A morphological and immunohistochemical study of the endoscopic ultrasound–fine-needle biopsy samples from solid pancreatic masses: a single center study

ALEXANDRU CONSTANTINESCU1,2, CRISTINA MĂDĂLINA ILIE-STAN2,3, VASILE ŞANDRU2,3, BOGDAN SILVIU UNGUREANU4, DAN IONUŢ GHEONEA5, TUDOREL CIUREA5, OANA MIHAELA PLOTOGEA2,3, CHRISTOPHER PAVEL2, VALENTIN ENACHE5, MIHAI ALEXANDRU Munteanu6, GABRIEL CONSTANTINESCU2,3

1) PhD Student, Doctoral School, University of Medicine and Pharmacy of Craiova, Romania
2) Department of Gastroenterology, Emergency Clinical Hospital, Bucharest, Romania
3) Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
4) Department of Gastroenterology, University of Medicine and Pharmacy of Craiova, Romania
5) Department of Pathology, Emergency Clinical Hospital, Bucharest, Romania
6) Department of Gastroenterology, Emergency County Hospital, Oradea, Romania

Abstract
Objective: The purpose of this study was to present the experience of a single center on endoscopic ultrasound–fine-needle biopsy (EUS–FNB) of pancreatic solid tumors amenable to immunohistochemistry (IHC) assay. Patients, Materials and Methods: Inclusion criterion for this prospective study was identifying patients with pancreatic solid tumors, by means of imaging methods, from January 2018 to February 2020, within the Department of Gastroenterology, Emergency Clinical Hospital, Bucharest, Romania. All patients underwent EUS–FNB and the harvested tissue was sent to the Department of Pathology for histopathological (HP) diagnosis and IHC assessment if tumoral origin remained undetermined. Results: A total of 57 patients were ultimately selected to take part in our study. We performed immunohistochemical analysis based on the morphological diagnosis of the pancreatic tumors and assessed cytokeratin (CK)7, CK20, caudal type homeobox 2 (CDX2), MutL homolog 1 (MLH1), MutS homolog (MSH)2, MSH6, postmeiotic segregation 2 (PMS2) for all histopathologically uncertain pancreatic ductal adenocarcinoma (PDAC) and chromogranin A, synaptophysin, pan-CK AE1/AE3 for pancreatic neuroendocrine tumors (pNETs). Cox hazard regression was performed to identify the factors influencing the survival rate. In univariate analysis, patient survival time was significantly associated with stage, location, surgical management and CK7 positivity. Our data show a statistically significant predictive relationship between stage (regional or metastatic) and hazard for survival ($p=0.015$), tumor localization in the tail ($p=0.015$) and radicality surgery ($p=0.015$) significantly decrease the survival of pancreatic cancer (PAC) patients. The presence of CK7 ($p=0.015$) significantly increases the survival of pancreas cancer patients. Conclusions: EUS–FNB has opened up a new path for pancreatic tumor diagnosis providing enough tissue for HP examination and IHC. A panel of several immunomarkers might aid in providing new therapies for PAC patients.

Keywords: pancreatic cancer, endoscopic ultrasound–fine-needle biopsy, immunohistochemistry.

§ Introduction
Pancreatic cancer (PAC) remains a major global burden with a rapid metastatic development and a grim prognosis [1]. Despite current advancements, no major breakthroughs were made for oncological therapy, especially due to late diagnosis, when only palliative management and nutrition therapy may be recommended [2]. With surgery as the only curative treatment and with a 5-year survival rate lower than 10%, PAC requires an early and precise diagnosis [3].

PAC diagnosis has also been a challenge over the years, due to the pancreatic retroperitoneal position. Along with the endoscopic ultrasound (EUS) introduction 40 years ago, the pancreato-biliary system became approachable, enabling new diagnostic and therapeutic procedures [4]. Fine-needle aspiration (FNA) was further established as the “gold standard” method for histopathological (HP) diagnosis of pancreatic tumors [5]. This procedure allows pancreatic access and helps clinicians provide an initial diagnostic and plan therapeutic decisions [6]. However, EUS–FNA may provide a cytological result with only a binary yield of positive or negative for malignancy [7]. Newly introduced needles for acquisition of core biopsy tissue, EUS–fine-needle biopsy (FNB), aid in sampling tissue with preserved architecture, amenable not only for HP analysis, but also for genetic and molecular diagnosis, or immunostaining of pancreatic malignancy samples [8, 9].

