ECRONICON OPEN ACCESS

EC GASTROENTEROLOGY AND DIGESTIVE SYSTEM

Research Article

# Experience Using Rapid On-Site Evaluation of Biopsies Obtained By EUS- Guided FNA Performed by Cytopathology-Trained Endoscopists

**Dínimo José Bolívar-Saenz[1]*, Lázaro Antonio Arango[2], Claudia Patricia Díaz Tobar[3], Ricardo González[4] and Edgar Julián Ferreira[5]**

*[1]General Surgeon of Universidad del Rosario, Fellow of the Clinical-Surgical Gastroenterology Program, University of Caldas, Manizales, Caldas, Colombia*

*[2]General Surgeon and Clinical Surgical Gastroenterologist, Chief of the Clinical-Surgical Gastroenterology Program, University of Caldas, Manizales, Caldas, Colombia*

*[3]General Surgeon and Clinical Surgical Gastroenterologist, University of Caldas, Manizales, Caldas, Colombia*

*[4]Anatomical and Clinical Pathologist, National University of Colombia. Professor at the Universidad Autónoma of Manizales. Manizales, Caldas, Colombia*

*[5]General Surgeon of Industrial University of Santander, Fellow of the Clinical-Surgical Gastroenterology Program, University of Caldas, Manizales, Caldas, Colombia*

***Corresponding Author**: Dínimo José Bolívar-Saenz, General Surgeon of Universidad del Rosario, Fellow of the Clinical-Surgical Gastroenterology Program, University of Caldas, Manizales, Caldas, Colombia.

**Received:** December 16, 2019; **Published:** January 18, 2020

## Abstract

**Background:** Biopsy by Fine-Needle Aspiration guided by ultrasonography (EUS-FNA) with rapid on-site evaluation (ROSE) by cytopathologists improves diagnostic capacity of the EUS.

**Objectives:** To assess whether the usefulness of ROSE performed by endoscopists with cytopathology training improves the adequacy of tissue sampling obtained by EUS-FNA.

**Methodology:** Between March and October 2015, 49 patients with solid or cystic lesions of the gastrointestinal tract were taken to EUS-FNA. Two endoscopists with short training in cytopathology performed the ROSE and they categorized samples obtained as adequate or not adequate; the results were compared with the evaluation of a pathologist, using the same criteria to assess concordance.

**Results:** A high concordance of a proper reading between the endoscopist and pathologist in first EUS-FNA (EUS-FNA 1) (Kappa agreement measure 81%, sig = 0.000) and second EUS-FNA (EUS-FNA 2) (Kappa agreement measure 78%, sig = 0.001) was found.

**Conclusion:** Endoscopists can acquire basic skills in cytopathology to perform the ROSE and the findings are consistent with those made by a pathologist. This strategy can contribute to obtaining adequate samples for cytopathology diagnosis and improve EUS-FNA diagnostic capacity. Basic cytopathology should be included as a subject in endosonography and endoscopy programs.

***Keywords:*** *EUS-FNA; ROSE; On-site Cytology; Endosonography*

## Introduction

The first report of a EUS-FNA of a pancreatic lesion was performed by Vilmann., *et al.* in 1992 [1]. Since then, pancreatic EUS-FNA has been developed with a high accuracy rate (80-90%) and a low complication rate (0 - 2.5%). Nowadays, this method for cytological

diagnosis has proven superior to conventional ultrasound (US)-guided biopsy, endoscopic retrograde cholangiopancreatography (ERCP), or computerized tomography (CT) [2].

