New approaches of early diagnosis of pancreatic cancer

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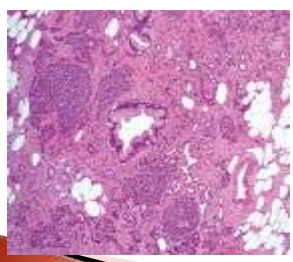
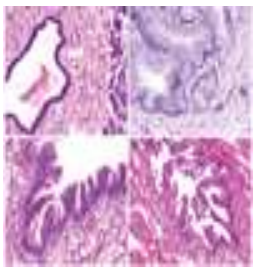
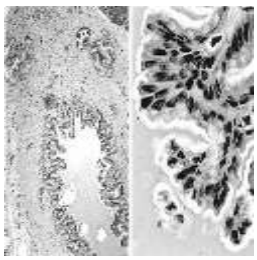
Epidemiology

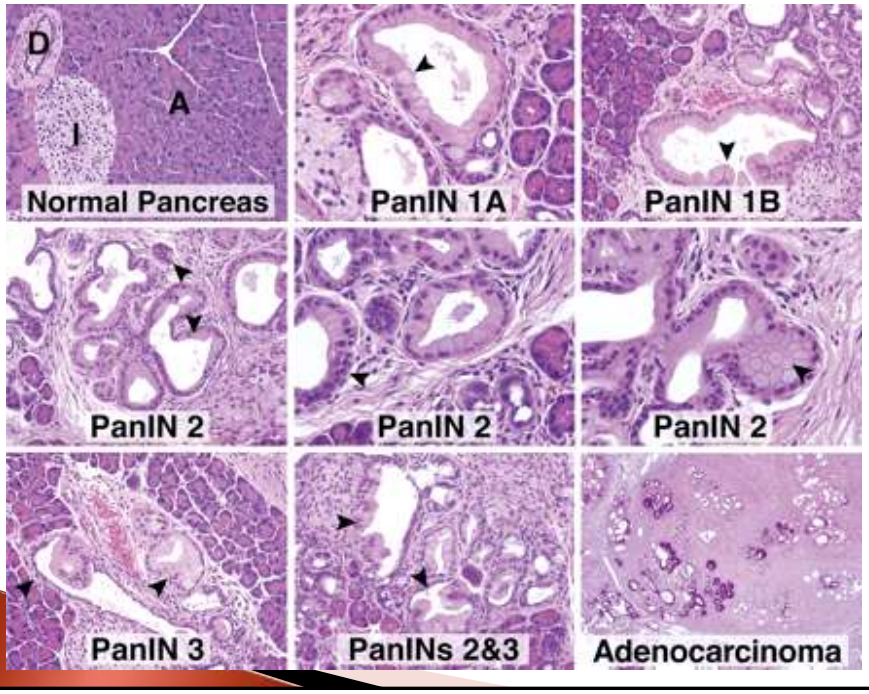
- The fourth leading cause of cancer-associated mortality,
  - High mortality, more than 35,000 deaths each year
- Early diagnosis is difficult
  - Most cases have mild symptoms before diagnosis at late-stage, locally advanced or metastatic disease
    - Cubill AL et al. Clin Bull 2008;8:91-99
- Radical operation is challenging
  - Only 15%-20% of pts. present with resecable disease
  - Only 15%-20% of surgically resected pts. survive to 5y
    - Smeenk HG et al.Langenbecks Arch Surg 2005, Sohn TA, et al. J Gastrointest Surg 2000

Precursor lesions
PanIN-noninvasive pancreatic intraepithelial neoplasis

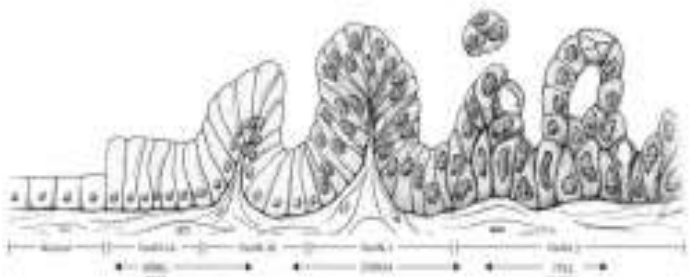
- Through the process of acinar-to-ductal metaplasia, pancreatic acinar cells give rise to PanIN

