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ENDOSCOPIC ULTRASOUND AND ELASTOGRAPHY IN DIAGNOSIS OF PANCREATIC NEUROENDOCRINE TMORS

DOCTORAL THESIS ABSTRACT

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Content

I. GENERAL PART

Since its introduction in 1992, endoscopic ultrasonography (EUS) combined with fine needle aspiration (FNA) has become the gold standard for the diagnosis of solid pancreatic lesions (SPL) [1]. In recent decades, the EUS of this method has increased significantly, surpassing traditional surgical biopsies due to its increased efficiency and safety [2], [3]. The diagnostic accuracy of EUS-FNA is greatly improved by the presence of a cytopathologist in the endoscopy room, who has a role in rapid on-site cytopathological evaluation (ROSE) [4]. The advantage lies in reducing inappropriate samples, but this is often limited by cost and availability [5]. In such cases, macroscopic quality assessment of the sampled material (MOSE) by an expert cytopathologist may represent a valuable alternative (24). More recently, biopsy needles (EUS-FNB) offer the possibility to obtain core samples, preserving the tissue architecture necessary for the diagnosis of complex pathologies, such as neuroendocrine tumors, with a comparable safety profile [6].

Currently, EUS, in association with fine needle aspiration (FNA) or fine needle biopsy (FNB), is the standard for the diagnosis of pancreatic lesions [7]. There is limited evidence-based data on differentiating solid neoplastic pancreatic lesions, particularly PNET, from other solid pancreatic lesions (SPL), such as pancreatic adenocarcinomas (PAC) or metastatic lesions of the pancreas [8].

In our department, we performed two prospective, observational cohort studies. One of them aimed to differentiate PNET from other SPL by examining specific pre-test elements such as the echoendoscopic characteristics of pancreatic lesions to which we also added risk factors and demographic characteristics. More specifically, we wanted to determine a prediction model for the final cytopathological or histopathological diagnosis of PNET based on the type of variables mentioned.

In the second study, the main objective was to evaluate the efficiency of EUS-FNA to obtain both cytological and histological material by preparing cell blocks for the diagnosis of SPL, in the presence of a dedicated cytopathologist to evaluate the samples macroscopically

(MOSE). The secondary objectives were to identify the factors that were associated with obtaining adequate biological material after EUS-FNA and the safety profile of this procedure.

II. PERSONAL CONTRIBUTIONS

1. A PREDICTION MODEL FOR DIAGNOSIS OF PANCREATIC NEUROENDOCRINE TUMORS BASED ON EUSD IMAGING FEATURES: A SINGLE CENTER EXPERIENCE

1.1. HYPOTHESIS

EUS, in combination with fine needle aspiration (FNA) or fine needle biopsy (FNB), is the standard for the diagnosis of pancreatic lesions [7]. There is limited evidence-based data on differentiating solid neoplastic pancreatic lesions, especially PNET from other solid pancreatic lesions (SPL), such as PAC or metastatic lesions of the pancreas [8]. The aim of this study was to differentiate PNET from other SPL by examining specific pre-test elements, including risk factors, demographic characteristics, and ultrasound features before cytopathological or histopathological diagnosis. These EUS tumor features could help the endoscopist to select the right needle to perform the tissue acquisition [7], [9].

1.2. OBJECTIVES OF THE STUDY

Although the current approach is EUSful, there is limited evidence of specific EUS features that can reliably differentiate PNET from other SPL such as PAC or pancreatic metastases before histopathological or cytological diagnosis. The present study focEUSd on the pre-test phase of the diagnostic process. By analyzing the characteristics of EUS together with the presence of a hypoechoic peripheral rim, but also together with risk and demographic factors, the development of a predictive model was aimed at guiding the initial assessment of pancreatic lesions. Hypoechoic rim, an ultrasound artifact that appears as a ring of low echogenicity around

the tumor, frequently observed in EUS [10], [11]. Although hypoechoic rim has been associated with various pancreatic tumors, including PNET, its efficacy in differentiating them from other SPL remains unclear. The study investigated the prevalence of this sign in PNET versus other SPL, evaluating it as a possible predictor for the diagnosis of PNET.

1.3. MATERIALS AND METHODS

The present study is a prospective observational cohort study over a period of 3 years between 2019 and 2021 and included all patients referred to our department for further investigation of suspected or previously diagnosed pancreatic masses based on CT or MR images who were performed EUS with elastography and fine needle aspiration (FNA). All patients with at least one SPL at the time of diagnosis had to be older than 18 years. Patients selected for the study were those in whom EUS-FNA was performed directly from the pancreatic lesion or adjacent lymphadenopathy.

The exclusion criteria were specifically the contraindications to EUS-FNA: coagulation disorders, anticoagulant or antiplatelet therapy, inaccessible lesion due to a large vessel or interposition of the pancreatic, bile duct, or a metastatic lesion). Also, patients who refEUSd informed consent for the study were not included.

A linear echoendoscope (EG-3870UTK, Pentax Medical), equipped with a Hitachi Arietta v70 processor or Hitachi EUB-6500HV, Tokyo, Japan, both with real-time elastography (EUS-E) function, was EUSd to perform the endoscopic procedure. the Doppler function. Fine aspiration aspiration (FNA) needles of 19 gauge (G), 22G, or 25G (EchoTip Ultra Endoscopic Ultrasound Needle; Cook Medical, Bloomington, IN, United States) were EUSd. Each procedure was performed by an experienced endoscopist while the patient was under deep Propofol sedation, assisted by an anesthesiologist. Airway intubation was not required in any of the patients examined. The examining physician selected the size of the FNA needle according to the location of the lesion and decided the number of needle passes. A cytopathologist was present in the endoscopy room to perform an immediate evaluation of the macroscopic appearance of the aspirate and slides. The procedure was completed after obtaining an adequate specimen confirmed by both the pathologist and the endoscopist.

The final diagnosis was established by histopathological and immunochemical analysis of the FNA (smear, cell block or embedded in paraffin) or the surgical specimen obtained later in the cases in which the surgical intervention was performed. Otherwise, namely in the absence of the histopathological diagnosis, it was established following the clinico-biological evolution over a period of at least 6 months in association with CT or MR imaging results. Case management was carried out according to the decision of the multidisciplinary committee for tumors.

The following data were collected prospectively: demographics (eg, age, sex), personal habits (eg, smoking and alcohol consumption), and history of diabetes; EUS procedure (eg FNA needle size, number of passes), EUS lesion characteristics (eg number, location, size - maximum diameter measured during EUS in millimetres, margins - well defined or irregular, echogenicity - hypoechoic or not, presence of a Doppler signal in the lesion suggesting tumor vascularization, detection of dilation of the main pancreatic duct (MPD) - head ≥3.5 mm or body ≥2.5 mm or tail >1.5 mm, elastography appearance - homogenous blue pattern or not, detection of vascular invasion (venous or arterial) and the presence of a hypoechoic ring delimiting the inner edges of the lesion (Figure 1.1.) EUS tumor characteristics were evaluated by the examining physician together with a trainee endoscopist.

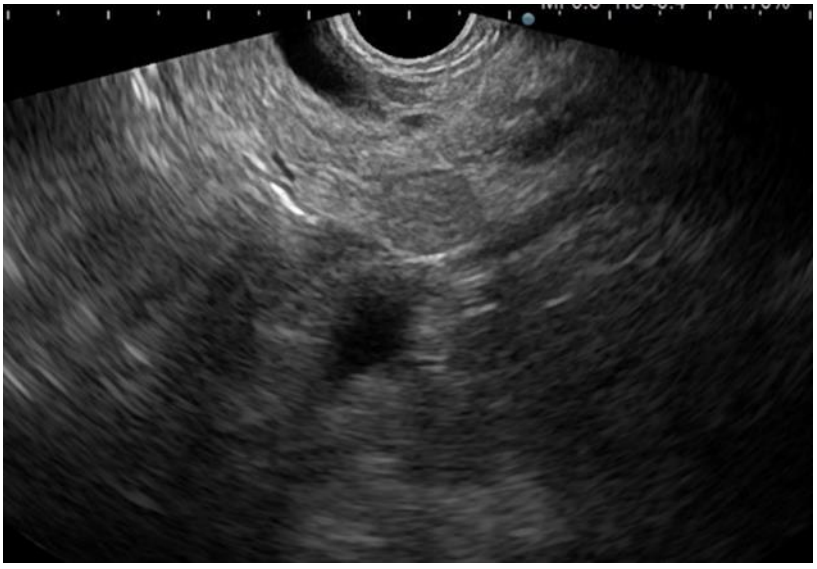

Figure 1.1. Pancreatic neuroendocrine tumor located caudally with peripheral hypoechoic rim. Personal archive

1.4. RESULTS

Study results are reported according to the STROBE guideline [12]. The study flow is represented in Figure 1.2. Among patients who were referred for EUS in our department, we included in the analysis only those in whom solid lesions were detected. All these patients were diagnosed with pancreatic masses following previous CT or MR imaging studies. Elastography and FNA were performed for all patients included in the study.

