University of Szeged

Albert Szent-Györgyi Medical School

Doctoral School of Theoretical Medicine

Early detection of pancreatic ductal adenocarcinoma – new opportunities of screening

PhD Thesis

Dóra Illés M.D.

Supervisor: László Czakó M.D., Ph.D., D.Sc.

Szeged

2024

Table of contents

1. List of publications

1.1 Related to this thesis

1.1.1 First Author

1. **Illés D, Terzin V, Holzinger G et al. New-onset type 2 diabetes mellitus – a high-risk group suitable for the screening of pancreatic cancer? *Pancreatology*. 2016;16(2):266-71. IF: 2.58 SJR indicator: Q2.**
2. Illés D, Torday L, Czakó L. A pancreascarcinoma korszerű kezelése. *Onkológia & hematológia*. 2016;5:22-30.
3. Illés D, Czakó L. A pancreascarcinoma korszerű kezelése. *Central European Journal of Gastroenterology and Hepatology*. 2016;2(4):176-182.
4. Illés D, Czakó L. A hasnyálmirigyrák Világnapja 11.21. – Lankadatlan éberség! *Lege Artis Medicinae*. 2019;29(12):643-646. SJR indicator: Q4.
5. Illés D, Czakó L. A diabetes és az emésztőrendszer betegségei: pancreatitis és emésztőrendszeri tumorok. *Magyar Belorvosi Archívum*. 2020;73:63–67.
6. **Illés D, Iványi E, Holzinger G et al. New Onset of DiabetEs in aSsociation with pancreatic cancer (NODES trial): Protocol of a Prospective, Multicentre Observational trial. *BMJ Open*. 2020;10(11):037267. IF: 2.692 SJR indicator: Q1.**

1.2 Not related to this thesis

1.2.1 First Author

1. Illés D, Urbán E, Lázár A et al. Az antibiotikumrezisztencia változása cholangitisben: klinikai tapasztalataink. *Orvosi Hetilap.* 2019;160(36):1437-1442.
2. Illés D, Czakó L. Rate of early hospital readmission in acute pancreatitis as a quality marker. *Orvosi Hetilap*. 2021;162(11):413-418.

1.2.2 Co-Author

1. Zsóri G, Terzin, Illés D et al. Effects of a continental climate on the prevalence and severity of acute non-variceal gastrointestinal bleeding. *Climate Research*. 2017;73(3):187-194.
2. Zsóri G, Czepán M, Illés D et al. Pancreatic diabetes mellitus and heavy drinking are risk factors for the development of liver fibrosis in alcoholic chronic pancreatitis. *Pancreatic Disorders and Therapy*. 2017;3:1000186.
3. Kui B, Illés D, Iványi E et al. Új megoldás egy régi problémára. Epeelfolyási zavar megoldása endoszkópos ultrahang-vezérelt epeúti drenázzsal. *Central European Journal of Gastroenterology and Hepatology*. 2018;4(4):816-822.
4. Zsóri G, Illés D, Terzin V et al. Exocrine pancreatic insufficiency in type 1 and type 2 diabetes mellitus: do we need to treat it? A systematic review. *Pancreatology*. 2018;18(5):559-565.
5. Lemes K, Illés D, Iványi E et al. Az idült hasmenés kóreredete és kezelése. *Magyar Belorvosi Archívum*. 2019;72(6):307-313.
6. Párniczky A, Lantos T, Illés D et al. Antibiotic therapy in acute pancreatitis: From global overuse to evidence based recommendations. *Pancreatology*. 2019;19(4):488-499.
7. Zsóri G, Illés D, Iványi E et al. In New-Onset Diabetes Mellitus, Metformin Reduces Fat Accumulation in the Liver, But Not in the Pancreas or Pericardium. *Metabolic Syndrome and Related Disorders*. 2019;17(5):289-295.
8. Tod P, Farkas N, Illés D et al. Initial Renal Function (eGFR) Is a Prognostic Marker of Severe Acute Pancreatitis: A Cohort-Analysis of 1,224 Prospectively Collected Cases. *Frontiers in Medicine*. 2021;8:671917.
9. Czakó L, Gyökeres T, Illés D et al. Epeút- és epehólyag-gyulladás: diagnosztikus kritériumok és terápia. *Orvosi Hetilap*. 2023;164:(20):770-787.
10. Tóth I, Ábrahám Sz, Illés D et al. Multidisciplinary management of acute cholecystitis during the COVID-19 pandemic. *Scientific Reports*. 2023;13(1):16257-64.
11. Karamya ZA, Kovács A, Illés D et al. Prevalence of Autoimmune Pancreatitis in Pancreatic Resection for Suspected Malignancy: A Systematic Review and Meta-analysis. *BMC Gastroenterology*. 2024 (in press).

1.2.2.1 Co-Author as a member of the Hungarian Pancreatic Study Group

1. Szentesi A et al. Analysis of Research Activity in Gastroenterology: Pancreatitis Is in Real Danger. *PLOS ONE.* 2016;11(10):0165244.
2. Kui B et al. The use of Early Achievable SeveritY (EASY) index is beneficial for rapid risk stratification in acute pancreatitis on hospital admission. *Pancreatology*. 2019;19:2-15.
3. Szentesi A et al. Multiple hits in acute pancreatitis: components of metabolic syndrome synergize each other's deteriorating effects. *Frontiers in Physiology*. 2019;10:1202.
4. Farkas N et al. A Multicenter, International Cohort Analysis of 1435 Cases to Support Clinical Trial Design in Acute Pancreatitis. *Frontiers in Physiology*. 2019;10:1092.
5. Halász A et al. Outcomes and timing of endoscopic retrograde cholangiopancreatography for acute biliary pancreatitis. *Digestive and Liver Disease*. 2019;51(9):1281-1286.
6. Hágendorn R et al. Development of disturbance of consciousness is associated with increased severity in acute pancreatitis. *Pancreatology*. 2020;20(5):806-812.
7. Demcsák A et al. Acid suppression therapy, gastrointestinal bleeding and infection in acute pancreatitis – An international cohort study. *Pancreatology*. 2020;20(7):1323-1331.
8. Mosztbacher D et al. Hypertriglyceridemia-induced acute pancreatitis: A prospective, multicenter, international cohort analysis of 716 acute pancreatitis cases. *Pancreatology.* 2020;20(4):608-616.
9. Nagy A et al. Glucose levels show independent and dose-dependent association with worsening acute pancreatitis outcomes: Post-hoc analysis of a prospective, international cohort of 2250 acute pancreatitis cases. *Pancreatology.* 2021;21(7):1237-1246.
10. Kiss S et al. Early prediction of acute necrotizing pancreatitis by artificial intelligence: a prospective cohort-analysis of 2387 cases. *Scientific Reports.* 2022;12(1):7827.

11. Ocskay K et al. Recurrent acute pancreatitis prevention by the elimination of alcohol and cigarette smoking (REAPPEAR): protocol of a randomised controlled trial and a cohort study. *BMJ Open*. 2022;12(1):050821.
12. Czapári D et al. Detailed characteristics of post-discharge mortality in acute pancreatitis. *Gastroenterology*. 2023;165(3):682-695.
13. Váncsa S et al. Metabolic-associated fatty liver disease is associated with acute pancreatitis with more severe course: Post hoc analysis of a prospectively collected international registry. *United European Gastroenterology Journal*. 2023;11(4):371-382.
14. Juhász MF et al. Invalidity of Tokyo guidelines in acute biliary pancreatitis: A multicenter cohort analysis of 944 pancreatitis cases. *United European Gastroenterology Journal*. 2023;11(8):767-774.

Scientometrics

Number of full publications	29
First author publications	8
Hirsch index	10
Number of independent citations	348

https://m2.mtmt.hu/gui2/?type=authors&mode=browse&sel=10058299&view=pubTable

2. List of abbreviations

ADA – American Diabetes Association

ADMP – antedecent diabetes mellitus in pancreatitis

BMI – body mass index

CA 19-9 – carbohydrate antigen 19-9

CFRD – cystic fibrosis related diabetes mellitus

CI – confidence interval

CP – chronic pancreatitis

CT – computed tomography

DEP – diabetes of the exocrine pancreas

DM – diabetes mellitus

eCRF – electronic case report form

EUS – endoscopic ultrasound

EUS-FNA – endoscopic ultrasound guided fine needle aspiration

FG – fasting glucose

GADA – glutamic acid decarboxylase antibodies

GCMS – gas chromatography mass spectrometry

GI – gastrointestinal

GIP – gastric inhibitory polypeptide

GLP-1 – glucagon like peptide -1

HbA1c – hemoglobin A1c

HOMA – homeostatic model assessment

HPLC – high performance liquid chromatography

IFG – impaired fasting glucose

IGF-1 – insulin-like growth factor-1

LCMS – liquid chromatography mass spectrometry

MS – mass spectrometry

NOD-PDAC – new-onset diabetes in pre-symptomatic pancreatic ductal adenocarcinoma

NPV – negative predictive value

PCRD – pancreatic cancer related diabetes mellitus

PDAC – pancreatic ductal adenocarcinoma

PDAC-T3cDM – pancreatic ductal adenocarcinoma related pancreatogenic diabetes

PEI – pancreas exocrine insufficiency

PP – pancreatic polypeptide

PPDM-A/C – post-pancreatitis diabetes mellitus – acute/chronic

PPV – positive predictive value

RR – relative risk

SD – standard deviation

SIR – standardized incidence ratio

T1DM – type-1 diabetes mellitus

T2DM – type-2 diabetes mellitus

T3cDM – pancreatogenic diabetes

US – ultrasound

3. Introduction

3.1. Importance of the topic

Among pancreatic malignancies, pancreatic ductal adenocarcinoma (PDAC) is the most common, accounting for more than 90% of exocrine pancreatic malignancies (1). Although it is a rare disease, with a lifetime prevalence of 1.39% (2), PDAC is a very aggressive disease with a poor prognosis: it develops asymptomatically or with asymptomatic symptoms for a long time, so that patients are diagnosed at a late, advanced stage, when the only curative therapy, surgical resection, is already impossible due to the presence of metastases and locoregional infiltration (3). Therefore, the mortality/morbidity rate of PDAC is ~1. The 5-year survival rate approached 10% for the first time in 2020, compared to 5.26% in 2000 (4), and this rate has hardly improved in the last 40 years (5). To make matters worse, the incidence of PDAC is increasing at a rate of 0.5% to 1% per year, and PDAC is projected to become the second leading cause of cancer death by 2030 (6).

Currently available cytotoxic regimens (fluorouracil, irinotecan, leucovorin, oxaliplatin or gemcitabine/nab-paclitaxel and nanoliposomal irinotecan/fluorouracil) for advanced disease are modestly effective, with a survival benefit of 2 to 6 months (1). The quality of life of these patients is usually very poor: anorexia, followed by weight loss and cachexia, weakens the immune system, leading to severe infections or even sepsis. Mechanical obstructions caused by PDAC tumour growth, such as biliary or gastrointestinal obstructions, lead to jaundice or gastric outlet syndrome, conditions that require hospitalization and often endoscopic surgery. Complications of advanced PDAC are extremely distressing for patients and are associated with significant hospital costs. All of this could be avoided if the disease were detected at an early stage when it is still operable.

Unfortunately, there is no effective screening program yet, although the success in improving the survival rate of PDAC depends to a large extent on the development of a screening program for the early detection of PDAC in the asymptomatic stage.

Given that PDAC is a rare disease with low lifetime-prevalence a population-wide screening would be ineffective and a huge financial burden on the healthcare system. Therefore, the recommendation for successful screening is that patients who are at high risk of PDAC should be screened (7). The clinical conditions predisposing PDAC are summarized in Table 1.