EUS–FNB could bridge the gap between endoscopic tissue acquisition, immunohistochemistry (IHC), and oncological therapy, thus providing new possible therapeutic targets. However, there are relatively few studies that assess diagnostic yield of the EUS–FNB in this aspect and even fewer that further immunoassay and discuss PAC samples [10, 11].

Aim
We propose a prospective study to evaluate the diagnostic and prognostic yield of EUS–FNB for the
**Immunohistochemical analysis of previously histopathologically uncertain pancreatic samples.**

### Patients, Materials and Methods

#### Study design

The following study is a prospective, single center study involving all patients diagnosed with pancreatic solid tumors within the Department of Gastroenterology, Emergency Clinical Hospital, Bucharest, Romania, from January 2018 to February 2020. The study was approved by the local Ethics Board and all patients signed an informed consent, according to the Helsinki Declaration. Following this, patients’ general information and technical data were included into a database dedicated exclusively for this study.

All patients underwent sectional diagnosis by means of computed tomography (CT) or magnetic resonance imaging (MRI). Afterwards, EUS–FNB for HP confirmation using a linear-array echoendoscope (GF-LUCT180 EVIS LUCERA, Olympus Medical System Corp., Tokyo, Japan) was carried out. Tissue acquisition was performed with a 22G Fransseintip needle (Acquire™, Boston Scientific Corp., Marlborough, MA, USA), by performing at least three passages through the tumor. The first step consisted of visualizing the tumor properly, with no interference of big vessels or other important structures with the needle trajectory. Lesions’ topographical and macroscopic EUS characteristics, such as location, diameter, adjacent lymph nodes, vascular extension, Doppler signal, transient elastography were considered for assessing puncture points (Figure 1, A and B).

#### Pathology examination

All EUS–FNB samples were fixed in 10% neutral buffered formalin solution for 72 hours and then processed for paraffin embedding. Next, 5 μm-thick sections were cut utilizing a microtome and routinely stained with Hematoxylin–Eosin (HE) and Giemsa. For immunostaining, selected sections were placed in a thermostat, at 37°C, dewaxed and hydrated. Antigen retrieval was performed by microwaving the sections in citrate pH 6 buffer for 20 minutes, then the sections were cooled down to room temperature (RT), incubated for 30 minutes in a 3% hydrogen peroxide solution at RT, followed by a 1% skimmed milk solution in phosphate-buffered saline (PBS) solution. All sections were incubated with specific primary antibodies at 4°C for the next 18 hours, utilizing optimized dilutions (Table 1). The next day, after thorough washing in PBS, the sections were incubated with species-specific biotinylated secondary antibodies (diluted as 1:100, Dako, Glostrup, Denmark), and then with a Streptavidin–Horseradish Peroxidase (HRP) (1:100, Dako), for 30 minutes each. Lastly, the signal was developed utilizing 3,3’-Diaminobenzidine (DAB), slides counterstained with Hematoxylin and coveredslipped with a xylene-based permanent mounting medium. The slides stained for the mismatch repair (MMR) proteins, cytokeratins (CKs), and neuroendocrine immunomarkers were used according to both paraclinical and HE or Giemsa results for positive and differential diagnosis.

### Table 1 – The primary antibodies utilized in the present study

| Name                | Description                  | Clone / Dilution | Producer       |
|---------------------|------------------------------|------------------|----------------|
| MLH1                | MutL homolog 1               | ES05 / 1:100     | Novocastra     |
| MSH2                | MutS homolog 2               | 25D12 / 1:100    | Novocastra     |
| MSH6                | MutS homolog 6               | PU29 / 1:100     | Novocastra     |
| PMS2                | Postmeiotic segregation 2    | M0R4G / 1:100    | Novocastra     |
| Chromogranin A      | –                            | DAK-A3 / 1:100   | Dako           |
| Synaptophysin       | –                            | DAK-SYNAP / 1:100| Dako           |
| CK AE1/AE3          | Pan-cytokeratin AE1/AE3      | M3515 / 1:100    | Dako           |
| CK7                 | Cytokeratin 7                | CV-TL 12/30 / 1:100| Dako          |
| CK20                | Cytokeratin 20               | M7019 / 1:100    | Dako           |
| CDX2                | Caudal type homeobox 2       | EP25 / 1:100     | Novocastra     |

Positive immunostaining was considered brown staining of the cytoplasm, cell membrane or nucleus. We estimated the number of positive cells by considering 0 for positivity of none or only rare single cells; 1+ for up to 50% positive tumor cells; 2+ for more or equal than 50–90% positive tumor cells; 3+ >90% of positive tumor cells.
Patients’ follow-up

All patients were monitored immediately after the EUS–FNB procedure, with no reports for immediate major complications. Afterwards, the patients were followed-up for prognosis and survival analysis. Based on tumoral staging and pathology characteristics, they followed specific oncological or surgical therapies.