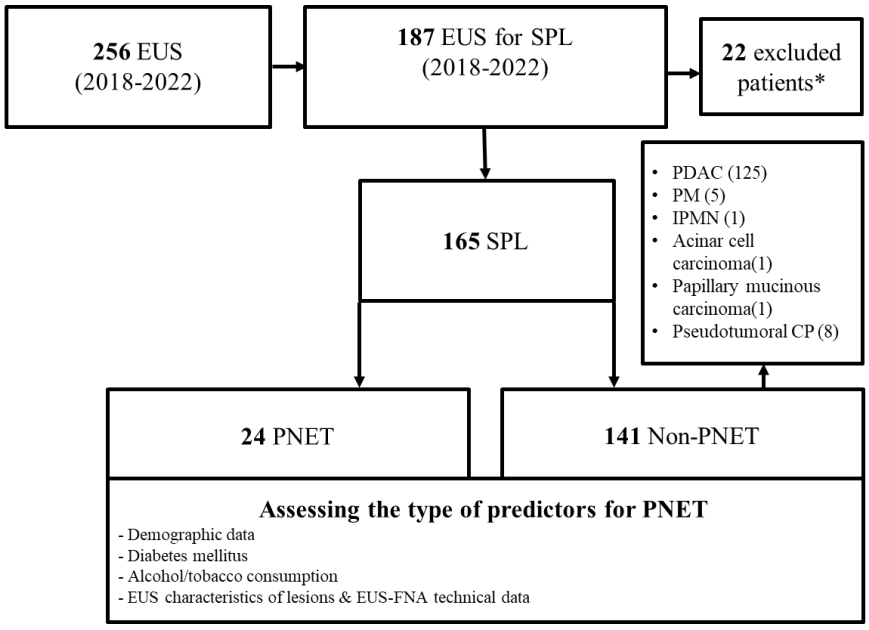

Figure 1.2. Flow chart illustrating the selection of the study population.

The histopathological result was the presence of tumor cells of pancreatic origin. Among patients with pancreatic tumors, the most common diagnoses were pancreatic ductal adenocarcinoma (PDA) (n=125) and PNET (n=24), followed by pancreatic metastases (n=5), intraductal papillary mucinous neoplasm of ductal branch type (IPMN) (n=1), acinar cell carcinoma (n=1) and papillary mucinous carcinoma (n=1). We also evaluated 8 patients previously diagnosed with chronic pseudotumoral pancreatitis, all of whom had negative cytology for neoplastic cells.

Descriptive analysis of the entire batch revealed the following data. In our study, PNETs were classified based on histopathological grading. Tumor grading was determined according to the WHO classification, which categorizes PNET into three grades (G1, G2, and

G3) based on the number of mitoses and the Ki67 index [13]. About half of the detected PNETs were G3 and poorly differentiated (NEC), while only 16.7% were with a degree of differentiation <2 mitoses/2 mm2, namely G1.

The study included 165 patients with a mean age of 63.55 years, of whom 43% were women and 57% were men. 35% of patients consumed alcohol, 27% were smokers, and 33% had type 2 diabetes. The lesions were located in the head/uncinate process (47%), body (41%), and tail (12%) of the pancreas, measuring average of 43.15 mm. Among patients, 19% had DPP dilatation, 43% vascular invasion, 7.3% multiple lesions, and 56% metastatic disease. On EUS, 66% of lesions were hypoechoic, 31% hypervascular, and 75% were blue on elastography. For EUS-FNA, the 22G needle was the most (58%). Surgical interventions were performed in 20% of patients.

The study identified several significant but also non-significant correlations between tumor characteristics, procedural variables and diagnostic outcomes. A weak but statistically significant negative correlation was observed between tumor size and needle thickness ($p = 0.027$), indicating that larger tumors were more frequently punctured with thinner needles, likely due to deep and distal location of them. A weak positive correlation was also found between tumor size and the number of FNA passes required ($p = 0.034$), suggesting that larger tumors often require more needle passes. In addition, a statistically significant negative correlation was observed between tumor size and the diagnosis of PNET ($p = 0.045$), indicating that larger tumors were less likely to be associated with PNET.

Regarding needle thickness, a significant positive correlation with the diagnosis of benign SPL was identified ($p = 0.007$), suggesting that thicker needles were more frequently EUSd in the diagnosis of benign SPL. On the other hand, no significant correlations were found between needle thickness and the diagnosis of PNET or malignant SPL.

Age also showed notable correlations with diagnostic outcomes. A significant positive correlation was found between age and diagnosis of malignant SPL ($p = 0.005$), indicating that older patients were more likely to have malignant SPL. In contrast, a significant negative correlation was observed between age and diagnosis of benign SPL ($p = 0.01$), suggesting that younger patients were more likely to have benign SPL. A point-biserial correlation between

age and the diagnosis of PNET showed a negative relationship (as patients' age increases, the probability of having PNET decreases), but statistically insignificant ($p = 0.094$).

The presence of peripheral hypoechoic rim was significantly associated with both homogeneous ($p = 0.011$) and hypervascular ($p = 0.013$) lesions, indicating that these tumor characteristics are strongly related to the presence of a hypoechoic SPL contour. However, no significant associations were found between hypoechoic contour and blue lesions on elastography ($p = 0.189$) or other variables such as tumor size, age, or Ki67 expression.

Comparative analysis of demographic data and personal history of patients with PNET vs non-PNET was performed using a simple univariate binomial logistic regression. Among patients diagnosed with PNET, the mean age was 60 years (±15.0), whereas non-PNET patients were older. The gender distribution was balanced in the group of patients diagnosed with PNET, and one third of the patients suffered from type 2 diabetes (33%). The majority (80%) were non-smokers and did not drink alcohol. None of the previously mentioned variables correlated with the diagnosis of PNET.

Also, in the comparative analysis of the characteristics of the lesions, no variable was correlated with the diagnosis of PNET, with the exception of tumor location. The mean size of the neuroendocrine lesions was 37 mm (±16.6), and most patients had a single pancreatic tumor (88%). Three patients were diagnosed with multiple pancreatic nodules, of which only one presented the diagnosis of multiple endocrine neoplasia type 1 (MEN-1), incidentally associating a ductal branch-type IPMN as a second lesion. Approximately half of the patients with PNET who were examined presented as metastatic disease, while in the case of patients with non-neuroendocrine lesions more than half (58%) presented with distant metastases. At the same time, half of them were diagnosed with locoregional invasion, thus suggesting the advanced stages of the patients' presentation.

The EUS features that were significantly associated with the diagnosis of PNET versus non-PNET were well-defined margins of the lesion (79% vs 26%, $p<0.001$), homogeneous appearance of the lesion (46% vs 9.9%, $p< 0.001$), the presence of small vessels inside the tumor (67% vs 25%, $p<0.001$) and the existence of a hypoechoic ring (46% vs 10%, $p<0.001$). The hypoechoic ring, often considered an ultrasound artifact, could represent a new specific feature of PNET, facilitating differentiation from other pancreatic lesions. Both the hypoechoic

appearance of the lesions and the homogenous blue appearance detected at EUS-E did not have a significant statistical value, not being considered predictive factors for the diagnosis of PNET.

Variables significantly associated with PNET tumor type were included in a multiple logistic regression. Using a forward selection algorithm, the best performing models were generated for each number of predictors EUSd, ranging from 1 to 6. The best performing model, with an accuracy of 89.1%, was the one that included 2 predictors: the homogeneous appearance of the lesion and the presence of the hypoechoic ring. The influence of the two predictors in the model was similar, with an OR of 6.34 (95% CI 2.21–18.3). On the other hand, the model that included all 6 predictors achieved a performance of 88%.

The model that demonstrated the best performance was the one that EUSd 2 predictors: the homogeneous appearance of the lesion and the presence of the hypoechoic ring. This model achieved an accuracy of 89.1%, indicating a high ability to differentiate between tumor types based on these factors. In the analysis, the influence of the two predictors was almost equivalent, each having a significant impact on the model, with an odds ratio (OR) of 6.34 (95% confidence interval: 2.21–18.3).

A second logistic regression analysis was performed to assess the influence of four variables on the diagnosis of PNET. The model was statistically significant ($p < 0.001$) and revealed that three variables (homogeneous lesion, hypervascular and hypoechoic rim) had a significant impact on the prediction of the diagnosis of PNET, increasing the probability of diagnosis by 4.05, 3 .33 and 4.17 times, respectively. The model presented an accuracy of 86.06%, slightly lower compared to the previous one, but with a specificity of 93.62%, and a lower sensitivity of 41.67%, indicating a better performance in identifying negative cases than the positive ones (Figure 1.4.).