Clinical conditions	Relative Risk (x)	Cumulative Risk at age 70 (%)	Affected gene
smoking	2.5		
chronic pancreatitis		15	
diabetes mellitus	2.2		
obesity		1.2	
Hereditary cancer syndromes			
• Peutz-Jeghers Syndrome	132	36	STK11/LKB1
• Hereditary atypical multiple mole melanoma	20-47	17	CDKN2A
• Hereditary breast/ovarium cancer	3-10	3-6	BRCA2
• Hereditary nonpolyposis colorectal cancer	9	<5	MLH1, MSH2, MSH6, PMS2
• Familial adenomatosus polyposis	4	<5	APC
• Fanconi anemia			PALB2
• Ataxia telangiectasia	3		ATM
• Li-Fraumeni syndrome	7		p53
Genetically predisposed chronic diseases			
• Hereditary pancreatitis	50-80	40	PRSS1/SPINK1
• Cystic fibrosis	5	<5	CFTR
Familial cumulation of pancreatic cancer			
• PDAC in 3< first-degree relatives	32	40	
• PDAC in 2 first-degree relatives	6.4	8-12	
• PDAC in 1 first-degree relative	4.5	2	

Table 1. *Clinical conditions (affected gene) predisposing-, and their relative risk causing pancreatic cancer.*

Among risk factors diabetes mellitus (DM) has the strongest link to PDAC: 40-65% of patients diagnosed with PDAC meet the criteria for DM (8) while genetic factors play a smaller role (9). DM is a disorder of the metabolism of carbohydrates, fats and proteins that is characterized by an absolute or relative lack of insulin. It counts as a worldwide epidemic with increasing incidence in recent decades: it is predicted that the number of people with DM will increase to

300 million by 2025 and 366 million by 2030 (10). This represents a huge number of new patients each year, so screening this large group for PDAC would not yet be cost-effective; further narrowing of the target group is needed by investigating the association between PDAC and DM.

3.2. Bidirectional connection/ dual causality with diabetes mellitus

The association between PDAC and DM has been known for decades (11). In epidemiological studies the increased incidence of gastrointestinal malignancies has been repeatedly observed in population with diabetes through the effect of insulin as a growth factor and the elevated level of mitogen cell proliferation-enhancing insulin-like growth factor-1 (IGF-1). The relative risk ranges from 1.3-4.7 (Table 2) (12-19).

Localization of malignancy	**Relative risk**
Esophagus	1.3
Stomach	1.7
Colon	1.5
Pancreas	4.7
Liver	2.3
Gallbladder	1.8
Biliary tract	4.2
All GI	1.5

Table 2. *Relative risk of gastrointestinal malignancies in patients diagnosed with DM (20).*

Interestingly, patients with short-term DM (<4 years) have more than 1.5-fold risk of displaying PDAC as compared with patients who are diabetic for more than 5 years (21). The risk of having PDAC is inversely proportional to the duration of DM (Table 3), being the highest at 1-year duration (22).

Duration of DM (years)	Ben et al. RR (95%CI) (22)	Huxley et al. RR (95%CI) (21)
<1	5.38 (3.49-8.3)	
1-4	1.95 (1.65-2.31)	2.05 (1.87-2.25)
5-9	1.49 (1.05-2.12)	1.54 (1.31-1.81)
10<	1.47 (0.94-2.31)	1.51 (1.16-1.96)

Table 3. *Risk of PDAC based on the duration of DM.*

In 85% of the PDAC cases the fasting blood glucose level is known to be elevated (23). Pannala et al. reported that the patients diagnosed with type-2 diabetes mellitus (T2DM) have an 8-fold risk of developing PDAC within 2-3 years from the diagnosis of T2DM relative to the general population (Figure 1) (24).

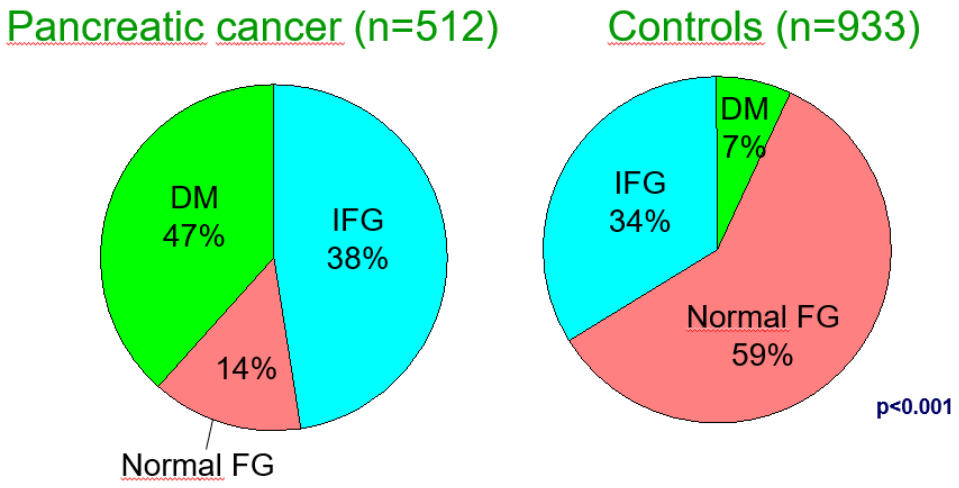


Figure 1. *Prevalence of DM in PDAC. Reproduced from Pannala et al. (24)*
FG – fasting glucose; IFG- impaired fasting glucose

Based on the above, the connection between PDAC and DM is bidirectional: the „long term" DM is a risk factor, the „short term" DM is a presumably paraneoplastic consequence of the PDAC (12). The exact mechanism of this paraneoplastic causal relationship is unknown. The „short term" DM was called „new-onset" DM in the literature. The precise definition for this entity is DM diagnosed within 36 months (23). Data on the incidence of PDAC in new-onset diabetes are scarce in the literature: 0.25%, 0.85% and 3.6% have been reported (13,25,26). *Patients with new-onset DM may be an appropriate group for pancreatic cancer screening, which should be statistically supported.*

Older age would further enrich the group eligible for screening for PDAC, as age is an independent risk factor for PDAC - in newly diagnosed diabetic patients over 50 years of age, the incidence of PDAC is approximately 1% (27).

It is important to emphasize that although the symptoms and clinical manifestations of 'simple' T2DM and PDAC-related DM are similar, there are fundamental differences between the two entities.

3.3 Pancreatogenic diabetes

For decades PDAC-related DM was presented in the literature as one of the manifestations of pancreatogenic diabetes (T3cDM). T3cDM is present if the glandular inflammation and the subsequent irreversible fibrotic damage or malignant destruction or partial/complete surgical resection of the pancreas result in islet cell loss (28). This pathomechanism explains the pancreatic exocrine insufficiency, the impaired incretin secretion and the lack of peripheral insulin resistency by the patients suffering from T3cDM. The clinical and laboratory findings in T2DM and T3cDM are depicted in Table 4. The main causes of T3cDM are summarized in Figure 2.

Parameter	T2DM	T3cDM
Ketoacidosis	Rare	Rare
Hypoglycemia	Rare	Common
Peripheral insulin sensitivity	Decreased	Normal or increased
Hepatic insulin sensitivity	Decreased	Normal or decreased
Insulin levels	High or “normal”	“Normal” or low
Glucagon levels	Normal or high	“Normal” or low
PP levels	Normal or high	Low or absent
GIP levels	Variable	Low
GLP-1 levels	Variable	Variable
Typical age of onset	Adulthood	Any
Typical etiology	Obesity, age	CP, cystic fibrosis, postoperative

Table 4. *Clinical and laboratory findings in T2DM and T3cDM. Adapted from Andersen (28). T2DM – type 2 diabetes mellitus, T3cDM – pancreatogenic diabetes, PP – pancreatic polypeptide, GIP – gastric inhibitory polypeptide, GLP-1 – glucagon-like peptide-1, CP – chronic pancreatitis*

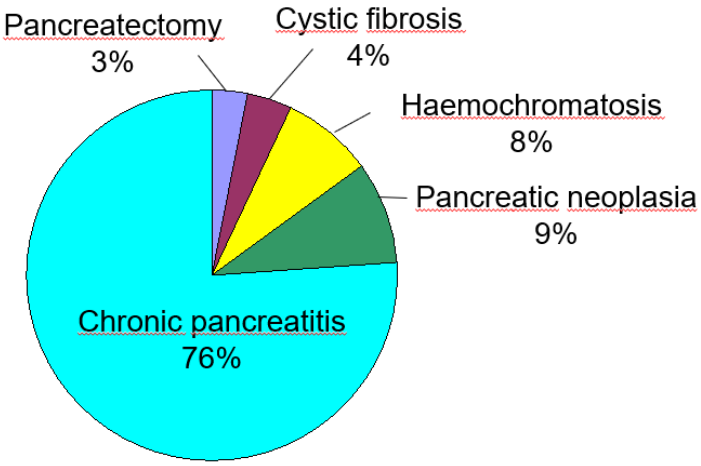


Figure 2. *Distribution of causes of T3cDM. Adapted from Andersen (28).*

The diagnosis of T3cDM requires the presence of pancreatic exocrine insufficiency (PEI), evidence of pathological pancreatic imaging and the absence of type-1 diabetes-associated autoimmune markers (Table 5); the diagnosis may be further supported by evidence of PP, incretin or insulin secretory defects in the absence of clinical or biochemical evidence of overt insulin resistance (28).

Major criteria (all must be fulfilled)
Presence of exocrine pancreatic insufficiency (according to monoclonal fecal elastase 1 or direct function tests).
Pathological pancreatic imaging (by endoscopic ultrasound, MRI, or computed tomography).
Absence of T1DM-associated autoimmune markers.
Minor criteria
Impaired β-cell function (e.g., as measured by HOMA-B, C-peptide/glucose ratio).
No excessive insulin resistance (e.g., as measured by HOMA of insulin resistance).
Impaired incretin (e.g., GIP) or PP secretion.
Low serum levels of lipid soluble vitamins (A, D, E, or K).

Table 5. *Diagnostic criteria for T3cDM. Adapted from Andersen (28)*
MRI – magnetic resonance imaging, T1DM – type 1 diabetes mellitus, HOMA – homeostasis model assessment, GIP - gastric inhibitory polypeptide, PP - pancreatic polypeptide

As mentioned above, the PDAC-related DM is a paraneoplastic phenomenon associated with the tumour of the pancreas. Previously, this type of diabetes was thought to develop as a consequence of tumour-induced destruction of the pancreas. However, in this case, a larger tumour involving rather the islet cell-rich body and tail region would need to be diagnosed in

patients with PDAC-related diabetes compared to non - diabetic PDAC patients. However, this has not been supported by observations (24).

Another hypothesis is that diabetes develops as a result of substances secreted by the tumour. This is supported by an experiment in which a supernatant derived from pancreatic cancer cell lines was injected daily intraperitoneally into immunodeficient mice, resulting in a significant increase in blood glucose concentrations and significantly reduced glucose tolerance compared to controls (29). In a study of 104 patients underwent resection because of pancreatic cancer, of whom 41 had DM at the time of surgery, it was found that 57% of the patients with new-onset DM had resolution of their DM postoperatively, whereas 100% of the patients with long-standing DM remained diabetic after pancreatic resection (24).

Based on the above, neither PEI nor pathological pancreatic imaging as diagnostic criteria for T3cDM are necessarily present in PDAC-related DM. In addition, PDAC-related DM accounts for only 9% of T3cDM cases. Therefore, the diagnostic criteria for T3cDM alone would not be effective in screening for PDAC-related DM, as these tests are difficult to obtain, expensive, burdensome for patients and therefore not suitable for screening.

3.4 Diabetes of the exocrine pancreas

The controversy that PDAC-related DM as a form of T3cDM does not necessarily shows the signs of PEI or pathological imaging highlighted the need to rethink the nomenclature of pancreatogenic or the former group „other specific types" of DM. Interestingly, it did not happen until 2017, when the first guideline by the United European Gastroenterology formally assessed the body of evidence on diabetes in diseases of the exocrine pancreas (DEP) (30). This group of diseases contains the post-pancreatitis (acute and chronic as well) diabetes mellitus (PPDM-A/C), cystic fibrosis-related diabetes (CFRD), pancreatic cancer-related diabetes (PCRD) and special entities such as antecedent diabetes mellitus in pancreatitis (ADMP) and new-onset diabetes in pre-symptomatic pancreatic ductal adenocarcinoma (NOD-PDAC) (31).

