SAHLGRENSKA ACADEMY

Endoscopic ultrasonography-guided fine-needle biopsy sampling for the preoperative grading of pancreatic neuroendocrine tumours

Degree Project in Medicine

Fredrik Bergstedt

Programme in Medicine

Gothenburg, Sweden 2019

Supervisor: Per Hedenström, MD, PhD

Medicinmottagningen,

Sahlgrenska Universitetssjukhuset

Table of contents

List of abbreviations

NET- Neuroendocrine tumour

panNET – Pancreatic neuroendocrine tumour

fpanNET- Functioning pancreatic neuroendocrine tumour

nfpanNET- Non-functioning pancreatic neuroendocrine tumour

MI - Mitotic index

Ki67% – Unit describing the rate of which cells divide

EUS – Endoscopic ultrasonography

FNA – Fine-needle aspiration

FNB – Fine-needle biopsy

US – Ultrasonography

CT – Computed tomography

MRI – Magnetic resonance imaging

GI- Gastrointestinal

Abstract

Title: Endoscopic ultrasonography-guided fine-needle biopsy sampling for the preoperative grading of pancreatic neuroendocrine tumours

Degree project, Programme in Medicine 2019

Author: Fredrik Bergstedt[1] **Project supervisor:** Per Hedenström[2]

Institute: [1]Sahlgrenska academy, Gothenburg University, Göteborg, Sweden. [2]Unit of Gastrointestinal Endoscopy, Sahlgrenska University hospital, Göteborg, Sweden

Introduction/Background

panNETs (Pancreatic neuroendocrine tumours) are rare, mostly indolent tumours. EUS (endoscopic ultrasonography) has amongst the highest sensitivity in diagnosing panNETs. However, EUS-FNA (EUS guided fine-needle aspiration) is non-appropriate for preoperative grading panNETs. EUS-FNB (EUS guided fine-needle biopsy) is comparable to EUS-FNA in the diagnosis of panNETs, but few studies have investigated EUS-FNB for the grading of panNETs.

Aims

The purpose of this study was to evaluate the capacity of EUS-FNB for grading panNETs in the pre-operative phase. In addition, we aimed to evaluate the diagnostic sensitivity of EUS-FNB and to analyse any factors with a potential impact the biopsy quality.

Material and methods

In a single-centre setting, patients with a suspected panNET referred to EUS 2012-2019 were eligible for study enrolment. Patients finally subjected to EUS-FNB were included, while patients with a final diagnosis other than panNET were excluded.

Results

49 cases, 46 unique patients (24 males and 22 females, mean age 61) were included in the study. EUS-FNB was diagnostic in 40 out of 49 cases (82%). No tested factor had a significant impact on the biopsy quality of EUS-FNB, with adequate quality being defined as a cell count of >1000. Twenty cases proceeded to surgery. Comparing the EUS-FNB specimens and the corresponding surgical specimens, there was a tumour grade concordance in 8/14 cases (57.1%). All cases that were discordant were due to under grading. Counting EUS-FNB sampling with a cell count > 2000 cells only (n=6), the concordance increased to 5/6 (83.3 %).

Discussion

EUS-FNB is sensitive for the diagnosis of panNETs, but only moderately accurate for the pre-operative grading of tumours. However, tumour grading seems more reliable in EUS-FNB specimens with a high tumour cell count. Therefore, EUS-FNB may play a future role in the preoperative management and prognostic risk assessment of panNETs. Further improvement of the needle design and additional studies are warranted.

Keywords

Endoscopic ultrasonography, fine-needle biopsy, pancreatic neuroendocrine tumour, Tumour grading, preoperative

Introduction

Pancreas

The pancreas is an organ that lies in the retroperitoneum, in close proximity to the duodenum and ventricle. The pancreas is anatomically divided into four parts – the head, neck, body, and tail of pancreas (Illustration 1). Functionally it is usually divided into two parts, the exocrine pancreas and the endocrine pancreas. The former produces and secretes hormones that digest and help the absorption of nutrients. The latter produces hormones that are responsible for the regulation of the blood sugar levels, i.e. Insulin and Glucagon. The first attempt of describing the endocrine part the pancreas was made in the late 19th century. It was attributed to the islets of Langerhans and shortly after it was concluded that the islets consisted of two cell types (it would later be discovered to be more cell types than two). The two cell types in the islets were named α- and β-cells. Thanks to the development of immunohistological staining, the function of the α- and β-cells could be described in the middle of the 20th century, i.e. the production of Insulin and Glucagon respectively. These pioneer studies have had a great impact on our understanding of the endocrine pancreas and any related pathology, i.e. DM (Diabetes Mellitus) (1). As with most other tissues of the human body, malignancy may arise from the endocrine pancreas. The most frequent tumour originating from the pancreas is pancreatic adenocarcinoma, accounting for around 90 % of all pancreas malignancies (2).

Illustration 1

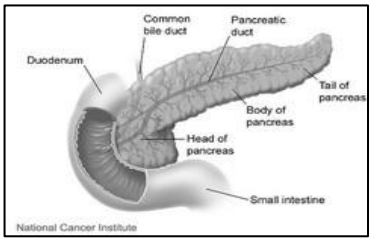


Illustration 1

An illustration on the anatomy of the pancreas with important anatomical structures pointed out. The neck of the pancreas is, however not indicated but is situated between the head and body of pancreas. Don Bliss (Illustrator), National Cancer Institute, September 20, 2007

Adenocarcinoma have a poor prognosis with 5-year overall survival rate of approximately 6% (3-5). However, the current study focuses on pancreatic neuroendocrine tumours which will be described in detail below.

Neuroendocrine Tumours of the Pancreas, Functional and Non-functional

Tumours arising from the endocrine part of the pancreas are called panNETs (pancreatic neuroendocrine tumours) previously known as *islet tumours*. They were called islet tumours as it was thought that they originated from the islets of Langerhans, but it has since then been concluded that they originate from pluripotent stem cells in the ductal epithelium. The tumours may arise from any anatomical part of the pancreas. One study, spanning 12 years, reported that 49.3 % of panNETs originated from the tail of the pancreas, 30.3% and 20.2 % originating from the head and the body respectively(6). This study does not seem to distinguish the neck of the pancreas from the body and head. Interestingly, one paper reports that neoplasms originating from the head are more likely to be malignant and to have poorer prognosis(7). There are several ways of which to grade and divide panNETs, one of which is the division into tumours that are functioning or so-called non-functioning. Whether or not they are classed as functioning are if they produce significant amounts of hormonally active peptides. There are also tumours that can be histologically confirmed to have risen from the endocrine part of pancreas but produce no hormone and are therefore called non-functioning panNETs. Important to note is that a tumour should only be classified as a functioning panNET if the excess hormone production is accompanied with a set of characteristic symptoms. That means that both the histological staining and the biological screening can point towards an overproduction of hormones and it not being a functional panNET if it's not accompanied with symptoms correlating with the overexpressed hormone(8). Most pancreatic neuroendocrine tumour cells have an over expression of somatostatin receptors at their cell membrane (9, 10), a fact that we take to our advantage in Somatostatin-scintigraphy, where a

somatostatin analogue is used to visualise somatostatin receptors. This method and other diagnostic means will be discussed in further detail in a later segment.

Functional Pancreatic Neuroendocrine Tumours - fpanNET

Insulinomas - The most common functional panNET is Insulinoma, making up 35-40% of fpanNETs (functional panNET)(11). Insulinomas have an annual incidence rate of 0.07 to 0.12/100,000(1, 12, 13). There is also a study which has retrospectively identified Insulinomas over a time span of 60 years, showing the incidence to be 0.4 per 100,000 person-years(14). Insulinomas are, however, benign in most cases, with a malignancy rate of <10 %(15, 16). Clinically, Insulinomas presents with "…the well-known symptoms associated with hypoglycaemia, especially after periods of fasting. Headache, weakness, dizziness, dysarthria, incoherence, convulsion and coma represent the most common symptoms which are due to the deleterious effects of hypoglycaemia on brain function"(1).

Gastrinomas - The second most frequent occurring functioning panNET is Gastrinomas(1). Gastrinomas have an incidence rate of 0.5-3/1000,000. Gastrinomas do not only occur in the pancreas. Roughly 50% are pancreatic, about 20 % are duodenal and the remaining split amongst other infrequent localisations(17). Gastrinomas are associated with Zollinger-Ellison syndrome, a syndrome described in the 1950s where ulcers in the small intestine can be attributed to an excess secretion of Gastrin(18). In contrast to Insulinomas, Gastrinomas have a higher malignancy rate, 60-90% at diagnosis(19).

There are more fpanNETs, such as VIPomas, Glucagonomas, Somatostatinomas, and more. They are, however, even more scarcely found than Insulinomas and Gastrinomas and will not be discussed further here.

Non-functional Pancreatic Neuroendocrine Tumours – nfpanNETs

Up to 90 %, however, of panNETs are hormonally silent and are classed as non-functional.(11, 20, 21). Non-functional panNETs are all panNETs without an accompanying syndromic hormone dysregulation. Because of its nature of not producing excess hormones, it can grow unnoticed until large in size. Symptoms are mostly caused by mass effects, meaning its size will limit space of organs surrounding it. Frequent symptoms include abdominal pain, jaundice or weight loss(22). Abdominal pain is the most common initial clinical symptom 23%(23). Because non-functioning panNETs often are asymptomatic in the early stages, up to 38.7 % of patients are diagnosed *en passant*, i.e. incidentally (24).

Grading of Pancreatic Neuroendocrine Tumours

Pancreatic neuroendocrine tumours can be graded and differentiated by several means, one being the now widespread TNM-system, which describes T-Tumour size, N – Number of lymph node metastases and M – distal metastases(25). Another grading scale widely used is the one written by WHO which divides panNETs into three groups dependent on Ki67 % or Mitotic Index (Illustration 2). The WHO-gradings depend on Ki 67 % or MI, both of which are measurements of cell division rate.