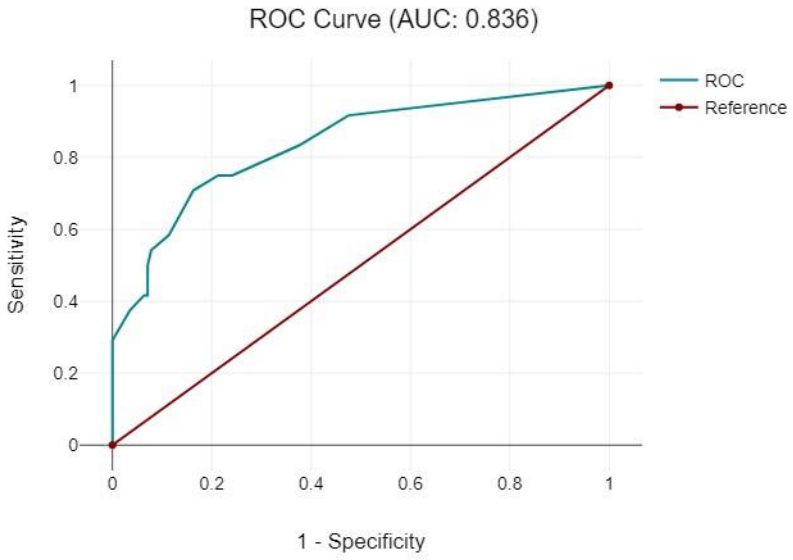

Figure 1.3. Area under the ROC curve = 8.4

1.5. DISCUSSION

Our study highlights that certain features obtained by EUS may serve as reliable predictors of PNET in patients with SPL. Key features such as well-defined lesion margins, vascularization patterns, hypoechoic texture, and the presence of a hypoechoic ring around the lesion were significantly associated with the diagnosis of PNET. Of note, the study also found that PNETs tend to be more commonly located in the distal pancreas, which heSPL differentiate them from other types of SPL.

A meta-analysis that evaluated the sensitivity, specificity, and accuracy of EUS-FNA for SPL detection included 31 studies, of which more than half were retrospective studies and only four were multicenter [14]. A strength of our study is its prospective design and uniform patient population, which consisted mainly of people with suspected SPL. It is noteworthy that our results showed that a younger age of the patients was associated with a higher probability of diagnosis of pancreatic neuroendocrine tumors (PNET) compared to non-PNET cases (mean age: 60 years vs. 64, 1 years), consistent with previous literature. In addition, a long-term study evaluating patients over 35 years of age identified PNET as the most common malignant tumor diagnosis [15].

The typical appearance of pancreatic neuroendocrine tumors (PNEP) on EUS examination is well known to be a well-demarcated, round, and homogeneous hypoechoic lesion [16], [17], [18]. Consistent with previous reports, the majority of PNETs evaluated in our study exhibited these four EUS features. In addition, less than half of PNET showed a hypoechoic ring demarcating the inner edge of the lesion, which emerged as an independent predictor of neuroendocrine tumor diagnosis. Acoustic shadowing, an artifact caused by impedance or refraction mismatches at tissue boundaries, heSPL diagnose conditions such as gallstones and pancreatic calcifications, but this phenomenon can produce a hypoechoic ring that outlines the inner edge of tumors [10], [11].

Criteria commonly EUSd to predict the behavior of PNET include factors such as tumor size, changes in size over time, morphological appearance, tumor grade, and Ki-67 expression [17], [19]. In this study, PNET showed significant variability in size (from 10 mm to 70 mm), highlighting the diversity of these tumor types. An important aspect observed is that more than two-thirds of patients diagnosed with PNET had high-grade tumors (G3), suggesting increased aggressiveness. Furthermore, as previously mentioned, half of the PNET studied were considered cancerous due to the presence of metastases, and two-thirds of these cases involved unresectable disease, thus contradicting the findings of other studies [20] [21]. This discrepancy could be explained by the limited availability of EUS in secondary medical centers and the steep learning curve associated with the EUS technique [7], [21], [22].

Also, vascular invasion, usually an indicator of poor prognosis, is rare in PNET and is more commonly associated with pancreatic ductal adenocarcinomas () [23], [24]. In the few cases of neuroendocrine tumors showing vascular invasion, most were larger than 40 mm in size, and only two cases were associated with pancreatic ductal dilatation. In contrast to PAC, which are commonly located in the head of the pancreas and are often related to dilatation of the pancreatic duct, PNET may have a slower and less aggressive course [23], [24]. In this study, half of the patients diagnosed with PNET had lesions located in the body of the pancreas, which explains the absence of dilatation of the pancreatic duct in these cases. This observation underscores the significant clinical differences between PNET and other types of pancreatic tumors, highlighting the need for a personalized diagnostic and therapeutic approach.

Qualitative elastographic assessment, although EUSful, is limited by its subjective nature, relying primarily on color patterns and uniformity of color distribution. A meta-analysis by Mei et al., which included 1,044 patients, investigated qualitative EUS elastography for the diagnosis of SPL, demonstrating a high sensitivity of 95% but a relatively low specificity of 67% [25]. In our study, 75% of PNET lesions showed a homogeneous blue appearance on elastographic evaluation, similar to that of non-PNET lesions, but this feature did not have a statistically significant correlation with the diagnosis of neuroendocrine tumor. The superiority of quantitative EUS-E was demonstrated in a study by Iglesias García et al., who reported a sensitivity of 100% and a specificity of 88% for distinguishing pancreatic adenocarcinoma from PNET, using an SR threshold set at 26, 6 [26].

Despite the trend toward fine needle biopsy (FNB), ESGE recommends both FNA and 25G/22G FNB for routine EUS-guided harvesting of solid masses and lymph nodes [27]. For obtaining a core specimen, 19G FNA or FNB needles or a 22G FNB needle are recommended. The 22G FNA needle is most commonly EUSd for non-PNETs, and the 19G needle is preferred for PNETs due to its ability to sample more tissue, although it is more difficult to EUS in certain locations such as the head of the pancreas [28]. The choice of needle for biopsy depends on the location of the lesion, the objective of the diagnosis and the experience of the medical team, having an impact on the quantity and quality of the tissue obtained [29].

2. ENDOSCOPIC ULTRASONOGRAPHY WITH FINE NEEDLE ASPIRATION: DIAGNOSIS OF SOLID PANCREATIC TUMORS THROUGH CYTOLOGICAL EXAMINATION AND CELL BLOCKS USING MOSE (SINGLE CENTER EXPERIENCE)

2.1. HYPOTHESIS

ROSE during EUS-FNA improves diagnostic accuracy by reducing inadequate sampling and the number of needle passes required [30], [31]. However, ROSE is often limited by cost and availability [32], [33]. An alternative approach, macroscopic quality assessment of material taken in situ (MOSE) by an expert cytopathologist, also improves diagnostic results

[33], [34], [35]. Although EUS-FNA has limitations in preserving the tissue architecture necessary to diagnose certain pathologies (eg, neuroendocrine tumors, autoimmune pancreatitis, gastrointestinal stromal tumors, or lymphomas), new biopsy needles (EUS-FNB) can obtain core tissue samples with fewer passages, maintaining a similar safety profile [7], [36], [37], [38], [39]. Cost considerations are particularly important in developing countries. Thus, the preparation of cell blocks obtained following conventional EUS-FNA, combined with MOSE, allows histological and immunohistochemical analysis, especially in the absence of ROSE or EUS-FNB [34], [40].

2.2. OBJECTIVES OF THE STUDY

The main objective of this study was to evaluate the effectiveness of EUS-FNA to obtain both cytological and histological material by preparing cell blocks for the diagnosis of SPL, in the presence of a dedicated cytopathologist to macroscopically evaluate the samples (MOSE) . The secondary objectives were to identify the factors that were associated with obtaining adequate biological material after EUS-FNA and the safety profile of this procedure.

2.3. MATERIALS AND METHODS

In the prospective observational cohort study, patients older than 18 years of age, who had at least SPL detected on a previous CT or MR imaging evaluation, admitted during 2021-2022, were included. Exclusion criteria were as follows: patients with cystic lesions, contraindications to EUS-FNA (eg, coagulation disorders, current EUS of anticoagulants or antiplatelet therapy, inaccessible lesions due to large vessels or pancreatic or bile duct interposition), patients not signed the informed consent prior to the EUS-FNA procedure.

The EUS-FNA procedure was performed by an experienced endoscopist using a linear echoendoscope (EG-3870UTK, Pentax Medical) equipped with a Hitachi Arietta v70 processor, Tokyo, Japan. The procedure involved deep sedation with Propofol administered by an anesthesiologist.

Various FNA needles (eg, 19G, 22G, or 25G) were EUSd to obtain the tissue. The examining physician selected the needle size based on the location of the lesion. A dedicated cytopathologist provided immediate evaluation of the specimen, and the tissue was recovered in formalin solution or on glass slides for further inspection. In most cases, at least two passes were attempted, with additional passes if no core tissue was visible [41]. Assessment of evidence adequacy is still a matter of debate, largely due to the lack of universally accepted standardized criteria [42]. In our study, an experienced cytopathologist performed macroscopic on-site evaluation (MOSE) and determined that a sample was considered suitable for cytology and cell block preparation if there was sufficient material on the smear slides or if core fragments were obtained , regardless of whether they contained blood clots or not (Figure 2.1.).

Figure 2.1. Microfragments from SPL acquired following EUS-FNA (24G)

This approach allowed for a more detailed assessment and ensured that the collected samples were of the necessary quality for further analysis, thus contributing to diagnostic accuracy. A sample was considered unsuitable for cell block preparation if no material was obtained after inserting the stylet through the needle or after flushing the needle with a 10 mL syringe.

After the procedure, cytological smears and cell block preparations were prepared and analyzed. The diagnosis of malignancy of SPL was based on the presence of malignant cells or

atypia associated with cells suspicious for malignancy. The final diagnosis of malignancy was based on one of the following: histology, if surgery was performed, IHC using cell blocks and cytologic smears, or CT/MR evidence of malignancy, consisting of the presence of regional or distant metastatic disease, or of local tumor infiltration in association with the clinico-biological evolution for a minimum of 6 months.

