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Elastography mean strain histogram value for the differential diagnosis of malignant pancreatic masses: a monocentric study

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Abstract (English)

Introduction

Endoscopic ultrasound (EUS) elastography is a recent ultrasound method used for the real-time visualization and evaluation of tissue elasticity. Qualitative and quantitative methods have been used, in particular in evaluation of pancreatic diseases and malignant lymph nodes, with interesting results regarding the accuracy and the differential diagnosis between malignant and benign masses. No consensus has been reached with regard to the superiority of different quantitative methods, but strain ratio and strain histogram (SH) remain the most used. SH corresponds to a graphical representation of the color distribution in a region of interest (ROI), and mean SH (mSH) value is a SH-derived quantitative measure of the global hardness in the evaluated ROI.

Materials and methods

The aim of the present study was to describe the different elastographic patterns of solid pancreatic masses (pancreatic adenocarcinoma, PA, and pancreatic neuroendocrine tumor, pNET) using the mSH value. A group of normal patients was enrolled to define the normal pancreatic elasticity.

This is a prospective, observational, monocentric study.

Experienced EUS examiners performed the endoscopic procedure and elastographic measures. Contrast enhancement was performed, and according to

the European Federation Societies of Ultrasound in Medicine and Biology guidelines and recommendations, 2 phases were defined for CE-US and CEH-EUS of the pancreas: an early and/or arterial phase (starting from 10 to 30 seconds) and a late and/or venous phase (from 30 to 120 seconds). Enhancement was evaluated in arterial phase. When the lesion resulted hypovascular (hypo-enhanced), the aspect was defined as typical for adenocarcinoma while an hypervascular lesion (with strong arterial hyper-enhancement) was considered typical of neuroendocrine tumors.

SH was calculated automatically by machine integrated software in a ROI manually selected by the operator. Four parameters were calculated using the histogram to quantify the elasticity of the pancreas: the mSH, the standard deviation of the histogram, the kurtosis and the skewness. Three different measures were performed by the same operator and the mean value of the previous described values was evaluated. After the elastographic measure a FNA was performed when a lesion was observed. We used the histology obtained by FNA or surgical specimens or the global assessment of cytology and imaging as reference standard for the diagnosis, except in case of normal examinations.

Results

A total of 88 patients were included: 9 normals, 45 PA, 9 pNET (5 grade 1, 4 grade2-3), 5 chronic pancreatitis, 20 others (IPMN, metastatic lesions, pancreatic cystoadenoma, etc). Only patients with normal pancreas, PA and pNET were

included in the statistical analysis. Mean age was 64 (range: 19-88). All the patients presenting a pancreatic lesion, except one, received the contrast. All the patients with pNET presented a typical hypervascular enhancement. On the other hand, of patients with histological proven pancreatic adenocarcinoma, only 37 presented a typical hypoenhancement, while 5 presented an hypervascularisation and 2 patients an atypical enhancement (represented by a partial arterial enhancement). Regarding elastography, we obtained a statistically significant difference between the malignant lesions (p-Net grade 2/3 and adenocarcinoma) and normal pancreas, considering all the parameters evaluated (mSH, standard deviation, kurtosis and skewness). No differences were found in elastographic measurement between p-NET and adenocarcinoma, neither between p-NET grade 1 (that, considering the good prognosis and slow evolution, could be considered neither as benign neither as malign) and p-NET grade 2/3. At univariate analysis we found that all the elastographic parameters measured were capable to differentiate benign versus malign disease, with a cut-off calculated with ROC-curves of 43.250 for mSH, 44.63 for standard deviation, 1.5 for the skewness and 5 for kurtosis. At multivariate analysis only mSH resulted statistically significative to distinguish benign versus malign lesions. At a model constructed by logistic regression (9.8958 – 0.3170*moyenne + 0.2989*SD + 0.7935*kurtosis – 7.0642*skewness) a cut off of 0.57 was found to distinguish benign versus malign lesions.

Conclusions

Mean strain histogram seems able to differentiate pancreatic cancer from benign pancreas. EUS elastography could potentially be used in negative EUS–FNA cases, which represent up to 25% of patients with focal masses. The presence of a strong suspicion of pancreatic cancer is indicated when combining meanSH, standard deviation, skewness and kurtosis in a statistical model we obtain a cut-off>0.57.

Abstract (Italian)

Introduzione

L'elastometria condotta tramite ecoendoscopia è una nuova tecnica utilizzata per la visualizzazione e la valutazione in real time dell'elasticità dei tessuti. È noto infatti che i tessuti patologici, quali ad esempio i tessuti tumorali, presentano un'elasticità diversa rispetto ai tessuti normali. Fino ad oggi sono state utilizzate tecniche di misurazione dell'elasticità sia di tipo qualitativo che di tipo quantitativo. Le misure qualitative sono però gravate da una scarsa riproducibilità. Per tale ragione esse sono state praticamente abbandonate afavore di misure quantitative. Non esiste un consenso circa la superiorità di un metodo quantitativo rispetto ad un altro, ma lo "strain ratio" (SR) e lo "strain histogram" restano le metodiche più utilizzate. Lo SH, utilizzato in questo studio, corrisponde a una rappresentazione grafica della distribuzione del colore in una determinata area di interesse, e il valore del mSH è una misura dell'elasticità globale in un'area di interesse prescelta, normalmente corrispondente alla lesione. L'accuratezza dell'elastometria è stata valutata nelle patologie pancreatiche e linfonodali, mostrando risulatati interessanti soprattutto nella diagnosi differenziale tra le masse benigne e le masse maligne.

Materiali e metodi

Lo scopo del nostro studio è la quantificazione del pattern elastografico nel pancreas normale, nell'adenocarcinoma pancreatico (AP) e nei tumori neuroendocrine (NET). Si tratta di uno studio prospettico, monocentrico e osservazionale. L'ecoendoscopia con contrasto è stata effettuata e, secondo le line guida della società Europea di ecografia, sono state individuate 2 fasi contrastografiche per lo studio del pancreas: la prima, dai 10 ai 30 secondi, denominata fase precoce o arteriosa e la seconda, tardiva o venosa, dai 30 ai 120 secondi. L'enhancement delle lesioni pancreatiche è stato valutato in fase arteriosa. All'ecoendoscopia con contrasto, l'aspetto tipico di AP è definito in presenza di ipo-enhancement, mentre l'aspetto tipèico in caso di NET è definite in presenza di iper-enhancement arterioso. Successivamente all'elastometria è stata eseguita un'elastografia. Lo SH è stato calcolato automaticamente da un software in un'area scelta manualmente dall'operatore. 4 parametri sono stati presi in considerazione: lo SH medio (Msh), la deviazione standard, la kurtosis e lo swekness. 3 misure differenti sono state effettuate dallo stesso operatore e il valore medio delle misure è stato preso in considerazione. Dopo la misura elastografica è stato eseguito il prelievo bioptico. Il risultato istologico ottenuto tramite biopsia è stato utilizzato come referenza per definire la natura della lesione, eccetto in caso di pancreas normale.

Risultati

Nello studio sono stati inclusi 88 pazienti: 9 pancreas normali, 9 NET, 45 AP, 5 pancreatiti croniche e 20 lesioni miste. Solo I pazienti con AP, NET e pancreas normale sono stati inclusi nell'analisi finale. Alla somministrazione di contrasto, i pazienti con NET hanno presentato un pattern ipervascolare dopo somministrazione di contrasto, mentre tra i pazienti con AP, solo 37 hanno presentato un pattern tipico ipovascolare, mentre 5 hanno presentato un pattern ipervascolare e 2 un enhancement atipico (arterioso parziale).

Per ciò che riguarda l'elastografia, abbiamo ottenuto una differenza statisticamente significativa tra il pancreas normale e le lesioni maligne, per tutti i 4 parametri valutati (media, deviazione standard, kurtosis e skewness). Invece, non abbiamo trovato una differenza statisticamente significativa tra i NET e l'AP. All'analisi univariata abbiamo trovato che tutti i parametri erano in grado di differenziare il pancreas benigno dal pancreas maligno, con un cut-off calcolato mediante la curva ROC di 43.250 per mSH, 44.63 per la deviazione standard, 1.5 per lo skewness and 5 per la kurtosis. All'analisi multivariata, solo il mSH è risultato statisticamente significativo. Abbiamo costruito un modello statistico tramite regressione logistica (9.8958 – 0.3170*mSH + 0.2989*SD + 0.7935*kurtosis – 7.0642*skewness), individuando un cut off di 0.57 per distinguere il pancreas normale dale lesioni maligne.

Conclusioni

Il mSH sembra efficace nella differenziazione tra lesioni pancreatiche maligne e pancreas benigno. Proponiamo pertanto l'uso dell'elastometria, e del nostro modello statistico, per definire le lesioni da operare o da sorvegliare in caso di biopsia negativa. Riteniamo infatti che, pur in caso di biopsia negativa, un indice elastografico superiore a 0.57 sia indicativo di una lesione maligna e pertanto necessiti di una presa in carico chirurgica.

Introduction

Differential diagnosis in focal pancreatic masses remains a main clinical problem in a high number of patients, even considering that pancreatic adenocarcinoma (PA) and neuroendocrine tumors (NET) present a different treatment and prognosis.

The gold standard tool for the differential diagnosis between these two histologically different cancers is the endoscopic ultrasound fine-needle aspiration (EUS-FNA) that presents the higher sensitivity and specificity compared to other diagnostic tools.

Unlikely, EUS-FNA is technically demanding, needs a prolonged learning curve and multiple punctures may be necessary to obtain a sufficient amount of tissue. Furthermore EUS-FNA can be associated to iatrogenic complications, such as acute iatrogenic pancreatitis, bleeding and infections. In addition, the sensitivity of cytology for malignancy is limited, and false-negative results are obtained in up to 20 % to 40 % of cases, requiring new techniques.

The rational of using elastometry in differential pancreatic masses diagnosis is the fact that pathological processes can induce alteration in tissue stiffness and that different tissues can be distinguished on the basis of their specific consistency.

Background

- **Pancreatic adenocarcinoma and neuro endocrine tumors**

Pancreatic adenocarcinoma (PA) is the fourth most common cause of cancer related death among men and women in United States. The peak of incidence is between the seventh and eighth decade of life.

The incidence of PA increased in USA in the decade between 1999 and 2008 and this is probably related to the increasing prevalence of obesity, life expectancy and others unknown factors. Unlikely, the PA related mortality remains stable.

Up to now, the known risk factors of PA are cigarette smoking, increased body max index (BMI), increased consumption of red/processed meat, occupational exposure to chemicals, such as beta-naphthylamine ans benzidine, and increased alcohol assumption. The role of diabetes mellitus is still debated, such as the role of antidiabetic drugs.

True familial PA is a rare entity, but a genetic predisposition is present in up to 5-10% of patients. The genetic cause of familial predisposition is frequently unknown but some familial well known cancer syndromes are associated with an increased risk. Between these syndromes there are Peutz-Jeghers syndrome and Lynch syndrome, both characterized by a colon cancer predisposition. An increased PA incidence has been found even in patients with BRCA2 (breast cancer susceptibility gene-2) mutations.

The role of the PA screening remains to be defined, but a recent PA screening project has been conducted in 225 asymptomatic high-risk individuals, screened with computer tomography (CT), or magnetic resonance imaging (MRI) or endoscopic ultrasounds (EUS). When the results of the 3 screening modalities were compared, EUS detected abnormalities in 45% of patients, compared with 33% and 11% for MRI and CT respectively (1).

