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Intermittent endoscopic ultrasound guided fine-needle aspiration for the diagnosis of solid pancreatic lesions. Pilot study

Raquel Herranz Pérez¹, Felipe de la Morena López¹, José Jiménez-Heffernan², Carlos Humberto Gordillo-Vélez², Lorena Vega Piris³, José Andrés Moreno Monteagudo³, Cecilio Santander¹

¹Department of Gastroenterology and Hepatology, Hospital Universitario de La Princesa, Instituto de Investigación Sanitaria Princesa (IIS-IP), CP 28006 Madrid, Spain.  
² Department of Anatomopathology, Hospital Universitario de La Princesa, Instituto de Investigación Sanitaria Princesa (IIS-IP), CP 28006 Madrid, Spain.  
³Methodological Support Unit, Department of Statistical Analysis, Hospital Universitario de La Princesa, Instituto de Investigación Sanitaria Princesa (IIS-IP), CP 28006 Madrid, Spain

Correspondence to:  
Raquel Herranz Pérez, Department of Gastroenterology, Hospital Universitario de La Princesa, C/ Diego de Leon nº62, 28006, CP 28033 Madrid, Spain.  
raquelherranzperez@gmail.com  
Telephone: +34915202200  
Fax: +34915204013

Keywords: Endoscopic ultrasound, pancreatic cancer, Fine-Needle Aspiration

List of abbreviations
CS continuous suction  
CT computed tomography  
ERCP endoscopic retrograde cholangiopancreatography  
EUS endoscopic ultrasound  
EUS-FNA endoscopic ultrasound fine-needle aspiration  
FNA fine needle aspiration
ABSTRACT

Background and purpose of the study: Endoscopic ultrasound guided fine-needle aspiration (EUS-FNA) is the method of choice for sampling pancreatic solid lesions. However, there is significant heterogeneity in terms of the technique used. Intermittent aspiration has not been evaluated in pancreatic solid lesions and could improve the diagnostic performance.

Methods: Single-blind, non-inferiority pilot study. Patients with solid pancreatic lesions and indication for EUS-FNA were prospectively included. Patients were randomly assigned to intermittent (IS) or continuous (CS) suction techniques. Diagnostic performance, cellularity, blood contamination and number of passes required to reach diagnosis were evaluated.

Main results: 33 patients were assigned to CS (16 patients) or IS (17 patients). Diagnostic performance was 87.5% for CS and 94.1% for IS (OR 2.29, 95%CI 0.19-27.99, p = 0.51). In the IS group samples had higher cellularity (OR 1.83, 95%CI 0.48-6.91, p = 0.37) and lower blood contamination (OR 0.38, 95%CI 0.09-1.54, p = 0.18). The number of passes required to reach diagnosis was 2.12 for CS and 1.94 for IS (p = 0.64). Liquid cytology was obtained in 73.3% of IS and 61.5% of CS (OR 1.72, 95%CI 0.35-8.50).

Conflict of interest: The authors declare no conflict of interest
Conclusions: The IS technique was not inferior to CS in terms of diagnostic accuracy in the evaluation of pancreatic solid lesions, with a tendency to obtain higher cellularity, lower blood contamination and frequent presence of cell block.

INTRODUCTION

Endoscopic ultrasound (EUS) has practically become an essential imaging technique for the study of biliopancreatic diseases, and has demonstrated greater diagnostic sensitivity than percutaneous ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET) and endoscopic retrograde cholangiopancreatography (ERCP) (1, 2). Endoscopic ultrasound fine-needle aspiration (EUS-FNA) is the technique of choice for sampling solid pancreatic lesions, with a sensitivity of 85%-89% and specificity of 96%-99% (3, 4). However, its diagnostic accuracy can be influenced by different factors such as the endoscopist expertise, location and type of lesion, presence of chronic pancreatitis, needle size, type of technique used, expertise of the cytopathologist and his or her presence in the room, among others (5-7). In fact, a systematic review assessed a sampling error rate of 4-45% in solid pancreatic lesions, 21-53% in cystic lesions and 6-14% in lymph nodes (8).