Accumulating evidence suggests that excessive intrapancreatic fat deposition (located outside the islets of Langerhans) plays a key role in the pathogenesis of these entities, which is also a non - characteristic feature in the pathogenesis of type 1 and type 2 DM. (32-37). For DEP, PEI should not be used as a diagnostic criterion as it is not a specific feature for PPDM or PCRD, it

is rather a strong risk factor for DEP. It is known that some patients with DM and exocrine pancreatic disease may not have visually remarkable imaging of the pancreas (38) therefore the pathological imaging of the pancreas is not required for DEP as a diagnostic criterion either. This nomenclature makes a clear distinction between „other specific types" of diabetes mellitus and helps to ensure the correct use of terms that have often been imprecise in the literature.

However, it is important to highlight that the two PDAC-related DEP entities, PCRD and NOD-PDAC are different: the latter is a condition that unfortunately only retrospectively can be recognized, as it happens in the asymptomatic stage of PDAC. Considering that the new nomenclature for DEP started to be used in 2021, in our own research, the group of NOD-PDAC patients is referred to as PDAC-T3cDM. This group would be ideal for PDAC screening. *Thus, among newly diagnosed diabetic patients differentiating "simple" T2DM from NOD-PDAC is important, but requires a new diagnostic approach.*

3.5 Current diagnostic opportunities for screening

As PDAC already causes symptoms at an advanced, unresectable stage, screening of early PDAC patients can be done using either blood analysis or imaging techniques, or a combination of both.

We should bear in mind that there are certain requirements to be met by screening methods: simple, quick to implement, widely available, repeatable/reproducible, painless, verified and affordable.

Among blood tests, measuring the serum level of tumourmarkers as possible screening modalities is promising, as these are cheap, easy to perform and widely available. Currently the carbohydrate antigen 19-9 (CA 19-9) is the only blood based biomarker in clinical use for PDAC.

CA 19-9 is a tumour-associated, but not tumour-specific epitope of sialyated Lewis A blood group antigen (39), which occurs in the epithelium of the salivary glands and in the ductal cells of the biliary tracts and is secreted by the exocrine pancreas too (40). However, false negative results can occur in Lewis antigen-negative individuals, who are unable to express this antigen. 5%–10% of the population have very low or even absent secretion of CA 19-9 (41). The serum level of CA 19-9 could be elevated in medical conditions such as liver cirrhosis, chronic

pancreatitis, cholangitis and other GI cancers (42). In addition, serum CA 19-9 shows increased levels in PDAC when the tumour is larger than 3 centimeters (43).

The sensitivity of this marker for PDAC is 70-90%, the specificity is 90%, the positive predictive value is 69% and the negative predictive value is 90% (40). CA 19-9 has been reported to discriminate between patients with pancreatic cancer and healthy controls (sensitivity 80.3%, 95% CI 77.7 to 82.6; specificity 80.2%, 95% CI 78.0 to 82.3) (44) and benign pancreatic disease (sensitivity 78.2%, 95% CI 72.3 to 80.4; specificity 82.8%) (45). However, even a test with a sensitivity and a specificity of 95% would yield numerous false-positive findings besides each true-positive result and it is *therefore recommended to combine CA 19-9 measurement with an other technique for screening* (46).

On the one hand, imaging is the gold standard for diagnosing PDAC. The first choice is transabdominal ultrasonography (US), but its sensitivity in diagnosing PDAC is only 50-70%. Its accuracy is low for tumours <1 cm, which are usually operable, and is negatively affected by obesity and meteorism (47).

Computer tomography (CT) has a better accuracy in diagnosing PDAC, but the low prevalence of PDAC and the radiation exposure associated with the modality prevent its use as a screening test.

Endoscopic ultrasound (EUS) or endoscopic retrograde cholangiopancreatography also have a high likelihood of correct diagnosis, but again the low prevalence of PDAC combined with the patient burden of endoscopic procedures precludes their use in screening.

Furthermore, it is not economically feasible to use CT or endoscopic imaging for screening, as these methods are associated with high costs to the healthcare system (48). *However, US is easy to use, widely available, non - invasive and relatively inexpensive, making it an ideal screening modality.*

On the other hand, as mentioned above, a blood test would be an ideal screening method. While much effort has been devoted to proteomic and genomic profiling and the identification of various protein and gene components of cancer (49-55), data on metabolic signatures in body fluids, cancer cells or tissues are still very limited.

Fortunately, metabolomics, including lipidomics, has recently become more feasible, allowing the identification of clinical metabolite biomarkers, and several small studies recruiting up to

50 patients per group suggest that metabolomics may be useful in the detection of pancreatic cancer. (56-58).

A biomarker panel consisting of nine metabolites plus the established protein CA 19-9 was recently identified by Mayerle et al. with 89.9% sensitivity, 91.3% specificity and 99.8% negative predictive value (NPV) for differentiating PDAC from chronic pancreatitis (Figure 3) (59).

Using the same methods, a biomarker panel was identified for the differential diagnosis of NOD-PDAC and non - cancer related diabetes. The metabolite signature needs to be validated in an independent test cohort. *Provided the biomarker is validated, the panel could be effective in screening for NOD-PDAC in the high-risk group of elderly patients with new-onset DM.*

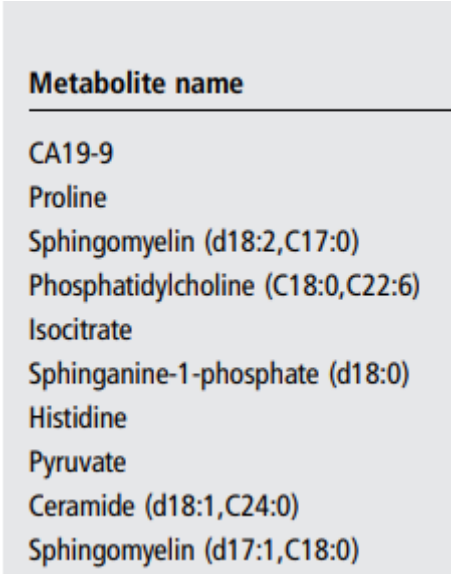


***Figure 3**. List of metabolites selected based on the multivariate elastic net analysis comprising the biomarker signature. Adapted from Mayerle (59).*

4. Aims

4.1 Study A - New-onset type 2 diabetes mellitus – a high-risk group suitable for the screening of pancreatic cancer? (HiRiPaC study)

In our first study, we aimed to provide statistical support that patients with new-onset DM may be an appropriate group for pancreatic cancer screening. We set out to determine the incidence of PDAC prospectively in new-onset T2DM patients. The screening method included the measurement of serum CA 19-9 levels combined with the performance of abdominal ultrasound (US).

4.2 Study B – New-Onset of DiabetEs in aSsociation with pancreatic ductal adenocarcinoma (NODES trial)

In our second study, we aim to differentiate 'simple' T2DM from NOD-PDAC in newly diagnosed diabetic patients by validating a biomarker panel as a screening method in the high-risk group of elderly patients with new-onset DM. In the study protocol article we summarized these aims as:

1. Estimate the incidence of PDAC in patients with new-onset diabetes.

2. Diagnose PDAC in an early operable stage.

3. Validate a biomarker that distinguishes patients with PDAC-caused T3cDM from patients with T2DM (48).

5. Patients and methods

5.1 Study A – HiRiPaC Study

5.1.1 Patients

Between March 2012 and October 2014, 115 consecutive patients with new-onset T2DM were enrolled in this prospective study by diabetologists at our clinic. The diagnosis of T2DM was

made according to the American Diabetes Association (ADA) criteria (60). New-onset DM was defined as DM diagnosed for the first time within the last 36 months prior to enrolment (23). Cases with T1DM and any type of symptoms suggestive of pancreatic disease were excluded. The duration of follow-up was 36 months from the first visit. All patients gave written informed consent to participate in the study.

The study protocol was in full compliance with the latest tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the University of Szeged (approval number 97/2012).

5.1.2 Methods

Serum CA 19-9 levels were measured and transabdominal US was performed at the first visit. In accordance with local laboratory standards, the cut-off serum CA 19-9 level was 27 U/mL. If the transabdominal US showed an abnormality (with either a normal or elevated CA 19-9 level), abdominal computed tomography (CT) was performed. Endoscopic ultrasound (EUS), EUS-guided fine needle aspiration (EUS-FNA) and surgical referral were performed if CT showed a pancreatic lesion. Abdominal CT was performed in the presence of an elevated serum CA 19-9 level without US abnormality. If the CT showed no lesion, the serum CA 19-9 level was repeated after 3 months. If the CA 19-9 level was normal and the US was negative, the CA 19-9 level was measured every 6 months and the US was performed every year. (Figure 4).

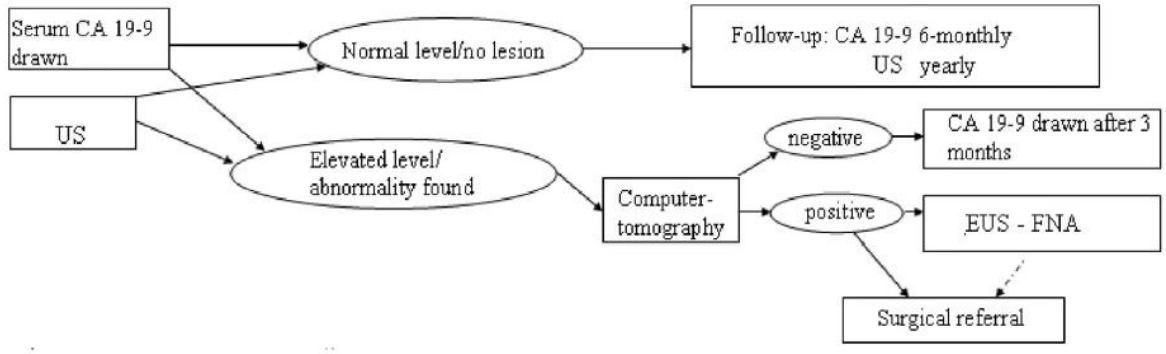


Figure 4. *'HiRiPaC Study' flow diagram.*

Potential risk factors for PDAC i.e. age >65 years, hereditary syndromes predisposing to PDAC and any first-degree relatives with PDAC were documented at the first visit along with body mass index (BMI; abnormally high if ≥ 25 kg/m2) and smoking status. Each of these criteria, if met, was given a score of one point.

The person-time incidence rate was calculated because we were comparing two populations in which exposures within subjects change over time (61). To assess the suitability of patients with new-onset T2DM as a risk group for PDAC, the standardized incidence ratio (SIR) was calculated using the person-time incidence based on our study and the age-adjusted incidence of PDAC in Hungary (9.3 cases/100.000 persons) (62).

The SIR is used to determine whether the incidence of cancer in a comparatively small, specific group is higher or lower than in the normal population (63). A SIR of 1 indicates that the number of cancer cases observed in the assessed population is equal to the number of cancer cases expected in the general population. An SIR > 1 indicates that there were more cancer cases than expected (64).

To assess the effectiveness of CA 19-9 and US as potential diagnostic tools for PDAC, sensitivity, specificity, positive and negative predictive values were calculated.

5.2 Study B – NODES trial

5.2.1 Patients

5.2.1.1 Design

NODES is a prospective, multicenter, observational cohort study aiming to validate a biomarker panel in the early stage of PDAC.

The inclusion criteria of this study are the following:

(1) Patients over 60 years of age.

(2) Diabetes diagnosed within 6 months (newly diagnosed) - diagnostical criteria are based on the Diabetes Control and Complications Trial (Table 6) (65).