Ductal adenocarcinoma and its variants account for over 90% of pancreatic malignancies. Presenting symptoms are not specific: weight loss, jaundice, pain, nausea, dyspepsia. All patients for whom there is a clinical suspicion of pancreatic cancer should undergo initial evaluation by CT or MRI and eventually to EUS. The role of EUS is more evident in staging the disease, providing complementary information regarding the vessels involvement, the distinction between benign and malignant stenosis, the cystic pancreatic lesions and the periampullary masses.

The biopsy is not required before surgery but it is required before neo-adjuvant therapy or exclusive medical therapy. The histological diagnosis is made by fine needle aspiration and/or biopsy by EUS or CT. EUS approach should be preferred because of better diagnosis, safety and lower complications.

PA is usually staged and graded according to the TNM classification, based on primary tumor extension (T), regional lymph nodes involvement (N) and distal metastasis presence (M). TNM staging and stage grouping are shown in **Table 1a and 1b**.

Table 1a:TNM staging of pancreatic cancer		
Primary tumor (T)	Tx	Primary tumor cannot be assesed
	T0	No evidence of a primary tumor
	Tis	Carcinoma in situ
	T1	Tumor limited to the pancreas, 2 cm or less in the greatest dimension
	T2	Tumor limited to the pancreas, more than 2 cm in the greatest diemsion
	T3	Tumor extends beyond the pancreas but without involvement of CA or the SMA
	T4	Tumor involves the CA or the SMA
Regional lymph nodes (N)	Nx	Regional lymph nodes cannot be assessed
	N0	No regional lymph nodes metastasis
	N1	Regional lymph nodes metastasis
Distal metastasis (M)	M0	No distant metastasis
	M1	Distant metastasis

Table 1b: Stage grouping			
Stage 0	Tis	N0	M0
Stage 1A	T1	N0	M0
Stage 1B	T2	N0	M0
Stage 2A	T3	N0	M0
Stage 2B	T1	N1	M0
	T2	N1	M0
	T3	N1	M0
Stage 3	T4	Any N	M0
Stage 4	Any T	Any N	M1

The therapeutical approach depends on the tumor resectability status, as defined below in the **Table 1c**:

Resectability Status	Arterial	Venous
Resectable	No arterial tumor contact (celiac axis [CA], superior mesenteric artery [SMA], or common hepatic artery [CHA]).	No tumor contact with the superior mesenteric vein (SMV) or portal vein (PV) or ≤180° contact without vein contour irregularity
Borderline Resectable[2]	Pancreatic head /uncinate process: • Solid tumor contact with CHA without extension to celiac axis or hepatic artery bifurcation allowing for safe and complete resection and reconstruction. • Solid tumor contact with the SMA of ≤180° • Presence of variant arterial anatomy (ex: accessory right hepatic artery, replaced right hepatic artery, replaced CHA and the origin of replaced or accessory artery) and the presence and degree of tumor contact should be should be noted if present as it may affect surgical planning. Pancreatic body/tail: • Solid tumor contact with the CA of ≤180° • Solid tumor contact with the CA of >180° without involvement of the aorta and with intact and uninvolved gastroduodenal artery [some members prefer this criteria to be in the unresectable category].	• Solid tumor contact with the SMV or PV of >180°, contact of ≤180° with contour irregularity of the vein or thrombosis of the vein but with suitable vessel proximal and distal to the site of involvement allowing for safe and complete resection and vein reconstruction. • Solid tumor contact with the inferior vena cava (IVC)
Unresectable[2]	• Distant metastasis (including non-regional lymph node metastasis) Head/uncinate process: • Solid tumor contact with SMA >180° • Solid tumor contact with the CA >180° • Solid tumor contact with the first jejunal SMA branch Body and tail • Solid tumor contact of >180° with the SMA or CA • Solid tumor contact with the CA and aortic involvement	Head/uncinate process • Unreconstructible SMV/PV due to tumor involvement or occlusion (can be due to tumor or bland thrombus) • Contact with most proximal draining jejunal branch into SMV Body and tail • Unreconstructible SMV/PV due to tumor involvement or occlusion (can be due to tumor or bland thrombus)

[1]Al-Hawary MM, Francis IR, Chari ST, et al. Pancreatic ductal adenocarcinoma radiology reporting template: consensus statement of the society of abdominal radiology and the American pancreatic association. Gastroenterology. 2014 Jan; 146 (1):291-304.
[2]Solid tumor contact may be replaced with increased hazy density/stranding of the fat surrounding the peri-pancreatic vessels (typically seen following neoadjuvant therapy); this finding should be reported on the staging and follow up scans. Decision on resectability status should be made in these patients, in consensus at multidisciplinary meetings/discussions

1) **Resectable disease**: surgery
2) **Borderline resectable disease**: chemotherapy (CTh) +/- surgery
3) **Locally advanced disease, unresectable**: radiotheraphy (RTh)/RTh-CTh. Sometimes a resection is possible after.
4) **Metastatic disease**: CTh, according to the OMS status.

Pancreatic neuroendocrine tumors (NET) origin from cells of the pancreatic endocrine system. They account for approximately 1% of pancreatic cancers by incidence and 10% by prevalence. The peak incidence of occurrence is between 40 and 69 years, but a significant number of patients is diagnosed before 35 years. Most of these tumors (estimated between 40 and 91%) are nonfunctional. In case of functional tumors, up to 70% are insulinomas, 15% glucagonomas, 10%

gastrinomas and somatostatinomas. The remaining tumors (5%) are VIPomas, PPomas and CCKomas.

The clinical symptoms depends on the secreted hormone. NET can occur in the context of Multiple Endocrine Neoplasia (MEN)-1 syndrome. In this case they are typically multiple and they require a different treatment from sporadic NET.

The diagnosis relies on CT or MRI scan and eventually on biochemical evaluation (symptom-guided). Serum chromogranine A and and pancreatic polypeptide may be tested as clinically appropriate. Somatostatine scintigraphy (SRS) and EUS are appropriate too.

The role of EUS in **non-functional** NET is particularly relevant for small tumors and EUS-FNAB has shown good results in confirming the diagnosis.

In case of **functional** NET, the role of EUS varies according to the histology and to the presence of syndromes such as the MEN-1 or the Zollinger Ellison Syndrome (ZES).

In case of ZES, EUS is indicated when cross CT-scan or MRI are negatives and surgery has been considered. In these cases, EUS is known to detect most pancreatic gastrinomas, while it misses up to 50% tumors in case of duodenal localization.

In case of MEN-1, EUS is more sensitive than cross-sectional imaging studies (CT, MRI, US) for the detection of small non-functional pNET, especially in the pancreatic head/body region and to determine their size. Furthermore, the presence of multiple pancreatic tumors with EUS is very suspicious of MEN1.

However, EUS is not generally recommended at present in all MEN-1 patients, because routine surgical resection of small NET (<2 cm) is not recommended and the EUS criteria on when to operate these patients are not established. Some experts recommended pancreatic EUS in selected MEN1 patients, especially if they have ZES or small non-functional NET on other imaging studies and are being followed without surgery, in an attempt to detect small nonfunctional tumors and to follow their growth in order to offer earlier surgery. In case of insulinoma, EUS is positive in 70–95% of cases and is the imaging study of choice if the other non-invasive studies are negative. Furthermore, EUS can help to determine if tumor enucleation is possible because it can evaluate the distance between the tumor and the pancreatic duct.

In case of more rare functional tumors, EUS is not universally recommended as a first-line procedure and it may be used in circumstances where CT, MRI and SRS-SPECT are inconclusive, especially preoperatively; in patients with liver metastasis, EUS is rarely necessary while it may be helpful in patients with large or aggressive tumors to more clearly define the tumor involvement where surgery is considered.

NET are staged and graded according to the same TNM classification as PA. The therapeutically approach depends more on the WHO 2010 classification, that distinguishes between well-differentiated and poorly differentiated NET, according to a grading scheme based on mitotic count or Ki67 index.

Low grade NET or **G1** present with a mitotic count < 2 per 10 high-power fields (HPF) and/or < 2% Ki67 index, intermediate grade or **G2** with a mitotic count 2–20 per 10 HPF and/or 3–20% Ki67 index and high grade NET or **G3** with a mitotic count >20 per 10 HPF and/or >20% Ki67 index. Low grade (G1) and intermediate grade (G2) are defined as well-differentiated NET while high grade (G3) as poorly differentiated neuroendocrine carcinoma (NEC). This classification should be used as a general guide. In fact it is recognized that occasionally a morphologically well differentiated NET may have a proliferation index by Ki67 index which technically falls into the high grade category. Clinical judgement should be used in such discordant cases. In general, this discordance should not cause a reclassification of a well differentiated NET into a poorly differentiated NEC (https://www.nccn.org/).

According to the French Oncological Digestive Guidelines (http://www.tncd.org/), the main factors associated to a bad prognosis are the low grade of differentiation, the high mitotic count and index and the metastatic disease. The disease treatment is different according to the disease grading and can be resumed as follows:

G1/G2 well differentiated:

1) Non-metastatic disease:
    - no NEM-1 associated: surgery. Except if lesion <2 cm in the head of pancreas, grade G1 and asymptomatic: follow up.

- NEM-1 associated: follow up. Except if lesion >2 cm and/or increasing in diameter, and/or if lympho-adenopathies are presents and/or if the disease is symptomatic (insulinoma, glucagonoma, VIPoma).

2) Metastatic:

- Primary and secondary lesions operable: surgery.
- Primary and/or secondary lesions not operable: evaluate lesions progression, treat symptoms and discuss primary tumor resection.

If non progressive disease: somatostatine analogs or follow up.

If progressive disease: anti-tumoral treatment (chemo-therapy or immuno-therapy).

G3 well differentiated:

- If good performance status and asymptomatic patient: like G1/G2
- If bad performance status/ symptomatic patient: like G3 scarcely differentiated

G3 scarcely differentiated:

1) **Non-metastatic disease:**
   - If complete resection is possible: surgery
   - If complete resection is not possible: medical therapy by chemo-therapy +/- radio therapy
2) **Metastatic disease:** Chemo-therapy in urgency

- **Endoscopic Ultrasounds (EUS), contrast enhancement EUS (CE-EUS) and EUS-elastography**

The endosonographic structure of the pancreas is uniformly iso/hyper-echoic compared to liver. The overall sensitivity and specificity of transabdominal ultrasonography (tUS) and its different modalities for the detection of pancreatic lesions is not yet established. In case of PA, the tUS typical finding corresponds to a hypoechoic lesion with badly defined margins, often with spiculas, with a tendency to alter the gland contour. At contrast enhancement tUS (CE-tUS), PA is characterized by hypo-enhancement. tUS can visualize NET lesions with a sensitivity of approximately 30–60%. Studies with CE-tUS have demonstrated that NET have hyper-vascularity compared with hypo-vascularity of adenocarcinoma, or iso-vascularity of pancreatitis. Individual patient factors such as obesity and intestinal air are the most frequent limitations in scanning of the pancreas.

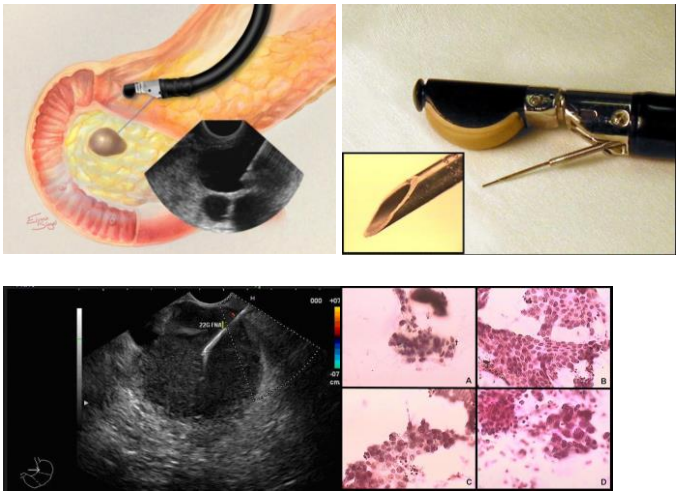
Endoscopic ultrasound (EUS) has evolved over time into a diagnostic and therapeutic modality that has a major clinical impact on both digestive and mediastinal diseases. Nevertheless, the main indication of EUS has remained the diagnosis and staging of pancreatobiliary diseases, for which EUS is currently considered to be the first-choice examination. An accurate diagnosis cannot always be determined using only conventional B-mode EUS imaging. In many

cases, EUS-guided fine needle aspiration (FNA) and/or biopsy (FNB) can provide a definitive diagnosis.