Several studies and meta-analyses have evaluated different technical aspects of EUS-FNA with the aim of optimizing this technique. Based on them, the European Society of Gastrointestinal Endoscopy (ESGE) published in 2017 a guideline with recommendations (9). For the puncture of solid lesions, the guideline recommends the use of 22G or 25G needle, with the option of fine needle aspiration (FNA) or fine needle biopsy (FNB). It recommends applying continuous suction with a 10 ml syringe, performing the fanning technique and neutralizing the negative pressure before removing the needle from the lesion. It makes no recommendation for or against the use of stylet. In addition, in the absence of a pathologist in the room, performing 3-4
passes with FNA or 2-3 with FNB is recommended.

Regarding the aspiration method, 3 types of techniques have been described: no aspiration, continuous suction (CS) or standard suction, and wet suction (WS). Standard aspiration is achieved by using luer-lock syringes which, connected to the proximal end of the echoendoscopy needle, provide a continuous suction effect generating a negative pressure inside the needle, and theoretically allow obtaining a larger sample. These suction systems can bet set at low (10 ml) or high (20-50 ml) pressure. Their effect on cost-effectiveness is not clear. While some studies show no significant differences with their use (10), others show greater diagnostic accuracy with the use of suction(7, 11) or suggest greater cost-effectiveness with the slow withdrawal of the stylet (12). On the other hand, WS has also demonstrated in some studies better results in terms of diagnostic accuracy than CS(13, 14). The intermittent suction (IS) technique has been described as a modality(15, 16), and some authors recommend performing FNA of thyroid nodules by a combination of puncture without aspiration followed by low-pressure aspiration(17). However, no prospective studies have been performed to evaluate its diagnostic accuracy in solid pancreatic lesions.

Therefore, based on the hypothesis that IS allows to reduce the number of passes to reach the diagnosis with respect to other methods, the main objective of our study was to determine whether IS technique could be equivalent or superior in terms of diagnostic performance to low-intensity CS in pancreatic solid lesions. As secondary objectives we evaluated the grade of cellularity and blood contamination, the achievement of liquid cytology, the number of passes necessary to reach diagnosis and the presence of complications.

**MATERIAL AND METHODS**

A non-inferiority, prospective, single-center, single-blind for the anatomopathologist, pilot study was performed. It was approved by the ethics committee and registered at clinicaltrials.gov (NCT03829748).

**Study population**
Patients with pancreatic solid lesion and indication for EUS-FNA referred to the digestive endoscopy unit of the Hospital Universitario de la Princesa over 12 months (March 2019 to March 2020) were prospectively and consecutively included. Exclusion criteria were inaccessibility for endoscopic ultrasound to the puncture site, unacceptable anesthetic risk (ASA IV), pregnancy, coagulopathy (INR>1.5), thrombocytopenia (<50000/mm3), non-suspension of anticoagulation or double antiplatelet therapy prior to the procedure. Patients were randomized IS or CS groups with a 1:1 ratio using the Epidat 4.0 informatics application by a statistician blinded to this study.

**Methods**

Patients underwent linear endoscopic ultrasound examination (GF-UTC 260 Olympus® Tokyo, Japan) performed by two expert endosonographists. The following characteristics were evaluated and collected: location, size, presence of necrosis and elastography pattern of the lesion. Puncture was then performed with a 25G needle (Expect Slimline 25G Boston Sci®) without stylet in 4 passes according to the assigned method, which was revealed during the procedure. The CS method consisted in applying a vacuum syringe with 10 ml once inside the lesion, followed by performing 15 passes using the fanning technique in each pass. In IS group the technique was modified by performing cycles of 5 passes alternating with opening and closing of the luer three times, intermittently generating negative pressure.