Statistical analysis

The age, gender, clinical presentation, paraclinical and laboratory findings were recorded. Mean, median and ranges were used to describe quantitative variables. Kaplan–Meier survival curves and the log-rank tests were used for survival analysis. All statistically significant parameters in the univariate analysis were introduced in a multivariate model to assess for independent predictors of survival rate. The GraphPad Prism 9.2.0 software (GraphPad Software, San Diego, CA, USA) was used with the threshold for statistical significance of 0.05.

Results

A total of 57 patients that met the inclusion criteria took part in our study. Tables 2 and 3 show the patients’ characteristics. Among the 57 subjects, 27 (47.4%) were males and 30 (52.6%) females, with a general mean age of 61.19 years [standard deviation (SD) 13.18 years; range 24–82 years]. Tumoral diameter ranged from 1.2 cm to 9 cm, with a median tumor size of 3.5 cm. EUS–FNB was performed in each case, without procedural complications reported neither during, nor after the intervention. With at least three passages per patient, enough tissue was collected for complete testing and HP examination, revealing 35 patients with pancreatic ductal adenocarcinoma (PDAC), one with solid pseudopapillary tumor, one with acinar cell carcinoma, 12 with pancreatic neuroendocrine tumor (pNET) and other eight with secondary tumors (two breast metastases, four lung metastases, two non-Hodgkin’s lymphoma type B). Our study highlights the aggressiveness of a pancreatic tumor, with most of the cases being diagnosed in an advanced stage either metastatic (54%) or locoregional with vascular invasion (42%). Thus, surgery was rarely used, with only six patients being subjected to radical surgery and four to palliation procedures.

Morphological analysis

Our data for HP assessment of PDACs (n=35) showed in most samples a tubulo-papillary pattern with disorganized and irregular architecture, atypical glandular proliferation with cubic-cylindrical cells, some with squamous-like, non-keratinized features and engulfed by necrotic detritus, fibrin, mucus, or acute inflammatory cells – macrophages, lymphocytes, as well as fibrino-hematic clots, alongside with benign-like structures of acinar or ductal-biliary phenotype. Cells showed slight to severe nuclear and cellular atypia, as well as atypical mitoses. Nuclei had visible nucleoli, atypical and polymorph, with vesicular, non-keratinized features and engulfed by necrotic detritus, with cubic-cylindrical cells, some with squamous-like architecture, atypical glandular proliferation in most samples a tubulo-papillary pattern with disorganized and irregular architecture, atypical glandular proliferation with cubic-cylindrical cells, some with squamous-like, non-keratinized features and engulfed by necrotic detritus, fibrin, mucus, or acute inflammatory cells – macrophages, lymphocytes, as well as fibrino-hematic clots, alongside with benign-like structures of acinar or ductal-biliary phenotype. Cells showed slight to severe nuclear and cellular atypia, as well as atypical mitoses. Nuclei had visible nucleoli, atypical and polymorph, with vesicular, enlarged profiles. Cytoplasm was overall more abundant than normal (Figures 2 and 3).

Table 2 – Cohort characteristics by type of cancer (n=57)

| Feature                | PDAC (n=35) | pNET (n=12) | Other tumors (n=18): 2 breast metastases, 4 lung metastases, 2 non-Hodgkin’s lymphoma type B, 1 acinar carcinoma, 1 solid pseudopapillary neoplasm |
|------------------------|-------------|-------------|-------------------------------------------------------------------------------------------------------------------------------------|
| Gender                 |             |             |                                                                                                                                     |
| • Male, n (percent)    | 20 (57%)    | 4 (33%)     | 3 (30%)                                                                                                                             |
| • Female, n (percent)  | 15 (43%)    | 8 (67%)     | 7 (70%)                                                                                                                             |
| Mean age, years (range)| 64.5 (26–80)| 59.8 (43–73)| 51.4 (24–82)                                                                                                                         |
| Mean diameter, cm (range)| 40 (20–90)  | 29 (12–50)  | 37 (27–70)                                                                                                                          |

n: No. of patients; PDAC: Pancreatic ductal adenocarcinoma; pNET: Pancreatic neuroendocrine tumor.

