nuclear size average likely accounts for the difference in categorization between atypical and suspicious. With relatively smaller nuclei in the atypical cohort, the pathologist likely had less confidence of a malignant category and, therefore, did not place these cases into the suspicious category.

A key feature commonly emphasized in the diagnosis of adenocarcinoma of the pancreatobiliary system is anisonucleosis. This is a variation in the size of individual nuclei within a group and generally quantitated by observation is multiple of size (×1 times, ×2 times, ×3 times, etc.). Observational experience has shown that benign BD epithelial cells will show virtually no difference and reactive reparative change tends to be more modest with 1–2 times variation. Adenocarcinoma tends to show a more pronounced variation of 3–4 times. The average nuclear area differential was statistically different with 69.7 µm² for the atypical and 88.4 µm² for the suspicious cohort (differential of 18.7 µm²) (P = 0.009). The more pronounced size differential measured in the suspicious cohort likely contributed to the pathologist degree of concern resulting in a suspicious category, instead of an atypical category.

Some of the other observational features of malignancy are more difficult to objectively quantify by image analysis. Focal crowding and overlap with the cellular disorder can be subtle and require more complex image analysis. Nuclear membrane irregularities and chromatin structure/pattern are important diagnostic features in the evaluation of malignancy. These data points are more difficult to quantify and are beyond the scope of most core image analysis software capabilities.

There is always variability of measures within a case and between cases when measured individually and in aggregate. When a large amount of information and data points are collected and aggregated, it can help in classification to set predetermined cut points to help interpret the findings. Within this cohort, certain patterns and grouping of data was observed and based on this stratification data points were assigned within the two classes that had statistical significance. For the average nuclear area, a data point of 70 µm² was set, and 5/17 atypical cases and 11/11 suspicious cases were above this number. Using a risk ratio calculation, an individual case above the data point had a risk ratio of 3.2. For the average nuclear differential, a data point of 75 µm² was set, and 6/17 atypical cases and 9/11 suspicious cases were above this number. Using a risk ratio calculation, an individual case above the data point had a risk ratio of 2.6. The assignment of stratification data and risk ratio has the potential to add additional information to the observational diagnostic interpretation, and in turn add to the information a clinician has in the evaluation of a biliary stricture that also includes clinical and radiographic findings.

CONCLUSION

The quantitative criteria findings as measured by image analysis on WSI showed that cases categorized as suspicious had more nuclear size pleomorphism (+18.8 µm²) and larger nuclei (+16.4 µm²) than those categorized as atypical. WSI and morphologic image analysis can demonstrate quantitative statistically significant differences between atypical and suspicious BD brushings and provide objective criteria that support the diagnosis of carcinoma.

Financial Support and Sponsorship
Nil.

Conflicts of Interest
There are no conflicts of interest.

REFERENCES

1. Jeung J, Patel R, Vila L, Wakefield D, Liu C. Quantitation of HER2/neu expression in primary gastroesophageal adenocarcinomas using conventional light microscopy and quantitative image analysis. Arch Pathol Lab Med 2012;136:610-7.
2. Nassar A, Cohen C, Agersborg SS, Zhou W, Lynch KA, Albilar M, et al. Trainable immunohistochemical HER2/neu image analysis: A multisite performance study using 260 breast tissue specimens. Arch Pathol Lab Med 2011;135:896-902.
3. Pantanowitz L, Sinard JH, Henricks WH, Fasth tee LA, Carter AB, Contis L, et al. Validating whole slide imaging for diagnostic purposes in pathology: Guideline from the College of American Pathologists Pathology and Laboratory Quality Center. Arch Pathol Lab Med 2013;137:1710-22.
4. Kothari S, Phan JH, Stokes TH, Wang MD. Pathology imaging informatics for quantitative analysis of whole-slide images. J Am Med Inform Assoc 2013;20:1099-108.
5. Park S, Pantanowitz L, Parwani AV. Digital imaging in pathology. Clin Lab Med 2012;32:557-84.
6. Hamilton PW, Wang Y, McCullough SJ. Virtual microscopy and digital pathology in training and education. APMIS 2012;120:305-15.
7. Harewood GC. Endoscopic tissue diagnosis of cholangiocarcinoma. Curr Opin Gastroenterol 2008;24:627-30.
8. Athanassiadou P, Grapsa D. Value of endoscopic retrograde cholangiopancreatography-guided brushings in preoperative assessment of pancreaticobiliary strictures: What’s new? Acta Cytol 2008;52:24-34.
9. Barr Fritcher EG, Kipp BR, Slezak JM, Moreno-Luna LE, Gores GJ, Levy MJ, et al. Correlating routine cytology, quantitative nuclear morphometry by digital image analysis, and genetic alterations by fluorescence in situ hybridization to assess the sensitivity of cytology for detecting pancreaticobiliary tract malignancy. Am J Clin Pathol 2007;128:272-9.
10. Osterheld MC, Andrejevic Blant S, Caron L, Braunschweig R, Dorta G, Bouzourene H, et al. Digital image DNA cytometry: A useful tool for the evaluation of malignancy in biliary strictures. Cell Oncol 2005;27:255-60.
11. Rumalla A, Baron TH, Leontovich O, Burgart LJ, Yacavone RF, Therneau TM, et al. Improved diagnostic yield of endoscopic biliary brush cytology by digital image analysis. Mayo Clin Proc 2001;76:29-33.
12. Vasilieva LE, Papadhimitriou SI, Dourakis SP. Modern diagnostic approaches to cholangiocarcinoma. Hepatobiliary Pancreat Dis Int 2012;11:349-59.
13. Binek J, Lindenmann N, Meyenberger CM, Hell M, Ulmer H, Spieler P, et al. Malignant biliary stenosis: Conventional cytology versus DNA image cytometry. Surg Endosc 2011;25:1808-13.
14. Levy MJ, Baron TH, Clayton AC, Enders FB, Gostout CJ, Halling KC, et al. Prospective evaluation of advanced molecular markers and imaging techniques in patients with indeterminate bile duct strictures. Am J Gastroenterol 2008;103:1263-73.
15. Collins BT, Collins LE. Assessment of malignancy for atypia of undetermined significance in thyroid fine-needle aspiration biopsy evaluated by whole-slide image analysis. Am J Clin Pathol 2013;139:736-45.
16. Lin F, Staerkel G. Cytologic criteria for well differentiated adenocarcinoma of the pancreas in fine-needle aspiration biopsy specimens. Cancer 2003;99:44-50.