Finally, the samples were obtained by introducing the stylet and were used to perform dry smears in separate numbered slides (passes 1-4) for Diff Quick staining, and by flushing with Thinprep® the remaining content for liquid cytology. Samples were later assessed by two expert cytopathologists, who were blinded to the method used. The samples were classified according to the Papanicolau classification(18). The grade of cellularity and blood contamination according to the criteria described in Table 1, the number of passes necessary to reach diagnosis and the representativeness for liquid cytology were also established.

Demographic variables (age and sex), lesion characteristics and the technique used were collected. In addition, anatomopathologic findings were recorded as previously described and the associated radiologic and surgical procedures were reviewed for
each patient. We used the following criteria to establish malignancy: positivity in
cytology or in the surgical specimen, progression of the lesion and/or metastatic
disease during follow-up, or death related to neoplastic complications up to 6 months
after diagnosis.

Statistical analysis

Quantitative variables were expressed as means ± standard deviation and categorical
variables as frequencies or percentages. The chi-square test was used for comparison
of qualitative variables. Comparison of means were analyzed by t-test for equal or
unequal variances as appropriate (after homocedasticity analysis with Levene’s test).
In order to evaluate the differences in diagnostic performance or representativeness,
the sensitivity after each pass was estimated, as well as the effect of both techniques
by means of odds rate (OR) and 95% confidence intervals (CI). The optimal number of
passes depending on the method was calculated assuming that the following passes
would not modify the additional diagnostic performance by more than 10% of the
cumulative performance. In addition, to compare the cellularity and blood
contamination, graded in three levels, between the two methods, an ordinal logistic
regression was performed. All variables that could potentially modify the diagnostic
performance of both methods were further assessed with univariate analysis and
followed by multivariate analysis by means of logistic regression analysis with the
STATA program (v13.0, StataCorp, College Station, Tex, USA).

RESULTS

Thirty-three patients with a total of 33 pancreatic solid lesions were included and
randomized to CS (16 patients) and IS (17 patients). The baseline characteristics of the
population and the lesions included are shown in Table 2. There were no significant
differences between both groups.

The diagnostic performance with CS was 87.5% and with IS 94.1%, although no
significant differences were found between the two techniques (OR 2.29, 95%CI
0.19-27.99, p = 0.51). In the IS group, samples presented higher cellularity (OR 1.83,
95%CI 0.48-6.91) and lower blood contamination (OR 0.38, 95%CI 0.09-1.54), but
neither of these parameters reached statistical significance ($p = 0.37$ and $p = 0.18$, respectively). The number of passes required to reach diagnosis was $2.12 \pm 1.26$ with CS and $1.94 \pm 0.97$ with IS ($p = 0.64$). After performing 3 passes, diagnosis was achieved in 100% of the samples obtained by IS and in 75% of those obtained by CS. After the fourth pass, cytological diagnosis was obtained in 100% of the samples from both groups. Liquid cytology was achieved in 73.3% of the samples obtained with IS and in 61.5% of those obtained with CS, although these differences were not significant (OR 1.72, 95% CI 0.35-8.50). No complications were recorded during or after the procedure. No differences were found for the rest of the variables studied (Table 3).

**DISCUSSION**

EUS-FNA is currently the technique of choice for obtaining samples from pancreatic solid lesions due to the proximity of the transducer to the lesion, the lower risk of seeding compared to radiologically guided punctures, its low complication rate and its high diagnostic accuracy(9, 19). The ideal technique must be safe, precise and achieve a high diagnostic performance. The latter is the most limiting factor in routine clinical practice. False negatives have a particular impact on patient management as a result of inadequate diagnostic-prognostic orientation, and are derived from failures in the puncture technique, inexperience of the endosonographist or the cytopathologist and/or the characteristics of the lesions. For this reason, multiple studies have been performed with the aim of improving diagnostic performance, mostly by modifying technical aspects such as the caliber or type of needle, and the puncture technique(13, 20).