PanIN1 PanIn-1,2 and3 PanIn3



Pancreatic Intraepithelial Neoplasia (PanIN)



Normal--PanIN1A-PanIn1B------PanIN-2----------PanIN-3
Her-2 --------- -----p16----------p53
K-ras---------- DPC4
----------BRCA2

Acquired Risk factors for PC

- Smoking (2x)
- Age
- Obesity
- Long lasting diabetes mellitus (2x)
- Chronic pancreatitis
- IPMN and MNC
- Occupational exposures?

Larsson SC et al. Br J Cancer 2005, Lin Y et al. J Gastroenterol 2005; Tada M et al. Clin Gastroenterol Hepatol 2006

IPMN and GNAS mutation

- **GNAS mutation is present exclusively in IPMN**
- GNAS and KRAS testing was performed on EUS-FNA pancreatic cyst fluid from 91 pancreatic cysts (41 IPMNs, 9 IPMNs with adenocarcinoma, 16 MCNs, 10 cystic pancreatic neuroendocrine tumors (PanNET), 9 serous cystadenomas (SCA), 3 retention cysts, 2 pseudocysts, and 1 lymphoepithelial cyst)
  - GNAS and KRAS mutations had 100% specificity and 65% sensitivity for mucinous differentiation.
  - Among IPMNs, mutations in either gene had 98% specificity and 84% sensitivity
    - Singhi AD et al. Clin Cancer Res 2014.
- 100% of intestinal type IPMNs demonstrated GNAS mutations compared to 51% of gastric IPMN, 71% of pancreatobiliary IPMNs, and 0% of oncocytic IPMNs.
  - Dal Molin M et al. Ann Surg Oncol, 2013.

IPMN

- PDAC-associated IPMN (branch type)
  - 9,5% of 94 pts. with IPMN was diagnosed with PDAC-associated IPMN
    - Yamaguchi K, Igakusion 2005.
- Malignisation of IPMN

Gene mutations

Rockacy MJ et al. Clin Gastroenterol Hepatol 2013
Mihaljevic AL et al. Pancreatology 2009

Gene mutation	PDAC	IPMN	MCN	SCN
K-ras	75-100%	0-75%(HGD)	20%(adenoame)-89%(carcinoame)	-
PT53	40-87%		0%(adnoame)-44%(carcinoame)	-
SMAD4	55-66%	+	+	-
BRCA2	+	-	-	-
GNAS	+	++	-	-
VHL	-	-	-	40%
p16,CDKN2A	40-100%	-	-	-
P15,CDKN2B	27-48%	-	-	-
FHIT	66-70%	-	-	-
STK1/LKB1	-	+	-	-
P13KCA	-	+	-	-

Serum biomarkers

- Classical tumor markers (CA 19-9, ACE) are not effective for the early detection of small PC
  - They have a contribution in prediction of invasive IPMN or concomitant ductal andenocarcinoma
- Elevated classical tumor marker levels indicate the presence of a significant number of cancer cells
- They are reliable parameters to determine disease progression during chemotherapy or recurrence after surgery

Tumoral markers in cystic neoplasms

- Tumoral markers from fluid (CA19-9, ACE, CA174-2)
  - **Can make the differation between benign and malign**
    - CEA and CA 19-9 are elevated in patients with malignant cysts (238±12.5 ng/ml and 222±31.5 U/ml, respectively) vs. benign lesions (34.5±3.7 ng/ml and 18.5±1.9 U/ml, respectively; $P<0.001$).
    - Based on these results, the sensitivity and specificity of CEA were 91.8 and 63.9% and of CA 19-9 were 81.3 and 69.4%, respectively.
    - Talar-Wojnarowska et al. Oncology Lett. 2013
- **Can not predict the progression from low dysplasia to high dysplasia or invasive pattern**
  - CA19-9 cut-off level of 37 units/ml specificity of 85,9% , NPV of 85,9%, PPV 74% and accuracy 81,7% for invasivity
    - Fritz S et al. Br J Surg. 2011 Jan;98(1):104-10