(3) The participants signed written informed consent.

Parameter	Value and unit	Description
Fasting plasma glucose	≥126 mg/dL (7.0 mmol/L)	Fasting is defined as no caloric intake for at least 8 hours.
2-hour plasma glucose	≥200 mg/dL (11.1 mmol/L)	Oral glucose tolerance test. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.
HbA1c	≥6.5% (48 mmol/mol)	The test should be performed in a laboratory using a method that is National Glycohemoglobin Standardization Program (NGSP) certified and standardised to the *Diabetes Control and Complications Trial* (DCCT) assay.

Table 6. *Diagnostic criteria of diabetes mellitus (65).*

Exclusion criteria are as follows:

(1) Continuous alcohol abuse;

(2) Chronic pancreatitis;

(3) Previous pancreas operation/pancreatectomy;

(4) Pregnancy;

(5) Present malignant disease; and

(6) Type 1 DM.

Patients with chronic pancreatitis were excluded because a metabolic signature that differentiates between chronic pancreatitis and patients with PDAC has already been published and is being further evaluated by the META-PAC consortium, while the present study aims to differentiate between patients with NOD-PDAC and new-onset diabetes due to other causes.

5.2.1.2 Sample size

Mayerle et al (59) found that the biomarker signature in question could distinguish patients with PDAC from those without with 89.9% sensitivity (marginal error 8.9%) and 81.3% specificity (marginal error 10.3%).

Chari et al (13) concluded that elderly subjects with new-onset DM have an eightfold higher risk of pancreatic cancer than a person of similar age and sex without DM. Considering the epidemiological data suggesting that the prevalence of PDAC in Hungary is significantly higher

than in other countries (5), we assumed a prevalence of 2% for PDAC. Based on these data, the sample size calculation suggests that 2661 patients would need to be enrolled to confirm or reject the hypothesis for the primary endpoint with a 10% drop-out rate, 80% power and 95% significance level.

5.2.1.3 Duration

The first recruitment centre was initialized on 1 July 2019. Start of patient recruitment: 31 January 2020. Planned end of recruitment: 30 January 2023. The recruitment period is planned to last 36 months and all enrolled patients will be followed for 36 months.

It is important to note that due to the COVID 19 pandemic, the dates of the study that were originally planned have had to be changed. The study is still ongoing. The delay of almost three years was caused by the reduced capacity of the health system as it tried to cope with the pandemic and is the consequence of central restrictions on outpatient care.

5.2.1.4 Clinical data and clinical end points

Essential baseline clinical data

Age, sex, body weight, body mass index, date of DM diagnosis, date of sampling, comorbidities, antidiabetic medication, clinical symptoms, histology and stage of PDAC were recorded.

Data collection by questionnaire and blood samples were taken from all patients. The questionnaires (form A at recruitment, form B at each follow-up visit - see Annex) were completed by each enrolled patient. Data were stored in a personalized electronic database (electronic case report form - eCRF).

Primary clinical end points

(1) The sensitivity, specificity, PPV, NPV and accuracy of the biomarker test.

Secondary end points

(1) Mortality rate of PDAC in patients with new-onset diabetes;

(2) The proportion of localized and resectable PDAC;

(3) Change in body weight before visit 1 and during visits 2–6;

(4) Change in fasting blood glucose and hemoglobin A1c (HbA1c) before visit 1 and during visits 2–6;

(5) Antidiabetic medications and the risk of PDAC;

(6) Presence of concomitant diseases;

(7) Smoking and alcohol intake;

(8) Incidence of PDAC in patients with new-onset diabetes;

(9) Cost-benefit analysis.

5.2.1.5 Ethics

The trial was registered on ClinicalTrials. gov (NCT04164602). The study has been approved by the Scientific and Research Ethics Committee of the Hungarian Medical Research Council (41085-6/2019). Protocol version: V1.0 08.01.2019.

5.2.2. Methods

5.2.2.1 Study protocol

Patients with DM were recruited by our diabetologist and collaborating general practitioners on the basis of a recent (<6 months) laboratory test. Visit 0 was scheduled within 2 weeks of referral. Patients who met the study entry criteria and who were not excluded were informed and offered to participate in the study, but a signed informed consent was required for inclusion.

Clinical data, body weight and worrisome features (unintentional weight loss: 5% of body weight within 6 months without knowing the reason, abdominal pain/discomfort, abnormal laboratory data, unstable glucose metabolism despite adequate diet and medical treatment and without intercurrent infection) were recorded at Visit 0, and a fasting blood sample was taken

for assessment of laboratory data and metabolomics. C-peptide and glutamic acid decarboxylase antibodies (GADA) were measured to classify diabetes at visit 0. Patients with type 1 DM were excluded. If worrisome features were present at Visit 0, magnetic resonance imaging (MRI) or EUS were performed. Unambiguous PDAC lesions (>1 cm or also seen on MRI) were referred to surgery for resection. Ambiguous lesions in the pancreas underwent EUS fine needle aspiration. PDAC was diagnosed by histological examination.

Visits 1-5 were scheduled every 6 months. Clinical symptoms, body weight, laboratory data (fasting blood glucose, HbA1c, liver and kidney function, lipids, blood count) were collected at each visit. Blood for biobank and CA19-9 were collected every 12 months. Follow-up was completed after 36 months (Figure 5).

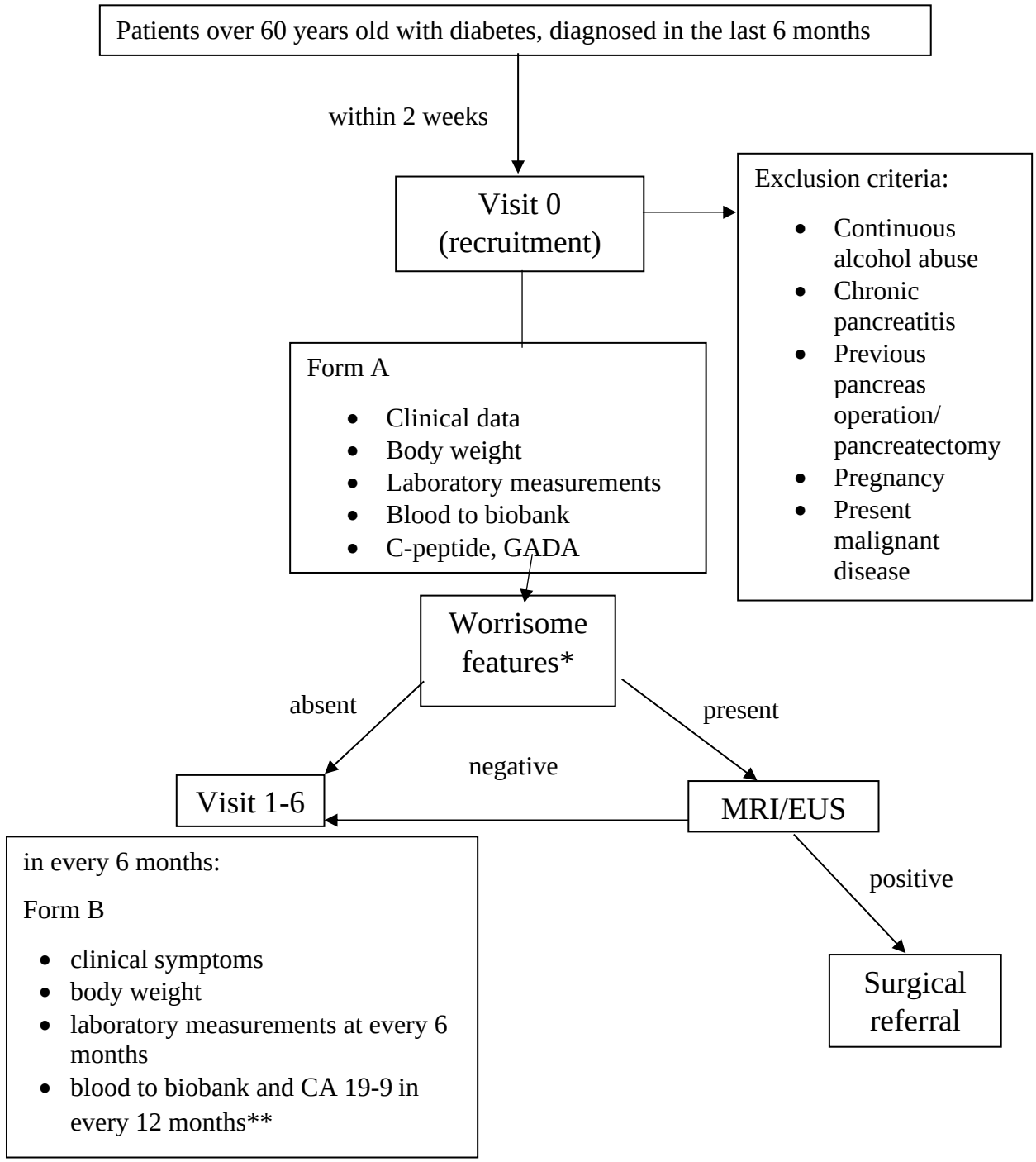


***Figure 5**. Flowchart of the study protocol*

** weight loss (except at visit0), abdominal pain/discomfort, abnormal laboratory data, unstable glucose metabolism despite the adequate diet and medical treatment and without intercurrent infection (except at visit 0).*

*** Fasted (overnight, at least 8 h) patients' blood samples at room temperature will be drawn into an EDTA tube. Within 2 h after blood draw samples will be at 19-21°C. After centrifugation, the supernatant is carefully removed. After that, the plasma is transferred in 0.5 ml aliquots to tubes and stored at -80°C, in a dedicated freezer (≤6 h from centrifuge to freezer).*
GADA - glutamic acid decarboxylase antibodies, MRI -magnetic resonance imaging, EUS – endoscopic ultrasound, CA 19-9 – carbohydrate antigen 19-9.

5.2.2.2 Biochemical methods

After informed consent, blood samples were collected from patients after fasting (overnight, at least 8 hours) in an EDTA tube. The blood tubes (9 mL) were centrifuged at 2000 × g for 10 minutes on a swing-out rotor within 2 hours of blood collection. Sample processing was performed at room temperature and the centrifuge was temperature controlled at 19°C-21°C. After centrifugation, the supernatant was carefully removed, transferred to a fresh 9 mL tube and gently mixed to homogenize any gradient that may have formed in the plasma supernatant. The plasma was then transferred in 0.5 mL aliquots to tubes (either Eppendorf Safe-Lock tubes 2 mL or Sarstedt screw-capped microtubes 2 mL) and stored at -80°C in a dedicated freezer (≤6 hours from centrifuge to freezer).

Biomarkers were determined by comparing metabolite levels in plasma samples from patients diagnosed with PDAC and cancer-free patients with diabetes. CA19-9 was measured centrally in a certified clinical laboratory using a cut-off of 37 U/mL as a classifier. The cost of the biomarker test, quality-adjusted life-years and incremental cost-effectiveness ratio are planned to be determined.

5.2.2.3 Metabolite profiling

- MxP global profiling

Two types of mass spectrometry (MS) analyses were applied. GC - MS (gas chromatography-MS; Agilent 6890 GC coupled to an Agilent 5973 MS System, Agilent, Waldbronn, Germany) and liquid chromatography - MS/MS (LC–MS/MS; Agilent 1100 high performance LC (HPLC)-System, Agilent, Waldbronn, Germany, coupled to an Applied Biosystems API4000 MS/MS-System, Applied Biosystems, Darmstadt, Germany) were used for a metabolite profiling approach (66).