Illustration 2

Classification/grade	Ki67 proliferation index	Mitotic index
Well-differentiated pancreatic neuroendocrine tumours		
G1 PanNET	<3%	<2
G2 PanNET	3–20%	2–20
G3 PanNET	>20%	>20
Poorly differentiated pancreatic neuroendocrine carcinomas		
PanNEC (G3)	>20%	>20

Illustration 2
Table of tumour grades based on the WHO grading system. M. Amin SE, F. Greene, D. Byrd, R. Brookland, M.K. Washington. AJCC Cancer Staging Manual: Springer International Publishing; 2017.

To determine the Ki 67 % or MI immunohistological stainings are used, that colour the cells that are undergoing cell division and you then count what portion of cells in the sample that are undergoing mitosis, to get a rate of which the tumour grows. Pancreatic neuroendocrine tumours with a MI (mitotic index) < 2% or a Ki67% <3 % is classified as a grade 1 tumour.

Grade 2 tumours are classified as tumours with a MI of 2-20 % or a Ki67 of 3-20 %. Lastly grade 3 tumours are classified as tumours with either a MI or Ki67% over 20 %.

WHO published an update of their guidelines of classifying panNETs in 2017 with further division of grade 3 tumours into two groups, distinguishing between well-differentiated and poorly-differentiated tumours. The tumours with well differentiated cells retain the name of panNET grade 3, but the poorly differentiated tumours are classified as panNECs (pancreatic neuroendocrine carcinoma).(26)

Epidemiology of Neuroendocrine Tumours of the Pancreas.

Neuroendocrine tumours of the pancreas are rare but indolent, in contrast to the adenocarcinomas of the pancreas. PanNETs make up 1-2 % of tumours originating from the pancreas(27). PanNETs constitute 7 % of all NETs(neuroendocrine tumours)(27). The survival rates for all panNETs, are 64 % after five years and 44 % after ten years(28). However, it is not quite fair to describe all panNETs under the same umbrella as different grades of panNET has proven to have different survival rates. One study reported 5-year survival rates of 97.5%, 87%, and 0% for G1, G2, and G3 panNETs, respectively(29).

Approximately 90% of panNETs are sporadic(1). There are inherited syndromes which encompasses increased probability of developing a panNETs, such as MEN 1 (multiple endocrine neoplasia type 1) and VHL (von Hippel Lindau). Women have greater 5-year overall survival, 70.8 % compared to 47.9 % for men(30). There are studies showing that panNETs are as prevalent as in 1.5 % of all autopsies(31), pointing towards the fact that these tumours might not be as scarce as previously thought and that they are, in a large portion of cases, subclinical. A study was published in 2008 on current epidemiology of neuroendocrine tumours (all types of neuroendocrine tumours). 35,825 cases of NET’s (neuroendocrine tumours) was retrospectively identified, diagnosed in the time span of 1973-2004. It was

concluded that the age adjusted incidence of NET's rose from 1.09/100,000 (1973) to 5.25/100,000 (2004)(32). This trend has also been confirmed by similar studies where the incidence reached as high as 6.98/100,000 (2012) in a study spanning 1973-2012(33). Important to note is that the increasing incidence of NETs is no doubt, to what extent is unknown, due to our ever-increasing ability to diagnose and find tumour diseases. Whatever the reason, the incidence of NETs is increasing, and we therefore seek further methods by which we can prognosticate and diagnose tumour diseases which are becoming growing problems in our population. As for panNETs, there is a review article done on approximately 200 papers describing the epidemiology of gastroenteropancreatic neuroendocrine tumours where it has been concluded that the incidence rate of panNETs has increased from 0.17/100,000 in 1973–1977 to 0.43/100,000 in 2003–2007 in the US population. A doubling of the incidence rate of panNETs in Norway from 0.15 to 0.3/ 100,000 in the timespan mid-1990s and the early 2000s was also seen(34).

Diagnostics and Imaging

CT and MRI - Conventional radiographic techniques, such as CT and MRI, has historically proven to have difficulty in elucidating smaller panNETs, especially fpanNETs as they tend to be smaller. Technology is, however, ever evolving and newer MRI- and CT-machines with higher resolution and multiphasic programs have improved their sensitivity. There is a study, comparing different CT-programs with EUS (endoscopic ultrasonography) in detecting insulinomas. Dual-phase thin-section multidetector CT, dual-phase multidetector CT without thin sections and sequential CT was compared with EUS. The reported sensitivity of the different techniques was 94.4 %, 57.1 % and 28.6 % respectively whilst EUS had a reported sensitivity of 93.8 % (40). This study sheds light on the potential underdiagnosis of smaller pancreatic neoplasms, but when applying multiphasic programs with appropriate protocols

and thin sections the sensitivity rises to a rate comparable to what today is considered the golden standard, EUS.

Somatostatin Analogue Techniques - Other diagnostic methods used are Somatostatin-scintigraphy and various PET-examination (positron emission tomography) techniques. These techniques take advantage of the fact that panNETs express Somatostatin receptors in >75 % of cases (10). A Somatostatin analogue, of which there are multiple, is introduced into the body. These markers are radioactive, and their activity can be detected by either a PET examination or by scintigraphy. These images, in conjunction with conventional imaging, can help determine if an uncertain mass on conventional imaging are to be suspected a neuroendocrine neoplasm. These techniques have their limitations as poorer differentiated tumours tend to lose their Somatostatin receptor positivity. Insulinomas have to a lesser extent expression of somatostatin receptors, therefore making them harder to visualise using these techniques (43). The sensitivity of PET is variously reported, ranging from 60-95 % (44-46). There is a review article published in 1994 where 83 % sensitivity for Somatostatin-scintigraphy was reported (47). The sensitivity is inferior compared to EUS and conventional imaging techniques but provides additional information when used in conjunction with other diagnostic methods.

Endoscopic Ultrasonography (EUS)

EUS (endoscopic ultrasonography) is a procedure where an ultrasonography probe is attached to an endoscopic instrument in order to visualise organs situated sub optimally for transcutaneous ultrasonography. The endoscopic instrument is introduced to the patient's ventricle and duodenum via the oesophagus. The pancreas, and other organs in proximity, are examined via ultrasonography through the ventricle or duodenal wall. This allows high resolution images without distortion of bones and gas that are problematic in transcutaneous ultrasonography. The first EUS-instrument was developed in the 1980's. EUS have become

widely used to examine tissues such as the pancreas(35). In the 1990's EUS-instruments with sampling possibilities were developed. EUS-FNA was introduced (endoscopic ultrasonography - fine-needle aspiration), where a needle capable of cytological aspiration was added to the instrument. Thereafter EUS-FNB (endoscopic ultrasonography - fine-needle biopsy) was developed to get a more representative sample of the tissue. Instead of a cytological needle, a biopsy needle was attached. There is a fine line to be considered here. The needle needs to be small enough to not frequently cause complications, i.e. perforation, infections, pancreatitis. The needle needs to be big enough to get representative biopsies for histological examination. There are several different needles on the market, and many more are being developed.

Illustration 3

Illustration 3
An endoscopic instrument with an ultrasonography probe and an FNB needle attached. The needle is a ProCore 22 Gauge needle. Alamoudi R. The smear layer in endodontic: To keep or remove – an updated overview. Endoscopic Ultrasound. 2014;3(2):71-81.

EUS in panNETs

EUS have proven to be a sensitive and specific imaging and diagnostic tool for panNETs. The pancreas anatomical position lends itself nicely to endoscopic ultrasonography due to its proximity to the duodenal and gastric wall. PanNETs has characteristically a hypoechoic echogenicity, are well vasculated, and are well defined, often round on the ultrasonic image. There is a study on evaluating the ability of finding neuroendocrine tumours with EUS in patients with clinical suspicion of a panNET. According to the study, CT (computed tomography) and US fail to localise the tumours in 40-60 % of cases. EUS reported to have a sensitivity of 82% and a specificity of 95%(36). There are several articles confirming the high sensitivity of EUS, i.e. another article reported 93 % sensitivity (37). Reviewing the field in large, EUS have shown to have a sensitivity of upwards of 90 % in detecting neuroendocrine neoplasms (21).

The next logical step after locating the tumour using EUS would be sampling of some sort and EUS-FNA (endoscopic ultrasonography - Fine-needle aspiration) have proven to have high sensitivity and specificity in diagnosing panNETs. A relatively large study spanning 11 years reported a sensitivity of EUS-FNA to 87 %, interestingly the sensitivity is a bit lower for fpanNETs (77%) and higher for nfpanNETs (91%)(38). Other papers report even greater sensitivity and specificity, 98.9% and 100% respectively(39). Due to the high sensitivity and specificity of EUS, it is today considered the golden standard in the diagnostic process of suspected panNETs(40).

There has been an increasing development of endoscopic biopsy needles recently. The initial reports for the first developed EUS-FNB needles, mainly the Trucut-needles, proved to be mostly inferior to EUS-FNA as it showed a sensitivity ranging from 45%-91%(41-43). Newer needles have been developed the last couple of years, and with them, the sensitivity has generally risen to about 80-85 %(44-46), whilst other articles report significantly poorer results(47).

Preoperative Estimation of the Ki67-index in panNETs

There has been, and is, a demand after a method by which tumour grade and more importantly Ki67% could be estimated already at the preoperative phase.

Although EUS-FNA has proven to be a valuable diagnostic tool, it does have its limitations. There are papers where authors have compared estimated Ki67 % on cytological samples collected via EUS-FNA to the Ki67 % calculated on pancreatic resections. Articles, in general, point towards that in smaller lesions, the estimation done on cytological material correlates quite well to that of a resection, but correlates poorer when lesions are larger(48-50). One paper reported moderate agreement (Cohen coefficient 0.434) of grading when comparing EUS-FNA to histological examination of resections(48). These number have, in

large, been reproduced in similar articles(49, 50). EUS-FNA tends to underestimate the Ki67 %, likely due to the samples being incohesive and small. Another possible reason is the fact that panNETs are heterogeneous and have what is usually referred to as Hot Spots, where the cell proliferation is at its highest. If the cytological material is not representative of a hot spot the Ki67 %, as well as the tumour grade, will be underestimated.