The degree of tumor differentiation [3], the experience of the endosonographist [4-6] and the ROSE [3,7-9] are factors that influence the EUS-FNA diagnosis ability of pancreatic masses. In some studies, EUS-FNA with ROSE has shown to have higher diagnostic accuracy than EUS-FNA without cytologic evaluation [3,7-9] the ROSE promotes the proper use of cytology to reduce the likelihood of false negatives and unsatisfactory aspirations, thus improving the sensitivity and accuracy of EUS-FNA [2]. According to Logrono and Waxman, the objectives of the EUS-FNA with ROSE are: a) adequacy of specimen and minimize the numbers of passes. This ensures that the target organ is sampled appropriately, obtaining a sufficient number of cells for reliable cytological evaluation; b) to assess the nature of the disease that is affecting the body. This allows taking behaviors that benefit the patient, such as making additional punctures in cases of lymphoma and histochemical or immunohistochemical studies for characterization of pancreatic tumors; and c) to conduct a preliminary diagnosis [10].

When EUS-FNA was introduced in our service in March 2012, endoscopists were aware of the usefulness of ROSE, but economic difficulties banned the availability of cytopathologists during biopsies. Therefore, the desire to improve the management of patients (and considering our gastroenterology service is a reference center of public and private institutions within and outside the city) led two endoscopists in January 2015 to undergo one-week training in basic cytopathology at a pathology center.

## Aim of the Study

The aim of this pilot study was to evaluate whether ROSE conducted by two endoscopists with training in cytopathology allows knowing if the samples obtained by EUS-FNA are adequate or not adequate for a cytological diagnosis and to compare if findings were consistent with those interpreted by a pathologist.

## Methodology

A concordance study was conducted in eighty patients, in the department of gastroenterology "Unión de Cirujanos" at Clínica de la Presentación in Manizales, Colombia, from March to October 2015.

The selection criteria were: 1) patients over 18 years of age; 2) confirmed mass presence by, at least, one diagnostic modality such as US, CT, magnetic resonance imaging (MRI), EUS, or digestive endoscopy; and 3) patients taken EUS-FNA with ROSE for pancreatic and upper gastrointestinal tract lesions. Exclusion criteria for performing EUS-FNA were coagulopathy (INR > 1.5), or thrombocytopenia (platelet count < 50,000 mcL) and inability to take samples due to vascular interposition. Patients undergoing EUS-FNA referred from other cities who could not be contacted for follow-up due to possible adverse events and those who did not consent to participate were excluded.

### Equipment and procedure

All procedures were developed under moderate sedation with propofol by two ultrasonography- expert endoscopists [11,12] with an EG-53OUT2 linear echoendoscope and a US SU-8000 processor (Fujinon, Tokyo, Japan). The needles used for FNA were 19, 22 and 25 G (Cook Medical, Winston Salem, NC and Boston Scientific, ExpectSlimline, USA). The imaging characteristics of the lesion were recorded. The EUS-FNA technique was standardized by the examiners, continuous suction was applied during the EUS-FNA, and the use of the stylus was performed in all cases (Figure 1). The number of passes was determined on the basis of previous publications in which the number of passes required to make a diagnosis in the absence of a cytopathologist in the room was reported (3.13). Two punctures and a maximum of 15 passes for each sample were used in this study, following the endosonographist's criteria. All patients were contacted 48 hours afterward to record adverse events.

### Evaluation of cytopathology

The sample obtained was extracted from the needle by introducing the stylet, thus spreading the material on two glass slides, which were affixed with alcohol-based spray for cytology and fitted with lamellae. The slides were labeled with the corresponding number of puncture and one that was colored with hematoxylin/eosin for interpretation on-site was selected (Figure 2).

