Performance of the Standard 22G Needle for Endoscopic Ultrasound-guided Tissue Core Biopsy in Pancreatic Cancer

Andrada Seicean1,2, Marcel Gheorghiu2, Teodor Zaharia2, Tudor Calinici1,2, Andrada Samarghitan2, Bogdan Marcus2, Simona Cainap1,4, Radu Seicean1,5

ABSTRACT

Background & Aim: Endoscopic ultrasonography (EUS) and EUS-guided fine-needle aspiration (EUS-FNA) are considered good tools for the diagnosis of pancreatic cancer and for obtaining material for cytology or histology. The accuracy of EUS-FNA can rise to 85-95%, but it is lower in cases with a chronic pancreatitis background or with previous biliary stenting. We aimed to establish the diagnostic yield of the visible length of the core biopsy samples in pancreatic cancer by using one single type of standard 22G needle and to evaluate the factors which can influence the results.

Method: EUS-FNA was performed by using a 22G standard needle on patients prospectively recruited with the suspicion of pancreatic masses on transabdominal ultrasound or CT scan over a period of eight months. The number of passes was limited by the length of the core obtained. The final diagnosis was based on EUS-FNA or hepatic biopsy for their metastasis or by follow up every three month by imaging methods.

Results: The study included 118 patients. Previous stents were present in 10 patients and chronic pancreatitis features were found in 3 patients. The procedure sensitivity was 89% and the global accuracy was 89%. The presence of biliary stents did not impede the accuracy of results. The number of passes did not influence the results.

Conclusions: The diagnostic rate of core biopsy by using 22G needles had a high accuracy and it is safe when the length of core dictates the number of passes. The presence of biliary stents did not influence the results.

Key words: endosonography – fine needle aspiration – core biopsy – 22G needle – pancreatic cancer – diagnosis – biliary stent.

Abbreviations: AUC: area under the curve; EUS: endoscopic ultrasonography; EUS-FNA: fine needle aspiration under endosonographic guidance; FNA: fine needle aspiration; INR: international normalised ratio; OR: odds ratio.

INTRODUCTION

Endoscopic ultrasound (EUS)-guided biopsy in pancreatic pathology is important for establishing the tumor type by using cytology or histology examination. The sample can be obtained by the tru-cut system, resulting in histology with maintained architecture, but these needles have difficulties in functioning in the antrum, fundus and duodenal bulb due to echoendoscope angulation [1]. Adequate samples are obtained in 80% of the cases, and the diagnostic rate is 83% [2]. Pro-core needles are also useful for obtaining histology from all parts of the pancreas (the core procurement rate is 88-100%), but they have a higher cost than the standard 22G needles [3, 4]. For the 22G core–needles the histologic core is obtained in 76-88% of cases [3-6], but for the 25G needles only in 32% of the patients [7]. However, their results seem similar to the core biopsy obtained with the standard fine needle aspiration (FNA) needle [4].

Another possibility of biopsy is based on the aspiration principle, by using endoscopic ultrasound fine needle aspiration (EUS-FNA) needles, with tissue architecture unpreserved. Usually the material obtained can serve for cytology, when the sample is arranged on a slide, with a sensitivity for diagnosis of 85-86% [8, 9] and limited value for immunohistochemistry. More rarely, the samples are analyzed...
as cell blocks, by concentrating the material flushed from the needle or as core biopsy, which offers an adequate sample of tissue, very useful for ancillary laboratory tests, especially in non-adenocarcinoma pancreatic masses. To obtain the core biopsy with standard FNA needles can be challenging and it depends on the needle size, with a procurement rate of 83-100% [4, 10]. However, the latest EUS-FNA guidelines encourage the procurement of histologic samples for each patient [11].

There are controversies about the number of passes in pancreatic masses for obtaining both cytology and core biopsies [11] and the length of the core has never been used for restricting them when the standard 22G needle has been used.

Until now it is not known whether the core biopsy diagnostic rate could be influenced by the mass features and if the results may differ between the various types of 22G needle.

