CLINICAL AT

Comparison of Methods to Detect Neoplasia in Patients Undergoing Endoscopic Ultrasound-Guided Fine-Needle Aspiration

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BACKGROUND & AIMS: Digital image analysis (DIA) and fluorescence in situ hybridization (FISH) can be used to evaluate biliary strictures with greater accuracy than conventional cytology (CC). We performed a prospective evaluation of the accuracy of CC, compared with that of DIA and FISH, in detection of malignancy in patients undergoing endoscopic ultrasonography (EUS) fine-needle aspiration (FNA). **METHODS:** We collected a minimum of 6 FNA samples from each of 250 patients during EUS. CC or DIA and FISH analyses were performed on every other specimen (from every other FNA pass); patients were randomly assigned to the first test performed. CC slides were reviewed by gastrointestinal cytopathologists who were blinded to all data. Findings from cytohistologic analysis, after a minimum 24-month follow-up period, were used as the standard (n = 202; median age, 65 years). **RESULTS:** Aspirates were collected from lymph nodes (n = 111), pancreas (n = 61), gastrointestinal lumen wall (n = 9), periluminal mass (n = 4), liver (n = 8), and miscellaneous sites (n = 9). Matched samples provided a mean of 3.2 passes for CC and 1.6 passes for DIA and FISH. The data indicate a potential lack of utility for DIA. The combination of CC and FISH detected malignancy with 11% greater sensitivity than CC alone (P = .0002), but specificity was reduced from 100% to 96%. **CONCLUSIONS: FISH analysis identifies neoplastic lesions with significantly greater sensitivity than CC in patients with diverse pathologies who underwent EUS with FNA, despite limited tissue sampling for FISH analysis.**

Keywords: Cancer Detection; Molecular Cytogenetic Marker; Diagnostic; Pathology.

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Endoscopic ultrasonography (EUS) is routinely used to evaluate intraintestinal and extraintestinal mass lesions and lymphadenopathy. The diagnostic accuracy of EUS-guided fine-needle aspiration (FNA) with conventional cytology (CC) is 60%–90%.[1–7] By enhancing diagnostic sensitivity, staging accuracy, and prognostic determination,[1,8] EUS FNA helps guide patient care and improves outcomes.[8–10] As a result, EUS FNA has become an essential component in the evaluation of a variety of gastrointestinal and nongastrointestinal disorders.

CC has high specificity but poor sensitivity.[1,11,12] This has driven the pursuit of new technologies such as digital image analysis (DIA) and fluorescence in situ hybridization (FISH) with potentially higher sensitivity to detect malignancy/neoplasia by assessing nuclear DNA content and the presence of aneusomy (ie, abnormal chromosome copy number), respectively.[13,14] These tests have the ability to identify malignant cells in samples of limited cellularity and yield greater diagnostic sensitivity than CC alone.[14,15] DIA and FISH were initially investigated for the detection of bladder cancer at our institution. However, because most solid tumors are characterized by numerical and structural chromosomal abnormalities,[16,17] DIA and FISH should also be able to detect cells that have chromosomal abnormalities consistent with neoplasia in exfoliative and aspiration gastrointestinal cytology specimens. We tested this hypothesis on endoscopic retrograde cholangiography (ERC) brush biopsy samples collected to evaluate indeterminate bile duct strictures and found that DIA and FISH provided greater diagnostic accuracy than CC in distinguishing benign from malignant strictures.[14,18–22] FISH is now a standard test used in our practice to help guide clinical decision making.

We speculated that assessing for numeric chromosomal alterations may also enhance the diagnostic accuracy of CC for samples collected at EUS FNA, based on the fact that numerical chromosomal alterations (aneuploidy) are observed among different cancer types. In a pilot study

Abbreviations used in this paper: CC, conventional cytology; DIA, digital image analysis; ERC, endoscopic retrograde cholangiography; EUS, endoscopic ultrasonography; FISH, fluorescence in situ hybridization; FNA, fine-needle aspiration; QNS, quantity not sufficient.

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involving 39 patients who underwent EUS FNA, we showed enhanced diagnostic accuracy for the composite DIA/FISH results over CC alone.[23] In the current study, we sought to more rigorously evaluate these techniques and test the hypothesis that the accuracy of the composite DIA/FISH result is greater than that of CC when applied to EUS FNA specimens from patients with a diverse spectrum of disease processes.

Patients and Methods

Patients

We prospectively enrolled patients referred for EUS FNA (1) who had known or suspected luminal or extraluminal malignancy and (2) in whom the endosonographer deemed the target lesion safe and feasible to allow the necessary study passes. Patients were excluded for the following reasons: (1) inability to provide informed consent, (2) anticipated unavailability or patients declined follow-up, and/or (3) coagulopathy (international normalized ratio >1.5) and/or thrombocytopenia (platelet count $<50 \times 10^9$/L).