High-risk stigmata and worrisome features of IPMN
Sindai criteria

- Cyst diameter larger than 3 cm
- Presence of mural nodule
- Thickened enhanced cyst walls
- Increasing size more than 2 mm/y
- Dysplasia on cytology
- Dilated MPD
- K-ras mutation in cystic fluid

- Tanka M et al, Pancreatology 2012
- Sadakari Y, Pancreas 2010,
- Khalid A, Gastrointest Endosc 2009,
- Nara S, Pancreatology 2009

Novel molecular markers

- Novel molecule expressed in PC and cystic neoplasms for screening and early detection of PC
  - Novel molecule to improve the sensitivity of cytology or biopsy
  - Novel transcriptomic or metabolomic biomarkers (e.g. blood, saliva samples)

Biomarkers in ERCP specimens

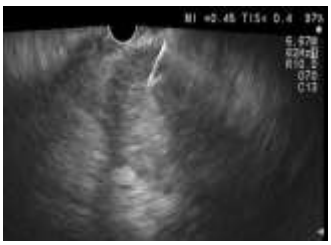
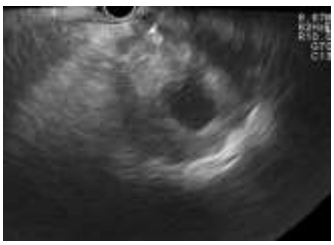
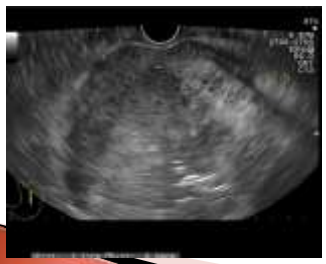
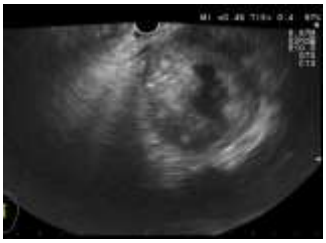
Endoscopic sampling of pancreatic juice:

- **Clacium binding protein S100P-expressed** exclusively in PC cells (high levels in PC and IPMN than CP)
  - Expression of S100P was found in PanIN-2 or-3
    - Ohuchida K, Clin Cancer Res 2006, Dowen SE et al Am J Pathol 2005
- **elevated expression of h-TERT** (human telomerase reverse transcriptase) in PC and malignant IPMN vs. CP
  - !false-positive results
- Presence of **K-ras mutation** in pancreatic juice has a marker of invasivity in pts. with IPMN (Wu et al, Gastroenterology 2013)

Direct forceps biopsy or brushing cytology from the stenotic pancreatic or bile duct

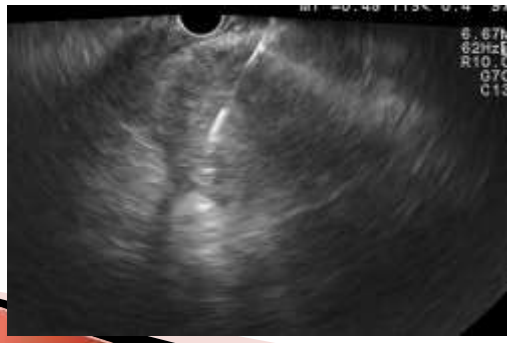
- **IHC of p53 protein** (brushing cytology) in PC vs. CP

Biomarkers in EUS-FNA specimens

Biomarkers in EUS-FNA specimens

- Salek et al., on 53 EUS-FNA specimens assessed **K-ras, p53, p16, Smad4 mutations** (Salek C et al. Anticancer Res 2009)
  - No any positive predictive factor for PC



Biomarkers in EUS-FNA specimens

- **IHC assessment of PC tissue provided several candidate molecules for the prediction of prognosis of PC pts.**
  - Higher expression of **CDCP1**-facilitate growth and migration of cancer cells
    - Correlated with the overall survival
  - **L1-CAM** (L1-cell adhesion molecule) expression
    - correlate with perneural invasion
  - Higher expression of **B7-H3** (co-stimulatory molecule for immune response) (Loos M, BMC cancer 2009)
    - correlates with a better postoperative prognosis
  - **hENT1** (human equilibrative nucleoside transporter 1) and gemcitabine sensitivity was established (Marechal R et al. Clin Cancer Res 2009)