There are, however, reports that when assessing cytological materials, sampled via EUS-FNA, with > 2,000 cells the concordance with histological examination of resections rose greatly(48). These reports have left us in demand after a method that can more accurately estimate the Ki 67% in the preoperative phase to prognosticate and point towards an adequate treatment.

It has been hypothesised that a biopsy, rather than an aspiration, might have higher sensitivity and specificity in grading and prognosticating panNETs. Few reports exist, however, on preoperative grading and Ki67 % estimation via EUS-FNB. One of few papers reports that the grading is in moderate concordance with histological examination of the resection, compared to FNA that shows a poor concordance of the same(51).

There is a lack of studies evaluating the use of EUS-FNB to grade panNETs, as most studies have focused on its diagnostic properties. An accurate tool that grades tumours with minimal invasiveness could come to have great implications in treatment, i.e. a large portion of patients might not have to undergo large surgery with not infrequent following complications. Tumours with a relatively low Ki67 % might instead be monitored with follow-up radiology or EUS. The Ki67 % of tumours may also have implications in choosing palliative care in patients with inoperable tumours. There has been a long ongoing debate on how to manage smaller, seemingly indolent, panNETs. This method, if proven adequately accurate, could put an end to this discussion as one could get a definitive answer on the tumours aggressiveness and with greater confidence choose to operate or not.

Kommenterad [FB1]:

Aims

The purpose of this paper was to investigate to what degree EUS-FNB can accurately grade pancreatic neuroendocrine neoplasms in the pre-operative phase using the WHO grading system.

Material and methods

Design and population

Patients referred to the Sahlgrenska university hospital endoscopy unit during 2012-2019 for a diagnostic EUS based on a clinical suspicion of panNET were eligible for study enrolment. The endoscopy unit of Sahlgrenska University hospital is a tertiary referral unit.

Inclusion Criteria

Patients over the age of 18, that were referred to EUS based on a clinical suspicion of a pancreatic neuroendocrine neoplasm were considered for enrolment. The suspicion may arise from an *en passant* find of a mass with radiological features of a panNET, i.e. hypoechoic, well vasculated, and often round formed mass in the pancreas. It may arise in patients with syndromes with symptoms characteristic for overproduction of certain hormones. It may also arise in patients with inexplicable jaundice, abdominal pain, or pancreatitis.

Exclusion Criteria

Patients unwilling to participate, patients with a final diagnosis other than panNET, and patients where no EUS-FNB was performed was excluded from this study.

Ethics

This study is approved by the regional ethic trial committee in Gothenburg. Written consent has been collected from all participating patients. The study is registered on clinicaltrials.gov. This study was performed in accordance with the Helsinki declaration. This study was performed in accordance with the human rights declaration published by FN.

The EUS procedure and EUS-FNB sampling

The endoscopic ultrasonography examination was done by either of two experienced endoscopic ultrasonographers. Patients were, for the most part, not heavily sedated. The endoscopist would then insert a linear endoscope into the mouth and would then venture down into the oesophagus and further down to the ventricle. The pancreas would then be examined with ultrasonography from both the ventricle and duodenum. Vascularity is examined with doppler. Additional features of interest are echogenicity and size. Eventual masses are punctured either via the ventricle (if the mass is in the body or tail of the pancreas) or the duodenum (if the mass is in the head of the pancreas) with a ProCore needle (mostly size 22 gauge) attached to the endoscopy instrument. The sample would then be grossly examined to determine if the biopsy seems representative, if not, more biopsies might be sampled. When taking multiple samples, the endoscopist would generally puncture in the shape of a fan to greater increase the chance of getting a representative sampling. The sample would then be placed in a container with formalin and sent to a pathologist. Often a cytological aspiration is also sampled to ensure diagnostic accuracy, which is done in the same manner, but a different needle type is used.

Histology

The EUS-FNB samples have initially been reviewed by pathologists in clinical practice. The samples with a panNET diagnosis were selected for inclusion in the study, then a single pathologist reviewed the samples to make sure that there were no pathologist dependent discrepancies. The original reviews were of varying quality and various histological markers have been used.

The included samples have been scanned of their histological glass and then imported into a computer. Two immunohistological stainings were used in assessing the samples, Ki67-

staining and Synpatophysin-staining. The three largest groups of tumour cells were then individually counted and a Ki67 % estimation was made, then a cell count and a Ki67 % estimation was done on the ten largest tumour cell groups as a whole. The Ki67% estimation was done with Visiopharm oncotopix. A photograph of the Synaptophysin staining was used in parallel to make sure that the counted tumour cell groups was actual tumour cells and not inflammatory cells that might be mistaken for tumour cells. EUS-FNB samples were considered malignant if it had the general morphological features of a panNET as well as at least one positive immunohistological marker pointing towards neuroendocrine heritage.

Follow-up and surgery

The patients were monitored and followed up by the referring clinicians. Clinical data was extracted at the end of the study period. Clinical data of interest was eventual tumour progress, recurrence of tumour growth, eventual adverse effects following the EUS-FNB procedure, and whether or not they had passed. Whether the patient had undergone additional biopsies on the tumour growth or on suspected metastases, as well as if the patient had undergone resecting surgery were also recorded. The surgical resections were considered the reference standard with which EUS-FNB samples were compared.

Data Collection

Apart from sampling pancreatic tissue from the patients, clinical data was also logged to get a better understanding to what might influence the biopsy quality, with adequate quality being set as a cell count of >1000. The data was collected from the patient's medical records. The data of interest were what diagnostic measures the patients had undergone before EUS, the location and radiological features of the lesion, the ultrasonic features gathered during EUS. Additional data of interest was age, sex, if the puncture is done through the ventricle or the duodenum, what material were used during the procedure i.e. what needle type, if clinical

symptoms associated with hormonally active tumours are present, histological features from pancreatic resections, if there were any complications following the procedure and follow-up time. This data will be presented in further detail in **Supplement 1**

Study outcomes

Primary outcome

- Accuracy of EUS-FNB in the grading of panNETs

Secondary outcomes

- EUS-FNB sensitivity for panNET

- Clinical features influencing biopsy quality

Statistical Analysis

Non-parametric tests were used in most cases. The Chi-square method was used in our calculations. The calculations were made in the program SPSS. P-values are generated by Fischer's exact two-sided test, a p-value of <0.05 was considered as statistically significant. Cohen's kappa values were used when comparing tumour grades between fine-needle biopsies and surgical resections. "Cohen's kappa statistic measures interrater reliability (sometimes called interobserver agreement). Interrater reliability, or precision, happens when your data raters (or collectors) give the same score to the same data item… The Kappa statistic varies from 0 to 1, where; 0 = agreement equivalent to chance. 0.1 – 0.20 = slight agreement. 0.21 – 0.40 = fair agreement. 0.41 – 0.60 = moderate agreement. 0.61 – 0.80 = substantial agreement. 0.81 – 0.99 = near perfect agreement 1 = perfect agreement. "(52).

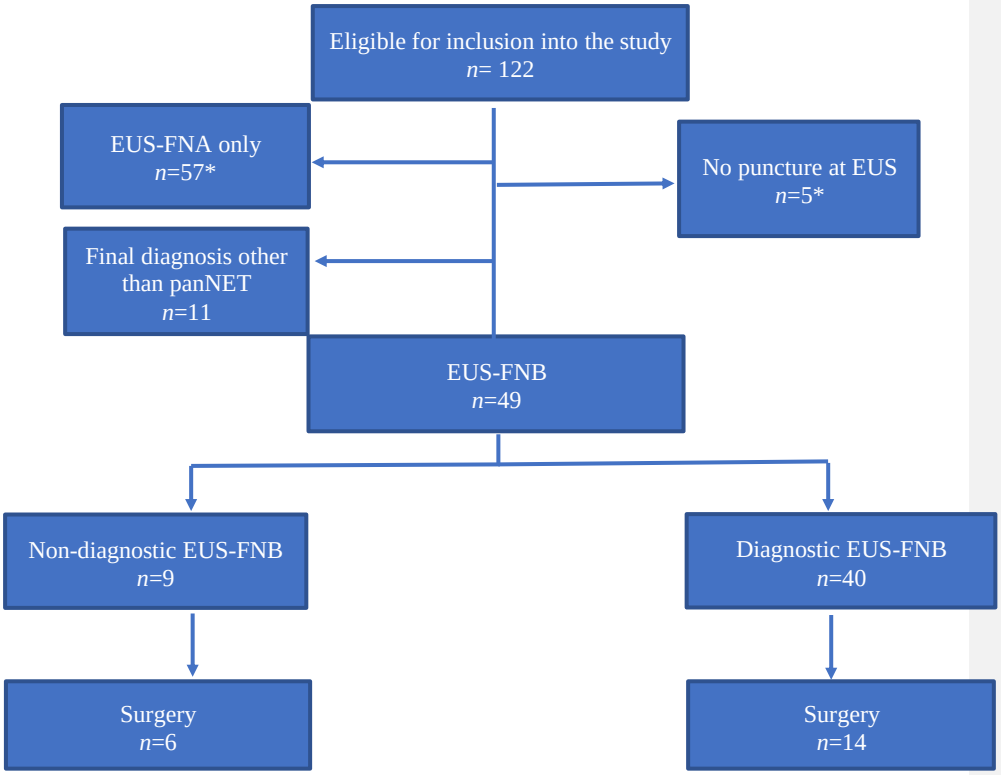
Kommenterad [FB2]:

Results

During the time period 2012-2019, there were 122 referrals, 110 unique patients, for EUS-FNB on the suspicion of panNETs. After exclusion, there were 49 (24 males and 22 females, mean age 61) referrals left, 46 unique patients, for inclusion in the study. 57 referrals were excluded because only fine-needle aspirations were performed and no fine-needle biopsy. 5 referrals were excluded because no puncture was performed. 11 referrals were excluded because the final diagnosis was not panNET. The average size of the panNETs were 30.1 ± 23.7 mm.