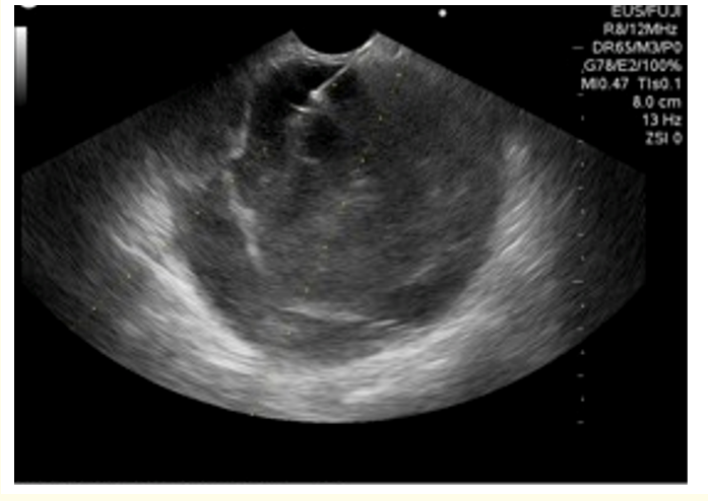

***Figure 1:*** *Endoscopic ultrasound image of a mass in the head of the pancreas. Fine needle aspiration of the mass.*

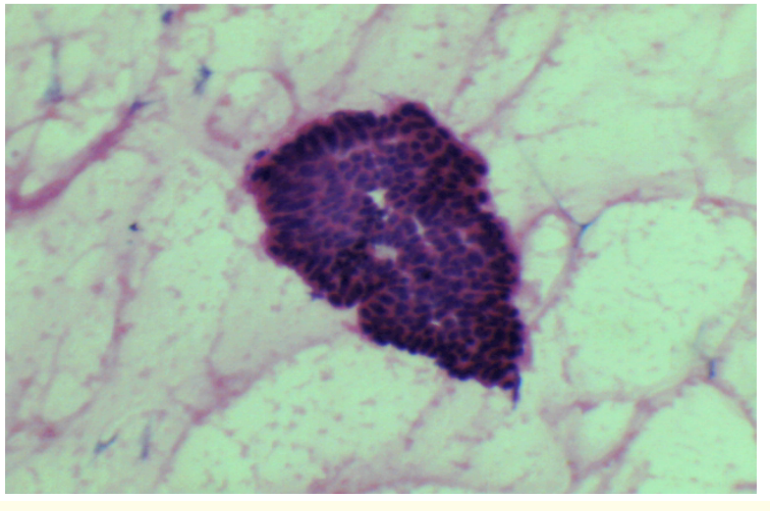

***Figure 2:*** *On-Site evaluation of a biliary epithelium obtained by CPRE. Monolayer sheet of uniform columnar cells with preservation of cohesion and polarity "honeycomb appearance" (hematoxylin/eosin x20).*

The ROSE was done by the two endoscopists trained in basic cytopathology. The training was provided before starting the study and carried out in the service of pathology at The Condes Clinic (Santiago de Chile, Chile) for one week; it included preparation and rapid coloring of the slides, cytologic evaluation, and the meaning of "adequate" and "inadequate" samples.

The impression on the "appropriate" nature of the samples was recorded taking into account the presence of the proper amount of cells (benign, atypical, or malignant) of the assessed body sufficient for evaluation. If the ROSE of the slide obtained in the first EUS-FNA revealed that the sample was adequate, the procedure was interrupted. If the sample was considered "inadequate," a second EUS-FNA was performed for additional material. If an adequate sample evaluated in the second slide was not obtained, no additional punctures were

performed, and the procedure was completed. The rest of the material obtained was affixed and sent to a pathology laboratory for institutional extra processing and subsequent analysis. In all cases, cell block (CB) was performed. Subsequently, the tested slides were sent to a pathologist with the patients' identification data with information on the endosonographic characteristics of the lesion for a second assessment using the same criteria. The pathologist did not know the result of the ROSE performed by the endoscopists.

### Statistical methods

In the case of categorical variables, frequencies and percentages were determined. In the case of numerical variables, averages, medians, and standard deviations (SD) were calculated according to relevance. The concordance analyses were performed using the Kappa agreement measure, as well as their confidence intervals.