Non-invasive biomarkers diagnosis of PC

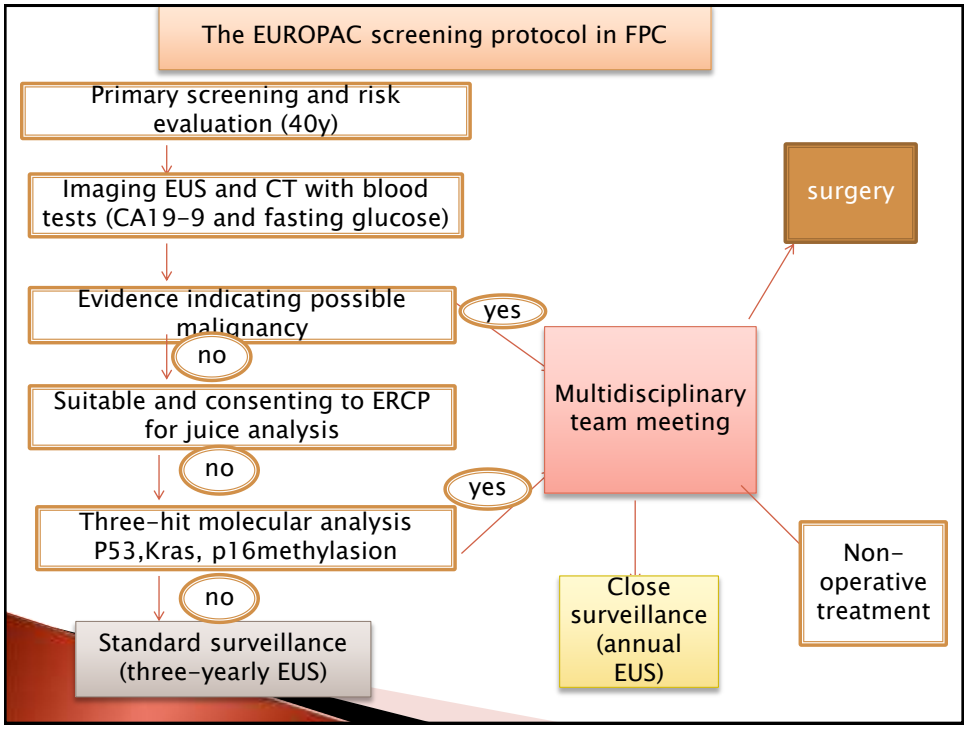
- microRNA plays a central role in the regulation of cellular functions such as migration, invasion and stem cell functions
- Zhang et al-using transcriptome profiles from saliva supernatants have found a Se 90% and Sp 95% for diagnosis of PC vs. normal or CP (Zhang L et al. Gastrenterology 2010)
  - 4 mRNA were combined to discriminate PC
- Altered microRNA expression can be detected in peripheral blood samples
  - miR-200a, miR-200b are highly expressed in pancreatic cancer cell lines and their expression levels are significantly elevated in the sera from PC pts.

Screening high risk patients

- Familial pancreatic cancer (2 first-degree relatives or 3 more distant relatives)(120x)
- Hereditary pancreatitis (BRCA2) (4-5x)
- Brest ovarian cancer syndrome (BRCA1, BRCA2)
- HNPCC
- FAMMM
- PJS, juvenile polyposis (132X)
- FAP

Methods of screening in high risk pts.

- Blood test (CA19-9, glucose test)
- EUS
- CT
- Molecular analysis (K-ras, p53, p16)



Screening for moderate risk patients?