The institutional review board granted approval for this prospective study and informed consent was obtained for all procedures, including DIA and FISH. Information concerning the presentation, clinical course, and outcomes was abstracted from the medical records and patient interviews. A patient was considered to have malignancy if there was (1) cytological and/or histologic evidence of malignancy based on material obtained via EUS FNA, ERC and tissue sampling, percutaneous biopsy, surgery, or autopsy; (2) a clinical course ($\geq$24 months) suggesting malignancy based on the presence of a new radiographic abnormality, including regional or distant mass (hepatic, pulmonary, or bone), mass infiltrating large blood vessels, or malignant-appearing lymphadenopathy with positive positron emission tomography imaging; or (3) cancer-related mortality. Designation of a lesion as benign required at least 24 months of follow-up and absence of any of the previously described criteria and/or follow-up imaging showing complete resolution of the abnormality. DIA and FISH findings were excluded from the medical records and did not affect patient care.

EUS FNA and Sample Processing

EUS FNA and CC, DIA, and FISH processing were performed with a 22-gauge needle (Echotip; Cook Medical, Winston-Salem, NC) using approximately 5 mL of negative pressure and standard techniques as previously described.[1,2,18,20,24–26] Six FNA samples were obtained from each patient during EUS using a 22-gauge needle (Echotip; Cook Medical). Additional passes were obtained at the discretion of the endosonographer and in-room cytotechnologist. CC or DIA/FISH analysis were performed on every other specimen (ie, every other FNA pass) with patients blindly randomized to the first test performed. Additional DIA/FISH passes were matched with additional CC passes in a 1:1 fashion as previously outlined. Primary statistical analysis included only matched samples. Our study protocol mandated that at least 3 passes be obtained each for CC and DIA/FISH. The DIA/FISH specimens were evenly divided for subsequent FISH and DIA analysis. Therefore, twice as much material was available for CC review as compared with DIA and FISH. This protocol was adopted to determine the accuracy of DIA and FISH sampling in a manner that limited additional passes to maintain safety. However, this approach biased sample acquisition and analysis in favor of CC. To help evaluate the impact of limiting DIA and FISH sampling relative to CC, the sample adequacy was graded for each specimen.

Although the study mandated 3 study passes for CC and DIA/FISH, additional passes could be obtained at the discretion of the endosonographer and in-room cytotechnologist. Additional DIA/FISH passes were matched by CC passes in a 1:1 fashion as previously outlined. To optimize patient care, the study allowed additional unmatched passes for CC. Primary statistical analysis included only matched samples. Gastrointestinal cytopathologists with expertise in each diagnostic modality reviewed the specimens while blinded to DIA/FISH results and follow-up. Specifically, CC, DIA, and FISH were each interpreted independently and without knowledge of the result for the other evaluated diagnostic modalities. All pathology interpretations and the assessment of the gold standard were conducted by different physicians who were completely blinded to the alternate data.

Dia

DIA is a form of cytologic analysis that quantifies cellular constituents by using spectrophotometry.[13] Small foci of tumor cells can be analyzed, unlike the large number of cells required for flow cytometry.[15] DIA processing uses the Feulgen reaction, which strips away non-nuclear material and hydrolyzes DNA into its constituent nucleic acids, which stoichiometrically bind to the Feulgen dye.[13] ThinPrep specimens (Hologic, Marlborough, MA) were prepared as previously described.[27] Up to 50 cells with the most nuclear atypia were selected for quantification using the CAS 200 image analyzer (Bacus Laboratories, Lombard, IL), which captures these cells and quantifies the optical density and compares these readings with the summed optical readings of the standard control. A video camera captured the transmitted light and converted the absorption values into an analog signal and "digitized" pixels of variable color.[28] DNA ploidy status was assigned based on a computer-generated histogram (Figure 1*A* and *B*). Cases were diagnosed as positive for malignancy if the histograms showed a clonal population of cells beyond a DNA index of 1.10 as previously described.[29]

Fluorescence In Situ Hybridization

FISH uses fluorescently labeled DNA probes to chromosomal centromeres or unique loci to detect cells that have numerical or structural abnormalities indicative of malignancy. The probe set used (UroVysion; Abbott Molecular Inc, Des Plaines, IL) targets centromeres of chromosomes 3 (CEP3), 7 (CEP7), and 17 (CEP17) and band 9p21 (P16/CDKN2A locus). Slides were processed and hybridized with the probe set as previously described.[17] The slides were assessed by scanning for cytologically atypical cells and by determining the number of CEP3, CEP7, CEP17, and 9p21 signals in those cells. Specimens were considered positive for malignancy if they showed gains of 2 or more chromosomes in 5 or more cells (ie, "polysomy"), homozygous chromosomal loss of the 9p21 locus in $>$20% of cells, a single copy of one chromosome (ie, "monosomy") in $>$20% of the cells, or gains of one chromosome in 10 or more cells. Hemizygous 9p21 (single copy) was equivocal and considered negative for data analysis (Figure 2*A* and *B*).

Statistical Analysis

We hypothesized that the diagnostic accuracy of the composite DIA/FISH result is greater than CC. Our specific aim was to determine the accuracy of CC versus the composite