This is illustrated in a flow chart (Flow chart 1) below. Baseline characteristics were as in **Supplement 1**.

Flow chart 1 – Inclusion and exclusion



Flow chart 1

A flowchart depicting the flow and distribution of referrals. All patients referred to the EUS unit on a suspicion of a panNET were considered eligible for inclusion. There are 122 referrals and 110 unique patients, some of the patients have been examined more than once have had different punctures done on them and therefore a single person might be part of multiple groups illustrated above.

Primary outcome - Preoperative grading of panNETs in EUS-FNB specimens

The EUS-FNB estimations on Ki67 % yielded 26 grade 1 tumours, ten grade 2 tumours and two grade 3 tumours, **Table 1**.

Table 1 – Tumour grades generated by EUS-FNB

Grades on EUS-FNB	***Cases***
Grade 1	26
Grade 2	10
Grade 3	2
*Grading not possible**	11
Total	49

Kommenterad [FB3]: Bytte från icke-diagnostiska eftersom det blir missförstånd med de två scanningarna som vi inte kunnat gradera

Table 1
A table illustrating the distribution of tumour grades yielded from the EUS-FNB sampling, using the WHO grading system. Grade 1 tumours include those with a Ki67% of 0-3%, grade 2 3-20%, and grade 3 >20 %.* 9 fine-needle biopsies were not diagnostic, 1 fine-needle biopsy could not be found, and 1 fine-needle biopsy had to unclear cellular outlinings for a Ki67 % estimation to be done.

In the cases where there were a diagnostic EUS-FNB and a corresponding surgery specimen the tumour grading was concordant in 57.1 % (8/14) of cases, Cohens kappa 0.263 (fair agreement), **Table 2**. The cases who were not in concordance with their corresponding surgical specimens were all due to under grading. Six EUS-FNB samples were classified as grade 1 tumours on the fine-needle biopsy whilst being grade 2 tumours in the surgical specimen PAD.

Table 2 – Comparison of tumour grades from EUS-FNB and surgical resections

	WHO-grade PAD surgery grade 1	***WHO-grade PAD surgery grade 2***	***WHO-grade PAD surgery grade 3***	***Total***
WHO-grade EUS FNB				
Grade 1	5	6	0	11
Grade 2	0	3	0	3
Grade 3	0	0	0	0
Total	5	9	0	14

Table 2
A crosstab depicting the tumour gradings of both EUS-FNB and of the surgical specimens to illustrate the concordance between the gradings. Concordance 57.1 %, Cohens kappa 0.263 (fair agreement).

Concordance was tested when excluding fine-needle biopsies with a cell count <1000, **Table 3**. When excluding the fine-needle biopsies with a cell count of <1000, the concordance rose to 70 % (7/10), Cohens kappa 0.400 (fair agreement). The cases that were not in concordance with their corresponding surgical specimens were due to under grading.

Table 3 - Comparison of tumour grades from EUS-FNB and surgical resections with exclusion of poor-quality biopsies

WHO-grade EUS-FNB when cell count > 1000	*WHO-grade PAD surgery grade 1*	*WHO-grade PAD surgery grade 2*	*WHO-grade PAD surgery grade 3*	*Total*
Grade 1	5	3	0	8
Grade 2	0	2	0	2
Grade 3	0	0	0	0
Total	5	5	0	10

Table 3
A crosstab depicting the tumour gradings of both EUS-FNB and of the surgical specimens, with the EUS-samples with cell counts <1000 excluded, to illustrate the concordance between the gradings. Concordance 70 %, Cohens kappa 0.400 (fair agreement).

Concordance was also further tested when excluding EUS-samples with a cell count of <2000, **Table 4**. The concordance was 83.3 % (5/6), Cohens kappa 0.571 (moderate agreement).

Table 4 - Comparison of tumour grades from EUS-FNB and surgical resections with inclusion only of biopsies with good quality

WHO-grade EUS-FNB when cell count > 2000	*WHO-grade PAD surgery grade 1*	*WHO-grade PAD surgery grade 2*	*WHO-grade PAD surgery grade 3*	*Total*
Grade 1	4	1	0	5
Grade 2	0	1	0	1
Grade 3	0	0	0	0
Total	4	2	0	6

Table 4
A crosstab depicting the tumour gradings of both EUS-FNB and of the surgical specimens, with the EUS-samples with cell counts <2000 excluded, to illustrate the concordance between the gradings. Concordance 83.3 %, Cohens kappa 0.571 (moderate agreement).

Secondary outcomes

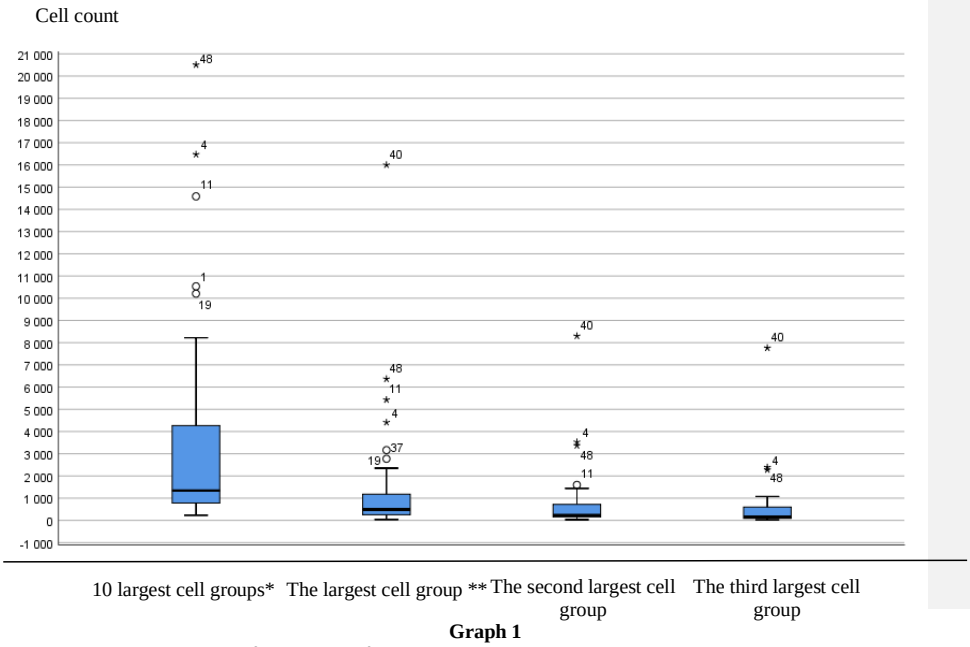
Out of 49 referrals, 40 of the EUS-FNB were diagnostic (82%). Ki67 % Estimations could be done on 38/40 diagnostic EUS-FNBs, one biopsy glass could not be found and therefore no estimation could be done, and one biopsy scanning proved to have to unclear cellular outlines to get a reliable Ki67% estimation.

Chi² calculations were done on characteristics potentially influencing the biopsy quality of EUS-FNB samplings, **Supplement 2**. Biopsy quality was considered as adequate if the cell count were >1000. The P-values are generated by Fischer's exact two-sided test. No tested factor proved to significantly influence the biopsy quality of EUS-FNB sampling.

EUS-FNB specimen cell count

The average cell count in the samples were 5642 ± 12682, when counting the ten largest cell groups. The EUS-FNB samples had cell counts of >1000 in 68.4 % of cases (26/38). Below is a boxplot (Graph 2) depicting the distribution of cell counts when counting the single largest cohesive cell group, the second largest, the third largest, and the ten largest cell groups counted together.

Graph 1 – Cell group sizes from fine-needle biopsies



Graph 1

A box plot depicting the spread of cell counts of the ten largest cell groups counted together, the single largest cell group, the second largest, and the third largest. * Two numbers are not visible in the plot as the spread was to large but are included in the calculations on the plot. The two numbers were 76968 and 61787. ** One number is not visible as the spread was to large to be easily visualised. The number was 55723.

The average Ki67% counted on the EUS-FNB samples were 0.045 ± 0.082. **Supplement 3** lists the Ki67% on the included cases. Ki67% is listed both when counting only the strongly stained cells for the Ki67 staining and when counting both the strongly and the weakly stained for Ki67.

Discussion

Tumour grade concordance

Few studies exist on the efficacy of pre-operative grading of panNETs using EUS-FNB. Linear regression calculations were tried to analyse if there is correlation between cell count and concordance of tumour grade, but the cell count spread was too large to interpret.

One study reports concordance of 83 % in tumour grading of EUS guided biopsies. The study used Ki67 % calculations from both cytological aspirations and histological biopsies for comparison with surgical specimens, but when both a cytological aspiration and a histological biopsy were present, they deferred to the biopsy. This study does not specify a Cohens Kappa coefficient or a similar coincidence variable. The material is of comparable size to this study, with 25 patients undergoing both EUS-guided puncture and surgery. There is neither a report of the biopsy quality in the samples making it hard to interpret if the cell count correlated with higher concordance in their material as it did in ours. In this study they also manually counted the cells that stained positively on photographed images instead of using a computer program. Most biopsies were performed with a 25-gauge needle (56).

This study reports overall higher concordance compared to our results, but there are key differences in the study design that needs to be considered as possible explanations for the differences. Manual counting on an image of the sample, has proven the most accurate method of estimating Ki67 % Indexes and by extension tumour grades(53). In our study we used a computer program called Visiopharm Oncotopix to calculate the Ki67% Index which might have influenced the concordance. There is no representation of the biopsy quality in their study which might influence the concordance if the cell samples are of higher quality.

Another potential influencing factor is that a different needle, both different in size and manufacturer, was used in the most passes in this study(54).