- Chronic pancreatitis
- Recent onset of diabetes mellitus
- Age over 50-60 year
- Obesity
- Smoking

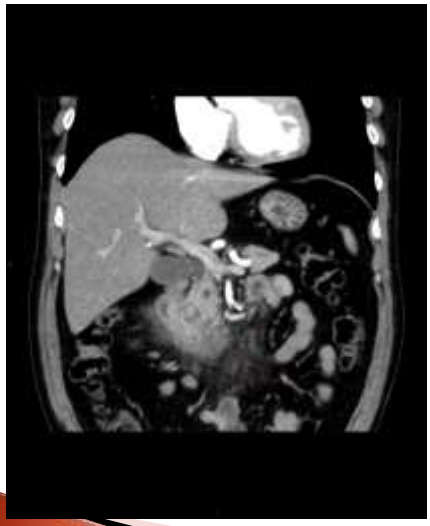
- If there are more than 1 risk factor

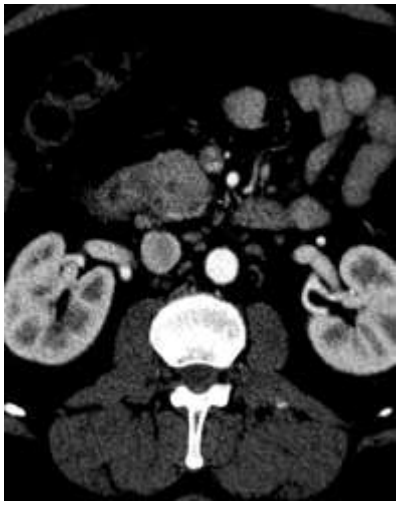
  - Is not cost-efficient

CV, male, 49 y

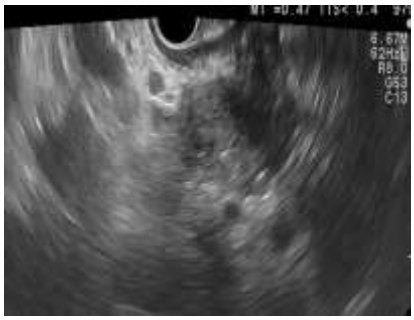
- Dyspepsia
- sclera jaundice
- Loss of 10 kilos in 2 months
- Upper GI endoscopy negative
- Pts. had an episode of acute pancreatitis 10 years before
- Heavy smoker

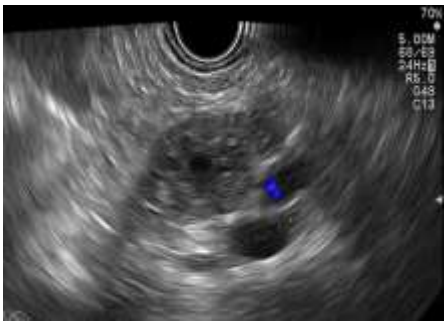
CT scan



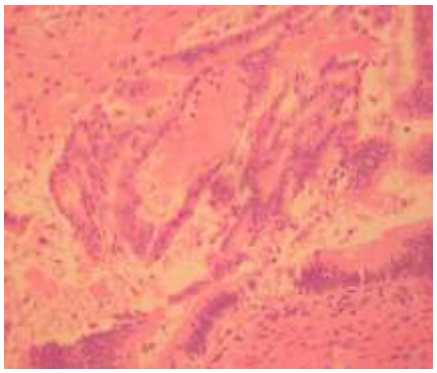


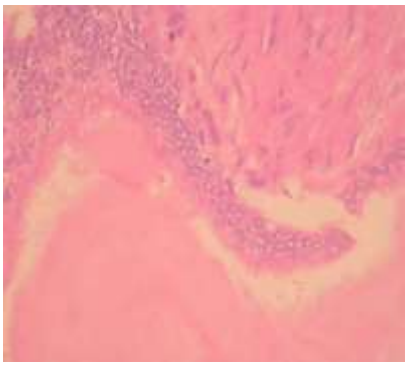
EUS-FNA





EUS-FNA, atypical cells, suspicion of neoplasia





What is the diagnosis?

- Pancreatic cancer?

or

- Inflammatory mass in the head of the pancreas (chronic pancreatitis)?

- Patient was operated
- Whipple resection was performed

Final diagnose

- Pseduotumoral chronic pancreatitis with PanIn1

Conclusions

- Novel molecular markers expressed in PC and cystic neoplasms were developed, use for screening and early detection of PC
- Combined diagnostic strategy including conventional cytology or biopsy and novel molecular markers will be beneficial for an accurate diagnose
- Screening for pancreatic cancer in high risk patients and in patients with more than 1 risk factor for PC are mandatory and needs to be implamentate in Romania