Table 3 – Clinicopathological features

| Feature                        | n (percent) |
|--------------------------------|-------------|
| Mean age, years (range)        | 61.19 (24–82)| |
| Gender                         |             |
| • Male                         | 27 (47%)    |
| • Female                       | 30 (53%)    |
| Type                           |             |
| • Pancreatic adenocarcinoma    | 35 (61%)    |
| • Pancreatic neuroendocrine tumor metastasis | 12 (21%) |
| • Small cell lung neuroendocrine tumor metastasis | 2 (4%) |
| • Pulmonary adenocarcinoma metastasis | 2 (4%) |
| • Breast neoplasm metastasis  | 2 (4%)      |
| • Non-Hodgkin’s lymphoma type B| 2 (4%)      |
| • Acinar carcinoma             | 1 (2%)      |
| • Solid pseudopapillary neoplasm| 1 (2%)    |
| Diabetes                       |             |
| • Present                      | 18 (32%)    |
| • Absent                       | 39 (68%)    |
| Tumor location                 |             |
| • Head                         | 43 (75%)    |
| • Body                         | 8 (14%)     |
| • Tail                         | 6 (11%)     |

n: No. of patients; TNM: Tumor, node, metastasis.

Regarding pNETs (n=12), their phenotype was primarily composed of monomorphic cells, with small to medium sized nuclei, without significant atypia or mitotic activity.
Nuclei were predominantly round, hyperchromic, without visible nucleoli. Neuroendocrine tissue was surrounded in most of cases by necrotic tissue, hematic clots or fibrin and areas of benign acinar structures; biliary or intestinal tissue were consistently present in the biopsies.

In the case of metastatic pulmonary neuroendocrine tumors \( (n=2) \), morphological analysis showed patterns consisting of round small-sized cells with hyperchromic nuclei, small cytoplasmatic volume, surrounded by necrotic and fibrino-hematic debris. The Ki67 proliferation index was significantly higher (closer to 90\%) compared to the primary pNETs (range 3–20\%).

The other pulmonary metastases \( (n=2) \) showed features of ductal architecture, with regularly disposed cells, without significant atypia, with pleomorphic, enlarged nuclei, and intertwined with hematic clots.

The single acinar cell carcinoma we encountered showed monomorphic cells, with minimal atypia, acinar (cribriform) architecture, without any significant mitotic activity.

The one solid pseudopapillary tumor was encountered in a very young woman, completely cured at the time of the publication, showed monomorphic cells, without significant atypia or mitotic activity, with a palisade cellular architecture along fibrovascular septa; no necrosis was noted. Nuclei were round to ovalar.

There were two cases of metastatic breast carcinoma which showed epithelioid cells with monomorphic, atypical nuclei. No mitotic activity was observed. Diagnostic was largely formulated based on patient history and IHC assessment.

On the lymphoma smears, we identified small-sized lymphocytes with hyperchromic nuclei and small cytoplasmatic volume.

**IHC assessment**

We performed an immunohistochemical analysis based on the initial morphological diagnosis of the pancreatic tumors, and we assessed carbohydrate antigen (CA)19-9, CK7, CK20, caudal type homeobox 2 (CDX2), MutL homolog 1 (MLH1), MutS homolog (MSH2), MSH6, post-meiotic segregation 2 (PMS2) for all pancreatic adenocarcinoma and chromogranin A, synaptophysin, pan-CK AE1/AE3 for pNET (Table 4). IHC was scored as +1 for focal positivity and incomplete coverage of the nuclei/membrane, as +2 for complete area coverage but with not maximum intensity, and as +3 for maximal area and signal intensity (Figures 4–7).

|                | CA19-9 | CK7 | CK20 | CDX2 | MLH1 | MSH2 | MSH6 | PMS2 |
|----------------|--------|-----|------|------|------|------|------|------|
| PDAC           | 1+     | 2+  | 3+   | 1+   | 2+   | 3+   | 1+   | 2+   |
|                | 7      | 11  | 12   | 5    | 8    | 14   | 1    | 4    | 3    | 1    | 2    | 2    | 3    | 7    | 9    | 4    | 5    | 6    | 4    | 5    | 6    | 3    | 6    | 8    |