There is one other study comparing concordance of EUS-FNB and EUS-FNA to surgical resections. 26 patients underwent both EUS-FNB and surgical resection in the material. They reported an overall concordance of 72 %, Cohens kappa 0.474 (moderate agreement). They report poor concordance between EUS-FNA and surgical specimens. In 65.7 % of cases, EUS-FNA yielded a sample in which a Ki67 % estimation could be done. Neither in this study is the biopsy quality presented. In this study they have mainly used two different types of biopsy needles, the ProCore needle (reverse bevel) and the SharkCore needle (Fork tip), manufactured by Cook Medicals and Medtronic respectively. They have presented separate concordances for the different needles, the Procore needle showed moderate agreement whilst the SharkCore showed a good agreement (Pearson's r-values of 0.521 and 0.788 respectively). In this study they have estimated the Ki67 % by manually counting the positively stained cells under high magnification with or without aid of a manual cell counter.

This study also reports quite high tumour grade concordance, but as in the case of the previously discussed study, there are some key differences in the study design. The absence of reported biopsy quality might, again, influence the concordance rate as the overall quality cannot be compared. As with the previously discussed study, they used manual counting in the Ki67 % estimation which has proven the most accurate (55). They used several different needle sizes and from different manufactures, which also might impact the relative discrepancies between their result and ours. This study illuminates that EUS-FNB has is superior in grading panNETs than is EUS-FNA.

All and all, the results of these studies, and ours, point in a common direction. EUS-FNB is a sensitive diagnostic tool. It also seems to have promising properties in grading panNETs. There is a trend, however, where EUS-FNB seems to underestimate the tumour grade. One possible explanation to the tendency to underestimate the grade might be the fact that panNETs usually have what is called hot spots, where the cell proliferation is at its highest. These hotspots might be missed in sampling resulting in a lower estimated grade.
The needle of choice seems to influence the success rate of the biopsy, needles of the SharkCore brand (Medtronic) seems more successful. Manual counting might be preferable to Visiopharm oncotopix.

There are more studies done on the grading possibilities of EUS-FNA than there are for EUS-FNB. The results are varied. One study reports the overall concordance to be 72 %. They also report that the grading was concordant in 95 % (21/22) of cases in lesions <20 mm, but discordant in 93.7 % of cases in lesions >20mm (51). There are other studies reporting concordance ratings ranging from 61-86 % (55-59). There is an ongoing discussion on EUS-FNA's tumour grading properties. A concern, that seems valid with the results above, is that EUS-FNA has a tendency to underestimate larger lesions. The reported concordances are varied and the reports at lower spectrum begs to question the efficacy of EUS-FNA.

Whilst EUS-FNB shows promising results in its grading properties, it seems not to have reached its full theoretical potential. We are still just scraping the surface on its properties, with further development and research we expect even greater results. EUS-FNB might not be obviously superior to EUS-FNA in tumour grading today, even though EUS-FNB seems to perform in EUS-FNA upper span of reported concordances of EUS-FNA, but as this research field still are in its early stages, we assume that with improvements it will surpass EUS-FNA.

Sensitivity

EUS-FNB has a high sensitivity in diagnosing panNETs. The sensitivity of EUS-FNB in our report is confirmed in several recent studies where the sensitivity has been reported ranging from 80-85 % (48-50). There have been varied reports on the sensitivity overall, the first reports showed EUS-FNB to be inferior to EUS-FNA (45-47) in diagnosing panNETs. With newer biopsy needles having been developed, the sensitivity rose with it. The sensitivity of EUS-FNB, in recent studies, seems comparable to that of EUS-FNA (42). One can assume that with further development of the biopsy needles and procedure techniques, even greater sensitivity and specificity can be accomplished, and it might surpass EUS-FNA. EUS-FNB should, in theory, generate a more representative tissue sample than does EUS-FNA and therefore, when perfected, reach uncontested levels of sensitivity and specificity. The biopsy needle used in the absolute majority of cases in this study was the ProCore 22-gauge needle (Cook medicals). Noteworthy is that, it is a fine line to cross when constructing these needles as they both need to generate as large a biopsy as possible and at the same time be as minimally invasive as possible to avoid complications in association with the puncture.

Factors influencing the success rate of EUS-FNB

No single clinical factor that we tested proved statistically significant. Multivariable calculations were made with similar results.

Two clinical factors came close to significance, the location of the tumour and the puncture route. Whilst not being significant, it is noteworthy since the tumour localisation determines what puncture route is taken. Puncture through the ventricle is generally preferred in lesions in the body and tail of the pancreas whilst puncture through the duodenum is generally chosen with lesions in the head of the pancreas. These results seem to infer that lesions that are easily accessible from the ventricle, generate higher quality biopsies than does lesions punctured via

the duodenum. One reason to why our results did not prove significant is almost assuredly our population size.

Reports on factors influencing the biopsy quality of either EUS-FNB or EUS-FNA are scarce. One study, however, reports that lesions size, lesion localisation, and if there was an on-site cytopathologist were significant in influencing diagnostic accuracy with P-values of <0.01, <0.03, and <0.01 respectively(60). This study has a much larger study population of 996 patients. This report is consistent with our result in that lesion localisation does significantly influence the success of EUS-guided sampling. The outcome in this study was diagnostic accuracy rather than a measure of cell count, as in our case, but one can assume that diagnostic accuracy goes hand in hand with adequately sized tissue samples. This study is also done on EUS-FNA rather than EUS-FNB. The success rate of EUS-FNA and EUS-FNB can, however, be assumed to be influenced by, in large, the same clinical factors. There is another report confirms significantly higher diagnostic accuracy in lesions located in the body and tail of the pancreas rather than the head(61).

Nevertheless, this is a subject that needs further research since little to none exist, especially on EUS-FNB.

Strengths and weaknesses

Our study has a lot of strengths. First of all, it is a prospective study which have its advantages. Enrolment is generally under a more controlled manner as the study design is decided on beforehand. Prospective studies generally have a lower risk of bias.
The study is also single centre which also provides a lot of advantages, and some downsides that we will cover shortly. The population is more easily overviewed as they are managed by the same institute. The medical records system is the same which makes it easier and more

reliable to extract clinically relevant data. The practitioners working under the same institution also means the diagnostics and treatment follows the same tradition. The EUS-procedure is also done by two experienced examiners, one of which has had part in training of the other. The fact that the examiners have similar training in the procedure and that the number of examiners is kept few means that the risk of inter-examiner discrepancies are as low as possible. We have also had a single pathologist, tied to the study, to do all the Ki67 % estimations on the EUS-FNB samples, and by extension tumour gradings on the EUS-FNBs. This means that inter-examiner discrepancies are not present. The Ki67 % estimations and the cell counting have been done in the same manner. We have also thoroughly extracted clinical data from the patient's medical records, made calculations on their potential influences on the biopsy quality, and presented them.

These kinds of statistics, on EUS-FNB specifically, are more or less non-existing in the research field today. Another strength of our study is that we have compared the concordance of tumour grading in the per-operative phase of EUS-FNB and surgical resections. This is also a field in which research is lacking. This might influence future studies on the field as we have problemised current short comings of the technique today as well as highlighted where it shines.

This study also has some weaknesses. The fact that it is a single-centre study is a double-edged sword as it has its downsides. It limits the populations size, something we have covered might be a reason for some statistical results not being significant. Single-centre studies also only sample patients from a single population and therefore might be less applicable on the large majority. The surgical resection details, such as the Ki67 % estimation and tumour gradings were extracted from the original pathologist reports. This project is not finished, as the plan is to have the same pathologist tied to the study to also review the surgical resection

specimens. This, however, was not permitted by the time restriction set by the degree project. This means that the tumour grades are not done in the same exact manner. The tumour gradings of EUS-FNB were done in the exact same manner by the same pathologist whilst the tumour gradings on the surgical resections were done by multiple different pathologist. The techniques of estimating the Ki67 % were not done consistently during the study period as some pathologist used manual counting, some used a technique called eye-balling, and some used computer programs like the one used in the EUS-FNB samples. The pathologist reports were also of varying quality. The reports were, however, generally of good quality as it is easier to estimate Ki67 % and tumour grades on an entire tissue as you don't get the problem with missed hot spots as discussed earlier.

Possible improvements

This study has a lot of good qualities but if we were to redo the study or make recommendations for future research there is a few things one might consider doing differently. One consideration is to make it a multiple-centre study to have a larger population which makes it easier to reach statistical significance. The best method of estimating Ki67%, and by extension tumour grade, should be closer considered. Manual counting on digital images might be superior to digitalised counting. One should also consider using different needle types, in our study the ProCore 22-gauge was mainly used. The ProCore needle proved adequate but still leaves much to be desired.

Implications and conclusions

This study has had great implications as it filled a whole in the research field. We have proved that EUS-FNB for pre-operative tumour grading is a subject worth exploring. Whilst not being perfected yet, it shows great promise and should come to have an important role to play

in clinical duty. The ability to reliably grade tumours that in the past have kept us unsure on how to treat them, is a great one. There is an ongoing debate on how to treat smaller, seemingly indolent tumours. One alternative is active monitoring, another is surgery. Pancreatic surgery is not something to take lightly as the anatomical position makes it hard to access. Pancreatic surgery is often massive, with not infrequent following complications. On the other hand, these are tumours where the intent for surgery, is cure. With reliable pre-operative tumour grading one can make an informed decision in whether to operate or not. Small tumours with confirmed lower cell proliferation rates may lend themselves better to active monitoring and patients will be spared from complicated, massive surgery.
This study also provides important ground laying research in the field on which future studies can build on and learn from. We have problemised the EUS-guided sampling procedure as it is now and provided guiding in where to continue on.

Whilst no clinical factor proved fully significant in influencing biopsy quality in our results, when taking into account results from similar reports, we have found factors that seem to influence the success or failure of EUS-guided sampling. We have highlighted that when puncturing tumours in the body and tail of the pancreas, via the ventricle, the outcome is greater than it is when puncturing tumours in the head of the pancreas via the duodenum. This might also come to influence clinical duty as it might prove only reliable to puncture tumours of certain locations in the pancreas. Where this to be true, the advantages of grading tumours in the pre-operative phase would only apply to tumours of certain locations but would still greatly influence the diagnostics and management of panNETs.

Populärvetenskaplig sammanfattning

Vävnadsprovtagning av bukspottskörteln genom magsäcken/tunntarmen för att kunna gradera hormoncellstumörer innan eventuell operation.