## Results

In total, 80 patients underwent EUS-FNAs. 31 EUS-FNAs and 16 ERCPs were excluded from the study (30 patients came from hospitals in different cities, and 17 patients did not give their consent). This analysis included a total of 57 patients, obtaining the sample for cytology was performed in 49 patients with EUS-FNA (86.0%) and 8 ERCP brushings (14.0%). With an average age of 59.3 years old (SD 15.1, range 20 - 83). 50.9% of patients were male. The average body mass index was 22.9 (SD 3.9; I range from 14.0 to 33.0). The most common forms of clinical presentation that prompted the consultation were weight loss, 52.6%; abdominal pain, 42.1%; and jaundice, 38.6%. 15.8% of the cases were asymptomatic. Pancreatitis and other symptoms such as anemia, cholangitis, diarrhea, dysphagia, and vomiting occurred in less than 5% of cases. Previous diagnostic methods that had confirmed the presence of mass were CT, 65.0%; MR, 26.3%; US, 8.8%; digestive endoscopy, 8.8%; EUS, 5.3%; and ERCP, 1.8%.

Endosonographic characteristics of the patients undergoing EUS-FNA are summarized in table 1.

| Variable | | Frequency | Percentage |
|---|---|---|---|
| Mass presence | | 46 | 93,9 |
| Mass location | Pancreas | 31 | 63,3 |
| | Head | 26 | 53,1 |
| | Body | 24 | 49,0 |
| | Uncinate | 15 | 30,6 |
| | Tail | 5 | 10,2 |
| | Stomach | 3 | 6,1 |
| | Retroperitoneum | 3 | 6,1 |
| | Another location | 12 | 24,5 |
| Type of mass | Solid | 31 | 63,3 |
| | Cystic | 8 | 16,3 |
| | Mixed | 10 | 20,4 |
| Mass Diameter (in mm.) | Media-DE | 36,80 | 25,40 |
| | Median | 35 | |
| | Range (minimum-maximum) | 7 | 140 |
| Diagnostic Impression | Adenocarcinoma | 12 | 24,5 |
| | BMPE Main Conduit | 6 | 12,2 |
| | GIST | 4 | 8,2 |
| | Lymphadenopathy | 4 | 8,2 |
| | Serous Cystadenoma | 3 | 6,1 |
| | Mucinous Cystadenoma | 2 | 4,1 |
| | Cystadenocarcinoma | 2 | 4,1 |
| | Cholangiocarcinoma | 1 | 2,0 |
| | Another diagnostic impression | 15 | 30,6 |
| Number of Patients | | 49 | |

***Table 1:*** *Endosonographic aspects of lesions in patients undergoing EUS-FNA.*

EUS-FNA 1 was successfully performed in all 49 cases. EUS-FNA 2 was performed in 18 patients. No adverse events occurred during the conduct of the proceedings. In table 2, the needle, the type of approach, the median number of passes conducted, and the number of slides sent to pathology for EUS-FNA 1 and 2 are described.

| Variable Frequency | | EUS-FNA 1(n = 49) | | EUS-FNA 2 (n = 18) | |
|---|---|---|---|---|---|
| | | Percentage | Frequency | Percentage | |
| Needle used | 19G | 12 | 24,5 | 8 | 16,3 |
| | 22G | 30 | 61,2 | 5 | 10,2 |
| | 25G | 7 | 14,3 | 5 | 10,2 |
| Approach | Transgastric | 24 | 49,0 | 10 | 20,4 |
| | Transduodenal | 22 | 44,9 | 8 | 16,3 |
| | Transesophageal | 3 | 6,1 | 0 | 0,0 |
| # of Passes | Median | 9 | | 15 | |
| | Minimum-Maximum | 5 | 15 | 5 | 15 |
| # Slides sent | Median | 3 | | 3 | |
| | Minimum-Maximum | 1 | 7 | 1 | 6 |

***Table 2:*** *Type of needle, approach, number of passes made, and number of slides (sent to pathology) of injuries from patients undergoing EUS-FNA 1 and 2.*

### Cytology results

A high concordance of an "appropriate" reading between the endoscopist and the pathologist in the EUS-FNA 1 (measure of agreement Kappa 81%, sig = 0.000) and EUS-FNA 2 (Kappa measure of agreement 78%, sig = 0.001) was found. Furthermore, a high concordance in the discovery of mucin by endoscopists and pathologists in the EUS-FNA 1 (Kappa agreement measure 85%, sig = 0.000) and EUS-FNA 2 (Kappa agreement measure 64% sig = 0.006) was found.