Sample fractionation and derivatization and detection technologies have been described previously (67-70). Proteins were removed from plasma samples (60 µl) by precipitation. Polar and non - polar fractions were then separated by the addition of water and a mixture of ethanol and dichloromethane for both GC - MS and LC - MS/MS analyses. For GC - MS analysis, the non - polar fraction was treated with methanol under acidic conditions to yield the fatty acid methyl esters derived from free fatty acids and hydrolyzed complex lipids. The polar and non - polar fractions were further derivatized with O – methyl - hydroxylamine hydrochloride (20

mg/ml in pyridine) to convert oxo groups to O - methyloximes and then with a silylating agent (N – methyl – N - (trimethylsilyl)trifluoroacetamide) prior to GC - MS analysis.

For LC-MS/MS analysis, both fractions were dried and then reconstituted in appropriate solvent mixtures. HPLC was performed by gradient elution with methanol/water/formic acid on reversed phase columns.

- MxP lipids

MxP lipids cover profiling of sphingolipids (ceramides, sphingomyelins and sphingobases). The metabolites were analyzed in a semiquantitative approach (i.e. relative to a pool). Total lipids were extracted from plasma by liquid/liquid extraction using chloroform/methanol. The lipid extracts were subsequently fractionated by normal phase liquid LC into different lipid groups according to the references (67-71). The fractions were analyzed by LC-MS/MS using electrospray ionization and atmospheric pressure chemical ionization with detection of specific multiple reaction monitoring transitions for sphingomyelins and ceramides, respectively.

5.2.2.4 Data normalization

Metabolite profiling based on a semiquantitative analytical platform results in relative metabolite levels ('ratios') to a defined reference. In order to support this concept and to allow comparison of different analytical batches, two different reference sample types were run in parallel throughout the process.

Firstly, a project pool was generated from aliquots of all samples and measured with four replicates within each analytical sequence of 24 samples. For all semiquantitative metabolites, the results of each analyte from each sample were normalized against the median of the corresponding analyte in the pool reference samples within each analytical sequence to provide pool-normalized ratios. This procedure compensated for interinstrumental and intrainstrumental variation, i.e. the variability that occurs when different analytical sequences are analyzed by different instruments.

Secondly, in order to allow experiment to experiment alignment of the semiquantitative data, the MxPool (a large pool of commercial human EDTA plasma suitable for alignment of MxP studies) was analyzed with 12 replicate samples and the pool normalized ratios were further normalized to the median of the MxPool samples, i.e. the ratios from this study were at the

same level and therefore comparable with data from other studies normalized to other aliquots of the same MxPool.

5.2.2.5 Data set analysis and normalization

Descriptive statistics - mean, median, SD, quartiles and relative frequency - relative risk (dichotomous variables), independent two-sample t-test (continuous variable) in case of normal distribution, and Mann-Whitney U test in case of non-normal distribution are planned to be performed. Logistic regression are used to explore predictive factors. Associated statistical analyses are performed with a 0.05 error probability (type I error probability).

Prior to statistical analysis, the ratios are log10 transformed to make the data distribution approximately normal. SIMCA-P v.14.0 (Umetrics AB, Umea, Sweden), TIBCO Spotfire v.7.12.0 and R v.3.3.4 are used for data analysis and visualization. First, exploratory multivariate analysis (principal component analysis) is applied to log10 transformed ratios scaled to unit variance. A simple linear model (ANOVA) with additional clinical information and potentially confounding factors such as 'disease', 'age', 'body mass index', 'sex' and 'sample storage time' as fixed effects is fitted to the data. The significance level is set at 5%.

The multiple testing problem for the number of metabolites is addressed by calculating the false discovery rate using the Benjamini and Hochberg method. To classify patients according to their metabolic profiles, a penalized logistic regression is fitted via the Elastic Net Algorithm using the R package glmnet (72). Equal penalties are used for both L1 and L2 norms. The cut-off previously established on the biomarker identification dataset is then applied to the test data without retraining and performance is measured in terms of area under the curve (AUC), sensitivity and specificity. Confidence levels for the AUC are calculated using the binormal model for the receiver operating characteristic curve. When sensitivity is fixed at a given value, PPV, NPV and accuracy become monotonic functions of specificity and CIs for these estimates are obtained by transforming the CI for specificity. CIs for sensitivity, specificity and accuracy are obtained for the cut-off prespecified in the training data using the Clopper and Pearson method for the binomial distribution. For PPV and NPV, CIs are obtained using the method of Gart and Nam (73) for ratios of binomial parameters as implemented in the R package pairwise CI (74). When comparing the biomarker and CA19-9 on the test data, differences in sensitivity and specificity are planned to test using McNemar's test.

6. Results

6.1 Study A HiRiPaC Study

A total of 115 patients with new-onset T2DM were included in the study (49 male, 66 female, mean age: 58 ± 11 years, range: 32-85 years). 7 patients were subsequently excluded for various reasons: 1 man had T1DM, 1 woman had polycystic ovary syndrome, and 5 patients later declined to participate. The mean time between diagnosis of T2DM and inclusion in the study was 3.5 ± 4.4 months (range: 0-20 months).

Three patients (2.78%) had a first-degree relative with PDAC. Of these, one patient had PDAC. Sixty-nine (64%) of the participants scored 1 point, 38 (35%) scored 2 points and 1 patient (1%) scored 3 points on our risk assessment system (Table 7). Patients had no specific symptoms suspicious for PDAC.

Patient characteristics	
Total no. of patients	*108*
Female, n (%)	*62 (57)*
Age, y, mean (SD)	*58 (±11)*
BMI, mean (SD)	*30.5 (±4.6)*
Ever smoker, n (%)	*34 (31.5)*
Positive family history for PDAC, n (%)	*3 (2.7)*
Hereditary syndromes predisposing for PDAC, n (%)	*0 (0)*
Risk points of patients with PDAC, mean (SD)	*2.34 (±0,58)*
Risk points of patients without PDAC, mean (SD)	*1.34(±0.48)*

Table 7. *Patient characteristics of study A.*
SD – standard deviation, BMI – body mass index, PDAC- pancreatic ductal adenocarcinoma

Serum CA 19-9 levels were elevated in 10 patients (9%) (mean: 52.613 ± 23.13 U/mL), but none of them had morphological abnormalities on US or CT.

Imaging studies revealed a pancreatic mass in three patients (2.78%) without an elevated serum CA 19-9 level. The mean age of patients with PDAC was 70 ± 7 years and their mean BMI was 30.1 ± 5.1 kg/m2 compared to 58 ± 11 years and 30.5 ± 4.6 kg/m2, respectively, for patients with T2DM only. The time between CA 19-9 test/US examination and CT examination was 1 ± 1.7 months.

The 3 cases of patients diagnosed with PDAC are discussed below.

Case 1

A 67-year-old non - smoking woman was diagnosed with T2DM 8 months before the first study visit. She had a positive history for various tumours (kidney, parotid and thyroid) and a first-degree relative with PDAC. Her BMI was 25.4 kg/m2. She scored 3 points (new-onset DM, age, positive family history for PDAC) on our risk assessment system. Her CA 19-9 level was normal. US showed a 30 mm hypoechogenic solid lesion, which was confirmed by CT. EUS-FNA showed pancreatic ductal adenocarcinoma. The patient was inoperable due to multiple metastases in the right lung. Chemotherapy induced regression and she continued treatment during the follow-up period (Figure 6).

Case 2

A 65-year-old man was diagnosed with T2DM 3 months before enrolment. His BMI was 29.4 kg/m2. He had not smoked for 7 years. The patient scored 2 points (new-onset DM, age) on our risk assessment system. The initial CA 19-9 level was 24.55 U/mL. The US findings were incomplete because the body and tail of the pancreas were not visible. 3 months after the US, a CT scan revealed an inhomogeneous hypodense mass with calcification in the tail, approximately 80 mm in size, with moderate infiltration of the peripancreatic area. Laparotomy was performed, but the tumour was inoperable due to advanced local invasion. Histological examination revealed ductal adenocarcinoma. At the 6-month follow-up, the CA 19-9 level was 25.90 U/mL. Chemotherapy was started and the patient had an overall survival of 32 months (Figure 6).

Case 3

A 78-year-old non - smoking woman was diagnosed with T2DM within 1 month prior to enrolment. Her BMI was 35.5 kg/m2. She scored 2 points (new-onset DM, age) on our risk assessment system. Her specific symptoms, such as weakness and weight loss, were thought to be the result of diabetes. Two weeks after her diagnosis of T2DM and enrolment in the study,

US revealed a 45 x 29 x 26 mm hypoechoic lesion in the tail. CT scan showed diffuse enlargement of the tail with a 56 x 31 x 43 mm hypodensity. The liver was inhomogeneous with multiple metastases measuring 8-25 mm. Histological examination of the US-guided biopsy revealed ductal adenocarcinoma. Chemotherapy was started, but unfortunately the patient died of PDAC only a few weeks after the diagnosis (Figure 6).

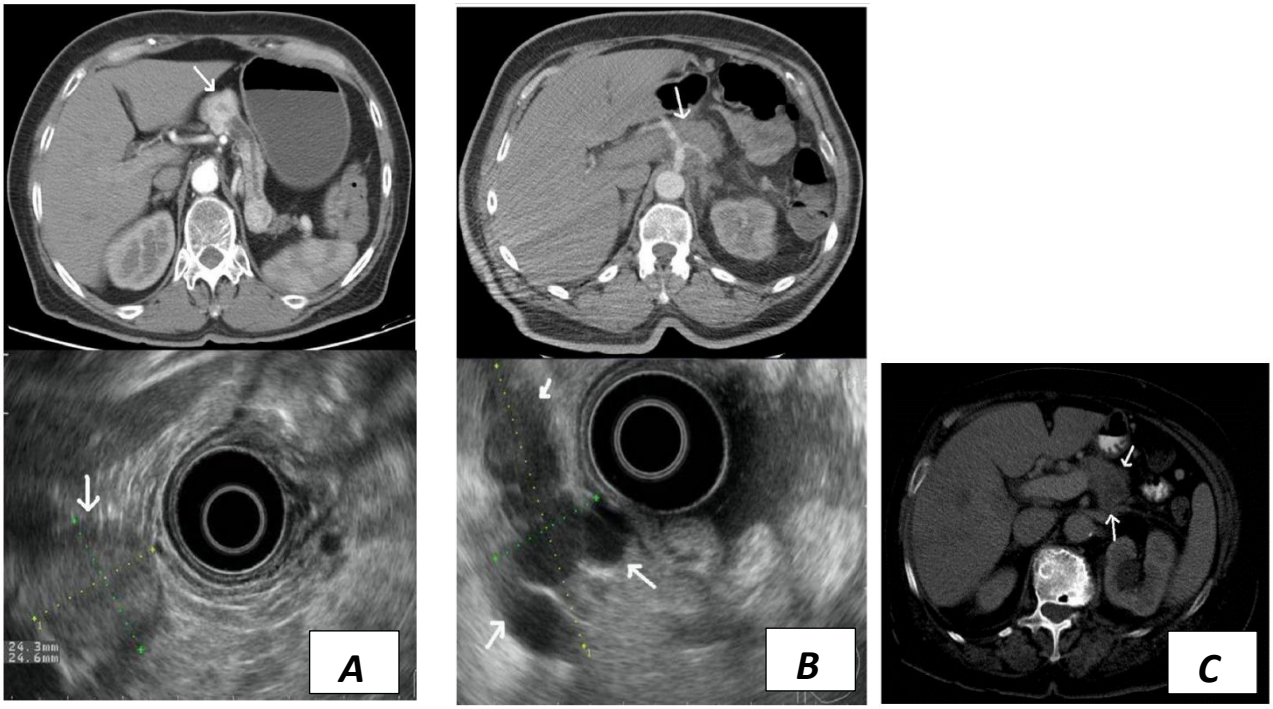


***Figure 6**. **A)** CT and EUS findings of case 1. A near 30 mm solid lesion in the head of the pancreas was revealed (arrows). **B)** CT and EUS findings of case 2. An inhomogeneous, about 80 mm in size hypodense mass was seen on CT with calcification in the tail and the peripancreatic area was moderately infiltrated. **C)** CT finding of case 3. An 56 x 31 x 43 mm hypodensity (arrows) was revealed with multiple liver metastases.*

The overall incidence of PDAC in our study was 2.78% (3/108). To calculate SIR the person-time incidence in our study was needed. The follow-up period of all participants was expressed in years and then summed up (162,42 years). The person-time incidence was (3/162,42*100) 1.847 cases/100personyear. The age-adjusted incidence in Hungary was 9.3/100.000 = 0.0093/100personyear. The value of SIR was 1.847/0.0093=198.6 (95% CI = 6.25 - 46.9). The effectiveness of the diagnostic modalities used in this study to screen for PDAC is shown in Table 8. Ten patients had elevated serum CA 19-9 levels, but none of them had PDAC. Two patients with PDAC had normal serum tumourmarker levels.