We aimed to establish the yield of the diagnostic sample of the length of the visible core biopsy samples in pancreatic cancer by using the same type of 22G needle, and the factors which can influence the results.

**PATIENTS AND METHOD**

This prospective study was conducted in a tertiary medical center from September 2014 to April 2015.

Patients between 18 and 90 years of age diagnosed with solid pancreatic masses on computer tomography (CT) were eligible for inclusion in the study; the solid component had to be > 80% of the total volume of the lesion. Patients were required to provide signed informed consent for EUS-FNA and inclusion in the study, according to the Helsinki guidelines and the local Ethical Committee.

Patients who declined to provide informed consent, patients with prior surgical treatment or chemo-radiotherapy for pancreatic disease, patients diagnosed with cystic pancreatic tumours or duodenal stenosis and patients lost from follow-up were excluded from the study. In addition, coagulopathy (INR >1.5) or trombocytopenia (< 60.000/mm³) represented exclusion criteria.

**Study design**

EUS assessment and EUS-FNA were performed on patients with a pancreatic mass. Core biopsy was sent for pathology assessment and examined by one pathologist blinded for the imaging results. The final diagnosis of malignancy was based on the first or second EUS-FNA results, on hepatic biopsy results or on the patients’ follow-up.

**EUS assessment**

All patients were first examined using a linear echoendoscope (GF-UCT140-AL5, Olympus Japan) in conjunction with the Aloka Alpha 5 or 7 ultrasound unit (Aloka, Japan). All the examinations were conducted by the same endoscopist (A.S.) with the patient under light sedation (intravenous midazolam). The following parameters were determined by linear EUS examination: the size of the pancreatic mass (maximum diameter), location of pancreatic mass, pancreatic morphology, vascularisation of focal pancreatic lesions using power Doppler, invasion of great vessels, aspect of surrounding lymph nodes, and left hepatic lobe structure. The EUS-FNA was done under continuous real-time ultrasound guidance, by using a 22-G needle (Expect, Boston Scientific) which was advanced into the lesion to obtain an aspirate of the tissue. The stylet was left inside the needle with slow pull out during the puncture, without suction, and the fanning technique was done whenever possible. Between one to three passes of EUS-FNA were made until the macroscopic length of the visible core was considered superior to 0.5 cm, based on previous reports [10, 12]. No cytopathologist was present in the room. The histological core was expelled by the reintroduction of the stylet and placed in 10% buffered formalin (Fig. 1). The specimen was carefully examined for the presence of a macroscopic visible core, which was defined as whitish or yellowish piece of tissue with an apparent bulk, and measured before deciding a subsequent pass. After FNA, the patients were observed for immediate adverse events for at least 2 hours, and contact was maintained for 24 hours after the procedure to monitor for moderate or severe adverse events (requiring any intervention or hospitalization, or death related) [13].

![Fig. 1. The core biopsy obtained by endoscopic ultrasound fine needle aspiration expelled in a formalin bottle.](image1)

**Histology assessment**

After fixation, the tissue was processed using the standard protocol for endoscopic biopsies (Fig. 2). The specimens were then processed by paraffin embedding with the usual staining (haematoxylin-eosin) and were examined by one pathologist (T.Z.), who was blinded to any clinical information (Fig. 3). Positive specimens were those categorized as unequivocally positive for malignancy. Only core biopsy was taken into consideration for this study and a specimen was deemed adequate for histological examination when it contained a coherent tissue sample from the target organ, which measured more than half of a field with a lengthwise magnification of 40x. Specimens that contained inadequate material or atypia were not excluded from our analysis, but they were considered negative in the sense that they could not provide a diagnosis of malignancy (i.e., in an intention-to-diagnose analysis).

![Fig. 2. The core biopsy measured before paraffin embedding.](image2)
Endpoints and definitions

The primary endpoint was to assess the overall accuracy for detection of malignancy using the 22G FNA needle. The second endpoint was to evaluate the factors associated with a false negative result.