No statistical relationship was found between the type of needle used or the involvement of the pancreas with respect to the fact that the sample was "adequate" or "inadequate" in the EUS-FNA 1 or 2. No relationship was found when the analysis was made according to the location of the mass in the pancreas, either.

## Discussion

EUS-FNA improves the diagnostic ability of the EUS by means of pathological findings. It's safe, fast, and it provides high accuracy in diagnosing gastric [14], pancreatic [2,15,16], bile, and liver tumors [17] (80 - 90% or more). Factors that contribute to false negatives are technical factors, patient factors and pathological factors, including the performance of ROSE and the availability of competent cytopathologists [2]. Two prospective studies [18,19] have stressed the importance of ROSE in EUS-FNA by cytopathologists, in determining cellularity, cell type and a preliminary diagnosis. Erickson states that feedback by cytopathologists increases the performance of EUS-FNA in 10 - 15% [7]. Unfortunately, the availability of cytopathologists to perform the ROSE is not universal because of the financial implications for health institutions [20].

To reduce the impact of the absence of cytopathologists on-site, several alternatives have been described, including the evaluation by cytotechnicians and gastroenterologists with training in cytopathology, with varying results. One study showed that the presence of trained cytotechnicians during EUS-FNAs allows a higher rate of appropriate samples and diagnostic accuracy compared to endosonographers, but not exceeding 68% and 75% respectively [21]. A prospective controlled study compares the identification of either appropriate or inappropriate samples obtained in 117 EUS-FNAs from 3 endosonographers and 1 cytotechnician, demonstrating greater diagnostic accuracy and rates of appropriate samples or inappropriate with trained cytologists (82%) compared to endosonographers (68 - 76%);

nonetheless, they concluded that it was premature to judge ROSE performed by endosonographers [22]. A retrospective study of 73 patients compared obtaining adequate specimens in two time periods: when ROSE was done by endosonographers (n = 38) and when it was conducted by cytopathologists (n = 35). Adequate specimens rates were similar between the 2 97.4 vs. 97.1% cohorts, respectively [2]. A retrospective study of 138 patients with solid pancreatic masses who underwent EUS-FNA found that the application of cytopathology criteria by two endosonographers improved diagnosis rates by 22% and decreased the inconclusive diagnoses by 18% [23]. Varadarajulu., *et al.* evaluated the effectiveness of an intensive, short training program in cytopathology of samples obtained by EUS-FNA, conducted in 6 endosonographers, by evaluating four performance measures: adaptation, interpretation, processing, and preliminary diagnosis; it was shown to be effective, with a 63% post-training improvement, a 95% efficiency in the interpretation of slides in *in vivo* cases and more importantly, the identification of appropriate samples was successful in 97% of cases [20]. Our pilot study showed that training in cytopathology allowed the endosonographers to perform the ROSE and to determine the adequacy of the samples, with a concordance of 81% when compared with what was assessed by a pathologist, which is high and significant, as other series reported [2,20,22].