The accuracy of **FNA** has been demonstrated to be very high, with sensitivity between 80% and 85%, and specificity approaching 100%; however, EUS-guided tissue sampling is technically demanding, and multiple punctures may be necessary to obtain a sufficient amount of tissue. In addition, the sensitivity of cytology for malignancy is limited, and false-negative results are obtained in up to 20 % to 40 % of cases.

FNB has not been shown to be superior to EUS-FNA for determining the etiology of pancreatic masses but should be considered if EUS-FNA is non-diagnostic and a histologic diagnosis is required. FNB is technically difficult for sampling of pancreatic head masses because of the stiffness of the needle and the acute angulation of the endoscope required for biopsy from this location. More-flexible needles have been developed recently that may circumvent this problem and allow better trans-duodenal sampling of pancreatic head masses that require core tissue to better determine the nature of the lesion. Potential adverse events from EUS-guided sampling of pancreatic masses include a 0.5% to 2% risk of pancreatitis or bleeding. Tumor seeding with EUS-FNA has been reported, but the risk appears to be exceedingly small, and reports are currently limited to isolated cases (2).

Figure 1: a) EUS trans-gastric puncture of a pancreatic lesion. **b)** EUS-needle. **c)** EUS-FNA. **d)** PA specimen at EUS-FNA



The development of contrast-enhancement EUS (CE-EUS) and elastography has allowed a better characterization of focal pancreatic masses, with possible implications in the management of patients with negative EUS-FNA and a strong suspicion of malignancy.

The **contrast enhancement- EUS (CE-EUS)** is not indicated to improve the detection of pancreatic lesions, but to improve the delineation and differential diagnosis of pancreatic lesions.

One of the fluoro-gas-containing contrast agents used in CE-EUS is Sonovue®, which consists of phospholipids-stabilized bubbles of sulfurhexafluoride (SF6). Sonovue® is isotonic, stable and resistant to pressure, with a viscosity similar to blood. It does not diffuse into the extravascular compartment remaining within

the blood vessels until the gas dissolves and is eliminated in the expired air (blood pool contrast agent). The safety profile of SonoVue showed a very low incidence of side effects; it is not nephrotoxic and the incidence of severe hypersensitivity is similar to other magnetic resonance imaging contrast agents. Moreover, Sono-Vue is approved for clinical use in EU countries.

The blood supply of the pancreas is entirely arterial, making contrast-enhanced examinations feasible and readily available.

Based on the European Federation Societies of Ultrasound in Medicine and Biology guidelines and recommendations, updated in 2008 and 2011, 2 phases were defined for CE-US and CEH-EUS of the pancreas: an early and/or arterial phase (starting from 10 to 30 seconds) and a late and/or venous phase (from 30 to 120 seconds) (3, 4).

An initial feasibility study showed that the specificity of the discrimination between benign and malignant focal pancreatic lesions is higher than 93.3% by using power Doppler contrast-enhanced EUS, as compared with 83.3% for conventional EUS.

PA is typically hypo-enhanced in all phases compared with the adjacent pancreatic tissue, possibly because of the desmoplastic reaction and low mean vascular density. The hypo-enhanced aspect of lesions based CE-EUS seemed highly sensitive and specific (higher than 90%) for adenocarcinoma in several published studies, even in small lesions (5-7). In clinical practice there is still the problem of discrimination of focal lesions in chronic pancreatitis to PA due to the

similar vascularization behavior. CE-EUS, however, provided the possibility to analyze the macrovessels (arterioles and venoules) of the pancreas for neovascularization patterns. In fact PA shows an irregular vessel system in contrast to a netlike homogenous vessel system with both arterial and venous vessels in chronic pancreatitis. Using these criteria a discrimination sensitivity and specificity of over 90% can be achieved.

NET present typically as hyperenhancing masses in the arterial phase, owing to their abundant arterial vascularization (8).

Typical PA and NET behavior at CE-EUS are shown in **Figure 2**.

Figure 2a: Typical aspect of hypo-enhanced PA at arterial phase

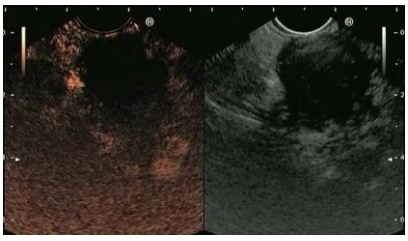
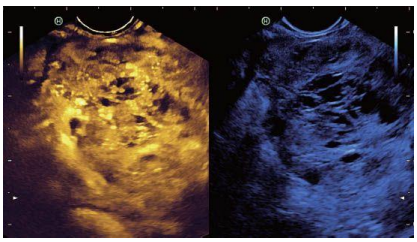
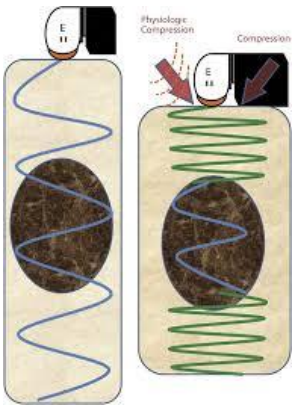


Figure 2b: Typical aspect of hyper-enhanced NET at arterial phase



EUS-elastography is a recent ultrasound method used for the visualization and evaluation of tissue elasticity in real-time (9). Ultra-sonographic (US) waves travel at different speeds through tissues of different stiffness. Compression of tissue changes its mechanical properties and its reflection of US waves. A lesion may deform to a different degree in response to compression than surrounding normal tissue. Elastography compares the spatial arrangement of the tissue and the velocity of US waves at rest and after compression. When the tissue of a lesion is harder compared with the surrounding normal organ, the echoes will be less distorted than in the surrounding tissues and, after compression, the harder tissue will be less distorted than the softer tissue. The consequence is that US waves will travel at a higher fast through the hard mass (**Figure 3**). Inflammation and neoplastic infiltration lead to changes in normal tissue structure causing hardening of the tissue and alteration of its elasticity (10).

Figure 3.



Elastography is broadly divided into two methodologies based on two different principles. One is “strain”, which is negatively correlated with tissue elasticity, and the other is “shear wave speed”, which is positively correlated with tissue elasticity. Currently, only strain elastography is available for use with EUS (11). Both longitudinal and radial echoendoscopes can be used for elastography, however, the former has the advantage that suspicious stiffer areas can be targeted for biopsy under direct visualization.

The elasticity modulus is a measure of ‘stress’ applied to tissue structures, relative to the ‘strain’ or deformation produced (real-time tissue elastography, RTE). Thus, by calculating the elasticity of tissue, it is possible to differentiate benign (soft) tissue from malignant (hard) tissue (12). Ultrasound elastography was previously used for the diagnosis of non-digestive as well as digestive tumors: breast lesions, prostate cancer, thyroid nodules, rectal tumors.

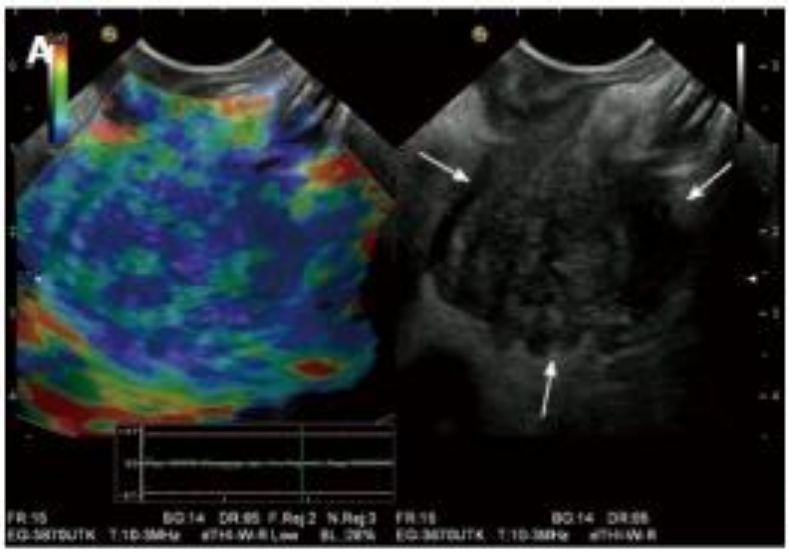
The pancreatic elastography can be performed by means of two different approaches, the percutaneous and the endoscopic. Most of the systems are set up to use a chromatic map (red–green–blue) in which hard tissue areas are shown in blue whilst soft tissue areas are displayed in red or green. The percutaneous approach is easy to perform, but has intrinsic limitations even related to the patient conformation and it is not really used. The echo-endoscopic approach is the most used nowadays for the elastographic study of the pancreas.

To obtain an elastographic image, echoendoscope is positioned and maneuvered according to the examined organs. The area to be evaluated is defined by a ROI (region of interest, color window). The size of the ROI that defines the elastographic image is very important. In fact the ROI should be sufficiently large to include both the pathological tissue under investigation and the surrounding reference tissue. The best image quality was recorded in phantom experiments when the lesion of interest covered 25%-50% of the ROI. In fact, when the ROI is too small, only the relative elasticity differences within the lesion is measured and displayed rather than the assessment of the lesion stiffness compared to the reference stiffness. Once the ROI is selected, a slight pressure can be applied by gently manipulating the probe. Normally very little additional compression is required, as the pressure difference from the pulsation of adjacent vessels is sufficient. The suitability of the elastographic signal is indicated by a numeric scale within the image. Elastographic and B-mode images are displayed simultaneously (13).

There are two types of evaluation methods for EUS-elastography: the qualitative and quantitative evaluation.

The **qualitative** method involves the evaluation of the *pattern* of the elastogram such as the *major color tone* and the *heterogeneity* of the color tones: elastography is shown in real time as transparent color images (red, green, and blue) in a region-of-interest box that is superimposed over the conventional gray-scale b-mode EUS image, similar to color Doppler images.