The US examination clearly showed the pancreatic mass in two of the three patients. In the remaining one hundred and five negative cases, the possibility of false-negative findings was excluded by follow-up.

CT was performed in eighteen patients (i.e. two patient with a positive US finding, ten with an elevated CA 19-9 level, four with incomplete US examinations and two with symptoms not suggestive of pancreatic disease). CT revealed a pancreatic mass in all three PDAC cases.

	Sensitivity	**Specificity**	**Positive predictive value**	**Negative predictive value**
CA 19-9	0%	90.4%	0%	97.9%
Transabdominal US	66.7%	100%	100%	99%
CT	100%	100%	100%	100%

Table 8*. Efficacy of screening modalities.*

6.2 Study B – NODES trial

As the NODES trial is still ongoing due to delays caused by the COVID 19 pandemic, no final results are available at the time of writing. Blood samples from 58 patients with new-onset DM are currently being analyzed in Germany. Of these, 4 patients (6.8%) had PDAC diagnosed within 6 months of DM diagnosis.

7. Discussion

PDAC has a poor prognosis due to its late diagnosis. Success in reducing the mortality rate of PDAC has been linked to the development of early detection and prevention programs. As the incidence of PDAC is low, screening should be limited to patients at high risk of developing PDAC (29). A meta-analysis showed that individuals with new-onset DM (<36 months after diagnosis) had a 50% increased risk of PDAC compared to those who had DM for >5 years (OR 2.1 vs. 1.5, P = 0.005) (21). Several studies have shown that DM is prevalent even in early stage PDAC (13, 24). In our prospective study, we demonstrated that the incidence of PDAC in patients with new-onset diabetes mellitus was 198.6-fold higher than in the normal

population. This is significantly higher than the previously reported 8-fold risk (24). In this retrospective study, Pannala et al. included patients diagnosed with PDAC and matched non-cancer patients. The presence of diabetes was not an inclusion criterion but a parameter studied. They included 512 patients with PDAC, of whom 41.6% were diabetic. Of these patients, 75% had new-onset diabetes. This means that this study did not distinguish between NOD-PDAC and T2DM cases. As a result, the cumulative risk of PDAC in this mixed DM group is lower than it would be in the NOD-PDAC group alone. In contrast, we included 108 new-onset diabetics in our study. During follow-up, we were only able to diagnose the NOD-PDAC patients because of the different study protocol. The limited number of cases could also cause some bias regarding the high value of the SIR.

New-onset T2DM can therefore be classified as a high-risk group for PDAC. Damiano et al. (16) reported a similar incidence (5.2%) of PDAC in patients hospitalized for newly diagnosed DM (less than 30 days), because the instability of the DM required insulin treatment.

Our study has provided evidence that screening is beneficial for the detection of PDAC in asymptomatic patients with new-onset DM. However, our results were also discouraging because all 3 PDAC cases diagnosed in our screening program were at an advanced, unresectable stage. A previous retrospective study showed that the mean interval between the onset of DM and the diagnosis of PDAC was 10 months (range 5 - 29 months) (15). The mean time between diagnosis of DM and enrolment of patients in our study was 3.5 ± 4.4 months in the whole screened population and 3.7 ± 4 months in patients finally diagnosed with PDAC. Therefore, the fact that advanced cases were diagnosed in our study cannot be explained by a longer interval between the onset of DM and inclusion in the study.

Prospective screening for PDAC by endoscopic retrograde pancreatography in patients with new-onset DM showed a very high incidence (13.9%) of PDAC. However, most of the diagnosed PDAC cases were unresectable, similar to our study (75). The time from onset of DM was longer in this study (9.2 months). Shortening the time to diagnosis of PDAC after diagnosis of DM or a more aggressive diagnostic approach may be beneficial.

Unfortunately, it appears that our protocol in the HiRiPaC study is not yet able to diagnose PDAC at an early stage when it is still resectable, although the results are based on extremely high SIR values, suggesting that patients with new-onset DM are an appropriate risk group for screening for PDAC.

Although the combination of serum CA 19-9 measurement and the use of transabdominal US as a screening tool for PDAC has been previously studied retrospectively, and this study showed that these tools together identified ductal adenocarcinoma of the pancreas with a sensitivity of 85.4%, which means that this combination is suitable for non-invasive screening of PDAC (76), we have confirmed that neither determining serum CA 19-9 levels nor performing transabdominal US are effective screening methods for detecting PDAC at an early stage.

CA 19-9 would be an ideal method for screening: it is easy to perform, widely available, repeatable, reliable, validated and cost-effective. However, we have shown that CA 19-9 is of no value in screening for PDAC in new-onset DM. Both the sensitivity and positive predictive value of CA 19-9 were zero, and the false positive rate was 9% in our study. The mean value of elevated CA 19-9 levels in our study was only 52.613 ± 23.13 U/mL. However, the optimal cut-off value of CA 19-9 to differentiate between benign and malignant pancreatobiliary disease has been shown to be 70.5 U/mL (82.1% sensitivity, 85.9% specificity, 81.3% positive predictive value and 86.5% negative predictive value) (77). Furthermore, CA 19-9 in diabetic patients can be considered as an indication of exocrine pancreatic dysfunction, and a higher cut-off value of CA 19-9 has been proposed to differentiate benign from malignant pancreatic disease (78). Serum CA 19-9 may be elevated not only in PDAC but also in biliary, hepatocellular, gastric, colorectal and non gastrointestinal tumours, liver disease and jaundice, leading to false-positive results. However, none of our patients with false-positive CA 19-9 results were found to have such diseases. Our results do not agree with those of Choe et al. (79) who reported that CA 19-9 alone is suitable for the identification of PDAC in patients with new-onset DM. Our results are more consistent with those of Zubarik et al. (80) who showed that the positive predictive value of CA 19-9 in patients with a positive family history of PDAC was only 3.7%.

Abdominal US would also be an ideal method for screening: it is easy to perform, widely available and inexpensive. However, the low sensitivity of US in our study suggests that it is not effective for screening. The effectiveness of abdominal CT in diagnosing PDAC was excellent in our study, but we could not prove that CT is an appropriate screening tool for early-stage PDAC. Furthermore, given the potential need for an extended screening program, CT is not recommended because of the risk associated with radiation exposure. If it is accepted that DM in PDAC is a paraneoplastic sign caused by the tumour itself, the search for tumour-produced diabetogenic factors (81) and their use as screening tools is needed.

A limitation of our HiRiPaC study is the small number of cases, and we therefore planned to further investigate the possibilities of early detection of PDAC.

To identify a higher proportion of cases with resectable PDAC, the risk group of newly diagnosed DM patients should be further narrowed.

Mizuno et al. found that weight loss and exacerbation of DM could be observed in DM patients 12 months prior to PDAC diagnosis. The timing of weight loss distinguishes two groups: patients with NOD-PDAC have already lost weight at the time of DM diagnosis and continue to lose weight until the cancer is diagnosed, whereas T2DM patients have already gained weight at the time of DM diagnosis and then stagnate or continue to gain weight (12). Furthermore, DM diagnosed in older individuals (>55 years) tends to be DM caused by PDAC, whereas diagnosis at a younger age is indicative of T2DM (12, 82), as seen in our study. However, Gupta et al. came to the opposite conclusion: younger age is a risk factor for NOD-PDAC (83).

At least one additional risk factor for PDAC, such as age, family history and obesity, was present in our patients diagnosed with PDAC in HiRiPaC study. A previous study showed that obesity and T2DM are independent risk factors for PDAC (84). Our results suggest that taking these factors into account may increase the yield of a screening program for PDAC.

Therefore, in the NODES trial we are studying elderly patients with new-onset diabetes mellitus using a biomarker panel (combining CA 19-9 with metabolites) to differentiate NOD-PDAC cases from T2DM patients. The expected positive endpoint of the NODES study is to validate this biomarker panel; whether it is suitable for early diagnosis of a mostly incurable, high mortality cancer, when surgery is still possible and the cancer can be cured. NOD-PDAC belongs to the T3cDM group, and T3cDM is the highest risk group for PDAC.

Unfortunately, it is still underdiagnosed in clinical practice - perhaps because its symptoms are very similar to those of T2DM and its diagnosis is based on complex, expensive tests that are not routinely available. Diagnosing patients with T3cDM based on these criteria would be extremely difficult and would not be a cost-effective screening method, which is not favorable.

While there are several pancreatic diseases that can cause T3cDM, the NODES study focuses only on the differences between NOD-PDAC and T2DM. In this way, this biomarker panel could be a diagnostic tool for the T3cDM subset NOD-PDAC. The test requires only a single blood sample, which means it is simple, repeatable, tolerable, minimally invasive, almost

painless, widely available and relatively inexpensive - it meets all the criteria for a screening method.

8. Conclusion

In conclusion, our prospective study demonstrated that the incidence of PDAC was significantly higher in patients with new-onset diabetes mellitus than in the normal population, making this group suitable for screening for PDAC. We confirmed that neither serum CA 19-9 nor transabdominal US, or both, are effective screening methods for the early detection of PDAC. In our study, abdominal CT is an effective imaging tool for the diagnosis of PDAC, but we were not able to prove that CT is an appropriate screening tool for early-stage PDAC.

The NODES study aims to validate a biomarker panel for the diagnosis of NOD-PDAC in elderly patients with new-onset diabetes, i.e. early detection of PDAC through surveillance of high-risk patients. This would increase surgical resection rates, cure rates and survival by 30%-40%. It would save lives, improve the wellbeing of the population and have a huge financial benefit: the increasing number of successful surgical interventions would lead to less need for chemotherapy and palliative interventions (such as stent implantation or gastroenteroanastomosis surgery) and reduce the burden of healthcare costs.

9. Novel observations

1. As statistically supported by our study, patients with new-onset diabetes mellitus are an appropriate group for pancreatic cancer screening.
2. CA 19-9 alone or in combination with transabdominal ultrasound is not effective for screening or diagnosing pancreatic ductal adenocarcinoma.
3. Transabdominal ultrasound is not effective for screening or diagnosing of pancreatic ductal adenocarcinoma.
4. Abdominal CT is a reliable imaging tool for the diagnosis of pancreatic ductal adenocarcinoma, although not in the early stages. It is therefore not suitable for screening, including radiation exposure.
5. By validating the biomarker panel studied in the NODES trial, the diagnosis of NOD-PDAC will allow us to screen for pancreatic ductal adenocarcinoma at an early stage in patients with new-onset diabetes mellitus.

10. Acknowledgements

First of all, I would like to thank my supervisor, **Prof. Dr. László Czakó**, for his guidance and support, without which this thesis would not have been possible. His knowledge and diligence are exemplary for me.

I would also like to thank **Prof. Dr. Csaba Lengyel, Prof. Dr. György Ábrahám** and **Prof. Dr. Tibor Wittmann**, the current and former heads of the Department of Medicine, for giving me the opportunity to work in their institutions.

Special thanks to **Dr. Viktória Terzin**, who has been there for me since the first day of my work as a student, providing me with help and information on how research should work.

I would like to thank all my colleagues and working group members, **Dr. Gábor Zsóri, Dr. Emese Iványi, Dr. Balázs Kui, Dr. Máté Tajti** for their cooperation and encouragement over the years.