In this study, pancreatic solid lesions (SPL) were classified as malignant if cyto-histopathology revealed malignancy or atypia with cells suspicious for malignancy. On the other hand, lesions categorized as "negative for malignancy" and "atypia" (without the presence of tumor cells) were considered non-malignant SPL [42].

2.4. RESULTS

The results obtained from the study are reported according to the STROBE guideline [13]. In the 107 patients with SPL who underwent the EUS-FNA procedure included in the study, the most common diagnosis was PAC (85.9%), followed by PNET (7.4%), chronic pseudotumoral pancreatitis (3, 7%), adenosquamous carcinoma (0.9%) and pseudopapillary solid tumor (0.9%).

The histopathological result obtained after surgery confirmed the diagnoses in 17 patients (15.8%). These included PAC (n= 12), PNET (n= 3), IPMN (n= 1) and pseudopapillary solid tumor (n= 1). Tissue acquisition by EUS-FNA provided diagnoses by cytology in 91 cases and by histopathology in 72 cases. IHC diagnosis of cell blocks was available in only 2 cases, both diagnosing pancreatic neuroendocrine tumors. Cell block preparation diagnosed malignancy in 6 cases where cytologic evaluation was nondiagnostic.

The mean age of the evaluated patients was 63.2 ± 8.9 years (range, 45 to 88 years), with an equal gender distribution. Of those diagnosed with PAC, 54.3% were female, while all patients diagnosed with PNET were male. More than half of the lesions were larger than 3 cm in diameter and were evenly distributed throughout the pancreas. About 13% of the lesions were less than 2 cm in size, and 33.6% were between 2 and 3 cm.

Regarding the location of SPL, the lesions were divided into two groups: those in the head and uncinate process of the pancreas (50.5%) and those in the body and tail of the

pancreas, the latter being easier to puncture by FNA [43] . The distribution of PAC was homogeneous throughout the pancreas, while the majority of PNETs had a corporeocaudal localization. In most cases, the 22G needle was EUSd (78.5%), regardless of SPL location. 19G needles were most often EUSd for lesions located corporeocaudally, due to the increased maneuverability of the needle at this level, while 25G needles were almost exclusively EUSd for the corporeocaudal SPL approach.

In 41 cases, the cytopathologist present in the endoscopy room considered that the samples obtained from the first two passes of the needle were inadequate or insufficient to provide adequate biological material. Most often, when an additional passage was required, 22G or 25G needles were EUSd. The Chi-square test revealed a statistically significant relationship between needle thickness and specimen suitability with a moderate association, thus suggesting that needle thickness influences the suitability of specimens assessed by MOSE ($p = 0.001$).

A point-biserial correlation analysis revealed a strong and statistically significant positive relationship ($p < 0.001$) between the number of needle passes and the suitability of samples assessed by MOSE. This indicates that a higher number of FNA passages is associated with an improved adequacy of samples. In 15.8% of procedures (n=17), no suitable material was obtained for the preparation of cell blocks after FNA. This result was not influenced by SPL location, but was more common when 25 G needles were EUSd (n=6).

Using MOSE, the endoscopist obtained adequate material for cytological smears in 98.1% of procedures. In 85.1% of FNA cases, smears were adequate, while 14.9% had insufficient cellularity. Of these, 12.3% still had sufficient material for cell block preparation. Overall, tissue obtained by EUS-FNA was suitable for cell block preparation in 74.7% of cases. The performance of cytological smears and preparation of cell blocks obtained by EUS-FNA was evaluated. Cytology alone achieved a sensitivity of 85.2% and an AUROC value of 0.92 for a definitive diagnosis of malignancy. Cell block preparation alone had a sensitivity of 88.4% and an AUROC value of 0.94. Specificity was 100% for both methods. The combined EUS of conventional cytology and cell block preparation outperformed either method alone, increasing the AUROC value to 0.95 ($p = 0.02$) (Figure 2.2.).

Notably, tumor location in the body or tail of the pancreas was significantly associated with a diagnosis of malignancy, with 55.43% of malignant tumors being located in these areas compared to only 13.33% of non-malignant tumors (p = 0.004).

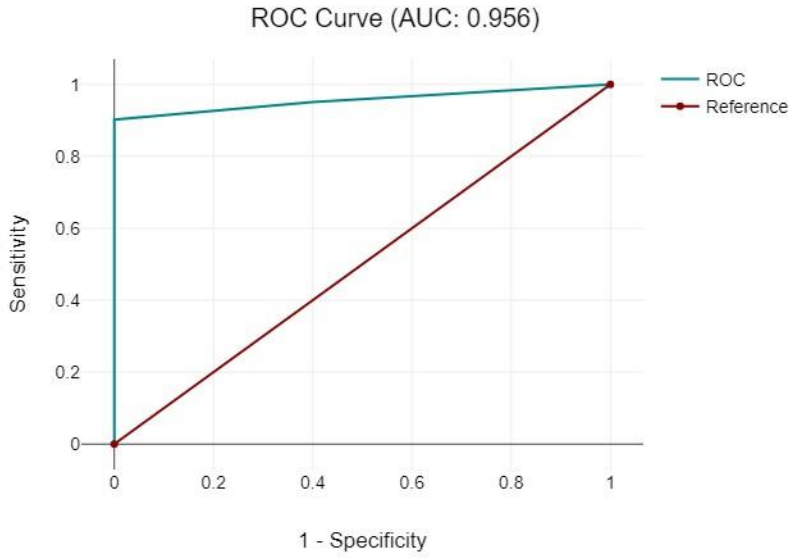

Figure 2.2. Predictive value of FNA cytology vs Cytology and Histology for the final diagnosis of malignant PSL (AUC =0.95) (p = 0.022)

Also, the presence of the cytopathologist in the endoscopy room, suggesting that the specimen was not adequate, was observed more frequently in non-malignant cases (66.70%) compared to malignant cases (33.70%), with a statistically significant difference (p = 0.01).

Tumor location and cytopathologist assessment of inadequate specimen were significantly associated with diagnosis of malignancy, whereas gender, number of needle passes, and lesion size were not significant predictors. EUS-FNA procedures had a good safety profile under deep sedation, but anesthesia-related complications occurred in 5.6% of cases and minor bleeding occurred in 1.8% of cases. Needle thickness was associated with an increased likelihood of complications ($p < 0.001$), and lesion location had a moderate association with complications ($p = 0.044$). No significant correlations were found between lesion size or final diagnosis and EUS-FNA complications.

2.5. DISCUSSION

According to guidelines based on limited evidence, the European Society of Gastrointestinal Endoscopy (ESGE) recommends that tissue acquisition by EUS should include histological preparations, such as cell blocks, rather than relying solely on cytology [7]. Recent studies indicate that cell block preparation is valuable for confirming malignancy when cell smears are insufficient [44]. In this context, our study aimed to evaluate the efficacy of both cytology and cell blocks derived from EUS-FNA samples, using 19G, 22G and 25G needles for the diagnosis of pancreatic neoplasms. This evaluation was performed in the presence of a dedicated cytopathologist who performed macroscopic on-site evaluations (MOSE).

Even though for an experienced cytopathologist, cytological detection of PAC is generally straightforward, it can sometimes be difficult due to factors such as hemorrhagic background, extensive necrosis, associated inflammation, intestinal epithelial cell contamination, or limited sampling [45], [46]. Assessment of appropriate evidence remains a debated issue, with standardization underway [42]. In our study, conducted with an experienced cytopathologist who performed MOSE, a sample was considered suitable for cytology and cell block preparation if there was material on the smear slides or if core fragments were obtained, with or without clots of blood. The overall cytological yield of EUS-FNA was 98.1%, exceeding the histological yield of 75.7%. Despite this high rate of adequate material obtained, compared to literature values of 70%–92%, [41], [47]. However, we had inconclusive cytologic diagnoses in 14.9% of cases due to limited sampling, with additional diagnostic challenges including chronic pseudotumoral pancreatitis and a pancreatic neuroendocrine tumor.

When smear slides do not contain sufficient biological material for interpretation, cell blocks can preserve cellular architecture, providing better HE staining, suitable serial sections to increase detection of malignant cells, and allowing IHC [7], [27], [48]. The suitability of the material obtained for the preparation of cell blocks in our study (75.7%) is comparable to that found in the study of Pausawasdi et al. (78.1%), where core tissue was obtained by EUS-FNA from various locations, including pancreas, lymph nodes, and massive intra-abdominal lesions with MOSE [40].

SPL are the most common targets of EUS-FNA for cytology, with reported sensitivity ranging from 73% to 90%, specificity from 95% to 100%, and accuracy from 81% to 95% [47], [49], [50] , [51]. In this study, malignancy or atypia with cells suspicious for malignancy on cyto-histopathology were considered positive for SPL malignancy, while “negative for malignancy” and “atypia” were classified as non-malignant [42]. Combining cytological analysis and cell blocks by EUS-FNA is ideal because it can be performed in a single exam with a single needle, significantly increasing the AUROC to 0.95 ($p=0.02$). These findings are consistent with ESGE recommendations that EUS-FNA should include cell block preparations [7].