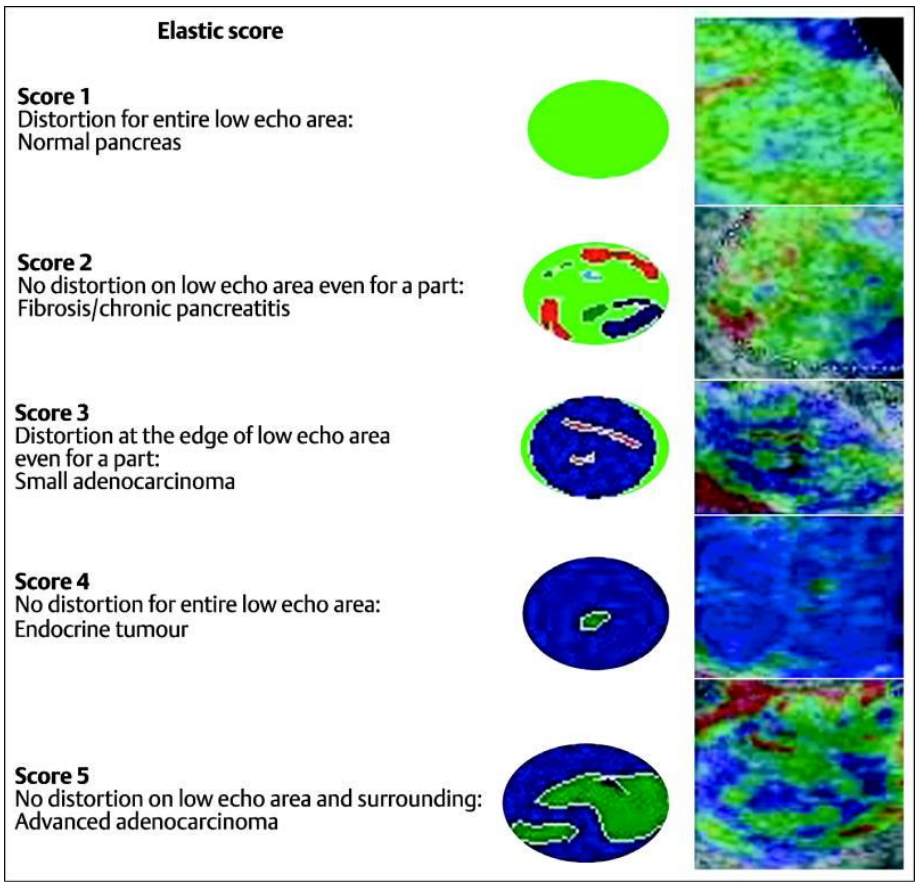
Figure 4. A pancreatic ductal adenocarcinoma is demonstrated as a heterogeneous hard (blue) pattern.



An elastic score was developed, based on the major color tone and the color tones' heterogeneity. Score 1 was for a homogeneous hypoechoic area (soft, green), corresponding to the normal pancreatic tissue. Score 2 was assigned to heterogeneous elastographic images, but still within the soft-tissue range (green, yellow, and red), corresponding to fibrosis while score 3 was assigned to elastographic images that were largely blue (hard), with minimal heterogeneity, corresponding to a small, early pancreatic adenocarcinoma (less than 25 mm in diameter). Score 4 was assigned to lesions with a hypoechoic region in the center, with a green appearance in a small area, surrounded by blue or harder tissue, and

was interpreted as typical of hypervascular lesion such as a neuroendocrine tumor or small pancreatic metastasis. Score 5 was assigned to lesions that are largely blue on the elastogram but with heterogeneity including softer tissue colors (green, red), representing necrosis, and so corresponding to advanced pancreatic adenocarcinomas (14). The score is shown in **Figure 5**.

Figure 5.



Another four score classification has been used by other authors, distinguishing homogeneous green pattern, heterogeneous green predominant pattern, heterogeneous blue predominant and homogeneous blue predominant pattern, corresponding respectively to normal pancreas, inflammatory pancreas, PA and malignant NET. (15).

Since elastographic images and movies represent a qualitative type output that entails a subjective interpretation by the examiner, human bias is always susceptible to interfere with the results and diagnoses, due to color perception errors, moving artifacts, or possible selection bias induced by the analysis of still images. More objective, computer-assisted semi-quantitative means of interpreting the results were developed, but these have the disadvantage of being labor-intensive and using third-party software that cannot be used in real time.

The **quantitative** evaluation involves image analysis techniques that are used to evaluate the characteristics of a lesion in a quantitative manner.

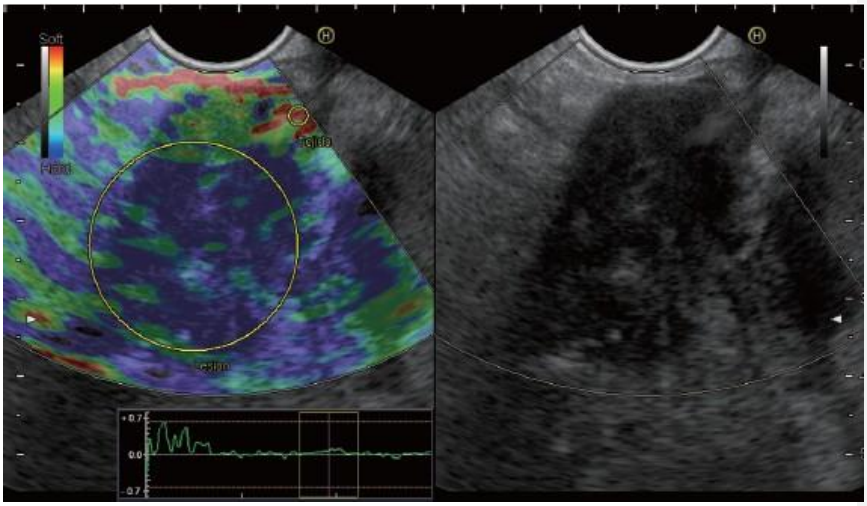
Three quantitative analysis methods have been used up to now: the strain ratio (SR), the strain histogram (SH) and the neural network. No consensus has been reached with regard to the superiority of each of these methods, but strain ratio and strain histogram remain the most used.

Because strain (deformation) is smaller in harder tissues and larger in softer tissues, the differences in strain (hardness) of the tissues can be quantified and showed in real-time sono-elastography (16).

The **SR** calculates the relative strain between two ROI, represented by the target area and a reference soft tissue area. Two different areas (A and B) are selected for quantitative elastographic analysis. Area A is selected so that it includes as much of the target lesion as possible without including the surrounding tissues. Area B is selected within a soft (red) reference area outside the target lesion, preferably the gut wall. The strain ratio is calculated as the quotient of B/A (17).

Figure 6. Several studies have been conducted, showing high but variable sensitivity and specificity of the strain ratio with a wide span of cut-off values delineating inflammatory from malignant pancreatic masses (18-20).

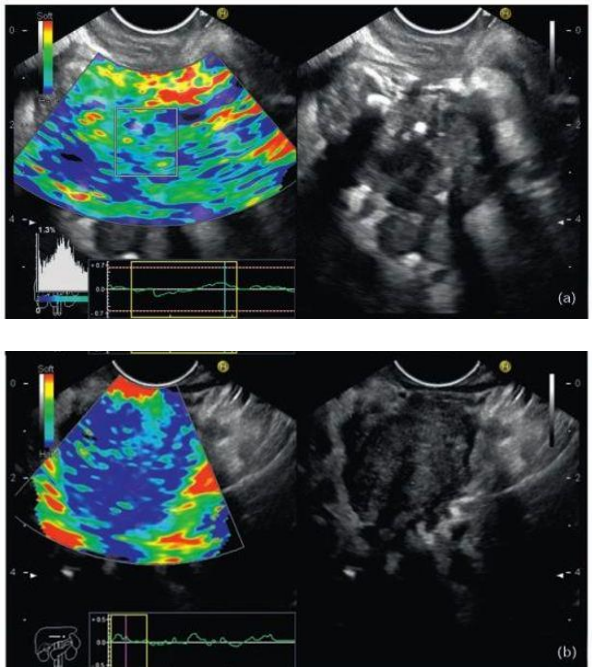
Figure 5. Quantitative EUS elastography based on strain ratio analysis of a solid pancreatic mass (in this case a pancreatic adenocarcinoma). Area A represents pancreatic parenchyma, and area B to correspond to a soft area from the gut wall. The B/A ratio is displayed at the bottom of the image.



The **SH** is the graphical representation of the color distribution (hues) in a selected image field; hue histograms are based on data for a manually selected ROI within the standard elastography image. The ROI has to be sufficiently large to contain the area under examination and enough surrounding tissue for comparison. The optimum ratio is approximately 50% lesion, 50% normal/surrounding tissue. Based on software analysis, each pixel in the ROI is displayed with a hue that represents the relative strain value (hardness) of the tissue. SH measures the strain values of elemental areas inside a ROI and divides the measurement range into intervals; if the strain value of an element falls into an interval, its initial area normalized by the initial total surface area is added to the running total of that interval; the total values of each interval are used to produce a graph and an average value.

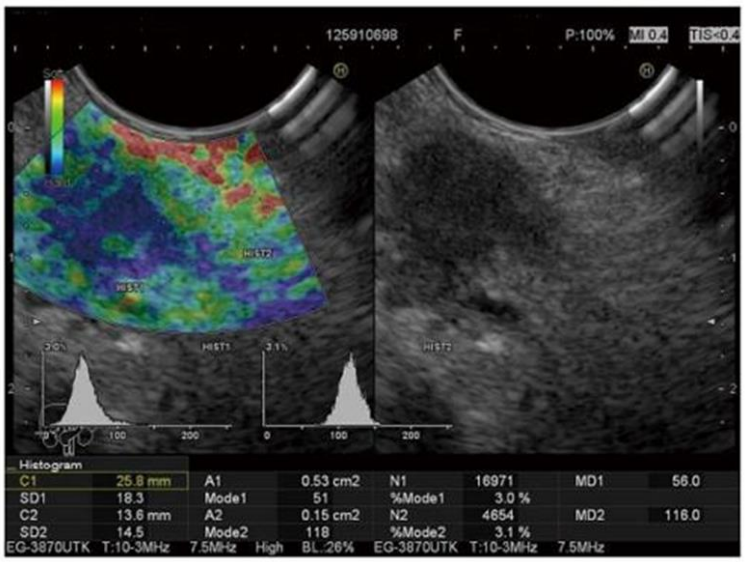
New technical developments allow averaging over several frames in order to calculate the mean hue histogram value that corresponds to global tissue elasticity within a selected area. Several studies have been conducted, showing high but variable sensitivity and specificity of the mean SH with a wide span of cut-off values delineating inflammatory from malignant pancreatic masses (6, 21, 22). An example of SH is shown in **Figure 6.**

Figure 6. Quantitative EUS elastography based on strain histogram analysis of a solid pancreatic mass (chronic pseudotumoral pancreatitis in the upper part and pancreatic adenocarcinoma in the lower part). The histogram (at the bottom of each figure) shows the elastographic colour dispersion in the region of interest and also gives the mean of elasticity (in a range from 0 to 255). In case of pseudotumoral chronic pancreatitis the aspect of hardness of at elastography is mixed (mostly green and blue), while in the case of PA is hard (blue).



A new variable was introduced by Opacic in 2015, the **strain histogram ratio** (SHR). This value was calculated by dividing the mean SH calculated over the mass and over an adjacent part of homogenous pancreatic tissue representing a reference area (23). An example of SH with SHR is shown in **Figure 7**.

Figure 7. Quantitative EUS elastography with SH ratio calculated dividing the mean SH obtained over the pancreatic mass (Mode 1) and the mean SH over an adjacent part of homogenous pancreatic tissue (Mode 2).



Aims of the study

The aim of the present study was to use the quantitative elastography (using the new technique of "strain histogram") to:

- quantify the EG pattern of normal pancreas.
- quantify the EG pattern of solid malignant pancreatic masses.
- differentiate within malignant pancreatic masses (adenocarcinoma vs NET).

Materials and methods

This was a prospective, consecutive, descriptive 18-months study comparing CE-EUS and elastography results for the characterisation of focal pancreatic masses, in comparison with the gold standard represented by pathology.

- **Inclusion criteria**
  - Patients diagnosed with solid pancreatic tumor masses, with cytological/histo-logical confirmation. Only patients presenting PA or NET were included in the final analysis. NET were defined by anatomo-pathologist according to their mitotic count and Ki67 into grade 1, grade 2 and grade 3.
  - Patients undergoing EUS-exploration during the study period because of extrapancreatic diseases and presenting with a normal pancreas at EUS were randomly selected and included as controls to describe the elastographic appearance of the healthy pancreas.
  - Age 18 to 90 years old, men or women.
  - From May 2014 to December 2015.
  - At the EUS Unit of the Department of Gastroenterology of the Institut Paoli Calmettes.
  - Signed informed consent for EG-EUS, CE-EUS and FNA biopsy.