Projektet syftade till att undersöka om en metod för vävnadsprovtagning genom magsäcken eller tunntarmen är träffsäker i att gradera hormoncellstumörer i bukspottskörteln. Det har länge funnits en metod som visat sig träffsäker i att diagnosticera hormoncellstumörer, där man sticker med en nål genom tunntarmen eller magsäcken in i bukspottskörteln och suger ut celler. Metoden har dock visat sig vara undermålig i att gradera tumörerna. De senaste åren har man utvecklat nålar som istället för att suga ut celler ur vävnaden, tar ett helt vävnadsprov för att få en mer representativ bild av tumörvävnaden. Det har visat sig att även dessa nålar har visat sig ha en hög träffsäkerhet i att diagnosticera hormoncellstumörer i bukspottskörteln. Det finns dock få gjorda studier på i vilken utsträckning dessa nålar kan gradera dessa tumörer korrekt.

hormoncellstumörer i bukspottskörteln är en minoritet av tumörer i bukspottskörteln och har betydande bättre prognos än andra tumörtyper i detta organ. Det är därför av stor nytta att kunna identifiera och gradera denna typ av tumör för att kunna avgöra vilka tumörer som lämpar sig för operation.

Vi har undersökt patienter som genomgått vävnadsprovtagning mellan åren 2012–2019. Vi har gått igenom patienternas journaler och letat efter kliniska uppgifter som kan ha påverkat varför ett vävnadsprov är framgångsrikt eller misslyckat. Vi hittade egentligen inga kliniska uppgifter som man inte helt och hållet kan utesluta beror på slumpen men en del faktorer som pekar mot att de har en påverkan på framgången av vävnadsprovtagningen. Faktorer såsom var i bukspottskörteln tumören sitter och huruvida man stack bukspottskörteln genom

antingen magsäcken eller tunntarmen är exempel på faktorer som pekade i riktningen i att de har en påverkan på framgången av vävnadsprovtagningen.

Vi har även undersökt i vilken utsträckning som vävnadsprovtagningen var korrekt i sin gradering av tumörtypen. Det visade sig att, i stort var gradering korrekt i 57,1 % av fallen. Vid bortselektion av vävnadsprover som var av dålig kvalité så var graderingen korrekt i 70 % av fallen. Slutligen, vid inklusion endast av vävnadsprover med god kvalité så var gradering korrekt i 83,3 % av fallen.

Vår slutsats är att detta är ett lovande område men att metoden är långt ifrån perfekt ännu och behöver utvecklas vidare. Det utvecklas ständigt nya nåltyper och vår data tyder på att om man får större vävnadsprover så blir graderingen mer korrekt. Nya nåltyper som tar större vävnadsprover behöver utvecklas men det är en balansgång då nålen samtidigt behöver vara tillräckligt liten för att inte orsaka skada och komplikationer när man sticker hål på tunntarmen, magsäcken och bukspottskörteln.

Acknowledgement

I would first and foremost want to thank Per Hedenström, the project supervisor. Per have taken countless hours out of his busy schedule to help supervise, answer questions, and be generally helpful. This project would not have happened without your guidance.

I would also like to thank Riadh Sadik, the secondary project supervisor. Riadh showed me the procedures of an EUS examination. Riadh also functions as an authority, together with Per Hedenstrom, on EUS and its current diagnostic properties.

I would also like to thank Alexander Borg Appelstrand, the pathologist tied to the project. Without your expertise this project wouldn't have happened.

I would like to thank my family for support and love, especially my fiancée who have shown endless patience with me during this period.

Lastly, I would like direct my thanks to something I cherish deeply, the black gold; coffee.

References

1. Rosa SL, Furlan D, Sessa F, Capella C. The Endocrine Pancreas. In: Lloyd RV, editor. Endocrine Pathology: Differential Diagnosis and Molecular Advances. New York, NY: Springer New York; 2010. p. 367-413.
2. Feldmann G, Beaty R, Hruban RH, Maitra A. Molecular genetics of pancreatic intraepithelial neoplasia. J Hepatobiliary Pancreat Surg. 2007;14(3):224-32.
3. Hiripi E, Gondos A, Emrich K, Holleczek B, Katalinic A, Luttmann S, et al. Survival from common and rare cancers in Germany in the early 21st century. Ann Oncol. 2012;23(2):472-9.
4. Sant M, Aareleid T, Berrino F, Bielska Lasota M, Carli PM, Faivre J, et al. EUROCARE-3: survival of cancer patients diagnosed 1990-94--results and commentary. Ann Oncol. 2003;14 Suppl 5:v61-118.
5. Karim-Kos HE, de Vries E, Soerjomataram I, Lemmens V, Siesling S, Coebergh JW. Recent trends of cancer in Europe: a combined approach of incidence, survival and mortality for 17 cancer sites since the 1990s. Eur J Cancer. 2008;44(10):1345-89.
6. Cienfuegos JA, Rotellar F, Salguero J, Ruiz-Canela M, Nunez Cordoba JM, Sola I, et al. A single institution's 21-year experience with surgically resected pancreatic neuroendocrine tumors: an analysis of survival and prognostic factors. Rev Esp Enferm Dig. 2016;108(11):689-96.
7. Badarna M, Percik R, Aharon-Hananel G, Uri I, Tirosh A. Anatomic site as prognostic marker of pancreatic neuroendocrine tumors: a cohort study. Eur J Endocrinol. 2019.
8. Kloppel G, Heitz PU, Capella C, Solcia E. Pathology and nomenclature of human gastrointestinal neuroendocrine (carcinoid) tumors and related lesions. World J Surg. 1996;20(2):132-41.
9. Reubi JC. Somatostatin and Other Peptide Receptors as Tools for Tumor Diagnosis and Treatment. Neuroendocrinology. 2004;80(suppl 1)(Suppl. 1):51-6.
10. Mizutani G, Nakanishi Y, Watanabe N, Honma T, Obana Y, Seki T, et al. Expression of Somatostatin Receptor (SSTR) Subtypes (SSTR-1, 2A, 3, 4 and 5) in Neuroendocrine Tumors Using Real-time RT-PCR Method and Immunohistochemistry. Acta Histochem Cytochem. 2012;45(3):167-76.
11. McKenna LR, Edil BH. Update on pancreatic neuroendocrine tumors. Gland Surg. 2014;3(4):258-75.
12. Cullen RM, Ong CE. Insulinoma in Auckland 1970-1985. N Z Med J. 1987;100(831):560-2.
13. Eriksson B, Oberg K, Skogseid B. Neuroendocrine pancreatic tumors. Clinical findings in a prospective study of 84 patients. Acta Oncol. 1989;28(3):373-7.
14. Service FJ, McMahon MM, O'Brien PC, Ballard DJ. Functioning insulinoma--incidence, recurrence, and long-term survival of patients: a 60-year study. Mayo Clin Proc. 1991;66(7):711-9.
15. Grant CS. Gastrointestinal endocrine tumours. Insulinoma. Baillieres Clin Gastroenterol. 1996;10(4):645-71.
16. Lo CY, Lam KY, Kung AW, Lam KS, Tung PH, Fan ST. Pancreatic insulinomas. A 15-year experience. Arch Surg. 1997;132(8):926-30.
17. Oberg K, Eriksson B. Endocrine tumours of the pancreas. Best Pract Res Clin Gastroenterol. 2005;19(5):753-81.
18. Zollinger RM, Ellison EH. Primary peptic ulcerations of the jejunum associated with islet cell tumors of the pancreas. Ann Surg. 1955;142(4):709-23; discussion, 24-8.
19. Ito T, Igarashi H, Jensen RT. Therapy of metastatic pancreatic neuroendocrine tumors (pNETs): recent insights and advances. J Gastroenterol. 2012;47(9):941-60.
20. Gorelik M, Ahmad M, Grossman D, Grossman M, Cooperman AM. Nonfunctioning Incidental Pancreatic Neuroendocrine Tumors: Who, When, and How to Treat? Surg Clin North Am. 2018;98(1):157-67.