In our study, the production of mucin by EUS-FNA of cystic neoplasms and determination of this in the sheets evaluated by endosonographers and cytopathologists was concordant in 85%. The presence of mucin could determine satisfaction of the sample despite not being accompanied by sufficient columnar or cuboidal cell elements. The string sign, carcinoembryonic antigen analysis, and molecular analysis are some alternatives described, which allow the classification of pancreatic mucinous lesions [24]. Therefore, in centers where there isn't a cytopathologist on-site, it is important to correlate the amount, the type of material obtained during the EUS-FNA, and the endosonographic findings [that is to say, the "type of injury (solid, cystic, mixed), the location, and the diagnostic printing"], which the pathologist must be informed of in order to improve the diagnostic accuracy and to avoid false negative samples interpreted as insufficient by the absence of cells, in relation to cystic tumors.

Cytological evaluation of bile brushing between the endosonographers and the pathologist allowed identifying satisfactory cellular elements, consistent in 7/8 patients. This allows us to suggest that the cytological evaluation could be applied to bile brushing and not exclusively to samples obtained by EUS-FNA, in order to increase diagnostic efficiency.

Published studies support that the CB of specimens obtained by EUS-FNA from pancreatic lesions increases the sensitivity of cytology from 79% to 90% and an accuracy of 81% to 91% [25]. Although the goal of our study was not to know the outcome of the CBs obtained, this was collected for all patients to increase the diagnostic efficiency of EUS-FNA without a cytopathologist on-site. In our opinion, visual assessment of sufficient tissue fillets immersed in the jar for CB is a subjective finding that may indicate the sample is adequate. The CB is technically easy; it does not adversely affect the results of the FNA procedure and it increases the likelihood of establishing a diagnosis in external pathology centers significantly.

This study has several limitations. It should be noted that it is not a formal diagnostic test study, and its intention was not to show how many cases are sufficient for an endoscopist trained in cytopathology to be able to independently evaluate a film, but to show that an endoscopist may be able to determine whether a sample is adequate. Although diagnostic performance can best be demonstrated through a greater number of procedures, the study design did not allow determining the impact of the learning curve, so its results are limited to determining consistency through it being compared with the opinion of a pathologist who evaluated the same slide chosen by the two endoscopists. We do not have the final concept by the external pathologists who processed and evaluated all the slides and CB, which would allow a closer approach to a diagnostic test design with determination of sensitivity, specificity, and predictive values.

## Conclusion

Our results demonstrate that endoscopists can acquire enough basic skills in cytopathology to perform the ROSE, and the findings are consistent with those made by a pathologist. This strategy can help improve diagnostic capacity of EUS-FNA and the usefulness of patients' outcomes, as well as reduce reliance on a cytopathologist during EUS-FNA. The authors suggest that endosonography and endoscopy programs should include a basic cytopathology course.

## Bibliography

1. Vilmann P., *et al*. "Endoscopic ultrasound with guided fine needle aspiration biopsy in pancreatic disease". *Gastrointestinal Endoscopy* 38 (1992): 172-173.

2. Hikichi T., *et al*. "Endoscopic ultrasound-guided fine-needle aspiration of solid pancreatic masses with rapid on-site cytological evaluation by endosonographers without attendance of cytopathologists". *Journal of Gastroenterology* 44.4 (2009): 322-328.

3. Erickson RA., *et al*. "Factors predicting the number of EUS-guided fine-needle passes for diagnosis of pancreatic malignancies". *Gastrointestinal Endoscopy* 51 (2000): 184-190.

4. Harewood GC., *et al*. "Influence of EUS training and pathology interpretation on accuracy of EUS-guided fine needle aspiration of pancreatic masses". *Gastrointestinal Endoscopy* 55 (2002): 669-673.

5. Eloubeidi MA and Tamhane A. "EUS-guided FNA of solid pancreatic masses: a learning curve with 300 consecutive procedures". *Gastrointestinal Endoscopy* 61 (2005): 700-708.

6. Mertz H and Gautam S. "The learning curve for EUS-guided FNA of pancreatic cancer". *Gastrointestinal Endoscopy* 59 (2004): 33-37.