EUS-FNA targeting SPL is a safe procedure with a complication rate ranging from 0.5% to 2.5%, which decreases to 0.6% to 1.1% when only major complications are considered (such as haemorrhage, acute pancreatitis and perforations) [52], [53], [54], [55], [56]. SPL ≤2 cm in diameter and pancreatic neuroendocrine tumors are risk factors for complications after EUS-FNA [57]. This study showed a similar safety profile with no major adverse events in patients undergoing EUS-FNA for SPL. The two cases of hemorrhage occurred in patients with small adenocarcinomas. Chen et al. found that the new FNB needles had a lower complication rate compared to EUS-FNA + ROSE for SPL because the former required fewer needle passes (2.3 vs. 3.0) and reduced procedure time (19 .3 min vs. 22.7 min) [39].

EUS-FNA achieves high diagnostic accuracy when an experienced cytopathologist is present for rapid on-site evaluation (ROSE), the absence of which can reduce the rate of cytological diagnoses by 10%-15% [30], [31], [39], [58]. However, in many countries, including Romania, financial constraints limit the EUS-FNB and ROSE. In this context, preparation of cell blocks using conventional EUS-FNA with MOSE is a viable option, allowing histological and immunohistochemical analysis, especially in the absence of ROSE or EUS-FNB [34], [40].

3. AN UNCOMMON CAUSE OF OBSTRUCTIVE JAUNDICE IN A NEWLY DIAGNOSED CELIAC DISEASE PATIENT: A CASE REPORT

3.1. INTRODUCTION

Celiac disease (CD) is characterized by intestinal malabsorption of nutrients after gluten ingestion, due to villus atrophy of the small intestinal mucosa [59], [60]. Patients show rapid clinical and histological improvement after strict adherence to a gluten-free diet (GFD), with clinical and histological relapse when gluten is reintroduced [59], [60].

Often, nutrient malabsorption can cause various extraintestinal manifestations, including anemia, osteopenia, or neurological manifestations (hypotonia, developmental delay, epilepsy and other seizure disorders, peripheral neuropathy, cerebellar ataxia) [61]. These manifestations have a negative impact on the patient's status so that they can delay the diagnosis of celiac disease (CD) [62], [63]. In addition, patients with BC with a long history or refractory disease are associated with an increased risk of malignancy [63], [64]. Small bowel carcinoma is the second most common malignancy after lymphoma in gluten-induced enteropathy [65]. Thus, in the case of patients with BC, who are subject to an increased risk of developing neoplasia, the diagnosis of cancer can be established not only during the follow-up of the patient, but also simultaneously (at admission or during the same month) and, more frequently, before for enteropathy to be discovered [66].

3.2. CASE REPORT AND DISCUSSION

A 35-year-old male epileptic patient was admitted with new-onset abdominal pain, vomiting, jaundice, pruritus, and weight loss. Abdominal ultrasound showed ductal criteria for chronic pancreatitis: irregular ductal contour, visible side branches, hyperechoic ductal margins, and dilated main duct. Blood tests indicated cholestasis, elevated transaminases, and a significant increase in lipase. Upper digestive endoscopy using a side-view endoscope revealed loss of duodenal folds with a scalloping appearance. Advancing towards the second part of the duodenum, this aspect became more irregular, with an infiltrative and stenotic appearance. Biopsies showed poorly differentiated duodenal adenocarcinoma with diffEUS areas of signet ring cells and marked villous atrophy, respectively: Corazza-Villanacci grade

B2. Anti-tissue transglutaminase antibodies were high titer positive, as was HLA DQ2. A cephalic duodenopancreatectomy was performed, with clear resection margins (G3, pT3N0M0). After surgery, in addition to remission of jaundice, the patient's neurological status improved considerably under optimized treatment and a gluten-free diet. At 6-month follow-up, there were no signs of residual tumor, the patient gained weight, US showed no signs of pancreatitis, and epileptic seizures were less frequent.

There is a 60- to 80-fold increased risk of small bowel carcinoma in patients with celiac disease (CD) [66], with long-standing or refractory disease further increasing the risk of malignancy [61], [63]. In this case, the morbidity was higher due to the development of episodes of acute pancreatitis against the background of chronic pancreatitis, caused by ampullary obstruction. CD patients have an increased risk of chronic and acute pancreatitis, but the intensity of these associations as well as the mechanisms involved are not very well clarified [67]. A retrospective Swedish study found that patients with CD had a 3-fold higher risk of developing pancreatitis, with a lower hazard ratio for gallstone-related AP compared to non-gallstone-related AP [68]. We believe that the association with the neurological disorder could be only coincidental, as imaging did not find occipital calcifications specific to a seizure syndrome associated with CD, described as early as 1970 [69].

6. CONCLUSIONS AND PERSONAL CONTRIBUTIONS

6.1. CONCLUSIONS

• The presence of metastatic disease in more than half of the studied cohort and the high degree of differentiation of PNET detected, underlines the late diagnosis of pancreatic neoplasms in a tertiary center in Romania.

• Larger PNET require more passes when FNA needles are EUSd.

• Larger SPL are less likely to be diagnosed as PNET, suggesting an inverse association between tumor size and the likelihood of a PNET diagnosis.

• Elderly patients are more likely to be diagnosed with malignant SPL, and younger patients are more likely to be diagnosed with chronic pseudotumoral pancreatitis. The relationship between age and the diagnosis of PNET is not statistically significant.

• Patients with SPL located corporeocaudally have a higher risk of PNET (or malignancy according to study 2).

• Malignant SPL require a higher number of FNA passes due to inadequate tissue sampling after two passes as judged by MOSE.

• Peripheral hypoechoic rim of SPL is more common in homogenous and hypervascular lesions.

• US imaging features: well-defined margins, homogeneous appearance, hypervascularization and peripheral hypoechoic rim are independent predictors for the diagnosis of PNET.

• The logistic model with as main predictors the homogeneous appearance of the lesion and the hypoechoic rim has an accuracy of 89.1% to identify PNET.

• Another logistic model based on the influence of the predictors of homogeneous lesion, hypervascularity and hypoechoic rim demonstrated a significant ability to predict the diagnosis of PNET, having an overall accuracy of 86.06%, with a high specificity but a low sensitivity.

• The developed models are easy to EUS by identifying the previously mentioned independent predictors. The clinical applicability is that during the evaluation of an SPL by EUS the identification of these predictors should encourage the examiner to EUS an FNB needle to obtain a tissue specimen suitable for IHC.

• Tissue acquisition by EUS-FNA with MOSE is effective and has high diagnostic accuracy if cytological examination is combined with histoptological examination of cell blocks.

• FNA cytology combined with cell block histology obtained from EUS-FNA with MOSE significantly improves diagnostic performance compared to using each method

separately. Diagnostic accuracy is confirmed by high values of sensitivity, specificity and AUC.

• EUS-FNA has a good safety profile with a low complication rate (1.8%). Complications are associated with needle thickness and lesion location, suggesting the need for increased caution in these cases.

• FNA cytology combined with cell block histology obtained from EUS-FNA with MOSE is recommended for routine clinical practice, especially in centers where ROSE is not available.

6.2. PERSONAL CONTRIBUTIONS

In our center's experience, we observed a significant association between the presence of peripheral hypoechoic rim and imaging features of SPL, such as homogeneous appearance and hypervascularity. This, together with EUS features already well documented in the literature for PNET (well-defined margins, homogeneous, hypoechoic and hypervascular appearance), suggests that the peripheral hypoechoic rim could be an important new predictive factor for the diagnosis of PNET. To date, to our knowledge, this aspect has not been reported in other studies.

The multivariate analysis performed in our study allowed the development of a prediction model for the diagnosis of PNET, integrating the peripheral hypoechoic rim as an independent predictive factor, alongside the other known predictors. The prediction models thus developed are easy to EUS in clinical practice, by simply identifying these factors during the evaluation of an SPL by EUS. In terms of clinical applicability, these findings suggest that when an examiner identifies specific features during a EUS evaluation of an SPL, he should be strongly encouraged to EUS a fine aspiration biopsy (FNB) needle to obtain a tissue specimen of sufficient quality for immunohistochemistry (IHC). This would greatly improve diagnostic accuracy and lead to appropriate patient management, thus contributing to better differentiation of PNET from other types of pancreatic lesions. The benefits of combining FNA cytology with cell block histology obtained by EUS-FNA with MOSE represent a significant contribution in at least local medical practice, demonstrating the clear improvement

in diagnostic performance over the separate EUS of each method. This emphasizes not only the superiority of the combined approach, but also its applicability in diverse clinical contexts, especially in centers where resources are limited.

Specifically, where one or those ETFs are not available, adopting this blending technique becomes essential. ROSE allows rapid on-site assessment of sample suitability, but not all centers have access to this service due to increased costs. In such situations, combining FNA cytology with cell block histology obtained by MOSE becomes a practical and effective solution to compensate for the lack of real-time cytological evaluation.

BIBLIOGRAPHY

[1] P. Vilmann, G. K. Jacobsen, F. W. Henriksen, and S. Hancke, "Endoscopic ultrasonography with guided fine needle aspiration biopsy in pancreatic disease," *Gastrointest Endosc*, vol. 38, no. 2, pp. 172–173, Mar. 1992, doi: 10.1016/S0016-5107(92)70385-X.