The present study was developed following the recommendations for the performance of EUS-FNA from the 2017 ESGE guideline(9). We decided to avoid stylet use because, although systematic reviews have not shown significant differences(21),
one study showed greater sample adequacy and less blood contamination(22). In addition, we chose 25G as cytology needle since a meta-analysis showed a higher sensitivity with 25G needles compared to 22G needles in pancreatic solid neoplasms (0.93, 95% CI 0.91-0.96 vs. 0.85, 95% CI 0.82-0.88; p = 0.0003) (23). We decided to apply vacuum syringe aspiration with 10 ml and compared the CS and IS techniques.

We obtained a diagnostic performance with the IS technique of 94.1% versus 87.5% with CS (OR 2.29, 95%CI 0.19-27.99, p = 0.51). Overall, it is estimated that the quality of an echoendoscopy unit can be considered high if its diagnostic performance is higher than 85%(24). Similar studies evaluating the diagnostic performance of the use of aspiration did not obtained superior results. Lee et al they obtained a diagnostic rate of 72.8% with aspiration vs. 58.6% without it (p = 0.001)(7), while Tarantino et al obtained 86.2% vs. 69.0% vs. 49.4% (p < 0.001) with the use of 20ml, 10ml and 0ml, respectively(11). Therefore, we can affirm that in our study the diagnostic performance was adequate with both techniques, with a tendency to obtain a higher diagnostic performance with IS, although without statistical significance.

Regarding the secondary objectives, we evaluated the adequacy of the sample by assessing cellularity and blood contamination, and found that samples from the IS group tended to have higher cellularity (OR 1.83, 95%CI 0.48-6.91, p 0.37) and lower blood contamination (OR 0.38, 95%CI 0.09-1.54, p 0.18), although without significant differences. The use of aspiration has been associated with a higher blood contamination, however, this does not seem to affect the diagnostic performance (7, 11). Another parameter analyzed was the number of passes required to reach a cytological diagnosis, which was 1.94 passes in the IS group and 2.12 in the CS group (p = 0.64). We achieved the diagnosis after the third pass in 100% of the samples obtained by IS and in 75% of those obtained with CS, and after the fourth pass in 100% of the samples in both groups. Previous studies have shown similar results, obtaining a diagnostic performance of 85% with ≤ 3 passes (25) and 96% with 4 passes(26). Finally, we evaluated the achievement of liquid cytology using Thinprep needle washing of the remaining content inside the needle after the fourth pass, which was 73.3% in samples obtained with IS and in 61.5% in those obtained with CS, although these differences were not significant (OR 1.72, 95%CI 0.35-8.50). Previous studies have found no
differences between cytological and histological preparations for malignant pancreatic solid lesions(9), and some studies have even demonstrated greater sensitivity with cytologic specimens(27). Therefore, the aim of our study was not to evaluate the collection of liquid cytology, but to determine the presence of remaining material after previous extraction with the stylet, finding the presence of representative material in > 50% of cases.

The main limitations of our study are the small sample size and its single-center nature of the study. Nevertheless, it is a pilot study aimed to assess the non-inferiority of the technique. EUS-FNA is a technique that has been the subject of numerous studies and meta-analyses demonstrating high diagnostic accuracy. A large sample size would be necessary to demonstrate an improvement.

In conclusion, the IS technique is non-inferior in terms of diagnostic performance and provides samples by EUS-FNA which show a tendency to present higher cellularity, lower blood contamination and frequent presence of liquid cytology in the diagnosis of pancreatic solid lesions compared to CS. Prospective studies with larger sample sizes are needed to confirm the superiority of this technique.