Table 4 – **Positivity rate of antibodies used on EUS–FNB specimens according to the tumor diagnosis**

- **CA19-9**: Carbohydrate antigen 19-9; **CDX2**: Caudal type homeobox 2; **CK**: Cytokeratin; **EUS–FNB**: Endoscopic ultrasound–fine-needle biopsy; **MLH1**: MutL homolog 1; **MSH**: MutS homolog; **PDAC**: Pancreatic ductal adenocarcinoma; **PMS2**: Postmeiotic segregation 2; **pNET**: Pancreatic neuroendocrine tumor.

![Figure 2](image1.png)

**Figure 2** – Hematoxylin–Eosin staining of a PDAC biopsy obtained through EUS–FNB (×100). EUS–FNB: Endoscopic ultrasound–fine-needle biopsy; PDAC: Pancreatic ductal adenocarcinoma.

![Figure 3](image2.png)

**Figure 3** – Giemsa staining of a PDAC biopsy obtained through EUS–FNB (×400).
Kaplan–Meier analysis for patients with adenocarcinoma

Cox hazard regression was performed to identify the factors influencing the survival rate. In univariate analysis, the patients’ survival span was significantly associated with tumor, node, metastasis (TNM) staging, type of surgery, location and CK7. Based on log-rank test (Table 5), it can be seen that the survival of a patient with PAC stage II would exceed that of a patient with stage IV. We obtained a statistically significant predictive relationship between stage (advanced locoregionally or metastatic) and hazard for survival ($p=0.015$). The location in tail ($p=0.015$) and radicality surgery ($p=0.015$) significantly decreased the survival of PAC patients. The presence of CK7 ($p=0.015$) significantly increases the survival of subjects with PAC (Figure 8; Figure 9, A–C).

Table 5 – Cox regression model for pancreatic adenocarcinoma

| Covariate                      | Univariate analysis | Multivariate analysis |
|--------------------------------|---------------------|-----------------------|
|                                | HR                  | p-value               | HR                  | p-value               |
| Gender                         | 0.64 (0.30–1.34)    | 0.233                 | –                   | –                     |
| Age                            | 1.03 (0.99–1.06)    | 0.101                 | –                   | –                     |
| Stage                          |                     |                       |                     |                       |
| • Metastatic vs. regional       | 3.01 (1.24–7.34)    | 0.015                 | 3.59 (1.37–9.37)    | 0.009                 |
| Tumor extension                |                     |                       |                     |                       |
| • II                           | 0.34 (0.13–0.88)    | 0.026                 | –                   | –                     |
| • III                          | 0.31 (0.09–1.08)    | 0.065                 | –                   | –                     |
| • IV                           | Ref.                | –                     | –                   | –                     |
The backward stepwise method extracted the least influencing factors so that the final model was obtained in step 4. Omnibus test of model coefficients ($\chi^2$-squared) = 19.152; df=2; $p$=0.024 showed that the most significant factors to the probability of death were stage (metastatic) and surgery (radicality). Based on the multivariate model, our cohorts’ hazard ratio (HR) of death of patients with radicality surgery was 70 times higher than that of those with no surgery (HR=70.36; $p$=0.004). In addition, the HR of death in patients with metastatic stage was 3.59 times more than those with regional stage (HR=3.59; $p$=0.009) (Table 6).

Survival was significantly longer for pNETs [mean 22.800, 95% confidence interval (CI): 17.673–27.927] than for PDAC (mean 11.069, 95% CI: 9.042–13.095) or other subtypes (mean 17.300, 95% CI: 9.785–24.815), with log-rank $p$=0.018.