I am grateful to **Prof. Dr. Péter Hegyi** and all the members of the **Hungarian Pancreatic Study Group** who allowed me to participate in several research projects at the same time.

Finally, to those to whom I owe the most. I will always be grateful to my brother **László** for being an inspiration that it's never too late to start or learn something new. I would like to thank my parents, **László** and **Ildikó**, for their endless love, support and encouragement. Without them, I would not be the person I am today. I dedicate this thesis to them.

11. References

1. Park W, Chawla A, O'Reilly EM. Pancreatic Cancer: A Review. *JAMA*. 2021;326:851-62.
2. Howlader N, Noone AM, Krapcho M et al. SEER Cancer Statistics Review, 1975-2018, National Cancer Institute. Bethesda, MD, https://seer.cancer.gov/csr/1975_2018/, based on November 2020 SEER data submission, posted to the SEER web site, April 2021.
3. Gillen S, Schuster T, Meyer Zum Büschenfelde C, et al. Preoperative/neoadjuvant therapy in pancreatic cancer: a systematic review and meta-analysis of response and resection percentages. *PLoS Med.* 2010;7:1000267.
4. Siegel RL, Miller KD, Fuchs HE, et al. Cancer statistics, 2021. *CA Cancer J Clin*. 2021;71:7–33.
5. Rawla P, Sunkara T, Gaduputi V. Epidemiology of pancreatic cancer: global trends, etiology and risk factors. *World J Oncol*. 2019;10:10–27.
6. Rahib L, Smith BD, Aizenberg R, et al. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. *Cancer Res*. 2014;74:2913–21.
7. Brand RE, Lerch MM, Rubinstein WS, et al. Advances in counselling and surveillance of patients at risk for pancreatic cancer. *Gut*. 2007;56:1460–9.
8. Ben Q, Cai Q, Li Z, et al. The relationship between new-onset diabetes mellitus and pancreatic cancer risk: a case-control study. *Eur J Cancer*. 2011;47:248-54.
9. Szmola R, Farkas G, Hegyi P, et al. Pancreatic cancer. Evidence based management guidelines of the Hungarian Pancreatic Study Group. *Orv Hetil*. 2015;156:326-39.
10. Balooch Hasankhani M, Mirzaei H, Karamoozian A. Global trend analysis of diabetes mellitus incidence, mortality, and mortality-to-incidence ratio from 1990 to 2019. *Sci Rep*. 2023;13:21908.
11. Aggarwal G, Rabe KG, Petersen GM, et al. New-onset diabetes in pancreatic cancer: a study in the primary care setting. *Pancreatology*. 2012;12:156-61.
12. Mizuno S, Nakai Y, Isayama H, et al. Risk factors and early signs of pancreatic cancer in diabetes: screening strategy based on diabetes onset age. *J Gastroenterol*. 2013;48:238-46.
13. Chari ST, Leibson CL, Rabe KG, et al. Probability of pancreatic cancer following diabetes: A population-based study. *Gastroenterology*. 2005;129:504-11.

14. Sah RP, Nagpal SJ, Mukhopadhyay D, et al. New insights into pancreatic cancer-induced paraneoplastic diabetes. *Nat Rev Gastroenterol Hepatol*. 2013;10:423-33.
15. Pelaez-Luna M, Takahashi N, Fletcher JG, et al. Resectability of presymptomatic pancreatic cancer and its relationship to onset of diabetes: a retrospective review of CT scans and fasting glucose values prior to diagnosis. *Am J Gastroenterol*. 2007;102:2157-63.
16. Damiano J, Bordier L, Le Berre JP, et al. Should pancreas imaging be recommended in patients over 50 years when diabetes is discovered because of acute symptoms? *Diabetes Metab*. 2004;30:203-7.
17. Illés D, Terzin V, Holzinger G, et al. New-onset type 2 diabetes mellitus-A high-risk group suitable for the screening of pancreatic cancer? *Pancreatology*. 2016;16:266-71.
18. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2010;33:62-9.
19. Ewald N and Bretzel RG. Diabetes mellitus secondary to pancreatic diseases [Type 3c]-are we neglecting an important disease? *Eur J Int Med*. 2013;24:203-6.
20. Illés D, Czakó L. A diabetes és az emésztőrendszer betegségei: pancreatitis és emésztőrendszeri tumorok. *Magy Belorv Arch*. 2020;73:63–7.
21. Huxley R, Ansary-Moghaddam A, Berrington de González A, et al. Type-II diabetes and pancreatic cancer: a meta-analysis of 36 studies. *Br J Cancer*. 2005;6(92):2076-83.
22. Ben Q, Xu M, Ning X, et al. Diabetes mellitus and risk of pancreatic cancer: A meta-analysis of cohort studies. *Eur J Cancer*. 2011;47:1928-37.
23. Aggarwal G, Kamada P, Chari ST. Prevalence of diabetes mellitus in pancreatic cancer compared to common cancers. *Pancreas*. 2013;42:198-201.
24. Pannala R, Leirness JB, Bamlet WR, et al. Prevalence and clinical profile of pancreatic cancer-associated diabetes mellitus. *Gastroenterology*. 2008;134:981-7.
25. Munigala S, Singh A, Gelrud A, et al. Predictors for pancreatic cancer diagnosis following new-onset diabetes mellitus. *Clin Transl Gastroenterol*. 2015;6:118.
26. Choe JW, Kim JS, Kim HJ, et al. Value of early check-up of carbohydrate antigen 19-9 levels for pancreatic cancer screening in asymptomatic new-onset diabetic patients. *Pancreas*. 2016;45:730–4.
27. Chari ST. Detecting early pancreatic cancer: problems and prospects. *Semin Oncol*. 2007;34:284-94.
28. Andersen DK, Korc M, Petersen GM, et al. Diabetes, Pancreatogenic Diabetes, and Pancreatic Cancer. *Diabete*s. 2017;66:1103-10.

29. Pannala R, Basu A, Petersen GM et al. New-onset diabetes: a potential clue tot he early diagnosis of pancreatic cancer. *Lancet Oncol.* 2009;10:88-95.
30. Löhr JM, Dominguez-Munoz E, Rosendahl J, et al. United European Gastroenterology evidence-based guidelines for the diagnosis and therapy of chronic pancreatitis (HaPanEU). *United European Gastroenterology J*. 2017;5:153–99.
31. Petrov MS, Basina M. Diagnosis of endocrine disease: Diagnosing and classifying diabetes in diseases of the exocrine pancreas. *Eur J Endocrinol*. 2021;184:151-63.
32. Singh RG, Cervantes A, Kim JU, et al. Intrapancreatic fat deposition and visceral fat volume are associated with the presence of diabetes after acute pancreatitis. American Journal of Physiology. *Gastrointestinal & Liver Physiology*. 2019;316:806–15.
33. Singh RG, Nguyen NN, DeSouza SV, et al. Comprehensive analysis of body composition and insulin traits associated with intra-pancreatic fat deposition in healthy individuals and people with new-onset prediabetes/diabetes after acute pancreatitis. *Diabetes, Obesity & Metabolism*. 2019;21:417–23.
34. Ko J, Skudder-Hill L, Cho J, et al. The relationship between abdominal fat phenotypes and insulin resistance in non-obese individuals after acute pancreatitis. *Nutrients.* 2020;12:2883.
35. Petrov MS. Harnessing analytic morphomics for early detection of pancreatic cancer. *Pancreas.* 2018;47:1051–54.
36. Sreedhar UL, DeSouza SV, Park B et al. A systematic review of intra-pancreatic fat deposition and pancreatic carcinogenesis. *Journal of Gastrointestinal Surgery*. 2020;24: 2560–69.
37. Löhr M, Goertchen P, Nizze H, et al. Cystic fibrosis associated islet changes may provide a basis for diabetes. An immunocytochemical and morphometrical study. Virchows Archiv. *Pathological Anatomy and Histopathology*. 1989;414:179–85.
38. Xiao AY, Tan ML, Wu LM, et al. Global incidence and mortality of pancreatic diseases: a systematic review, meta-analysis, and meta-regression of population-based cohort studies. Lancet. *Gastroenterology & Hepatology*. 2016;1:45–55.
39. Tempero MA, Uchida E, Takasaki H, et al. Relationship of carbohydrate antigen 19-9 and Lewis antigens in pancreatic cancer. *Cancer Res*. 1987;47:5501-3.
40. Kim JE, Lee KT, Lee JK, et al. Clinical usefulness of carbohydrate antigen 19-9 as a screening test for pancreatic cancer in an asymptomatic population. *J Gastroenterol Hepatol.* 2004;19:182-6.

41. Luo G, Fan Z, Cheng H, et al. New observations on the utility of CA19-9 as a biomarker in Lewis negative patients with pancreatic cancer. *Pancreatology*. 2018;18:971-6.
42. Duffy MJ, Sturgeon C, Lamerz R, et al. Tumor markers in pancreatic cancer: a European Group on Tumor Markers (EGTM) status report. *Ann Oncol*. 2010;21:441–7.
43. Illés Dóra, Czakó László. A hasnyálmirigyrák Világnapja 11.21. – Lankadatlan éberség! *Lege Artis Medicinae*. 2019;29(12):643-6.
44. Gui JC, Yan WL, Liu XD. CA19-9 and CA242 as tumor markers for the diagnosis of pancreatic cancer: a meta-analysis. *Clin Exp Med*. 2013;14:225–33.
45. Poruk KE, Gay DZ, Brown K, et al. The clinical utility of CA 19-9 in pancreatic adenocarcinoma: diagnostic and prognostic updates. *Curr Mol Med*. 2013;13:340–51.
46. Okusaka T, Yamada T, Maekawa M. Serum Tumor markers for pancreatic cancer: the dawn of New Era? *J Pancreas* (Online). 2006;7:332-6.
47. Rickes S, Unkrodt K, Neye H, et al. Differentiation of pancreatic tumours by conventional ultrasound, unenhanced and echo-enhanced power Doppler sonography. *Scand J Gastroenterol*. 2002;37:1313–20.
48. Illés D, Iványi E, Holzinger G, et al. New Onset of DiabetEs in aSsociation with pancreatic ductal adenocarcinoma (NODES Trial): protocol of a prospective, multicentre observational trial. *BMJ Open*. 2020;0:037267.
49. Jenkinson C, Earl J, Ghaneh P, et al. Biomarkers for early diagnosis of pancreatic cancer. *Expert Rev Gastroenterol Hepatol*. 2015;9:305–15.
50. Majumder S, Chari ST, Ahlquist DA. Molecular detection of pancreatic neoplasia: current status and future promise. *World J Gastroenterol*. 2015;21:11387–95.
51. Gerdtsson AS, Malats N, Säll A, et al. A multicenter trial defining a serum protein signature associated with pancreatic ductal adenocarcinoma. *Int J Proteomics*. 2015;2015:587250.
52. Fiedler GM, Leichtle AB, Kase J, et al. Serum peptidome profiling revealed platelet factor 4 as a potential discriminating Peptide associated with pancreatic cancer. *Clin Cancer Res.* 2009;15:3812–9.
53. Jiang JT, Wu CP, Deng HF, et al. Serum level of TSGF, CA242 and CA19-9 in pancreatic cancer. *World J Gastroenterol*. 2004;10:1675–7.
54. Kawa S, Tokoo M, Hasebe O, et al. Comparative study of CA242 and CA19-9 for the diagnosis of pancreatic cancer. *Br J Cancer*. 1994;70:481–6.