[2] A. Facciorusso *et al.*, "Diagnostic Yield of Endoscopic Ultrasound-Guided Liver Biopsy in Comparison to Percutaneous Liver Biopsy: A Two-Center Experience," *Cancers (Basel)*, vol. 13, no. 12, p. 3062, Jun. 2021, doi: 10.3390/cancers13123062.

[3] H. Okasha *et al.*, "Endoscopic ultrasound-guided fine needle aspiration versus percutaneous ultrasound-guided fine needle aspiration in diagnosis of focal pancreatic masses," *Endosc Ultrasound*, vol. 2, no. 4, p. 190, 2013, doi: 10.4103/2303-9027.121239.

[4] S. Sundaram, "Rapid on-site evaluation (ROSE) versus macroscopic on-site evaluation (MOSE) for endoscopic ultrasound-guided sampling of solid pancreatic lesions: a paired comparative analysis using newer-generation fine needle biopsy needles," *Ann Gastroenterol*, 2023, doi: 10.20524/aog.2023.0790.

[5] T. Khoury and W. Sbeit, "Cost-effectiveness of rapid on-site evaluation of endoscopic ultrasound fine needle aspiration in gastrointestinal lesions," *Cytopathology*, vol. 32, no. 3, pp. 326–330, May 2021, doi: 10.1111/cyt.12962.

[6] I. Levine and A. J. Trindade, "Endoscopic ultrasound fine needle aspiration *vs* fine needle biopsy for pancreatic masses, subepithelial lesions, and lymph nodes," *World J Gastroenterol*, vol. 27, no. 26, pp. 4194–4207, Jul. 2021, doi: 10.3748/wjg.v27.i26.4194.

[7] M. Polkowski *et al.*, "Technical aspects of endoscopic ultrasound (EUS)-guided sampling in gastroenterology: European Society of Gastrointestinal Endoscopy (ESGE) Technical Guideline – March 2017," *Endoscopy*, vol. 49, no. 10, pp. 989–1006, Oct. 2017, doi: 10.1055/s-0043-119219.

[8] V. Gagovic *et al.*, "Endoscopic Ultrasound Fine-Needle Aspiration Characteristics of Primary Adenocarcinoma versus Other Malignant Neoplasms of The Pancreas," *Canadian Journal of Gastroenterology*, vol. 26, no. 10, pp. 691–696, 2012, doi: 10.1155/2012/761721.

[9] I. Saizu, "A Model to Predict Diagnosis of Pancreatic Neuroendocrine Tumors Based on EUS Imaging Features," *Acta Endocrinologica (Bucharest)*, vol. 19, no. 4, pp. 407–414, 2023, doi: 10.4183/aeb.2023.407.

[10] R. Steel, T. L. Poepping, R. S. Thompson, and C. Macaskill, "Origins of the edge shadowing artefact in medical ultrasound imaging," *Ultrasound Med Biol*, vol. 30, no. 9, pp. 1153–1162, Sep. 2004, doi: 10.1016/j.ultrasmedbio.2004.07.014.

[11] J. M. Rubin, R. S. Adler, J. B. Fowlkes, and P. L. Carson, "Phase cancellation: A cause of acoustical shadowing at the edges of curved surfaces in B-mode ultrasound images,"

Ultrasound Med Biol, vol. 17, no. 1, pp. 85–95, Jan. 1991, doi: 10.1016/0301-5629(91)90013-M.

[12] S. Cuschieri, "The STROBE guidelines," *Saudi J Anaesth*, vol. 13, no. 5, p. 31, 2019, doi: 10.4103/sja.SJA_543_18.

[13] I. D. Nagtegaal *et al.*, "The 2019 WHO classification of tumours of the digestive system," *Histopathology*, vol. 76, no. 2, pp. 182–188, Jan. 2020, doi: 10.1111/his.13975.

[14] G. Chen, S. Liu, Y. Zhao, M. Dai, and T. Zhang, "Diagnostic accuracy of endoscopic ultrasound-guided fine-needle aspiration for pancreatic cancer: A meta-analysis," *Pancreatology*, vol. 13, no. 3, pp. 298–304, May 2013, doi: 10.1016/j.pan.2013.01.013.

[15] M. Redelman, H. M. Cramer, and H. H. Wu, "Pancreatic fine-needle aspiration cytology in patients < 35-years of age: A retrospective review of 174 cases spanning a 17-year period," *Diagn Cytopathol*, vol. 42, no. 4, pp. 297–301, Apr. 2014, doi: 10.1002/dc.23070.

[16] M. A. Khashab *et al.*, "EUS is still superior to multidetector computerized tomography for detection of pancreatic neuroendocrine tumors," *Gastrointest Endosc*, vol. 73, no. 4, pp. 691–696, Apr. 2011, doi: 10.1016/j.gie.2010.08.030.

[17] F. A. F. Figueiredo *et al.*, "Pancreatic Endocrine Tumors," *Pancreas*, vol. 38, no. 8, pp. 947–953, Nov. 2009, doi: 10.1097/MPA.0b013e3181b365db.

[18] S. G. Krishna *et al.*, "Diagnostic Differentiation of Pancreatic Neuroendocrine Tumor From Other Neoplastic Solid Pancreatic Lesions During Endoscopic Ultrasound-Guided Fine-Needle Aspiration," *Pancreas*, vol. 45, no. 3, pp. 394–400, Mar. 2016, doi: 10.1097/MPA.0000000000000488.

[19] I. Bartolini, L. Bencini, M. Risaliti, M. N. Ringressi, L. Moraldi, and A. Taddei, "Current Management of Pancreatic Neuroendocrine Tumors: From Demolitive Surgery to Observation," *Gastroenterol Res Pract*, vol. 2018, pp. 1–12, Jul. 2018, doi: 10.1155/2018/9647247.

[20] B. Lawrence, B. I. Gustafsson, A. Chan, B. Svejda, M. Kidd, and I. M. Modlin, "The Epidemiology of Gastroenteropancreatic Neuroendocrine Tumors," *Endocrinol Metab Clin North Am*, vol. 40, no. 1, pp. 1–18, Mar. 2011, doi: 10.1016/j.ecl.2010.12.005.

[21] M. Polkowski *et al.*, "Learning, techniques, and complications of endoscopic ultrasound (EUS)-guided sampling in gastroenterology: European Society of Gastrointestinal Endoscopy (ESGE) Technical Guideline," *Endoscopy*, vol. 44, no. 02, pp. 190–206, Feb. 2012, doi: 10.1055/s-0031-1291543.

[22] G. Johnson *et al.*, "Curriculum for ERCP and endoscopic ultrasound training in Europe: European Society of Gastrointestinal Endoscopy (ESGE) Position Statement," *Endoscopy*, vol. 53, no. 10, pp. 1071–1087, Oct. 2021, doi: 10.1055/a-1537-8999.

[23] K. Öberg and B. Eriksson, "Endocrine tumours of the pancreas," *Best Pract Res Clin Gastroenterol*, vol. 19, no. 5, pp. 753–781, Oct. 2005, doi: 10.1016/j.bpg.2005.06.002.

[24] N. V. Adsay, A. Andea, O. Basturk, N. Kilinc, H. Nassar, and JeanetteD. Cheng, “Secondary tumors of the pancreas: an analysis of a surgical and autopsy database and review of the literature,” *Virchows Archiv*, vol. 444, no. 6, Jun. 2004, doi: 10.1007/s00428-004-0987-3.

[25] M. Mei, J. Ni, D. Liu, P. Jin, and L. Sun, “EUS elastography for diagnosis of solid pancreatic masses: a meta-analysis,” *Gastrointest Endosc*, vol. 77, no. 4, pp. 578–589, Apr. 2013, doi: 10.1016/j.gie.2012.09.035.

[26] J. Iglesias–Garcia, J. Larino–Noia, I. Abdulkader, J. Forteza, and J. E. Dominguez–Munoz, “Quantitative Endoscopic Ultrasound Elastography: An Accurate Method for the Differentiation of Solid Pancreatic Masses,” *Gastroenterology*, vol. 139, no. 4, pp. 1172–1180, Oct. 2010, doi: 10.1053/j.gastro.2010.06.059.

[27] J.-M. Dumonceau *et al.*, “Indications, results, and clinical impact of endoscopic ultrasound (EUS)-guided sampling in gastroenterology: European Society of Gastrointestinal Endoscopy (ESGE) Clinical Guideline – Updated January 2017,” *Endoscopy*, vol. 49, no. 07, pp. 695–714, Jul. 2017, doi: 10.1055/s-0043-109021.

[28] A. Laquière *et al.*, “19 G nitinol needle versus 22 G needle for transduodenal endoscopic ultrasound-guided sampling of pancreatic solid masses: a randomized study,” *Endoscopy*, vol. 51, no. 05, pp. 436–443, May 2019, doi: 10.1055/a-0757-7714.

[29] A. Facciorusso *et al.*, “Comparative accuracy of needle sizes and designs for EUS tissue sampling of solid pancreatic masses: a network meta-analysis,” *Gastrointest Endosc*, vol. 90, no. 6, pp. 893-903.e7, Dec. 2019, doi: 10.1016/j.gie.2019.07.009.