- **Exclusion criteria**
  - Prior surgical treatment with curative intent or chemo-radiotherapy.
  - Patients diagnosed with mucin producing tumors, pancreatic cystic tumors, etc.

- **Data collection**
  - Personal data (name, surname, age, weight, admission date, diagnosis at admission).
  - Lesions (presence/absence, position and size, histology).

- **Imaging tests**

  All patients with a suspicion of pancreatic masses underwent EUS with FNA with sequential CE-EUS and EG-EUS.

  - **EUS** was performed with linear instrument with complete examinations of the pancreas. Tumor characteristics (echogenicity, echostructure, size) were described as well as presence/absence of power Doppler signals. EUS-FNA was performed in all pancreatic masses with at least three passes.
  - **CE-EUS** was performed with a two panel image (conventional gray-scale B-mode EUS image on the right side and contrast harmonic image on the left side).

The starting point of the timer was considered the moment of intravenous contrast injection (Sonovue 4.8 mL). The whole movie of one minute (T0-T60s) was recorded on the embedded HDD of the ultrasound system, for later analysis.

We defined the enhancement as "typical of PA" when lesion appeared as hypovascularized, "typical of NET" when lesions appeared as hypervascularized and atipycal when the presented a weekly enhancement in the early phase (from 10 to 30 seconds from the injection of SonoVue and flushing).

- **EUS-EG** was performed during usual EUS examinations, with three movies of 10 seconds recorded on the embedded HDD in order to minimize variability and to increase repeatability of acquisition.

  A two panel image with the usual conventional gray-scale B-mode EUS image on the right side and with the elastography image on the left side was used.

  The region of interest for EUS-EG was chosen as preferably larger than the focal mass (approximately 50%-50%), in order to include the surrounding structures. If the focal mass was larger than 3 cm, part of the mass was included in the ROI, as well as the surrounding structures (preferably avoiding large vessels). Very large ROI for the elastography calculations were avoided due to the risk of appearance of side artifacts.

The following pre-settings were used: elastography colour map 1, frame rejection 2, noise rejection 2, persistence 3, dynamic rage 4, smoothing 2, blend 50%.

Strain histograms (SH) was measured; with three measurements made and recorded on the embedded HDD. The following 4 parameters were calculated using the histogram to quantify the elasticity of the pancreas: **mean** (the arithmetic mean on this histogram, with a higher value indicating soft tissue), classified in a scale from 0 to 255; **standard deviation** (a measure of the spread of values on the histogram, with a higher value indicating heterogeneous hardness); **skewness** (a measure of the asymmetry of the histogram, with a higher value indicating hard tissue) and **kurtosis** (a measure of the ‘‘peakedness’’ of the histogram, with a higher value indicating a concentration of specific hardness)

Interpretation of strain histogram

The **x-axis** in a hue histogram represents tissue elasticity expressed numerically, and the height of the spikes displayed in the **y-axis** indicates the number of pixels of each elasticity level found in the ROI.

- **Final diagnosis**

Histology of surgical specimens was considered as the reference method in operated cases.

A positive cytology for malignancy and compatible EUS and CT imaging were considered the reference method in unresectable tumors.

Statistical analysis

All results were expressed as mean ± standard deviation (SD).

Differences between the patients with pancreatic cancer and normal pancreas were performed by the two-sample *t*-test (two independent samples). Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of EG-EUS were determined in comparison with the final diagnosis.

Diagnostic accuracy of quantitative EUS–elastography based on strain histogram for detecting malign lesions was calculated after drawing the corresponding receiver operating characteristic (ROC) curve analysis, with specific values for the area under the curve, the significance of the area, and confidential intervals.

Based on ROC curve analysis, a cut-off point was selected as the point of the curve closest to the upper left corner of the graphic, where sensitivity and specificity are equal to 1.0.

Sensitivity (SS), specificity (SP), positive and negative predictive values, and positive and negative likelihood ratios are also expressed in the analysis.

The calculation of the overall accuracy (ACC) of the prediction was based on previous parameters.

The positive likelihood ratio is calculated as sensitivity/1 - specificity. The negative likelihood ratio is calculated as 1- specificity/sensitivity.

The overall accuracy is calculated as the sum of the positive and negative predictive values.

Statistical significance is set to $p < 0.05$, with all confidence intervals expressed at the 95% level.

Results

- **Patients' characteristics**

A total of 80 patients were included in the study. Of these only 63 were included in the statistical analysis. The other patients were excluded because they presented pancreatic lesions different from PA and NET (intrapapillary mucinous neoplasia, lesions of chronic pancreatitis, others).

Patient's characteristics are described in **Table 2**.

Table 2: Patients caracteristics

	All (n=63)	**Normals pancreas (n=9)**	**Pancreatic adenocarcinoma (n=45)**	**Pancreatic NET (n=9)**
Sex, M (%)	32	4	25	3
Age, mean (range)	64 (19-88)	51 (19-69)	68(48-88)	57(20-76)
Height, mean (range): m	1,6 (1,5-1,8)	1,6 (1,5-1,7)	1,6 (1,5-1,8)	1,6 (1,5-1,8)
Weight, mean (range): Kg	70 (44-128)	80 (48-103)	68 (44-128)	72 (58-110)
BMI, mean (range)	25 (16-43)	28 (19-40)	24 (16-41)	25 (19-43)
Smoking, number (%)	14	0	13	1
Alcool, number (%)	3	0	3	0
Lesion position:		n.a.		
head	20		19	1
body	22		18	4
tail	12		8	4
Lesion max diameter, mean (range)	35 (9-76)	n.a.	36 (15-60)	31 (9-76)

In 4 patients, these was the second (or more) EUS + FNA, due to non-conclusive or false negative results in previous FNA.

Within PA group, 4 patients presented, according to TNM staging, a stage 1 PA, 5 patients a stage 2a PA, 2 patients a stage 2b PA, 7 patients a stage 3 PA and the last 27 patients a stage 4 PA.

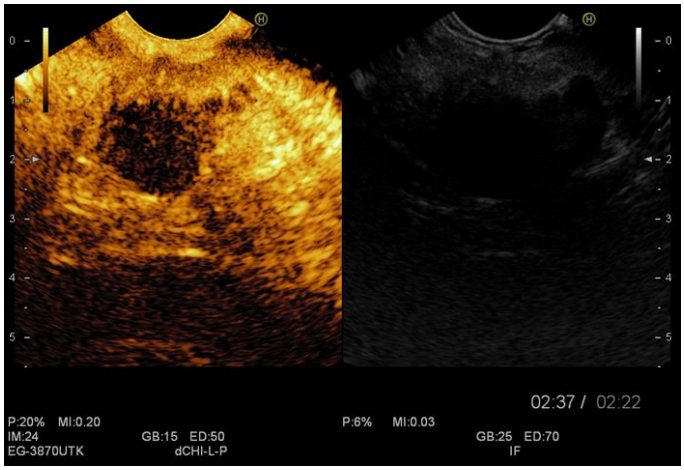
Within NET group, 4 patients presented at histology a grade 1 tumor, while 5 patients a grade 2/3.

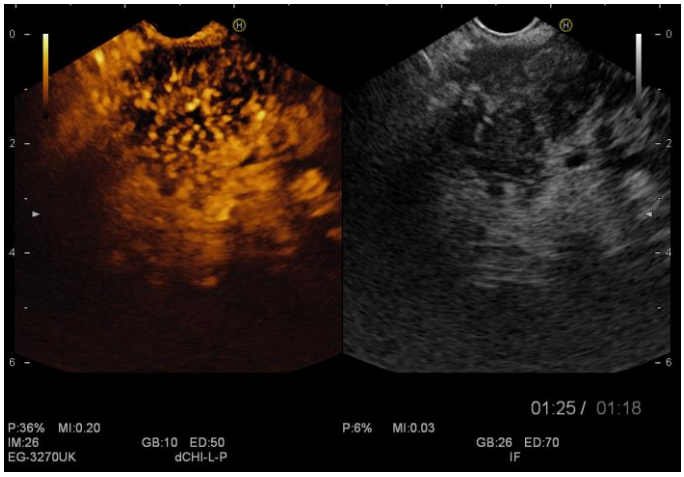
- **CE-EUS**

CE-EUS was performed in all the 54 patients presenting a pancreatic mass. Enhancement behavior was compared with histological results. Between patients with a histological diagnosis of PA, 15% presented an atypical enhancement for PA. All patients with NET presented a "typical for NET" enhancement (**Figure 8**). Results are shown in **Table 3**.

Table 3: CE-EUS. Pancreatic adenocarcinoma vs. P-NET			
	All (n=54)	**Pancreatic adenocarcinoma (n=45)**	**Pancreatic NET (n=9)**
Hypoenhanced: typical of pancreatic adenok	38	38	0
Hyperenhanced: typical of NET	14	5	9
Atypical	2	2	0

Figure 8. A typical PA (on the top) and NET (on the bottom) behavior at CE-EUS.

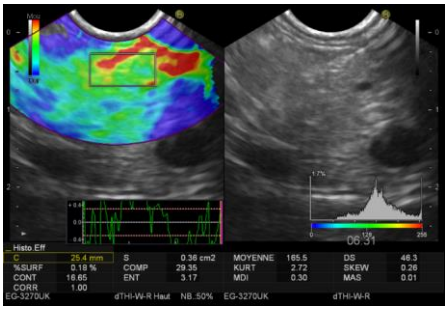


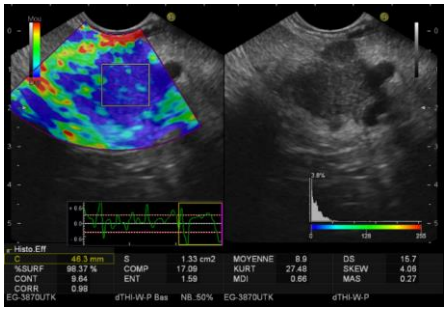

0
2
4
6
01:25 / 01:18
P:36% MI:0.20
IM:26
EG-3270UK
GB:10 ED:50
dCHI-L-P
P:6% MI:0.03
GB:26 ED:70
IF

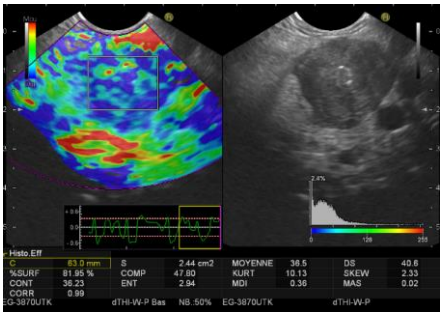
- **Elastrography**

Elastography was performed in all patients. A strain histogram of a patients with normal pancreas, of a patients with NET and of a patient with PA are shown in **Figure 9.**

Figure 9. Normal pancreas (top), PA (middle) and NET (bottom) aspect are shown.







- **Comparison between normal pancreas and malignant pancreatic masses**

A comparison between normal pancreas and malignant pancreatic masses (PA + NET grade 2/3) was performed, showing a statistically significant difference in all the parameters examined. Data are shown in **Table** 4

Table 4. EUS-EG. Normal pancreas vs. Malignant pancreatic masses.			
Paramethers	**Normal pancreas**	**Malignant pancreatic lesions (adenocarcinoma + NET 2/3)**	**p-values**
Mean SH	95.2	30.04	<0.001
Standard Deviation	51.31	34.98	<0.001
Skewness	0.78	2.19	<0.001
Kurtosis	4.16	10.12	<0.001

The univariate analysis was performed, and the diagnostic accuracy of all the quantitative EUS–elastographic measured was calculated. Data are shown on

Table 5.