55. Schultz NA, Dehlendorff C, Jensen BV, et al. MicroRNA biomarkers in whole blood for detection of pancreatic cancer. *JAMA*. 2014;311:392–404.
56. Kobayashi T, Nishiumi S, Ikeda A, et al. A novel serum metabolomics-based diagnostic approach to pancreatic cancer. *Cancer Epidemiol Biomark Prev*. 2013;22:571–9.
57. Nishiumi S, Shinohara M, Ikeda A, et al. Serum metabolomics as a novel diagnostic approach for pancreatic cancer. *Metabolomics.* 2010;6:518–28.
58. Ritchie SA, Chitou B, Zheng Q, et al. Pancreatic cancer serum biomarker PC-594: Diagnostic performance and comparison to CA19-9. *World J Gastroenterol*. 2015;21:6604–12.
59. Mayerle J, Kalthoff H, Reszka R, et al. Metabolic biomarker signature to differentiate pancreatic ductal adenocarcinoma from chronic pancreatitis. *Gut.* 2018;67:128-37.
60. American Diabetes Association. Executive summary: standards of medical care in diabetes - 2009. *Diabetes Care*. 2009;32(Suppl.1):6-12.
61. Becher H, Winkler V. Estimating the standardized incidence ratio (SIR) with incomplete follow-up data. *BMC Med Res Methodol*. 2017;17(1):55.
62. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. GLOBOCAN 2012 v1.1, cancer incidence and mortality worldwide: IARC Cancer-Base No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2014. Available from:http://globocan.iarc.fr [accessed 16.01.15].
63. New Jersey Department of Health & Senior Services Cancer Epidemiology Services. Fact sheet: explanation of standardized incidence ratios.
Internet:
https://nj.gov/health/ceohs/documents/eohap/haz_sites/passaic/pompton_lakes/pompton_lakes_fs_sir.pdf
64. Explanation of a Standardized Incidence Ratio (SIR) and 95% Confidence Interval Source: Massachusetts Department of Public Health. Bureau of environmental health assessment. December 1998.
Internet:
https://www.mass.gov/doc/appendix-a-explanation-of-a-standardized-incidence-ratio-sir-and-95-confidence-interval-0/download
65. American Diabetes Association. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2018. *Diabetes Care*. 2018;41:13–27.

66. Jung K, Reszka R, Kamlage B, et al. Tissue metabolite profiling identifies differentiating and prognostic biomarkers for prostate carcinoma. *Int J Cancer*. 2013;133:2914–24.
67. Christie WW. Rapid separation and quantification of lipid classes by high performance liquid chromatography and mass (light-scattering) detection. *J Lipid Res.* 1985;26:507–12.
68. Mutch DM, Fuhrmann JC, Rein D, et al. Metabolite profiling identifies candidate markers reflecting the clinical adaptations associated with Roux-en-Y gastric bypass surgery. *PLoS One.* 2009;4:7905.
69. Roessner U, Wagner C, Kopka J, et al. Technical advance: simultaneous analysis of metabolites in potato tuber by gas chromatography-mass spectrometry. *Plant J*. 2000;23:131–42.
70. van Ravenzwaay B, Cunha GC-P, Leibold E, et al. The use of metabolomics for the discovery of new biomarkers of effect. *Toxicol Lett*. 2007;172:21–8.
71. Schmidt H, Schmidt R, Geisslinger G. LC-MS/MS-analysis of sphingosine-1-phosphate and related compounds in plasma samples. *Prostaglandins Other Lipid Mediat*. 2006;81:162–70.
72. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B.* 1995;57:289–300.
73. Gart JJ, Nam J. Approximate interval estimation of the ratio of binomial parameters: a review and corrections for skewness. *Biometrics*. 1988;44:323–38.
74. Scherer R, Schaarschmidt F, Prescher S, et al. Simultaneous confidence intervals for comparing biodiversity indices estimated from overdispersed count data. *Biom J*. 2013;55:246–63.
75. Ogawa Y, Tanaka M, Inoue K, et al. A prospective pancreatographic study of the prevalence of pancreatic carcinoma in patients with diabetes mellitus. *Cancer*. 2002;94(9):2344-9.
76. Bovo P, Rigo L, Togni M, et al. Rapid diagnosis of pancreatic cancer by combination of ultrasonography and serum tumour markers CA 19-9 and CA 50. *Ital J Gastroenterol.* 1993;25:477-81.
77. Morris-Stiff G, Teli M, Jardine N, et al. CA19-9 antigen levels can distinguish between benign and malignant pancreaticobiliary disease. *Hepatobiliary Pancreat Dis Int.* 2009;8(6):620-6.

78. Uygur-Bayramicli O, Dabak R, Orbay E, et al. Type 2 diabetes mellitus and CA 19-9 levels. *World J Gastroenterol*. 2007;28(13):5357-9.
79. Choe JW, Kim HJ, Kim JS, et al. Usefulness of CA 19–9 for pancreatic cancer screening in patients with new-onset diabetes. *Hepatobiliary & Pancreatic Diseases International*. 2018;17:263-8.
80. Zubarik R, Gordon SR, Lidofsky SD, et al. Screening for pancreatic cancer in a high-risk population with serum CA 19-9 and targeted EUS: a feasibility study. *Gastrointest Endosc*. 2011;74:87-95.
81. Basso D, Greco E, Fogar P, Pucci P, Flagiello A, Baldo G, et al. Pancreatic cancerderived S-100A8 N-terminal peptide: a diabetes cause? *Clin Chim Acta*. 2006;372:120-8.
82. Hart PA, Kamada P, Rabe KG, et al. Weight loss precedes cancer-specific symptoms in pancreatic cancer-associated diabetes mellitus. *Pancreas*. 2011;40(5):768-72.
83. Gupta S, Vittinghoff E, Bertenthal D, et al. New onset diabetes and pancreatic cancer. *Clin Gastroenterol Hepatol*. 2006;4(11):1366-72.
84. Suceveanu AI, Suceveanu AP, Catrinoiu D et al. Diabetes mellitus, obesity and chronic pancreatitis? Independent risk factors for pancreatic adenocarcinoma (PAC) in Romanian Black Sea coast area. *Pancreatology*. 2015;15:86.

12. Annex

NODES FORM A

1. Patient personal details

Institute:
Doctor code
Blood sample code:
Date of blood sampling:

Insurance number:
Name:
Date of birth:
Age:
Gender: female / male
Race: Asian-Indian / White / Black / N/A
Date of diabetes diagnosis:
Inclusion date:

2. Inclusion criteria

Patients older than 60 years	YES	NO
Newly diagnosed diabetes mellitus (in the last 6 months)	YES	NO
Written informed consent signed	YES	NO
One „NO" is present = DO NOT INCLUDE!		

3. Exclusion criteria

Continuous alcohol abuse (weekly, daily)	YES	NO
Chronic pancreatitis	YES	NO
Previous pancreas operation/pancreatectomy	YES	NO
Pregnancy	YES	NO
Present malignant disease	YES	NO
Type-1 diabetes mellitus	YES	NO
One „YES" is present = EXCLUDE!		

4. Details from the medical history

Alcohol consumption: yes / no
If yes: frequency: N/A / occasionally/monthly
Smoking: yes / no
if yes: amount (cigarettes/day):……
for how many years? ……
if not: Did you smoke earlier? yes/no/ N/A
if yes: amount (cigarettes/day):……
for how many years?……
How long ago did you stop smoking? ……..

Antidiabetic treatment – if already taken

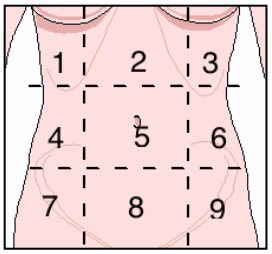
Name of medication:……..
active substance:……
dose: ……
dose without unit (number only!)
unit: g / mg / IU
how many times per day (e.g. 3): ……
Method of administration: oral / subcutan

Insulin: yes / no
dosage
Name of the medication:……
Dosage (00:00 – 23:00)

5. Status on admission

Body weight:…… kg Body height:…… cm
Calculated BMI:

6. Complains, symptoms



Abdominal pain: yes / no
If yes: since when (hours):……..
type: N/A / cramping / dull / sharp
intensity (1-10):……..
location: diffuse / localised
Please mark the location!
radiation:……..

Appetite: good / retained / bad

Weight loss: yes / no
If yes: How long did it take? (weeks):……
How much (kg):……

7. Laboratory parameters on admission

White blood cell (WBC) count (G/l)	
Hemoglobin (g/l)	
Hematocrit (%)	
Thrombocyte (G/l)	
ASAT/GOT (U/l)	
ALAT/GPT (U/l)	
Gamma GT (U/l)	
Total bilirubin (umol/l)	
Direct/Conjugated bilirubin (umol/l)	
Alkaline phosphatase (U/l)	
Cholesterol (mmol/l)	
Triglyceride (mmol/l)	
Glucose (mmol/l)	

Blood urea nitrogen (mmol/l)	
Creatinine (umol/l)	
HbA1c (%)	
CA 19-9 (U/ml)	
Fe (µmol/l	
Sedimentation rate (mm/h)	
Na (mmol/l)	
K (mmol/l)	

8. Imaging examinations needed **yes / no**

Weight loss	**YES**	**NO**
Abdominal pain/discomfort	**YES**	**NO**
Abnormal laboratory data	**YES**	**NO**
Two "YES"es are present = worrisome features -> EUS/MRCP!		

EUS: yes / no
If yes: Description:
……

MRCP: yes / no
If yes:
Description:……

Other examinations happened :

Abdominal ultrasonography: yes / no
If yes: Description:
……

Abdominal Computed Tomography: yes / no
If yes: Description:
……

9. Date of next visit:

NODES FORM B

1. Patient personal details

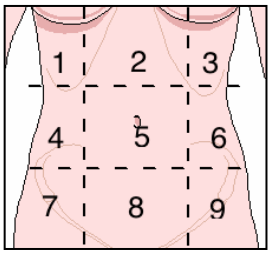
Institute:
Doctor code
Biomarker yes no

Insurance number:
Name:
Date of birth:
Visit No.:
Date of visit:

2. Status on admission

Body weight:…… kg **Body height:** …… cm
Calculated BMI:

3. Complains, symptoms



Abdominal pain: yes / no
If yes: since when (hours):……..
type: N/A / cramping / dull / sharp
intensity (1-10):……..
location: diffuse / localised
Please mark the location!
radiation:……..

Appetite: good / retained / bad
Weight loss: yes / no
If yes: How long did it take? (weeks):……
How much (kg):…….

4. Laboratory parameters on admission

White blood cell (WBC) count (G/l)	
Hemoglobin (g/l)	
Hematocrit (%)	
Thrombocyte (G/l)	
ASAT/GOT (U/l)	
ALAT/GPT (U/l)	
Gamma GT (U/l)	
Total bilirubin (umol/l)	
Direct/Conjugated bilirubin (umol/l)	
Alkaline phosphatase (U/l)	
Cholesterol (mmol/l)	
Triglyceride (mmol/l)	
Glucose (mmol/l)	
Blood urea nitrogen (mmol/l)	
Creatinine (umol/l)	
HbA1c (%)	
CA 19-9 (U/ml)	
Fe (µmol/l	
Sedimentation rate (mm/h)	

Na (mmol/l)	
K (mmol/l)	

5. **Imaging examinations needed** yes / no

Weight loss	**YES**	**NO**
Abdominal pain/discomfort	**YES**	**NO**
Abnormal laboratory data	**YES**	**NO**
Unstable glucose metabolism*	**YES**	**NO**
One "YES" is present = worrisome features -> EUS/MRCP!		

* despite the adequate diet and medical treatment and without intercurrent infection

EUS: yes / no
If yes: Description:
……

MRCP: yes / no
If yes: Description:
……

Other examinations happened :

Abdominal ultrasonography: yes / no
If yes: Description:
……

Abdominal Computed Tomography: yes / no
If yes: Description:
……

6. **Applied therapy/changes in therapy**

Name of medication:……..
active substance:……
dose: …….
dose without unit (number only!)
unit: g / mg / IU
how many times per day (e.g. 3): …….
Method of administration: oral / subcutan

<u>Insulin:</u> yes / no
dosage
Name of the medication:……
Dosage (00:00 – 23:00)

7. **Date of next visit:**