BIBLIOGRAPHY


**TABLE 1:** Adequacy of the sample

<table>
<thead>
<tr>
<th>Celularity</th>
<th>Adequacy</th>
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<tr>
<td>Scant</td>
<td>&lt; 10 cell clusters</td>
</tr>
<tr>
<td>Adequate</td>
<td>10-20 cell clusters</td>
</tr>
<tr>
<td>Excelent</td>
<td>&gt; 20 cell clusters</td>
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</table>

<table>
<thead>
<tr>
<th>Blood contamination</th>
<th>Adequacy</th>
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</thead>
<tbody>
<tr>
<td>Low</td>
<td>Contamination present in &lt; 25% of the slide</td>
</tr>
<tr>
<td>Moderate</td>
<td>Contamination present in 25-50 % of the slide</td>
</tr>
<tr>
<td>High</td>
<td>Contamination present in &gt; 50 % of the slide</td>
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### TABLE 2: Baseline characteristics of the study population and the lesions included

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Continuous suction (N = 16)</th>
<th>Intermittent suction (N = 17)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female, n. (%)</td>
<td>7 (43.7) /9 (56.3)</td>
<td>7 (41.2) /10 (58.8)</td>
<td>0.881</td>
</tr>
<tr>
<td>Mean age, years (± SD)</td>
<td>70.1 ± 12.3</td>
<td>69.5 ± 12.5</td>
<td>0.892</td>
</tr>
<tr>
<td>Lesion location, n. (%):</td>
<td>11 (68.8)</td>
<td>12 (70.6%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Head</td>
<td>3 (18.7)</td>
<td>3 (17.6%)</td>
<td></td>
</tr>
<tr>
<td>Body</td>
<td>2 (12.5)</td>
<td>2 (11.7%)</td>
<td></td>
</tr>
<tr>
<td>Mean size, mm (± SD)</td>
<td>29.9 ± 16.9</td>
<td>25.8 ± 8.3</td>
<td>0.375</td>
</tr>
<tr>
<td>Necrosis/cystic spaces, n. (%)</td>
<td>9 (56.2%)</td>
<td>5 (29.4%)</td>
<td>0.166</td>
</tr>
<tr>
<td>Elastography, mean SR (± SD)</td>
<td>25.9 ± 40.6</td>
<td>23.2 ± 29.2</td>
<td>0.829</td>
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</tbody>
</table>

SD: standard deviation
### TABLA 3: Final results comparing continuous and intermittent suction

<table>
<thead>
<tr>
<th>Lesions (N = 33)</th>
<th>Continuous suction (N = 16)</th>
<th>Intermittent suction (N = 17)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papanicolau classification, n. (%)</td>
<td></td>
<td></td>
<td>0.601</td>
</tr>
<tr>
<td>I: Non-diagnostic</td>
<td>1 (6.2)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>II: Negative for malignancy</td>
<td>1 (6.2)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>III: Atypical</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>IV: Neoplastic: benign</td>
<td>1 (6.2)</td>
<td>1 (5.9)</td>
<td></td>
</tr>
<tr>
<td>V-VI: Suspicious of malignancy or malignant</td>
<td>13 (81.3)</td>
<td>16 (94.1)</td>
<td></td>
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<tr>
<td>Diagnostic performance, n. (%)</td>
<td>14 (87.5)</td>
<td>16 (94.1)</td>
<td>0.601</td>
</tr>
<tr>
<td>Cellularity, n. (%)</td>
<td></td>
<td></td>
<td>0.154</td>
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<tr>
<td>Scant</td>
<td>5 (31.2)</td>
<td>1 (5.9)</td>
<td></td>
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<tr>
<td>Adequate</td>
<td>6 (37.5)</td>
<td>11 (64.7)</td>
<td></td>
</tr>
<tr>
<td>Excellent</td>
<td>5 (31.2)</td>
<td>5 (29.4)</td>
<td></td>
</tr>
<tr>
<td>Blood contamination, n. (%)</td>
<td></td>
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<td>0.439</td>
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<tr>
<td>Low</td>
<td>4 (25)</td>
<td>8 (47.1)</td>
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</tr>
<tr>
<td>Moderate</td>
<td>10 (62.5)</td>
<td>8 (47.1)</td>
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<tr>
<td>Significant</td>
<td>2 (12.5)</td>
<td>1 (5.9)</td>
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<tr>
<td>Nº passes for diagnosis, n ± SD</td>
<td>3.1 ± 1.3</td>
<td>1.0 ± 0.8</td>
<td>0.640</td>
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