Table 5: EUS-elastography. Normal pancreas vs malignant pancreatic masses. Univariate analysis

Parametheres	Odds ratio [CI 95%]	AUC [CI 95%]	Cut-off	p-values
Mean SH	0.928 [0.890 ; 0.967]	0.933 [0.869 ; 0.997]	43.250	0.0004
Standard Deviation	0.906 [0.852 ; 0.963]	0.813 [0.709 ; 0.918]	44.633	0.0015
Skewness	7.316 [2.573 ; 20.804]	0.876 [0.762 ; 0.991]	1.505	0.0002
Kurtosis	1.616 [1.199 ; 2.178]	0.882 [0.766 ; 0.998]	5.080	0.0016

A cut-off was calculated for each measure to differentiate between malignant and benign pancreatic masses, showing to be statistically significant. Data are shown on **Figures 10-13**.

Figure 10. Comparison between normal pancreas and malignant pancreatic masses, Mean SH

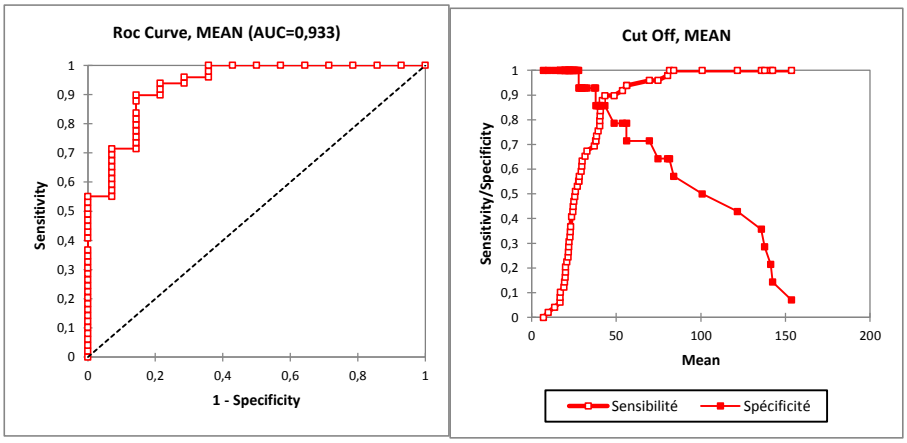


Figure 11. Comparison between normal pancreas and malignant pancreatic masses, Standard Deviation SH

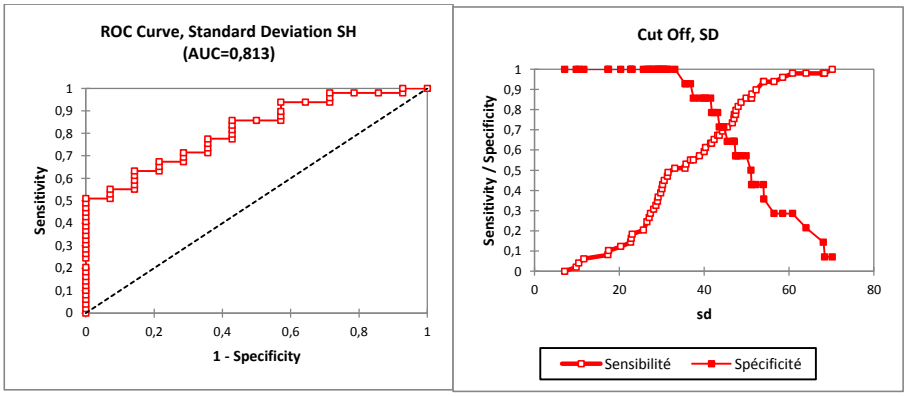


Figure 12. Comparison between normal pancreas and malignant pancreatic masses, Shewness SH

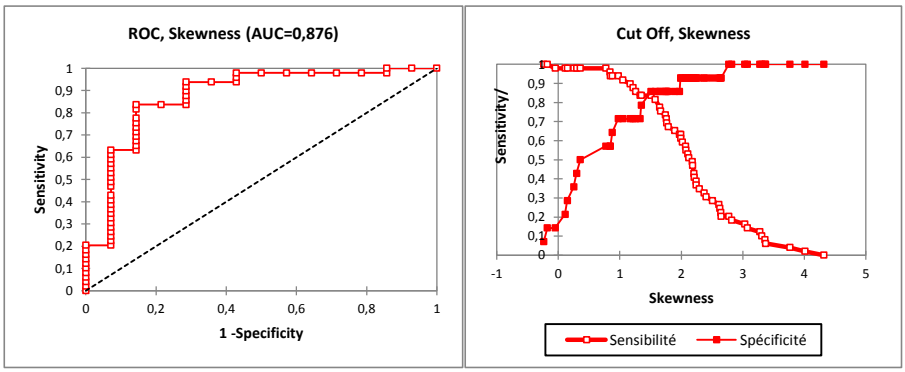
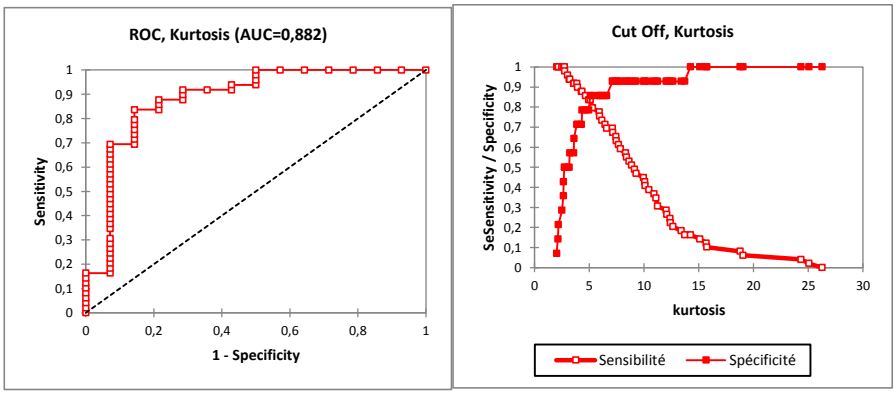


Figure 13. Comparison between normal pancreas and malignant pancreatic masses, Kurtosis SH



At multivariate analysis only the mean SH resulted as statistically capable to differentiate between benign and malignant pancreatic masses. Data are shown on **Table 6**.

Table 6: EUS-elastography. Normal pancreas vs malignant pancreatic masses. Multivariate analysis		
Paramethers	**Odds ratio [CI 95%]**	**p-values**
Mean SH	0.728 [0.550 ; 0.965]	0.0270
Standard Deviation	1.348 [0.988 ; 1.841]	0.0598
Skewness	<0.001 [<0.001 ; 11.141]	0.2027
Kurtosis	2.211 [0.652 ; 7.495]	0.1439

At logistic regression a statistical model was constructed to differentiate normal pancreas and malignant pancreatic masses.

The model is the following:

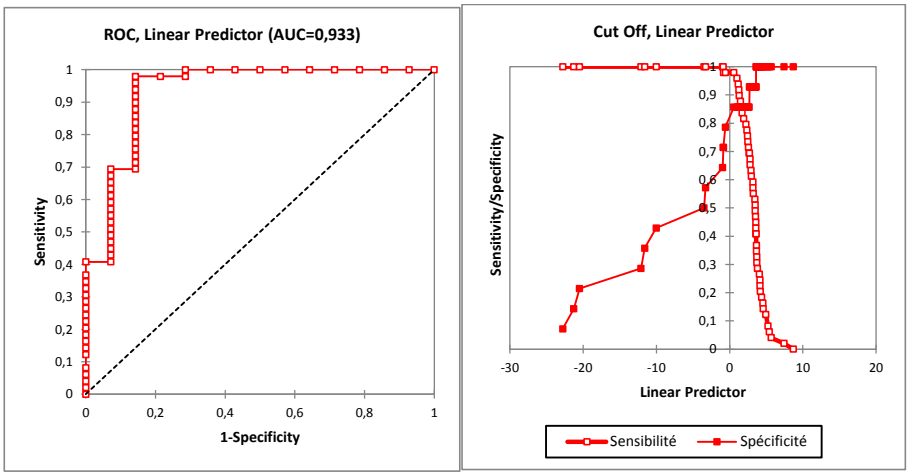
9.8958 – 0.3170*mean + 0.2989*SD + 0.7935*kurtosis – 7.0642*skewness.

AUC: 0.933 [0.850 ; 1.000]

A cut-off of 0.570 was detected, with lower or equal values corresponding to benign pancreas and higher value corresponding to malignant pancreas.

Using this model, we found a sensibility, specificity and accuracy of 95.23%, 97.96% and 85.71% respectively. Data are shown on **Figure 12.**

Figure 12. Linear Predictor



- **Comparison within malignant pancreatic masses (PA and NET)**

A comparison within malignant pancreatic masses was therefore performed, showing no statistically significant differences to distinguish PA from malignant NET. Data are shown on **Table 7.**

Table 7. EUS-EG. PA vs. Malignant p-NET.			
Paramethers	**Pancreatic adenocarcinoma**	**Malignant NET (grade 2/3)**	**p-values**
Mean SH	29.94	31.10	0.63
Standard Deviation	35.28	31.62	0.72
Skewness	2.25	1.56	0.307
Kurtosis	10.35	7.51	0.36

A cut-off was calculated for each measure to differentiate within malignant PA and NET, showing to be not statistically significant. Data are shown on **Figures 13-16**.

Figure 13. Comparison within malignant pancreatic masses (PA and NET grade 2/3), Mean SH.

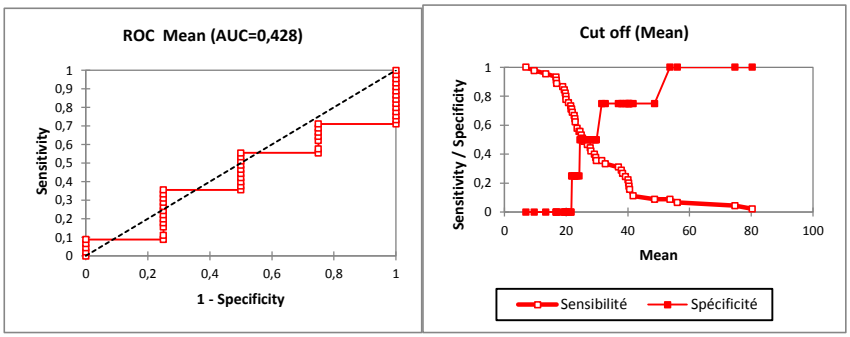


Figure 14. Comparison within malignant pancreatic masses (PA and NET grade 2/3), SD SH.

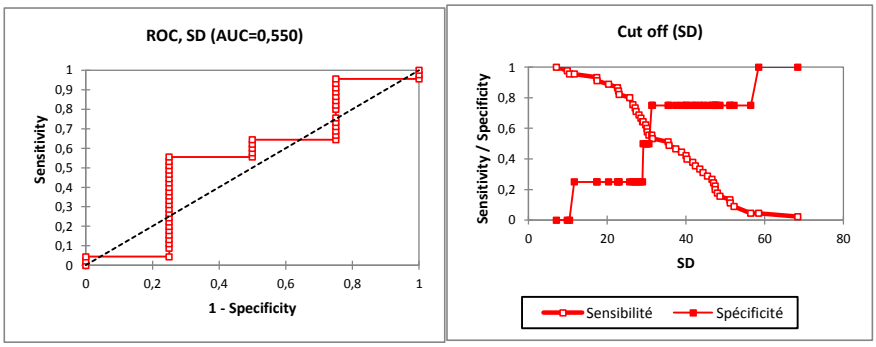


Figure 15. Comparison within malignant pancreatic masses (PA and NET grade 2/3), Skewness SH.