The final diagnosis was established by the EUS-FNA pathology result; a second EUS-FNA was proposed if results were non-conclusive with a high susceptibility of malignancy, or by hepatic biopsy of their metastasis or they were followed up to 6 months by clinical examination and abdominal ultrasound at 3-month intervals, with repeated spiral CT / EUS if needed.

Statistics

Cross-tabular analyses were performed, accompanied by the \( \chi^2 \) test. Non-parametric tests were used to establish the difference between the groups (Mann-Whitney U-test). The area under the curve (AUC) was calculated, the 95% confidence intervals (95% CI) were determined, and the cut-off and performance quantifiers such as sensitivity and specificity were calculated. The required total sample size for a 90% power of the \( \chi^2 \) test and a moderate effect size (\( w=0.3 \)), computed using GPower v3.1.9.2 software [14] was of 117 subjects. All continuous data were expressed as mean ± standard deviation. The statistical analyses were made using R v3.2.1 software.

RESULTS

Patients’ characteristics

There were 118 patients who fulfilled the inclusion criteria. Their characteristics are showed in Table I. EUS-FNA was feasible in all cases and it was performed via stomach (n=41) or via duodenum (n=77). The median number of passes for each lesion was 2 (interquartile range 2-3). Visible core biopsy was obtained in all cases by using one pass (n=48), two passes (n=59) or three passes (n=11). No adverse events associated with EUS-FNA were reported.

The final diagnosis was obtained from the first EUS-FNA in 96 patients, from the second EUS-FNA in 8 patients, from the hepatic biopsy in 3 patients and by follow-up in the remaining 11 patients.

There were 107 malignant masses comprising 103 adenocarcinomas, 2 malignant neuroendocrine tumours and 2 metastases (colon cancer and sarcoma) and 11 benign masses interpreted as chronic pancreatitis nodules.

Diagnosis of malignancy yield of EUS-FNA

After the first EUS-FNA, the pathologist considered the sample insufficient in 1 case, without histology core but with atypia in 6 cases, with negative core histology in another 15 cases and with positive core histology in 96 cases. There were 11 true negative cases and 11 false negative cases. The sensitivity was 0.89 (95%CI: 0.911-0.939), specificity was 1.00 (95%CI: 0.735-1), positive predictive value was 1.00 (95%CI: 0.97-1), negative predictive value was 0.5 (95%CI: 0.435-0.632), global accuracy was 0.89 (95%CI: 0.794-0.944). A second EUS-FNA was performed in 8 patients from the 11 false negative cases who accepted to repeat the procedure and they were all positive for malignancy, raising the sensitivity to 0.93 (0.911-0.939), the negative predictive value to 0.63 (95%CI: 0.435-0.632) and the global accuracy to 0.94 (95%CI: 0.894-0.944).

Patient-related factors influencing the EUS-FNA results

The age and the transduodenal approach were not significant factors in obtaining the correct diagnosis (Table II). Males had a false negative result more frequently, with an odds ratio, OR=5.1 (CI95%:0.96-36.26, p=0.02). The location of the pancreatic masses did not influence the results (p>0.05)(Table II). The sensitivity, specificity, positive predictive value, negative predictive value, accuracy rate for EUS-FNA diagnosis of the masses in the head and the body of the pancreas was 0.90, 1.00, 1.00, 0.48, 0.92 and 0.89, 1.00, 1.00, 0.62, and 0.90, respectively.

The T stage on CT scan did not influence the results (p >0.05). Neither the presence of chronic pancreatitis changes nor the biliary stents had a significant influence on the EUS-FNA results (Table II).