21. Bilimoria KY, Tomlinson JS, Merkow RP, Stewart AK, Ko CY, Talamonti MS, et al. Clinicopathologic features and treatment trends of pancreatic neuroendocrine tumors: analysis of 9,821 patients. J Gastrointest Surg. 2007;11(11):1460-7; discussion 7-9.
22. Cloyd JM, Poultsides GA. Non-functional neuroendocrine tumors of the pancreas: Advances in diagnosis and management. World J Gastroenterol. 2015;21(32):9512-25.
23. Zhang M, Zhao P, Shi X, Zhao A, Zhang L, Zhou L. Clinicopathological features and prognosis of gastroenteropancreatic neuroendocrine neoplasms in a Chinese population: a large, retrospective single-centre study. BMC Endocr Disord. 2017;17(1):39.
24. Zerbi A, Falconi M, Rindi G, Delle Fave G, Tomassetti P, Pasquali C, et al. Clinicopathological features of pancreatic endocrine tumors: a prospective multicenter study in Italy of 297 sporadic cases. Am J Gastroenterol. 2010;105(6):1421-9.
25. M. Amin SE, F. Greene, D. Byrd, R. Brookland, M.K. Washington. AJCC Cancer Staging Manual: Springer International Publishing; 2017.
26. Inzani F, Petrone G, Rindi G. The New World Health Organization Classification for Pancreatic Neuroendocrine Neoplasia. Endocrinol Metab Clin North Am. 2018;47(3):463-70.
27. Lawrence B, Gustafsson BI, Chan A, Svejda B, Kidd M, Modlin IM. The epidemiology of gastroenteropancreatic neuroendocrine tumors. Endocrinol Metab Clin North Am. 2011;40(1):1-18, vii.
28. Ekeblad S, Skogseid B, Dunder K, Öberg K, Eriksson B. Prognostic Factors and Survival in 324 Patients with Pancreatic Endocrine Tumor Treated at a Single Institution. Clinical Cancer Research. 2008;14(23):7798-803.
29. Bu J, Youn S, Kwon W, Jang KT, Han S, Han S, et al. Prognostic factors of non-functioning pancreatic neuroendocrine tumor revisited: The value of WHO 2010 classification. Ann Hepatobiliary Pancreat Surg. 2018;22(1):66-74.
30. Caldarella A, Crocetti E, Paci E. Distribution, incidence, and prognosis in neuroendocrine tumors: a population based study from a cancer registry. Pathol Oncol Res. 2011;17(3):759-63.
31. Griruelius L, Hultquist GT, Stenkvist B. Cytological differentiation of asymptomatic pancreatic islet cell tumours in autopsy material. Virchows Archiv A. 1975;365(4):275-88.
32. Yao JC, Hassan M, Phan A, Dagohoy C, Leary C, Mares JE, et al. One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. J Clin Oncol. 2008;26(18):3063-72.
33. Dasari A, Shen C, Halperin D, Zhao B, Zhou S, Xu Y, et al. Trends in the Incidence, Prevalence, and Survival Outcomes in Patients With Neuroendocrine Tumors in the United States. JAMA Oncol. 2017;3(10):1335-42.
34. Fraenkel M, Kim M, Faggiano A, Herder WWd, Valk GD. Incidence of gastroenteropancreatic neuroendocrine tumours: a systematic review of the literature. 2014;21(3):R153.
35. DiMagno EP, DiMagno MJ. Endoscopic Ultrasonography: From the Origins to Routine EUS. Digestive Diseases and Sciences. 2016;61(2):342-53.
36. Rosch T, Lightdale CJ, Botet JF, Boyce GA, Sivak MV, Jr., Yasuda K, et al. Localization of pancreatic endocrine tumors by endoscopic ultrasonography. N Engl J Med. 1992;326(26):1721-6.
37. Anderson MA, Carpenter S, Thompson NW, Nostrant TT, Elta GH, Scheiman JM. Endoscopic ultrasound is highly accurate and directs management in patients with neuroendocrine tumors of the pancreas. Am J Gastroenterol. 2000;95(9):2271-7.
38. Pais SA, Al-Haddad M, Mohamadnejad M, Leblanc JK, Sherman S, McHenry L, et al. EUS for pancreatic neuroendocrine tumors: a single-center, 11-year experience. Gastrointestinal Endoscopy. 2010;71(7):1185-93.
39. Krishna SG, Bhattacharya A, Li F, Ross WA, Ladha H, Porter K, et al. Diagnostic Differentiation of Pancreatic Neuroendocrine Tumor From Other Neoplastic Solid Pancreatic Lesions During Endoscopic Ultrasound-Guided Fine-Needle Aspiration. Pancreas. 2016;45(3):394-400.

40. Plöckinger U, Rindi G, Arnold R, Eriksson B, Krenning EP, de Herder WW, et al. Guidelines for the Diagnosis and Treatment of Neuroendocrine Gastrointestinal Tumours. Neuroendocrinology. 2004;80(6):394-424.
41. Sakamoto H, Kitano M, Komaki T, Noda K, Chikugo T, Dote K, et al. Prospective comparative study of the EUS guided 25-gauge FNA needle with the 19-gauge Trucut needle and 22-gauge FNA needle in patients with solid pancreatic masses. J Gastroenterol Hepatol. 2009;24(3):384-90.
42. Wittmann J, Kocjan G, Sgouros SN, Deheragoda M, Pereira SP. Endoscopic ultrasound-guided tissue sampling by combined fine needle aspiration and trucut needle biopsy: a prospective study. Cytopathology. 2006;17(1):27-33.
43. Itoi T, Itokawa F, Sofuni A, Nakamura K, Tsuchida A, Yamao K, et al. Puncture of solid pancreatic tumors guided by endoscopic ultrasonography: a pilot study series comparing Trucut and 19-gauge and 22-gauge aspiration needles. Endoscopy. 2005;37(4):362-6.
44. Fabbri C, Luigiano C, Maimone A, Tarantino I, Baccarini P, Fornelli A, et al. Endoscopic ultrasound-guided fine-needle biopsy of small solid pancreatic lesions using a 22-gauge needle with side fenestration. Surg Endosc. 2015;29(6):1586-90.
45. Hucl T, Wee E, Anuradha S, Gupta R, Ramchandani M, Rakesh K, et al. Feasibility and efficiency of a new 22G core needle: a prospective comparison study. Endoscopy. 2013;45(10):792-8.
46. Lee YN, Moon JH, Kim HK, Choi HJ, Choi MH, Kim DC, et al. Core biopsy needle versus standard aspiration needle for endoscopic ultrasound-guided sampling of solid pancreatic masses: a randomized parallel-group study. Endoscopy. 2014;46(12):1056-62.
47. Strand DS, Jeffus SK, Sauer BG, Wang AY, Stelow EB, Shami VM. EUS-guided 22-gauge fine-needle aspiration versus core biopsy needle in the evaluation of solid pancreatic neoplasms. Diagn Cytopathol. 2014;42(9):751-8.
48. Boutsen L, Jouret-Mourin A, Borbath I, van Maanen A, Weynand B. Accuracy of Pancreatic Neuroendocrine Tumour Grading by Endoscopic Ultrasound-Guided Fine Needle Aspiration: Analysis of a Large Cohort and Perspectives for Improvement. Neuroendocrinology. 2018;106(2):158-66.
49. Larghi A, Capurso G, Carnuccio A, Ricci R, Alfieri S, Galasso D, et al. Ki-67 grading of nonfunctioning pancreatic neuroendocrine tumors on histologic samples obtained by EUS-guided fine-needle tissue acquisition: a prospective study. Gastrointestinal Endoscopy. 2012;76(3):570-7.
50. Weynand B, Borbath I, Bernard V, Sempoux C, Gigot JF, Hubert C, et al. Pancreatic neuroendocrine tumour grading on endoscopic ultrasound-guided fine needle aspiration: high reproducibility and inter-observer agreement of the Ki-67 labelling index. Cytopathology. 2014;25(6):389-95.
51. Leeds JS, Nayar MK, Bekkali NLH, Wilson CH, Johnson SJ, Haugk B, et al. Endoscopic ultrasound-guided fine-needle biopsy is superior to fine-needle aspiration in assessing pancreatic neuroendocrine tumors. Endosc Int Open. 2019;7(10):E1281-e7.
52. To SH. Cohen's Kappa Statistic December 8, 2014 [Available from: https://www.statisticshowto.datasciencecentral.com/cohens-kappa-statistic/.
53. Reid MD, Bagci P, Ohike N, Saka B, Erbarut Seven I, Dursun N, et al. Calculation of the Ki67 index in pancreatic neuroendocrine tumors: a comparative analysis of four counting methodologies. Mod Pathol. 2015;28(5):686-94.
54. Di Leo M, Poliani L, Rahal D, Auriemma F, Anderloni A, Ridolfi C, et al. Pancreatic Neuroendocrine Tumours: The Role of Endoscopic Ultrasound Biopsy in Diagnosis and Grading Based on the WHO 2017 Classification. Digestive Diseases. 2019;37(4):325-33.
55. Laskiewicz L, Jamshed S, Gong Y, Ainechi S, LaFemina J, Wang X. The diagnostic value of FNA biopsy in grading pancreatic neuroendocrine tumors. Cancer Cytopathol. 2018;126(3):170-8.
56. Diaz Del Arco C, Diaz Perez JA, Ortega Medina L, Sastre Valera J, Fernandez Acenero MJ. Reliability of Ki-67 Determination in FNA Samples for Grading Pancreatic Neuroendocrine Tumors. Endocr Pathol. 2016;27(4):276-83.

57. Farrell JM, Pang JC, Kim GE, Tabatabai ZL. Pancreatic neuroendocrine tumors: accurate grading with Ki-67 index on fine-needle aspiration specimens using the WHO 2010/ENETS criteria. Cancer Cytopathol. 2014;122(10):770-8.
58. Jin M, Roth R, Gayetsky V, Niederberger N, Lehman A, Wakely PE, Jr. Grading pancreatic neuroendocrine neoplasms by Ki-67 staining on cytology cell blocks: manual count and digital image analysis of 58 cases. J Am Soc Cytopathol. 2016;5(5):286-95.
59. Weiss VL, Kiernan C, Wright J, Merchant NB, Coogan AC, Shi C. Fine-Needle Aspiration-Based Grading of Pancreatic Neuroendocrine Neoplasms Using Ki-67: Is Accurate WHO Grading Possible on Cytologic Material? J Am Soc Cytopathol. 2018;7(3):154-459.
60. Hijioka S, Hara K, Mizuno N, Imaoka H, Bhatia V, Mekky MA, et al. Diagnostic performance and factors influencing the accuracy of EUS-FNA of pancreatic neuroendocrine neoplasms. Journal of gastroenterology. 2016;51(9):923-30.
61. Hijioka S, Hara K, Mizuno N, Imaoka H, Bhatia V, Mekky MA, et al. Diagnostic performance and factors influencing the accuracy of EUS-FNA of pancreatic neuroendocrine neoplasms. J Gastroenterol. 2016;51(9):923-30.