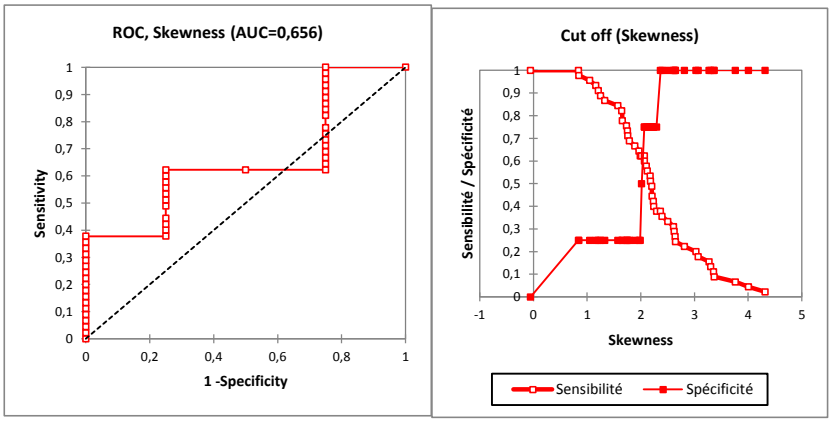
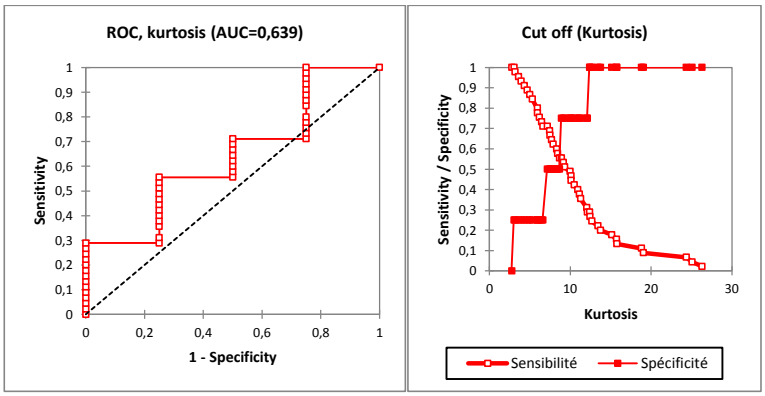


Figure 16. Comparison within malignant pancreatic masses (PA and NET grade 2/3), Kurtosis SH.



Conclusions

EUS represents one of the most reliable and accurate techniques for the diagnosis and staging of pancreatic cancer. However, the differential diagnosis of solid pancreatic masses remains a challenge in clinical work.

For that reason, EUS-FNA has been introduced, obtaining high specificity (close to 100%) and good sensibility (from 80 to 85%). On the other way, EUS-FNA is associated with several difficulties such as the prolonged learning curve and the need, in same case, of several punctures to obtain an adeguate sample. Furthermore EUS-FNA can be associated to iatrogenic complications, such as acute iatrogenic pancreatitis, bleeding and infections.

CE-EUS has improved the differential diagnosis within malignant pancreatic masses. Several studies have been conducted to evaluate the efficacy of CE-EUS in the diagnosis of pancreatic neoplasia.

For PA, studies showed a sensitivity of 91%, a specificity of 93% with positive and negative predictive value of 100 and 88% respectively (7).

CE-EUS is very advantageous for the detection of NET, that can present variably as hypo to iso to hyper-echoic. Classically, these tumors are hyper-vascular, therefore clearly visible after the contrast administration as a hyper-enhancing mass at the arterial phase. The presence of filling defects within an enhancing pancreatic lesion frequently corresponds to hemorrhage or necrosis of malignant diseases at pathological examination. This may have a potential role in differentiating benign versus malignant NET. The ability to distinguish a NET by

imaging, without the need for FNA, is useful for several reasons. In fact, therapeutic enucleation procedures may be hampered if FNA is performed. Secondly, adequate tissue acquisition may be difficult in small lesions. The decision for or against FNA depends on the single patient, as there may be prognostic implications to cytology results (24).

Differential diagnosis between PA and NET at CE-EUS remains still difficult. Even in our experience, we found that 15.5% of patients with histological diagnosis of PA presented a partial enhancement or a hyper-enhancement at arterial phase.

The limitations of FNA and CE-EUS have required the development of new techniques.

For that reason EUS-elastography has been developed, at the beginning as a qualitative technique and now as a quantitative measure.

The first study on **qualitative** elastometry was published in 2006 by Giovannini et al. In this study 24 patients with a pancreatic mass and 25 patients with lymph nodes underwent EUS examination and elastography. Masses or lymph nodes that appeared mostly blue (harder) were considered to be malignant, while others were regarded as benign. The final diagnosis was based on the histological assessment of the FNA samples and surgical specimens, when available. The sensitivity and specificity of elastography in the diagnosis of malignant lesions were 100% and 67%, respectively. In the same study the author developed a five score classifications, based on elastographic findings, able to

distinguish between normal pancreas, fibrotic pancreas (corresponding to chronic pancreatitis), neuro endocrine tumors and initial or advanced pancreatic adenocarcinoma (14).

In a subsequent multi-centric study published by the same author, the sensitivity, specificity, positive and negative predictive values of qualitative EUS elastography to differentiate benign from malignant pancreatic masses were evaluated, resulting 92.3%, 80.0%, 93.3% and 77.4% respectively, with a global accuracy of 89.2%. The authors affirmed that the goal of elastography was not to replace citologycal or histological confirmation. Instead, the information obtained could be complementary to the conventional EUS imaging and could orientate further exploration rather than immediate surgery or conservative management in case of repeated negative EUS-FNA (25).

Another 4 variables score (based on colour predominance pattern) was created by other authors. Based on this score the diagnostic sensitivity, specificity and overall accuracy of EUS elastography for diagnosing malignancy corresponded to 100%, 85.5% and 94%, respectively (15).

Similar studies were conducted showing a sensitivity between 41 and 100%, a specifity between 53 and 85%, a positive and negative predictive values around 90% and an accuracy between 45 and 94% (15, 26-28). In particular results from a study by Janssen et al were particularly disappointing, and the authors concluded that chronic pancreatitis and hard malignant tumors cannot be distinguished by elastography, probably due to their similar fibrous structure (26). On the contrary,

a more recent study conducted by Iglesias-Garcia et al. showed a very good correlation between observers by analyzing the videos recorded from 258 patients with chronic pancreatitis and pancreatic cancer (21). A review of the literature regarding qualitative elastometry is shown in **Table 8**.

Table 8. Review of the litterature. QUALITATIVE elastography.

		N of total patients	N of patients with pancreatic masses	Sensitivity	Specificity	PPV	NPV	Accuracy	Interobserver agreement (k)
Giovannini M, Endoscopy 2006	monocentric	49	24	100	67	n.a.	n.a.	n.a.	n.a.
Janssen J, Gastrointestin Endosc 2007	monocentric	73	33	93,8	65,4	n.a.	n.a.	73,5	n.a.
Hirche TO, Endoscopy 2008	monocentric	80	70	41	53	n.a.	n.a.	45	n.a.
Giovannini M, World Journal Gastroenterology 2009	multicentric	222	(72 adenocarcinoma, 16 NET, 3 metastatic tumours, 30 chronic pancreatitis)	92,3	80	93,3	77,4	89,2	0,785
Iglesias-Garcia J, Gastrointest Endosc, 2009	monocentric	150	130	100	85,5	90,7	100	94	0,772

Considering the limitations related to the qualitative, mostly due to perception errors and the inability of the human eye to completely characterize all color hues, the **quantitative** elastometry has been introduced.

The first study was published by Saftoiu et al in 2008. Sixty-height patients were enrolled, of whom 22 presented a normal pancreas and the others chronic pancreatitis (11), PA (32) and NET (3). A post-processing software analysis was used to examine the EUS elastography movies by calculating the strain histograms mean value of each individual image. Data that were further subjected to an extended **neural network analysis** to differentiate benign from malignant patterns. Based on a cutoff of 175 for the mean strain histogram value recorded on the region of interest, the sensitivity, specificity, and accuracy of differentiation of benign and malignant masses were 91.4%, 87.9%, and 89.7%, respectively. The positive and negative predictive values were 88.9% and 90.6%, respectively (22).

Others studies used the mean **strain histogram** value, showing a sensitivity between 75 and 91%, a specificity between 78 and 95%, a positive and negative predictive values around 90% and 70% respectively and an accuracy between 79 and 89% (6, 21).

The first study using **strain ratio** was conducted by Iglesias-Garcia in 2010. One-hundred and six patients were enrolled, of whom 49 presented pancreatic adenocarcinoma (49), and the others inflammatory mass (27), malignant NET (6), metastatic oat-cell lung cancer (2), pancreatic lymphoma (1),

and pancreatic solid pseudopapillary tumor (1). The strain ratio was significantly higher among patients with pancreatic malignant tumors compared with those with inflammatory masses. The sensitivity and specificity of strain ratio for detecting pancreatic malignancies were 100% and 92.9%, respectively (area under the receiver operating curve, 0.983) (29).

Others studies used the strain ratio, with cut off ranging from 3.17 to 6.04, showing a sensitivity between 86 and 100%, a specificity between 33 and 92%, a positive and a negative predictive values of 89% and 60% respectively and an accuracy of 81% (19, 20, 30, 31).

In 2015 Opacic et al. introduced the SH ratio (SHR), a new variable calculated by dividing the mean SH over a lesion and the mean SH over a reference area. The traditional mean SH showed in this study a high sensitivity in pancreatic malignant tumor detection but disappointingly low specificity. Slight improvements in specificity and accuracy were achieved using the SHR. The SHR reached 98% sensitivity, 50% specificity and an overall accuracy of 69% (95%CI: 63%-70%). The positive and negative predictive values were 92% and 100%, respectively (23).

A review of the literature regarding quantitative elastometry is shown in **Table 9**.

Table 9: Review of the litterature. QUANTITATIVE elastography.

		Evaluation	N of total patients	N of patients with pancreatic masses	Sensitivity	Specificity	PPV	NPV	Accuracy	Interobserver agreement (k)	notes
Saftoiu A, Gastrointestinal Endoscopy 2008	monocentric	neural network analysis + strain histogram (cut off: 175)	68	35 (32 adenocarcinoma 3 NET)	91,4	87,9	88,9	90,6	89,7	n.a.	
Iglesias-Garcia J, Gastroenterology, 2010	monocentric	strain ratio (cut off: 6,04)	86	86 (49 adenocarcinoma, 27 inflammatory mass, 6 NET 6 mts, 1 lymphoma, 1 solid pseudopapillary tumor)	100	92,9	n.a.	n.a.	n.a.	n.a.	
Saftoiu A, Gastrointestinal Endoscopy 2010	monocentric	contrast-enhanced PDVI (cut off 20%) + strain histogram (cut off: 175)	54	54 (33 adenocarcinoma 21 chronic pancreatitis)	75,8	95,2	96,2	71,4	83,3	n.a.	pseudotumoral pancreatitis and pancreatic cancer
Itokawa F, J Gastroenterol 2011	monocentric, retrospective	strain ratio	109	81 (72 adenocarcinoma, 9 NET)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
Saftoiu A, Endoscopy 2011	multicentric, prospective	strain histogram (cut off strain histogram: 185)	258	258 (211 adenocarcinoma, 47 chronic pancreatitis)	79,2	78,7	94,4	45,7	79,1	n.a.	pseudotumoral pancreatitis and pancreatic cancer
Dawwas MF, Gastrointestinal Endoscopy 2012	monocentric	strain ratio (cut off: 6,45)	104	104 (74 adenocarcinoma, 11 NET, 2 mts, 17 pancreatitis)	100	16,7	86,1	100	86,5	n.a.	
Hocke M, Z Gastroenterol 2012	monocentric	?	58	58 (19 adenocarcinoma 39 chronic pancreatitis)	94,7	33,4	n.a.	n.a.	n.a.	n.a.	pseudotumoral pancreatitis and pancreatic cancer
Lee Th, Clin Endosc 2013	monocentric	strain ratio (no cut off)	35	15 (14 adenocarcinoma 1 pseudocyst)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	between pancreatic masses and controls pancreatitis and pancreatic cancer
Dyrla P, Prz Gastroenterol. 2015	monocentric	n.a.	80	54 (34 adenocarcinoma, 20 pancreatic pseudotumors)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
Kongkam P, J Gastroenterol Hepatol 2015	monocentric	strain ratio (cut off: 3,17)	38	38 (23 adenocarcinoma, 5 NET, 1 MTS, 2 pancreatic pseudotumor, 3 AIP, 4 others)	86,2	66,7	89,3	60	81,6	n.a.	
Opacic D, World J Gastroenterol 2015	monocentric	strain histogram ratio (cut off : 1,153)	149	105 (58 adenocarcinoma, 47 pancreatic pseudotumor)	98	50	92	100	69	n.a.	