### Table 6

| Covariate | Univariate analysis | Multivariate analysis |
|-----------|---------------------|-----------------------|
|           | **HR** | **p-value** | **HR** | **p-value** |
| **Surgery** | | | | |
| • No | Ref. | – | – | – |
| • Laparoscopy | 0.29 (0.07–1.28) | 0.102 | 0.30 (0.07–1.30) | 0.11 |
| • Palliation | 0.45 (0.13–1.52) | 0.198 | 0.44 (0.13–1.54) | 0.19 |
| • Radicality | 29.68 (1.85–475.12) | 0.017 | 70.36 (3.96–1249.4) | 0.004 |
| **Diabetes** | | | | |
| 1.02 (0.48–2.16) | 0.961 | – | – |
| **Diameter** | | | | |
| 1.02 (0.99–1.05) | 0.205 | – | – |
| **Location** | | | | |
| • Body | Ref. | – | – | – |
| • Head | 2.48 (0.73–8.49) | 0.147 | – | – |
| • Tail | 7.3 (1.49–35.9) | 0.014 | – | – |
| CK7 | 0.15 (0.03–0.78) | 0.024 | – | – |
| CK20 | 0.66 (0.25–1.77) | 0.411 | – | – |
| CDX2 | 0.19 (0.02–1.45) | 0.108 | – | – |
| CA19-9 | 1.36 (0.51–3.64) | 0.545 | – | – |
| MLH1 | 0.85 (0.33–2.21) | 0.744 | – | – |
| MSH2 | 0.85 (0.33–2.21) | 0.744 | – | – |
| MSH6 | 0.85 (0.33–2.21) | 0.744 | – | – |
| PMS2 | 0.85 (0.33–2.21) | 0.744 | – | – |

CA19-9: Carbohydrate antigen 19-9; CDX2: Caudal type homeobox 2; CK: Cytokeratin; HR: Hazard ratio; MLH1: MutL homolog 1; MSH: MutS homolog; PMS2: Postmeiotic segregation 2; Ref: Reference group.

**Figure 8** – Kaplan–Meier curve. Survival by type of cancer identified by immunohistochemistry. PAC: Pancreatic adenocarcinoma; pNET: Pancreatic neuroendocrine tumor.

**Figure 9** – Kaplan–Meier curve. Probability of survival of pancreatic cancer patients with adenocarcinoma according to tumor local extension (A), surgery (B) or stage (C).
Traditionally, HP diagnosis of solid pancreatic tumors was performed by EUS–FNA, through cytological examination, direct smears, cytospin [14]. Rapid on-site evaluation (ROSE) was also proposed to faster analyze the smears and repeat the EUS–FNA if necessary [15]. However, randomized studies have shown that this approach may not be so efficient, and it does not significantly improve EUS sampling resultant [16]. More recently, confocal laser endomicroscopy has also been tested as a rapid diagnostic tool for freshly harvested pancreatic samples [17, 18]. In the last few years, EUS–FNB was introduced and proved to be successful in providing enough tissue for HP assessment [19]. Generally, EUS–FNA yields mainly aspiration fluid, which is directly ejected on smears and, if enough material is present, it is stored in a container and fixed in formalin for further processing as a paraffin-embedded cellular block. On the other hand, when performing EUS–FNB, tissue fragments are stored after each needle passage and then pushed out directly into the container, thus enabling the classical protocol for core biopsies.

This study presents a single centers’ experience with EUS–FNB of solid pancreatic lesions, followed by HP assessment. Adequate tissue samples were obtained for all included patients, with three passes performed for each patient, which translates into a high HP accuracy. While some studies recommend two passes with or without ROSE, our results based on EUS–FNB with three passes per patient regards this protocol as safe and efficient for a complex HP analysis, including IHC, pointing a 100% sample adequacy for biopsies collected. Our results are similar with other studies, showing that the Franseen-tipped needle (Acquire™ from Boston Scientific) has a relevant applicability in pancreatic lesions, and it clearly features improved tissue acquisition in comparison to EUS–FNA needles [20–22]. Nonetheless, the most important characteristic of an EUS–FNB needle is not the needle diameter, which is similar to an FNA one, but the amount of tissue obtained which may relate to a core biopsy. Roughly, the specimens are more easily obtained due to the cutting system, especially if a sharper needle is used and thus tissue is more likely to be harvested, enabling HP diagnosis.

The primary endpoint of this study was evaluating the morphological and immunohistochemical statistical relation to several patient-related features like survivability, TNM staging, tumoral diameter, surgery. Our analysis proved that IHC may easily be performed on EUS–FNB samples. Furthermore, we did not encounter any adverse events during the procedures, and state it as safe, with practically zero morbidity rate.

HP outcome of the studied cohort proved once again the HP diversity of lesions found in the pancreas, which included besides 35 PDACs, also 12 pNETs, eight secondary tumors, one solid pseudopapillary tumor and one acinar cell carcinoma.