---

Table I. The characteristics of the 118 patients included in the study.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Age mean ± SD (min, max), years</th>
<th>Male:female ratio</th>
<th>Mass location (n)</th>
<th>Mass size, long axis (mm), mean ± SD</th>
<th>Necrosis (n, %)</th>
<th>Biliary stent(n, %)</th>
<th>Chronic pancreatitis features (n, %)</th>
<th>Stage T assessed by EUS (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>63.25±9.77 (40-83)</td>
<td>1:1</td>
<td>Head + uncinate process</td>
<td>77</td>
<td>25 (21.1)</td>
<td>11 (9.3)</td>
<td>3 (2.5)</td>
<td>T1: 4, T2: 4, T3: 45, T4: 65</td>
</tr>
<tr>
<td></td>
<td>SD: standard deviation</td>
<td></td>
<td>Body</td>
<td>33</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tail</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>35.6±11.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

Fig. 3. Pancreatic cancer fragment obtained from core-histology (H&E, x40). The FNA cylinder contains loose connective tissue and fibrous connective tissue; in the central area, eosinophilic areas with necrosis and malign epithelial cells with advanced atypia. Malignant cells are either isolated or in small hollow structures (ducts). On the right, a fragment of gastric antrum mucosa.
The number of passes related to the EUS-FNA results

There was no influence of the number of passes on the diagnostic rates (p>0.05). There were 48 patients with only one pass (40 true malignant, 5 true benign, 3 false benign), 59 patients with two passes (48 true malignant, 6 true benign, 5 false benign), 11 with three passes (8 true malignant, 3 false benign). When we compared the results of one single pass with 2 or 3 passes results, the OR was 0.525 (95%CI: 0.088–2.945). When one or two passes results were compared to three passes results, the OR was 1.65 (95%CI: 0.562-4.8).

DISCUSSION

There has been no published study assessing the core biopsy results in pancreatic cancer with the limitations of the number of passes by the size of the fragment sampled.

Patients with pancreatic cancer have a poor prognosis with a median survival of 4-6 months and a <5% 5-year survival rate [15]. Tissue confirmation of malignancy, which is useful for differential diagnosis or for palliative purposes [16], does not influence survival [17] and this was the reason for performing EUS-FNA in resectable cases in this study by using the standard 22G needle which permits a biopsy from any part of the pancreas. Its efficiency in diagnosis seems similar with a pro-core 22G, but with at least two passes [4, 18]. When the duodenal stenosis is present, the entire evaluation of the lesion is difficult, the position of the needle is unstable and the core may contain more pancreatic normal tissue than tumor tissue, related to the passage through the isthmus of the pancreas. In this case, the visible core may not represent the tumor tissue harvested. This was the reason for excluding duodenal stenosis from the present study.

It is not known if there are differences in obtaining core biopsy with different types of standard 22G needles, the reported diagnostic rate being 84-87% [19]. Most studies assessing histology with a standard 22G needle have utilised other types than we used in this study.

A first paper concluded that macroscopically visible material collected may be considered as a valid alternative to the on-site presence of the pathologist with the need of a higher number of passes for the standard needle (three) compared to the pro-core needle, but with 88% diagnostic accuracy [18]. The authors used both core biopsy and cytoblock for the diagnosis, so we do not know how much a contribution the core biopsy made by itself. A second study, focused on the 22G FNA standard needle, with a median of two passes, showed that the accuracy for histology was 82% and the sensitivity was only 81%, in the conditions of splitting samples for two cytology examinations and for histology, a fact which could diminish the diagnostic yield [20]. A similar accuracy (82.5%) was obtained in a third study with two passes of FNA. The sample quality for histological analysis (diagnostic cell clusters, appropriate architecture) differed between two experienced endosonographers, proving the importance of the technique in core sampling [6]. Previously, a diagnosis sensitivity rate of 60-73% by using another 22G needle [10, 21, 22] was obtained. Only one study used the same type of 22G FNA needle as we did, in a small number of patients; the procurement of core biopsy was 100%, but the optimal histological core was 66.7% [4].