Supplements

Supplement 1 – Baseline characteristics

Kommenterad [FB4]:

CLINICAL DATA	WHOLE POPULATION	SURGICAL
TOTAL NUMBER OF PATIENTS	49	20
SEX		
MEN	25 (51%)	12 (60%)
WOMEN	24 (49%)	8 (40%)
AGE AT EUS (YEARS)		
MEAN ± SD	71 ± 14.4	56.9 ± 13.9
MIN AGE	23	31
MAX AGE	87	74
FOLLOW UP (MONTHS)		
MEAN ± SD	26.4 ± 23.4	16 ± 19
MIN NUMBER	1	1
MAX NUMBER	68	43
PRE-EUS INVESTIGATION		
CT	41 (84 %)	16 (80%)
MR	22 (45%)	14 (70%)
US	12 (24%)	5 (25%)
PET	9 (18%)	3 (15%)
ERCP/MRCP	7 (14%)	2 (10%)
EUS	9 (18%)	4 (20%)
EUS-FNA	7 (14%)	2 (10%)
SCINTIGRAFI	9 (18%)	4 (20%)
POSITION ON IMAGING		
CAUDA	18 (37%)	10 (50%)
CORPUS	13 (27%)	4 (20%)
NECK	1 (2%)	0 (0%)
CAPUT	23 (47%)	7 (35%)
SIZE ON IMAGING (MM)		
MEAN ± SD	32.7 ± 25.67	32.8 ± 21.2
MIN SIZE	5	5
MAX SIZE	128	80
POSITION ON EUS		
NECK	19 (39%)	11 (55%)
CORPUS	14 (29%)	6 (30%)
NECK	5 (10%)	1 (5%)
CAPUT	21 (43%)	7 (35%)
SIZE ON EUS (MM)		
MEAN ± SD	30.1 ± 23.7	32.4 ± 16.1
MIN SIZE	8	9
MAX SIZE	150	60
DENSITY ON EUS		
SOLID	35 (71%)	14 (70%)
CYSTIC	4 (8%)	3 (15%)
SEMI-CYSTIC	12 (24%)	6 (30%)
VASCULARITY ON EUS		
HIGH	44 (90%)	17 (85%)
LOW	1 (2%)	0 (0%)
UNCERTAIN	4 (8%)	3 (15%)
ECHOGENICITY ON EUS		
HIGH	45 (92%)	19 (95%)
LOW	0 (0%)	0 (0%)
HETEROGENEOUS	3 (6%)	1 (5%)
ISOGENIC	1 (2%)	0 (0%)
EUS SAMPLING		
FNB	10 (20%)	3 (15%)
FNB + FNA	39 (80%)	17 (85%)
PUNCTURE ROUTE		

VENTRICLE	28 (57%)	14 (70%)
DUODENUM	21 (43%)	6 (30%)
FNB-NEEDLE (GAUGE)		
19	1 (2%)	1 (5%)
20	2 (4%)	1 (5%)
22	43 (88%)	18 (90%)
25	2 (4%)	0 (0%)
22-SIDEPORT	1 (2%)	0 (0%)
FNB NUMBER OF PASSES		
MEAN ± SD	1.9 ± 0.7	1.9 ± 0.7
MIN NUMBER OF PASSES	1	1
MAX NUMBER OF PASSES	3	3
PAD QUALITY		
ADEQUATE	19 (39%)	6 (30%)
SPARSELY	27 (55%)	11 (55%)
NOTHING	3 (6%)	3 (15%)
FNA-NEEDLE (GAUGE)		
22	3 (8%)	1 (6%)
25	34 (84%)	15 (83%)
22 PROCORE	3 (8%)	2 (11%)
FNA NUMBER OF PASSES		
MEAN ± SD	2.5 ± 0.7	2.6 ± 0.8
MIN NUMBER OF PASSES	1	1
MAX NUMBER OF PASSES	4	4
PAD IMMUNOHISTOLOGICALLY STAINED		
YES	43 (88%)	17 (85%)
NO	6 (12%)	3 (15%)
PAD CONCLUSIVE FOR DIAGNOSIS		
YES	40 (82%)	15 (75%)
NO	9 (18%)	5 (25%)
PAD REPORT		
MALIGNANT	39 (80%)	14 (70%)
BENIGN	0 (0%)	0
UNCERTAIN	9 (18%)	6 (30%)
NON-DIAGNOSTIC	1 (2%)	0
PAD RESECTION		
MALIGNANT	19 (95%)	19 (95%)
BENIGN	1 (5%)	1 (5%)
CLINICALLY FUNCTIONING TUMOUR		
NON-FUNCTIONAL (NF)	48 (98%)	19 (95%)
FUNCTIONAL – INSULIN	1 (2%)	1 (5%)
FUNCTIONAL – GASTRIN	0	0
FUNCTIONAL – ACTH	0	0
FUNCTIONAL – OTHER	0	0
HISTOLOGICALLY FUNCTIONING		
NON-FUNCTIONAL (NF)	25 (51%)	13 (65%)
FUNCTIONAL – INSULIN	2 (4%)	2 (10%)
FUNCTIONAL – GASTRIN	1 (2%)	1 (5%)
FUNCTIONAL – GLUCAGON	12 (24%)	3 (15%)
FUNCTIONAL – OTHER	4 (8%)	1 (5%)
COMPLICATIONS DUE TO EUS-SAMPLING		
BLEED	0 (0%)	0 (0%)
PANCREATITIS	0 (0%)	0 (0%)
OTHER	0 (0%)	0 (0%)
FOLLOW UP		
ALIVE WITHOUT TUMOUR DISEASE	15 (31%)	15 (75%)
ALIVE WITH REMAINING TUMOUR	27 (55%)	4 (20%)
DECEASED	7 (14%)	1 (5%)

Supplement 1

Clinical data collected from patients' medical records. One column, the one named "whole population", represents all patients included in the study. The other column represents the patients that underwent surgical treatment.
* There are more tumour types, when adding them up, than there are patients included in the study, this is because some of the tumours seemingly produced more than one hormone.

Kommenterad [FB5R4]: Lade till procentsatser

Supplement 2 – Clinical factors influencing the biopsy quality

Factors influencing EUS-FNB	*Cell count <1000*	*Cell count >1000*	*Total*	*P-value*
Sex				0.321
Male	4	21	25	
Female	7	17	24	
Total	11	38	49	
EUS-positioning				0.06
Tail	0	14	14	
Body	4	9	13	
Neck	0	2	2	
Head	7	13	20	
Total	11	38	49	
EUS-solidity				0.79
Solid	8	24	32	
Cystic	0	1	1	
Semisolid necrotic	3	13	16	
Total	11	38	49	
EUS-echogenicity				0.214
Hypoechogenous	9	36	45	
Heterogenous	1	2	3	
Isoechogenous	1	0	1	
Total	11	38	49	
Puncture route				0.096
Transgastric	4	25	29	
Transduodenal	7	13	20	
Total	11	38	49	
FNB number of passes				0.462
1	2	11	13	
2	8	19	27	
3	1	8	9	
Total	11	38	49	
Tumour Size				0.171
<20 mm	3	21	24	
>20 mm	8	17	25	
Total	11	38	49	

Supplement 2

A table depicting different Chi² calculations done on different factors that might influence the success or failure of a EUS-FNB pass. The cut off for a successful pass has been set to >1000 cells in the sample for these calculations

Supplement 3 – Cell count and Ki67 % estimations

EUS-Biopsies	*Ki67% Index Strongly stained*	*Ki67% Index Strongly and weakly stained*	*Total cell count*
EUS-FNB case # 1	0.39	0.43	10535
EUS-FNB case # 2	0.01	0.01	350
EUS-FNB case # 3	0	0	944
EUS-FNB case # 4	0.3	0.37	16473
EUS-FNB case # 5	0	0	1012
EUS-FNB case # 6	0	0	232
EUS-FNB case # 7	0.01	0.01	1605
EUS-FNB case # 8	Non-diagnostic	Non-diagnostic	Non-diagnostic
EUS-FNB case # 9	0.12	0.14	779
EUS-FNB case # 10	0.02	0.02	1919
EUS-FNB case # 11	0.05	0.06	14585
EUS-FNB case # 12	0.01	0.02	1341
EUS-FNB case # 13	0	0.01	2648
EUS-FNB case # 14	0	0.01	769
EUS-FNB case # 15	0.01	0.01	1313
EUS-FNB case # 16	0.03	0.03	1180
EUS-FNB case # 17	0.01	0.02	561
EUS-FNB case # 18	0.05	0.05	515
EUS-FNB case # 19	0.02	0.03	10206
EUS-FNB case # 20	0.05	0.07	2016
EUS-FNB case # 21	0.02	0.02	840
EUS-FNB case # 22	*	*	*
EUS-FNB case # 23	Non-diagnostic	Non-diagnostic	Non-diagnostic
EUS-FNB case # 24	Non-diagnostic	Non-diagnostic	Non-diagnostic
EUS-FNB case # 25	0.05	0.06	2608
EUS-FNB case # 26	Non-diagnostic	Non-diagnostic	Non-diagnostic
EUS-FNB case # 27	Non-diagnostic	Non-diagnostic	Non-diagnostic
EUS-FNB case # 28	Non-diagnostic	Non-diagnostic	Non-diagnostic
EUS-FNB case # 29	0.2	0.21	691
EUS-FNB case # 30	0.04	0.04	686
EUS-FNB case # 31	0.01	0.02	1118
EUS-FNB case # 32	Non-diagnostic	Non-diagnostic	Non-diagnostic
EUS-FNB case # 33	0.01	0.02	10606
EUS-FNB case # 34	**	**	**
EUS-FNB case # 35	0	0.01	3585
EUS-FNB case # 36	0.02	0.03	4265
EUS-FNB case # 37	0.07	0.07	13709
EUS-FNB case # 38	0.02	0.02	5766
EUS-FNB case # 39	Non-diagnostic	Non-diagnostic	Non-diagnostic
EUS-FNB case # 40	0.01	0.02	76968
EUS-FNB case # 41	0.00	0.02	257
EUS-FNB case # 42	0.03	0.04	563
EUS-FNB case # 43	0.01	0.01	3660
EUS-FNB case # 44	Non-diagnostic	Non-diagnostic	Non-diagnostic
EUS-FNB case # 45	0	0	4850
EUS-FNB case # 46	0	0.01	1174
EUS-FNB case # 47	0.02	0.02	1229
EUS-FNB case # 48	0.01	0.02	61787
EUS-FNB case # 49	0.01	0.01	1682

Supplement 3

A table with all EUS-FNB punctures included in the study. It encompasses 49 different, specific EUS-FNB puncture occasions on 46 different unique patients. The Ki67% Indexes are counted on the ten largest cell groups in the samples. Two different Ki67% Indexes are depicted, one counted only with the strongly stained for Ki67 and the other includes the weaker stained for Ki67. *The biopsy glass has gone missing and no Ki67 % estimation could be done. ** The cellular outlines were to unclear and a Ki67% estimation could not be done.