We decided to test CKs (CK7, CK20, CDX2) and MMR proteins on EUS–FNB PAC samples, thus ensuring a more relevant immunohistochemical diagnosis. These CKs have been associated with periampullary carcinomas, whereas for pancreatic tissue their contribution to disease outcome is limited [23]. Commonly, CK7 may also be found in the gastrointestinal tract, urinary and respiratory tract, as well as breast and genital tract [24, 25]. Other tested CKs, CDX2, which is mostly being used for identification of adenocarcinoma of unknown origin, and CK20, which is not usually found in pancreatic tissue require further extensive assessments [26, 27]. Also, the MMR proteins (MLH1, MSH2, MSH6 and PMS2) are considered to be a predicting factor for survivability in the case of PAC patients [28]. However, our results show that by providing a univariate analysis, patient’s survival was only associated with tumoral location and its staging, surgical parameters and only CK7 positivity (p=0.015). Among all, metastatic stage, and type of surgery, among all elements, were found to be more associated with a grim prognosis.

PNETs were identified in 12 cases and were found to stain positive for synaptophysin and chromogranin A, two diagnostic immunomarkers strongly recommended.

### Table 6 – Cox regression model for pancreatic cancer patients with neuroendocrine tumors

| Covariate | HR (95% CI) | p-value |
|-----------|------------|---------|
| Gender    | 0.58 (0.04–9.29) | 0.698   |
| Age       | 1.04 (0.86–1.26) | 0.655   |
| Stage     |            |         |
| Local     | Ref. –     |         |
| Metastatic| 0.01 (0.00–13×10°) | 0.667   |
| Regional  | 0.01 (0.00–20×10°) | 0.491   |
| Tumor extension | 0.01 (0.00–20×10°) | 0.491   |
| Surgery   |            |         |
| No        | Ref. –     |         |
| Laparoscopy| 0.01 (0.00–13×10°) | 0.667   |
| Radicability| 0.01 (0.00–20×10°) | 0.491   |
| Diabetes  | 3.5 (0.22–55.7) | 0.381   |
| Diameter  | 0.99 (0.87–1.12) | 0.865   |
| Location  |            |         |
| Body      | Ref. –     |         |
| Head      | 38.41 (0.00–19×10°) | 0.722   |
| Tail      | 1 (0.00–2×10°) | 1       |
| Chromogranin A | 22.93 (0.00–8×10°) | 0.844   |
| Synaptophsin | – – – – – | – – – – – |
| Pan-CK AE1/AE3 | 30.08 (0.00–44×10°) | 0.639   |

CK: Cytokeratin; HR: Hazard ratio; Ref: Reference group.
for this type of tumor. Further on, we also tested pan-Ck AE1/AE3, which is an immunomarker of epithelial differentiation and usually labels 50% of the tumors [29] and found its expression in about 66.7% of the cases.

**Study limitations**

Our study has several limitations. Mainly, the number of patients is relatively small, and this might be considered a drawback; however, the diversity of pancreatic solid tumors included may be more relevant. We’ve only tested a few immunohistochemical markers and for sure a larger panel could have been more useful to determine the patients’ prognosis [30]. Moreover, we did not have a parallel FNA cohort to highlight the advantages brought about using a FNB for solid pancreatic tumors.

Most patients arrived in advanced stages, either regional or metastatic, when surgery is no longer an option, adding the idea of early, precise diagnosis as the bedrock for oncological management. The new available needles help for the road for further targeted therapies in the future to come and the immunostaining studies, along with molecular or genetic studies should become a common ground in the following years for all PAC patients.

**Conclusions**

EUS–FNB has opened up a new path for pancreatic tumor diagnosis, especially because most patients are diagnosed in advanced stages when they require only oncological management. The new available needles help enhance the diagnostic yield by providing enough tissue for IHC which will allow testing for new potential oncological therapies.

**Conflict of interests**

The authors declare that they have no conflict of interests.

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Corresponding author
Bogdan Silviu Ungureanu, Teaching Assistant, MD, PhD, Department of Gastroenterology, University of Medicine and Pharmacy of Craiova, 2 Petru Rareș Street, 200349 Craiova, Dolj County, Romania; Phone +40723–178 763, e-mail: bogdan.ungureanu@umfcv.ro

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