These accuracy results were similar to our data (89%), which in turn, are similar to the results of a 22G histology needle sampling of an intraabdominal mass [5]. The procurement of visible core was obtained in all cases in our

<table>
<thead>
<tr>
<th>Patients’ feature</th>
<th>EUS-FNA result</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>True malignant</td>
<td>True benign</td>
</tr>
<tr>
<td>Age (years)</td>
<td>&lt; 70</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>&gt;70</td>
<td>30</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>51</td>
</tr>
<tr>
<td>Approach</td>
<td>Transduodenal</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>Transgastric</td>
<td>32</td>
</tr>
<tr>
<td>Tumor location</td>
<td>Head</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>Body</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Tail</td>
<td>7</td>
</tr>
<tr>
<td>Necrosis</td>
<td>Absent</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>19</td>
</tr>
<tr>
<td>Tumor size</td>
<td>&lt;20 mm</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>&gt;20 mm</td>
<td>90</td>
</tr>
<tr>
<td>Chronic pancreatitis</td>
<td>Absent</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>1</td>
</tr>
<tr>
<td>Biliary stent</td>
<td>Absent</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>6</td>
</tr>
</tbody>
</table>
study, in the literature being reported in 83 - 100% of cases [4, 6, 10, 22]. Conversely, there is one paper showing a diagnostic rate of 34% based on core biopsy with a 22G needle and five passes [23].

In the present study, the number of passes was empirically limited by the macroscopic length of the core and it was a maximum of three, as proved in other studies [24]. It was based on previous research comprising different lesions sampled with a 19G needle. Those results showed that a core length longer than 4 mm was enough for the diagnosis in 92% of cases compared to 40% when the core was less than 4 mm [12]. Another study reported that the median core length with 22G needle was 6 mm for a diagnostic accuracy of 92%, although the specimen was adequate only in 83% [10]. In the case of the 25G needle, the gross visual inspection was inefficient [7]. We could not demonstrate that the number of passes influenced the results, so we considered the quantity of the tissue sampled was more important in restricting the passes.

The main limitation of the study is that this was conducted in a single institution, which may not simulate the practices in other centers. Another limitation was the single experienced operator performing EUS-FNA and the single very experienced pathologist analyzing the samples. The samples were not analyzed separately for each pass, so we cannot establish the diagnostic rate on each pass. We did not use any cytology assessment in this study because this is the local hospital policy.

One important problem remains: the negative predictive value, which was only 50% at the first attempt, and it increased to only 63% after the repeated EUS-FNA. A similarly low negative predictive value (64%) was found previously in a study using core biopsy and cytoblock preparation [6]. To increase this value, a higher number of passes [11], a proper selection of adequate material [6] or guidance under contrast enhancement can be performed [21]. Other reports showed that repeating the EUS-FNA in the case of an initially negative cytology increased the yield of diagnosis [25-27], as in our group of patients, where the diagnostic rate increased to 96%, despite some delay for obtaining the final diagnosis.

The accuracy of EUS-FNA and cytologic analysis was lower in other studies where chronic pancreatitis features were present [28], which can impose the increase in the number of passes [29]. This was not proved in our group, perhaps due to the fact that there were only three patients with chronic pancreatitis features. The presence of biliary plastic stents has been reported to diminish the rate of EUS-FNA [30], but it did not influence our results, although biliary stents were seen in about 10% of our cases, as in the other study [30]. As already known from the cytology assessment, the diagnostic accuracy of core biopsy is not related to the mass size or the presence of necrosis inside, facts previously reported also by our group [21, 31-33].

CONCLUSION

We demonstrated that the length of the core is a useful tool for restricting the number of EUS-FNA passes in pancreatic masses with high diagnostic rate. Mass size or the presence of necrosis or biliary stents did not influence the results. Further multicentric comparative studies using different standard 22G needles should demonstrate if there are any differences in obtaining core biopsy during endosonography sampling.

Conflicts of interest: No conflict to declare.

Authors’ contribution: A.S., A.S., B.M. and R.S. included the patients; A.S. performed endoscopic ultrasonography examination; T.Z. performed histological assessment, T.C. and S.C. the biostatistical analysis, and M.G., A.S., B.M., R.S. collected data and followed up the patients; A.S., M.G., T.Z., T.C., S.C., R.S. prepared the draft. All authors revised the draft and approved the final version of the manuscript.

REFERENCES