[30] J. Iglesias-Garcia *et al.*, “Influence of On-Site Cytopathology Evaluation on the Diagnostic Accuracy of Endoscopic Ultrasound-Guided Fine Needle Aspiration (EUS-FNA) of Solid Pancreatic Masses,” *American Journal of Gastroenterology*, vol. 106, no. 9, pp. 1705–1710, Sep. 2011, doi: 10.1038/ajg.2011.119.

[31] S. Hébert-Magee *et al.*, “The presence of a cytopathologist increases the diagnostic accuracy of endoscopic ultrasound-guided fine needle aspiration cytology for pancreatic adenocarcinoma: a meta-analysis,” *Cytopathology*, vol. 24, no. 3, pp. 159–171, Jun. 2013, doi: 10.1111/cyt.12071.

[32] L. J. Layfield, J. S. Bentz, and E. V. Gopez, “Immediate on-site interpretation of fine-needle aspiration smears,” *Cancer*, vol. 93, no. 5, pp. 319–322, Oct. 2001, doi: 10.1002/cncr.9046.

[33] T. ITOI *et al.*, “HISTOLOGICAL DIAGNOSIS BY EUS-GUIDED FINE-NEEDLE ASPIRATION BIOPSY IN PANCREATIC SOLID MASSES WITHOUT ON-SITE CYTOPATHOLOGIST: A SINGLE-CENTER EXPERIENCE,” *Digestive Endoscopy*, vol. 23, no. s1, pp. 34–38, May 2011, doi: 10.1111/j.1443-1661.2011.01142.x.

[34] A. Yamabe *et al.*, “Efforts to improve the diagnostic accuracy of endoscopic ultrasound-guided fine-needle aspiration for pancreatic tumors,” *Endosc Ultrasound*, vol. 5, no. 4, p. 225, 2016, doi: 10.4103/2303-9027.187862.

[35] I. Cazacu, A. Luzuriaga Chavez, A. Saftoiu, P. Vilmann, and M. Bhutani, “A quarter century of EUS-FNA: Progress, milestones, and future directions,” *Endosc Ultrasound*, vol. 7, no. 3, p. 141, 2018, doi: 10.4103/eus.eus_19_18.

[36] A. Rana and S. Rana, “Endoscopic ultrasound-guided tissue acquisition: Techniques and challenges,” *J Cytol*, vol. 36, no. 1, p. 1, 2019, doi: 10.4103/JOC.JOC_146_18.

[37] A. Aadam *et al.*, “A randomized controlled cross-over trial and cost analysis comparing endoscopic ultrasound fine needle aspiration and fine needle biopsy,” *Endosc Int Open*, vol. 04, no. 05, pp. E497–E505, May 2016, doi: 10.1055/s-0042-106958.

[38] L. Tian *et al.*, “Evaluation of 22G fine-needle aspiration (FNA) versus fine-needle biopsy (FNB) for endoscopic ultrasound-guided sampling of pancreatic lesions: a prospective comparison study,” *Surg Endosc*, vol. 32, no. 8, pp. 3533–3539, Aug. 2018, doi: 10.1007/s00464-018-6075-6.

[39] Y.-I. Chen *et al.*, “Endoscopic ultrasound (EUS)-guided fine needle biopsy alone vs. EUS-guided fine needle aspiration with rapid onsite evaluation in pancreatic lesions: a multicenter randomized trial,” *Endoscopy*, vol. 54, no. 01, pp. 4–12, Jan. 2022, doi: 10.1055/a-1375-9775.

[40] N. Pausawasdi, P. Hongsrisuwan, W. V. Chalermwai, A. S. Butt, K. Maipang, and P. Charatchareonwitthaya, “The diagnostic performance of combined conventional cytology with smears and cell block preparation obtained from endoscopic ultrasound-guided fine needle aspiration for intra-abdominal mass lesions,” *PLoS One*, vol. 17, no. 3, p. e0263982, Mar. 2022, doi: 10.1371/journal.pone.0263982.

[41] K. Möller *et al.*, “EUS-guided FNA of solid pancreatic masses: high yield of 2 passes with combined histologic-cytologic analysis,” *Gastrointest Endosc*, vol. 70, no. 1, pp. 60–69, Jul. 2009, doi: 10.1016/j.gie.2008.10.008.

[42] M. B. Pitman and L. J. Layfield, “Guidelines for pancreaticobiliary cytology from the Papanicolaou Society of Cytopathology: A review,” *Cancer Cytopathol*, vol. 122, no. 6, pp. 399–411, Jun. 2014, doi: 10.1002/cncy.21427.

[43] M. J. Chung *et al.*, “Clinical and Technical Guideline for Endoscopic Ultrasound (EUS)-Guided Tissue Acquisition of Pancreatic Solid Tumor: Korean Society of Gastrointestinal Endoscopy (KSGE),” *Gut Liver*, vol. 15, no. 3, pp. 354–374, May 2021, doi: 10.5009/gnl20302.

[44] M. Mallik, K. Kapila, A. Mohanty, S. Inamdar, A. AlAli, and A. Al Naseer, “Endoscopic ultrasound-guided fine needle aspiration cytology of pancreatic adenocarcinomas revisited. A detailed cytological analysis,” *J Cytol*, vol. 38, no. 1, p. 31, 2021, doi: 10.4103/JOC.JOC_177_20.

[45] E. Vigliar, G. Troncone, U. Bracale, A. Iaccarino, V. Napolitano, and C. Bellevicine, “<scp>CD</scp> 10 is EUSful to identify gastrointestinal contamination in endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) cytology from pancreatic ductal

adenocarcinoma," *Cytopathology*, vol. 26, no. 2, pp. 83–87, Apr. 2015, doi: 10.1111/cyt.12148.

[46] A. Ieni, P. Todaro, S. F. Crinò, V. Barresi, and G. Tuccari, "Endoscopic ultrasound-guided fine-needle aspiration cytology in pancreaticobiliary carcinomas: diagnostic efficacy of cell-block immunocytochemistry," *Hepatobiliary & Pancreatic Diseases International*, vol. 14, no. 3, pp. 305–312, Jun. 2015, doi: 10.1016/S1499-3872(15)60367-8.

[47] T. H. Kim, K. H. Choi, H. S. Song, J. W. Kim, and B. J. Jeon, "Histology Combined with Cytology by Endoscopic Ultrasound-Guided Fine Needle Aspiration for the Diagnosis of Solid Pancreatic Mass and Intra-Abdominal Lymphadenopathy," *Gut Liver*, vol. 7, no. 5, pp. 605–610, Sep. 2013, doi: 10.5009/gnl.2013.7.5.605.

[48] B. E. Bluen *et al.*, "Accuracy and Quality Assessment of EUS-FNA: A Single-Center Large Cohort of Biopsies," *Diagn Ther Endosc*, vol. 2012, pp. 1–7, Oct. 2012, doi: 10.1155/2012/139563.

[49] A. Facciorusso, S. P. Sunny, V. Del Prete, M. Antonino, and N. Muscatiello, "Comparison between fine-needle biopsy and fine-needle aspiration for EUS-guided sampling of subepithelial lesions: a meta-analysis," *Gastrointest Endosc*, vol. 91, no. 1, pp. 14-22.e2, Jan. 2020, doi: 10.1016/j.gie.2019.07.018.

[50] H. Li, W. Li, Q.-Y. Zhou, and B. Fan, "Fine needle biopsy is superior to fine needle aspiration in endoscopic ultrasound guided sampling of pancreatic masses," *Medicine*, vol. 97, no. 13, p. e0207, Mar. 2018, doi: 10.1097/MD.0000000000010207.

[51] P. A. van Riet, N. S. Erler, M. J. Bruno, and D. L. Cahen, "Comparison of fine-needle aspiration and fine-needle biopsy devices for endoscopic ultrasound-guided sampling of solid lesions: a systemic review and meta-analysis," *Endoscopy*, vol. 53, no. 04, pp. 411–423, Apr. 2021, doi: 10.1055/a-1206-5552.

[52] M. A. Eloubeidi *et al.*, "Endoscopic Ultrasound–Guided Fine Needle Aspiration Biopsy of Patients With Suspected Pancreatic Cancer: Diagnostic Accuracy and Acute and 30-Day Complications," *American Journal of Gastroenterology*, vol. 98, no. 12, pp. 2663–2668, Dec. 2003, doi: 10.1111/j.1572-0241.2003.08666.x.

[53] C. P. Raut *et al.*, "Diagnostic Accuracy of Endoscopic Ultrasound–Guided Fine-Needle Aspiration in Patients With Presumed Pancreatic Cancer," *Journal of Gastrointestinal Surgery*, vol. 7, no. 1, pp. 118–128, Jan. 2003, doi: 10.1016/S1091-255X(02)00150-6.

[54] G. C. Harewood and M. J. Wiersema, "Endosonography-guided fine needle aspiration biopsy in the evaluation of pancreatic masses," *Am J Gastroenterol*, vol. 97, no. 6, pp. 1386–1391, Jun. 2002, doi: 10.1111/j.1572-0241.2002.05777.x.

[55] F. Gress *et al.*, "EUS-guided fine-needle aspiration of the pancreas: Evaluation of pancreatitis as a complication," *Gastrointest Endosc*, vol. 56, no. 6, p. a129602, Dec. 2002, doi: 10.1067/mge.2002.129602.

