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For diagnosing pancreatobiliary cancer ...Brush Cytology Gets a Big Boost From Genotyping

San Francisco—Researchers have found that by genotyping suspicious tissue specimens they can add new luster to brush cytology and perhaps eliminate questionable diagnoses. This may come as good news to gastroenterologists who recognize brush cytology—which boasts 100% specificity—as the gold standard for diagnosing pancreatobiliary cancer. But the technique is tarnished by a dismal sensitivity rate of only 50% to 60%.

The high false-positive rate associated with brush cytology, and cytology reports that frequently describe specimens as "suspicious," "atypical," or "probably reactive but benign," leave gastroenterologists in an informational lurch, forcing them to formulate treatment plans based on incomplete information.

"You want a firm diagnosis in order to optimize therapy, avoid undertreatment of potentially malignant disease and avoid overtreatment of benign reactive conditions," said Sydney Finkelstein, MD, associate professor of pathology at the University of Pittsburgh Medical Center.

Missed Readings Affect Treatment Decisions

The problem can greatly influence survival. For example, if a physician chooses to conservatively monitor a patient based on uncertain cytologic findings, a delay in treatment can be critical if cancer is present.

But don't fault pathologists, who want nothing more than to provide accurate information and are loath to label pancreatic and bile duct cells malignant based solely on how they look under the microscope, said Dr. Finkelstein at the 2002 Digestive Disease Week meeting. "If we're not 100% comfortable that the changes are clearly from a malignancy, we'll call it 'suspicious' or 'atypical.' But if I can take these same cells and demonstrate that mutations are or are not present, I can provide objective information needed for the basis of a definitive treatment plan."

Specimens from the bile and pancreatic ducts can confound pathologists because obstructions often irritate epithelial cells, causing them to appear malignant when analyzed visually. In the pancreatic duct, the problem is augmented by exposure to digestive enzymes that cause lining cells to assume abnormal characteristics. "They may look malignant, but in reality they may not be," said Dr. Finkelstein.

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Brush Cytology

Soon, however, genetic testing may pave the way for an unprecedented level of diagnostic accuracy that relies on genetic markers, potentially reducing the diagnostic uncertainty to near zero.

When Dr. Finkelstein and his colleagues isolated specimen cells that pathologists considered suspicious and screened them for mutational changes, they found that nearly all of the samples deemed atypical or inconclusive by pathologists contained allelic loss and could be diagnosed as neoplastic and not reactive.

They concluded that microdissection-based, broad-panel, allelic-loss marker analysis can be reliably performed on ERCP brush cytology specimens to improve diagnostic accuracy without jeopardizing morphologic analysis. The value of other genetic tests intended to discern mutagenicity in pancreatobiliary tissue, such as the *K-ras* mutational analysis, and p53 immunostaining and telomerase RNA detection, remains questionable. The technique may prove highly beneficial for the subgroup of brush cytology samples that are deemed highly suspicious but not diagnostic of cancer, James Farrell, MD, told *Gastroenterology & Endoscopy News*.

"This is the first time I've seen a technique in which several technologies—laser-capture microdissection combined with molecular analysis—are used for the diagnosis of biliary tract cancer," said Dr. Farrell, director of endoscopic ultrasound at the University of California, Los Angeles School of Medicine's Division of Digestive Diseases. "They actually isolated individual cells that look potentially malignant and confirmed their impression by molecular means. That's a feature that's not present in similar studies. It's unclear, though, what role [the technique] will have if the yield of epithelial cells from brush cytology is low. Endoscopists can't relax in their attempt to harvest an adequate number of cells for analysis."

Dr. Farrell added that the study numbers were small, but still sufficient to prove that the underlying principles of combining advanced molecular analysis with standard endoscopic techniques are sound and improve diagnostic capabilities.

The Study

The study involved 12 patients with surgically proven pancreatobiliary cancer. Each underwent preoperative cytology brushing and had a resected specimen available for comparative analysis. In six cases, the cytology reports were interpreted as inconclusive. In the other six cases, cytologic interpretation was assessed as positive. At least two individual aggregate samples of 50 to 200 representative cells were microdissected and separately genotyped from each cytology sample. Two sampling methods were used: a collective assembly method and an individual cell-cluster method. Non-neoplastic control cells were also microdissected from cytologic slides or from corresponding serial unstained sections of the surgically resected tissue specimens.

In all six patients whose cytology specimens were described as atypical or inconclusive, investigators found multiple mutational alterations, enabling them to objectively diagnose the condition as neoplastic and not reactive. All positive and suspicious cytology samples displayed at least three acquired mutations, and in all cases mutations in cytologic specimens matched those of tissue

specimens. All of the subjects who had suspicious findings eventually developed cancer. No mutations were found in any of the non-neoplastic cellular samples, which were known to be cancer-free.

Augment, Not Replace

Dr. Finkelstein emphasized that the experimental technique is designed to augment, not replace, cytology. "We're simply extending the diagnostic reach and saying to the pathologist, 'go ahead, look at the tissue and indicate to us the areas you're most concerned about.'" he said. "Then we'll microdissect those worrisome cells off the cytology glass slide and analyze them for a panel of 15 potential mutations."

"Clean" samples—benign, reactive, noncancerous alterations—will show no mutations. "At this time, our experience has indicated to us that three mutations may be expected to be present, permitting an objective diagnosis of cancer," said Dr. Finkelstein. He predicted that genetic testing won't be used when cytology clearly shows cancer or when the cells are clearly not malignant, but only in the gray zone where the changes are worrisome or even slightly suspicious.

—*Steve Frandzel*

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