7. Erickson RA. "EUS-guided FNA". *Gastrointestinal Endoscopy* 60 (2004): 267-279.

8. Yamao K., *et al*. "Endoscopic ultrasound-guided fine-needle aspiration biopsy (EUS-FNAB): past, present, and future". *Journal of Gastroenterology* 40 (2005): 1013-1023.

9. Klapman JB., *et al*. "Clinical impact of on-site cytopathology interpretation on endoscopic ultrasound guided fine needle aspiration". *The American Journal of Gastroenterology* 98 (2003): 1289-1294.

10. Logrono R and Waxman I. "Interactive role of the cytopathologists in EUS-guided fine needle aspiration: an effective approach". *Gastrointestinal Endoscopy* 54 (2001): 485-490.

11. DiMaio CJ., *et al*. "EUS core curriculum". *Gastrointestinal Endoscopy* 76.3 (2012): 476-481.

12. Wani S., *et al*. "Quality indicators for EUS". *Gastrointestinal Endoscopy* 81.1 (2015): 67-80.

13. LeBlanc JK., *et al*. "Optimal number of EUS-guided fine needle passes needed to obtain a correct diagnosis". *Gastrointestinal Endoscopy* 59 (2004): 475-481.

14. Mehmood S., *et al*. "On site cytopathology evaluation and ancillary studies beneficial in EUS-FNA of pancreatic, mediastinal, intra-abdominal, and submucosal lesions". *Diagnostic Cytopathology* 43.4 (2015): 278-286.

15. Takahashi K., *et al*. "Differential diagnosis of pancreatic cancer and focal pancreatitis by using EUS-guided FNA". *Gastrointestinal Endoscopy* 61 (2005): 76-79.

16. Wakatsuki T., *et al*. "A comparative study of diagnostic value of cytologic sampling by endoscopic ultrasonography-guided fine-needle aspiration and that by endoscopic retrograde pancreatography for the management of pancreatic mass without biliary stricture". *Journal of Gastroenterology and Hepatology* 20 (2005): 1701-1711.

17. Hammoud GM., *et al*. "Usefulness of endoscopic ultrasound-guided fine needle aspiration in the diagnosis of hepatic, gallbladder and biliary tract Lesions". *World Journal of Gastrointestinal Oncology* 6.11 (2014): 420-429.

18. Wiersema MJ., *et al*. "Endosonography-guided real-time fine-needle aspiration biopsy". *Gastrointestinal Endoscopy* 40 (1994): 700-707.

19. Chang KJ., *et al*. "Endoscopic ultrasound-guided fine-needle aspiration". *Gastrointestinal Endoscopy* 40 (1994): 694-699.

20. Varadarajulu S., *et al*. "Training endosonographers in cytopathology: improving the results of EUS-guided FNA". *Gastrointestinal Endoscopy* 81.1 (2015): 104-110.

21. Petrone MC., *et al*. "Does cytotechnician training influence the accuracy of EUS-guided fine needle aspiration of pancreatic masses?" *Digestive and Liver Disease* 44 (2012): 311-314.

22. Savoy AD., *et al*. "Can endosonographers evaluate on-site cytologic adequacy? A comparison with cytotechnologists". *Gastrointestinal Endoscopy* 65 (2007): 953-957.

23. Hayashi T., *et al*. "Rapid on-site evaluation by endosonographer during endoscopic ultrasound-guided fine needle aspiration for pancreatic solid masses". *Journal of Gastroenterology and Hepatology 28* (2013): 656-663.

24. Samarasena JB., *et al*. "Endoscopic ultrasonography-guided fine-needle aspiration of pancreatic cystic lesions: a practical approach to diagnosis and management". *Gastrointestinal Endoscopy Clinics of North America* 22.2 (2012): 169-185.

25. Kim JH., *et al*. "Incremental value of cell block preparations over conventional smears alone in the evaluation of EUS-FNA for pancreatic masses". *Hepatogastroenterology* 61.135 (2014): 2117-2122.

**Volume 7 Issue 2 February 2020**