[56] M. A. Eloubeidi, A. Tamhane, S. Varadarajulu, and C. M. Wilcox, “Frequency of major complications after EUS-guided FNA of solid pancreatic masses: a prospective evaluation,” *Gastrointest Endosc*, vol. 63, no. 4, pp. 622–629, Apr. 2006, doi: 10.1016/j.gie.2005.05.024.

[57] A. Katanuma *et al.*, “Factors Predictive of Adverse Events Associated with Endoscopic Ultrasound-Guided Fine Needle Aspiration of Pancreatic Solid Lesions,” *Dig Dis Sci*, vol. 58, no. 7, pp. 2093–2099, Jul. 2013, doi: 10.1007/s10620-013-2590-4.

[58] M. A. Eloubeidi *et al.*, “Endoscopic ultrasound-guided fine-needle aspiration in the diagnosis of foregut duplication cysts: the value of demonstrating detached ciliary tufts in cyst fluid.,” *Cancer*, vol. 102, no. 4, pp. 253–8, Aug. 2004, doi: 10.1002/cncr.20369.

[59] W. Holtmeier and W. F. Caspary, “Celiac disease,” *Orphanet J Rare Dis*, vol. 1, no. 1, p. 3, Dec. 2006, doi: 10.1186/1750-1172-1-3.

[60] M. F. Kagnoff, “Celiac disease: pathogenesis of a model immunogenetic disease,” *Journal of Clinical Investigation*, vol. 117, no. 1, pp. 41–49, Jan. 2007, doi: 10.1172/JCI30253.

[61] N. Zelnik, A. Pacht, R. Obeid, and A. Lerner, “Range of Neurologic Disorders in Patients With Celiac Disease,” *Pediatrics*, vol. 113, no. 6, pp. 1672–1676, Jun. 2004, doi: 10.1542/peds.113.6.1672.

[62] A. Pithawa, “Sleisenger and Fordtran’s Gastrointestinal and Liver Disease: pathophysiology, diagnosis, management,” *Med J Armed Forces India*, vol. 63, no. 2, p. 205, Apr. 2007, doi: 10.1016/S0377-1237(07)80085-2.

[63] A. Saizu, I. Bancilă, G. Becheanu, M. Dumbrava, and C. Gheorghe, “An unusual etiology of obstructive jaundice in a newly diagnosed celiac disease patient,” *Journal of Gastrointestinal and Liver Diseases*, vol. 30, no. 1, 2021, doi: 10.15403/jgld-3343.

[64] M. Ivanova, L. Bottiglieri, E. Sajjadi, K. Venetis, and N. Fusco, “Malignancies in Patients with Celiac Disease: Diagnostic Challenges and Molecular Advances,” *Genes (Basel)*, vol. 14, no. 2, p. 376, Jan. 2023, doi: 10.3390/genes14020376.

[65] F. Pelizzaro *et al.*, “The Risk of Malignancies in Celiac Disease—A Literature Review,” *Cancers (Basel)*, vol. 13, no. 21, p. 5288, Oct. 2021, doi: 10.3390/cancers13215288.

[66] P. H. R. Green, A. T. Fleischauer, G. Bhagat, R. Goyal, B. Jabri, and A. I. Neugut, “Risk of malignancy in patients with celiac disease,” *Am J Med*, vol. 115, no. 3, pp. 191–195, Aug. 2003, doi: 10.1016/S0002-9343(03)00302-4.

[67] J. F. Ludvigsson, S. M. Montgomery, and A. Ekbom, “Risk of Pancreatitis in 14,000 Individuals With Celiac Disease,” *Clinical Gastroenterology and Hepatology*, vol. 5, no. 11, pp. 1347-1353.e2, Nov. 2007, doi: 10.1016/j.cgh.2007.06.002.

[68] O. Sadr–Azodi, D. S. Sanders, J. A. Murray, and J. F. Ludvigsson, “Patients With Celiac Disease Have an Increased Risk for Pancreatitis,” *Clinical Gastroenterology and Hepatology*, vol. 10, no. 10, pp. 1136-1142.e3, Oct. 2012, doi: 10.1016/j.cgh.2012.06.023.

[69] J. K. VISAKORPI, P. KUITUNEN, and P. PELKONEN, “INTESTINAL MALABSORPTION: A CLINICAL STUDY OF 22 CHILDREN OVER 2 YEARS OF AGE,” *Acta Paediatr*, vol. 59, no. 3, pp. 273–280, May 1970, doi: 10.1111/j.1651-2227.1970.tb09003.x.

List of published papers

Articles published in extenso as first author in specialist journals

1. **Saizu Ionuț Adrian**, Cotruta B, Iacob RA, Bunduc S, Saizu RE, Dumbrava M, Pietrareanu C, Becheanu G, Grigorie D, Gheorghe C. A MODEL TO PREDICT DIAGNOSIS OF PANCREATIC NEUROENDOCRINE TUMORS BASED ON EUS IMAGING FEATURES. Acta Endocrinol (Buchar). 2023 Oct-Dec;19(4):407-414.

DOI: 10.4183/aeb.2023.407. Epub 2024 Jun 24. PMID: 38933251; PMCID: PMC11197819.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC11197819/pdf/ACTA-19-407.pdf

2. **Saizu Ionuț Adrian**, Bancila I, Becheanu G, Dumbrava M, Gheorghe C. An Unusual Etiology of Obstructive Jaundice in a Newly Diagnosed Celiac Disease Patient. J Gastrointestin Liver Dis. 2021 Mar 12;30(1):15.

DOI: 10.15403/jgld-3343. PMID: 33723549.

https://www.jgld.ro/jgld/index.php/jgld/article/view/3343/1680

Articles published as co-author

1. Ocskay K, Juhász MF, Farkas N, Zádori N, Szakó L, Szakács Z, Szentesi A, Erőss B, Miklós E, Zemplényi A, Birkás B, Csathó Á, Hartung I, Nagy T, Czopf L, Izbéki F, Gajdán L, Papp M, Czakó L, Illés D, Marino MV, Mirabella A, Małecka-Panas E, Zatorski H, Susak Y, Opalchuk K, Capurso G, Apadula L, Gheorghe C, **Saizu Ionuț Adrian**, Petersen OH, de-Madaria E, Rosendahl J, Párniczky A, Hegyi P; Hungarian Pancreatic Study Group. Recurrent acute pancreatitis prevention by the elimination of alcohol and cigarette smoking (REAPPEAR): protocol of a randomised controlled trial and a cohort study. BMJ Open. 2022 Jan 4;12(1):e050821.

DOI: 10.1136/bmjopen-2021-050821. PMID: 34983758; PMCID: PMC8728419.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8728419/pdf/bmjopen-2021-050821.pdf

2. Nagy R, Ocskay K, Sipos Z, Szentesi A, Vincze Á, Czakó L, Izbéki F, Shirinskaya NV, Poluektov VL, **Saizu Ionuț Adrian**, Gheorghe C, Hegyi P. et al. Discharge protocol in acute pancreatitis: an international survey and cohort analysis. Sci Rep. 2023 Dec 13;13(1):22109.

DOI: 10.1038/s41598-023-48480-z. PMID: 38092809; PMCID: PMC10719286.

https://www.nature.com/articles/s41598-023-48480-z

3. Vadan R, Iacob R, Costache R, Stroie T, **Saizu Ionuț Adrian**, Iacob S, Gheorghe L, Diculescu M, Gheorghe C. Inflammatory Bowel Disease Management in a Romanian Tertiary Gastroenterology Center: Challenges of the COVID-19 Pandemic. J Gastrointestin Liver Dis. 2020 Dec 12;29(4):549-553.

DOI: 10.15403/jgld-3183. PMID: 33331349.

https://www.jgld.ro/jgld/index.php/jgld/article/view/3183/1640

4. Les A, Iacob R, Saizu R, Cotruta B, **Saizu Ionuț Adrian**, Iacob S, Gheorghe L, Gheorghe C. Bowel Ultrasound: a Non-invasive, Easy to EUS Method to Predict the Need to Intensify Therapy in Inflammatory Bowel Disease Patients. J Gastrointestin Liver Dis. 2021 Dec 21;30(4):462-469.

DOI: 10.15403/jgld-3726. PMID: 34752586.

https://jgld.ro/jgld/index.php/jgld/article/view/3726/1769

5. Radu EC, **Saizu Ionuț Adrian**, Grigorescu RR, Croitoru AE, Gheorghe C. Metastatic neuroendocrine pancreatic tumor - Case report. J Med Life. 2018 Jan-Mar;11(1):57-61. PMID: 29696066; PMCID: PMC5909947.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5909947/pdf/JMedLife-11-057.pdf

Posters at scientific events organized by international professional associations

Saizu Ionuț Adrian, G Becheanu , M Dumbrava , R Costache , R Iacob , C Gheorghe. WHICH EUS FEATURE BEST PREDICT THE DIAGNOSIS OF PANCREATIC NEUROENDOCRINE TUMORS - prezentare ePoster. ESGE Days 2020 April 23, 2020. Endoscopy 2020; 52(S 01): S318.

DOI: 10.1055/s-0040-1705025

https://www.thieme-connect.de/products/ejournals/html/10.1055/s-0040-1705025