In our study we evaluated the capacity of 4 derived SH values (mean SH, standard deviation, skewness and kurtosis), singularly and combined, to differentiate between normal pancreas and malignant pancreatic masses. At univariate analysis all the 4 different parameters resulted statistically effective in the differential diagnosis. At multivariate analysis only mean SH reached the statistically difference, confirming the results of previous studies.

We even evaluated if SH variables were capable to differentiate within malignant pancreatic masses (between PA and NET), but results were non statistically significant at both univariate and multivariate analysis. We believe that our results could have been influenced by the limited number of NET enrolled in the study and that this statement should be confirmed by a larger size study.

The real innovation of our study is the construction of a statistical model that integrated the 4 quantitative variables obtained by SH to differentiate malignant pancreatic masses from normal pancreas. We obtained a cut-off of 0.57. This cut-off seems to be capable to distinguish benign pancreas and malignant pancreatic masses, with a very high sensitivity and specificity.

The main limitation of our study was, as already said, the low number of patients enrolled with NET. This is absolutely concordant with the real life, where NET account for only 1% of pancreatic cancer by incidence and PA as 90%, and with population of previous published studies. We decided not to include in our study degenerated IPMNs or other cystic malignant lesions due to their appereance at elastography as artifact, *i.e.*, BGR (blue-green-red) artifact. Unlikely, the use of

elastography for IPMNs seems limited, but the number of EUS + FNA for IPMNs degeneration's surveillance is increasing in parallels with the increased discover of these lesions.

Based on the results of our study, a strong suspicion of pancreatic cancer should be retained for values of the strain histogram multivariate model analysis higher than 0.57. We believe that actual knowledge on elastography is still insufficient to replace FNA or FNB with this technique. Therefore we believe that EUS-EG should be used in case of patients with pancreatic masses but multiple negative EUS–FNA (up to 25% of FNA), and/or atypical CE-EUS (hyper-enhancement or late enhancement).

We propose this model: patients with negative EUS–FNA (and eventually an atypical CE-EUS) and value lower than 0.57 should be followed up; patients with negative EUS–FNA (and eventually an atypical CE-EUS) and value higher than 0.57 should repeat EUS–FNA and/or undergo surgery.

Our results should be validated by a multicentre high volume study, even comparing benign and malignant pancreatic masses.

Future developments of EUS elastography for pancreatic related pathologies concern the elastography-guided biopsy of the pancreas and the cancer staging Evenmore EUS elastography is very sensitive in detecting even very small lesions and could improve the accuracy of EUS +/- CE-EUS. The additional value of elastography combined with other techniques such as contrast-

enhanced endoscopic ultrasound (CE-EUS), fusion imaging or 3D elastography examinations might also be feasible.

References

1. Canto MI, Hruban RH, Fishman EK, Kamel IR, Schulick R, Zhang Z, et al. Frequent detection of pancreatic lesions in asymptomatic high-risk individuals. Gastroenterology. 2012;142(4):796-804; quiz e14-5.

2. Committee ASoP, Eloubeidi MA, Decker GA, Chandrasekhara V, Chathadi KV, Early DS, et al. The role of endoscopy in the evaluation and management of patients with solid pancreatic neoplasia. Gastrointestinal endoscopy. 2016;83(1):17-28.

3. Claudon M, Cosgrove D, Albrecht T, Bolondi L, Bosio M, Calliada F, et al. Guidelines and good clinical practice recommendations for contrast enhanced ultrasound (CEUS) - update 2008. Ultraschall in der Medizin. 2008;29(1):28-44.

4. Piscaglia F, Nolsoe C, Dietrich CF, Cosgrove DO, Gilja OH, Bachmann Nielsen M, et al. The EFSUMB Guidelines and Recommendations on the Clinical Practice of Contrast Enhanced Ultrasound (CEUS): update 2011 on non-hepatic applications. Ultraschall in der Medizin. 2012;33(1):33-59.

5. Dietrich CF, Ignee A, Braden B, Barreiros AP, Ott M, Hocke M. Improved differentiation of pancreatic tumors using contrast-enhanced endoscopic ultrasound. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2008;6(5):590-7 e1.

6. Saftoiu A, Iordache SA, Gheonea DI, Popescu C, Malos A, Gorunescu F, et al. Combined contrast-enhanced power Doppler and real-time

sonoelastography performed during EUS, used in the differential diagnosis of focal pancreatic masses (with videos). Gastrointestinal endoscopy. 2010;72(4):739-47.

7. Hocke M, Schulze E, Gottschalk P, Topalidis T, Dietrich CF. Contrast-enhanced endoscopic ultrasound in discrimination between focal pancreatitis and pancreatic cancer. World journal of gastroenterology. 2006;12(2):246-50.

8. Dietrich CF, Sharma M, Hocke M. Contrast-enhanced endoscopic ultrasound. Endoscopic ultrasound. 2012;1(3):130-6.

9. Frey H. [Realtime elastography. A new ultrasound procedure for the reconstruction of tissue elasticity]. Der Radiologe. 2003;43(10):850-5.

10. Pedrosa MC, Barth BA, Desilets DJ, Kaul V, Kethu SR, Pfau PR, et al. Enhanced ultrasound imaging. Gastrointestinal endoscopy. 2011;73(5):857-60.

11. Ohno E, Kawashima H, Hashimoto S, Goto H, Hirooka Y. Current status of tissue harmonic imaging in endoscopic ultrasonography (EUS) and EUS-elastography in pancreatobiliary diseases. Digestive endoscopy : official journal of the Japan Gastroenterological Endoscopy Society. 2015;27 Suppl 1:68-73.

12. Garra BS, Cespedes EI, Ophir J, Spratt SR, Zuurbier RA, Magnant CM, et al. Elastography of breast lesions: initial clinical results. Radiology. 1997;202(1):79-86.

13. Cui XW, Chang JM, Kan QC, Chiorean L, Ignee A, Dietrich CF. Endoscopic ultrasound elastography: Current status and future perspectives. World journal of gastroenterology. 2015;21(47):13212-24.

14. Giovannini M, Hookey LC, Bories E, Pesenti C, Monges G, Delpero JR. Endoscopic ultrasound elastography: the first step towards virtual biopsy? Preliminary results in 49 patients. Endoscopy. 2006;38(4):344-8.

15. Iglesias-Garcia J, Larino-Noia J, Abdulkader I, Forteza J, Dominguez-Munoz JE. EUS elastography for the characterization of solid pancreatic masses. Gastrointestinal endoscopy. 2009;70(6):1101-8.

16. Dietrich CF, Saftoiu A, Jenssen C. Real time elastography endoscopic ultrasound (RTE-EUS), a comprehensive review. European journal of radiology. 2014;83(3):405-14.

17. Iglesias-Garcia J, Lindkvist B, Larino-Noia J, Dominguez-Munoz JE. Endoscopic ultrasound elastography. Endoscopic ultrasound. 2012;1(1):8-16.

18. Iglesias-Garcia J, Larino-Noia J, Abdulkader I, Forteza J, Dominguez-Munoz JE. Quantitative endoscopic ultrasound elastography: an accurate method for the differentiation of solid pancreatic masses. Gastroenterology. 2010;139(4):1172-80.

19. Itokawa F, Itoi T, Sofuni A, Kurihara T, Tsuchiya T, Ishii K, et al. EUS elastography combined with the strain ratio of tissue elasticity for diagnosis of solid pancreatic masses. Journal of gastroenterology. 2011;46(6):843-53.

20. Dawwas MF, Taha H, Leeds JS, Nayar MK, Oppong KW. Diagnostic accuracy of quantitative EUS elastography for discriminating malignant from benign solid pancreatic masses: a prospective, single-center study. Gastrointestinal endoscopy. 2012;76(5):953-61.

21. Saftoiu A, Vilmann P, Gorunescu F, Janssen J, Hocke M, Larsen M, et al. Accuracy of endoscopic ultrasound elastography used for differential diagnosis of focal pancreatic masses: a multicenter study. Endoscopy. 2011;43(7):596-603.

22. Saftoiu A, Vilmann P, Gorunescu F, Gheonea DI, Gorunescu M, Ciurea T, et al. Neural network analysis of dynamic sequences of EUS elastography used for the differential diagnosis of chronic pancreatitis and pancreatic cancer. Gastrointestinal endoscopy. 2008;68(6):1086-94.

23. Opacic D, Rustemovic N, Kalauz M, Markos P, Ostojic Z, Majerovic M, et al. Endoscopic ultrasound elastography strain histograms in the evaluation of patients with pancreatic masses. World journal of gastroenterology. 2015;21(13):4014-9.

24. Mohamed RM, Yan BM. Contrast enhanced endoscopic ultrasound: More than just a fancy Doppler. World journal of gastrointestinal endoscopy. 2010;2(7):237-43.

25. Giovannini M, Thomas B, Erwan B, Christian P, Fabrice C, Benjamin E, et al. Endoscopic ultrasound elastography for evaluation of lymph nodes and pancreatic masses: a multicenter study. World journal of gastroenterology. 2009;15(13):1587-93.

26. Janssen J, Schlorer E, Greiner L. EUS elastography of the pancreas: feasibility and pattern description of the normal pancreas, chronic pancreatitis, and focal pancreatic lesions. Gastrointestinal endoscopy. 2007;65(7):971-8.

27. Hirche TO, Ignee A, Barreiros AP, Schreiber-Dietrich D, Jungblut S, Ott M, et al. Indications and limitations of endoscopic ultrasound elastography for evaluation of focal pancreatic lesions. Endoscopy. 2008;40(11):910-7.

28. Dietrich CF, Hirche TO, Ott M, Ignee A. Real-time tissue elastography in the diagnosis of autoimmune pancreatitis. Endoscopy. 2009;41(8):718-20.

29. Iglesias-Garcia J, Larino-Noia J, Dominguez-Munoz JE. [Elastography in the evaluation of chronic pancreatitis]. Gastroenterologia y hepatologia. 2011;34(9):629-34.

30. Lee TH, Cho YD, Cha SW, Cho JY, Jang JY, Jeong SW, et al. Endoscopic ultrasound elastography for the pancreas in Korea: a preliminary single center study. Clinical endoscopy. 2013;46(2):172-7.

31. Kongkam P, Lakananurak N, Navicharern P, Chantarojanasiri T, Aye K, Ridtitid W, et al. Combination of EUS-FNA and elastography (strain ratio) to exclude malignant solid pancreatic lesions: A prospective single-blinded study. Journal of gastroenterology and hepatology. 2015;30(11):1683-9.