

# PhD THESIS

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## **THEORETICAL AND PRACTICAL INNOVATIONS IN THE TREATMENT OF PANCREAS DISEASES: INVESTIGATION OF PACAP AND PAC1 RECEPTOR EXPRESSION, DEVELOPMENT OF POSTOPERATIVE COMPLICATIONS.**

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## **Introduction**

The pancreas is perhaps one of the most complex organs in the human body. It has both endocrine and exocrine functions, which are involved in the digestive processes and the hormonal functioning of the body at many points. The exocrine pancreas is almost vital for the proper functioning of the body's metabolism through a number of enzymes, endocrine pancreas acts both on its own and in regulating several other organs in the body through the production of various hormones. Inbalance can have very serious consequences. Acute inflammation can, in severe cases, even lead to a life-threatening condition. Chronic pancreatitis affects more and more people, often causing malabsorption or can be starting point for diabetes. Chronic inflammation of the pancreas also plays a risk-increasing role in the development of pancreatic cancer. Pancreatic cancer is the 12th most common cancer in the world, currently the 4th most common cause of death, and is projected to be the 2nd most common cause of death by 2030. At the time of discovery, patients are often at an advanced stage, patients have a poorer physical condition from the beginning, and due to the often late discovery, the five-year chance of survival in patients with pancreatic tumors is very low (around 5%). Diagnosis is complicated by similar symptoms of inflammation and tumor, and to date there are no sufficiently specific and sensitive tumor markers for screening.

The stimulatory or inhibitory role of PACAP (Pituitary Adenilate-Cyclase Activating Polypeptide) and its receptors in tumorigenesis has been described in many organs. For endocrine and exocrine pancreatic tumors, this is not yet clear. In one part of my dissertation, I aimed to examine this.

Pancreatic surgeries are very stressful procedures for the patient and often a major professional challenge for the operator. The development of postoperative complications and the factors influencing the development (pancreatic tissue quality, choice of type of pancreatic anastomosis, use of abdominal drain) are still only partially clarified, in the other part of the dissertation I aimed to investigate these factors.

## **Tumor of the exocrine pancreas**

Pancreatic cancer (PC) is the 12th most common cancer in the world. Histologically, PC can be divided into exocrine tumors, which account for approximately 90% of cases and endocrine tumors, which occur in approximately 10%. The vast majority (90%) of exocrine tumors are ductal adenocarcinoma (PDAC), with a lower incidence of cystic tumors: IPMN (Intraductal Papillary Mucinosus Neoplasm), SPT (Solid Pseudopapillary Tumor), serous cystadenoma (benign lesion), mucinous cystic neoplasia (a lesion prone to malignancy). In the case of PC, the expected five-year survival is less than 5%, and in more than 80% of patients, regional lymph node involvement or distant metastasis can be detected at the time of diagnosis. According to the WHO data, the PC is the 13th most common cause of death in Hungary, with 1.89% of all annual deaths due to PC in 2018.

In case of a lesion in the head of the pancreas, pancreatoduodenectomy (conventional Kausch-Whipple operation or pylorus preserving pancreatoduodenectomy), in the case of the tail region, the so-called distal pancreatic resection (with or without spleen removal), in case of central localisation total pancreatectomy is required. In non-resectable cases, the goal is to provide passage or eliminate the patient's icterus (biliodigestive anastomosis).

Systemic (chemo) therapy is needed for locally advanced or metastatic PC: monotherapy with Gemcitabine or combination of Gemcitabine with albumin-bound (Nab) Paclitaxel or cisplatin. The combination of FOLFIRINOX treatment can show effective results in case of metastatic, locally advanced or non-resectable PC. These combinations can be used also for neoadjuvant (induction) treatment. Immunotherapy also promises good results in systemic therapy: pembrolizumab, a PD-1/PDL-1 inhibitor, can be used in microsatellite unstable cases, in the presence of a metastatic, BRCA-mutated cases, and ineffective platinum-based therapy, PARP inhibitor olapanib maintainer therapy can be admitted.

## **Tumor of the endocrine pancreas**

The so-called Neuroendocrine tumors (NETs) are clinically classified based on the effect and amount of the produced hormone. The neuroendocrine tumors of the pancreas are called islet cell tumors or pancreatic neuroendocrine tumors (PNETs). Islet cell tumors account for only about 3% of all pancreatic tumors, the expected prognosis is more favorable than exocrine pancreatic tumors. 40-50% of PNET patients present clinical symptoms, these are called hormonally active tumors, the other half of the patients are the non-functioning tumor group. The frequency and overall incidence of all NETs, including PNETs, is increasing year by year. Blood glucose imbalance is a condition that affects many people around the world, in the background often with disorder of insulin production or effect. Insulin-producing tumor (insulinoma) is one of the functioning PNET tumors, it is rare (with an incidence of around 0.4%) and, like other islet cell tumors, have a benign morphology. Insulinoma is the most common cause of hypoglycemia associated with high endogenous insulin levels, in some cases as part of MEN-1 (Multiple Endocrine Neoplasia) syndrome. The diagnostic triad (Whipple's triad) is characterized by fasting-provoked hypoglycaemia, blood sugar level below 50 mg/dl at the onset of symptoms and reduction of these symptoms after administration of sugar. In most of the cases, it can be treated with enucleation (but in severe cases of malignant insulinoma, extended pancreatic resection may be required, with simultaneous oncology therapy). Tumor localization and other tumor surgical guidelines define the operation technique and extent same as in case of exocrine and other endocrine pancreatic tumors: if tumor is benign and non-infiltrating, enucleation is sufficient. Diazoxide, also used as an antihypertensive drug, had leading role in the treatment of malignant insulinoma until the 1950s. In the 1990s, the focus shifted to the SS analog Octreotide, which reduces plasma insulin concentrations, in the last decade in combination with cytoreductive therapy (temozolomide + capecitabine or cisplatin/carboplatin + etoposide). In advanced cases, the mTOR inhibitor everolimus can preserve a longer progression-free survival, in these cases control of excessive hormone production can be better. The same effectiveness can be noticed using the tyrosine kinase inhibiting sunitinib.

## **Operations of the pancreas, complications**

During pancreatic surgery, an upper curved (subcostal) incision is most often recommended for open exploration, however, if the patient had previously a longitudinal incision, median laparotomy can be used, combined with an additional transverse incision over the umbilical region. In all cases, mobilization of the duodenum according to Kocher is essential for correct palpation of the head of the pancreas. The anterior surface of the pancreas can be explored after cutting the gastrocolic ligament. In case of pancreatoduodenectomy (pancreas head and body operations), removing the gallbladder and transection (as well as exploration) of the common bile duct are practically essential parts of the procedure, in cases of left side resection (body-tail) and total pancreatectomy splenectomy is often needed. Based on the pathological and oncological principles, adequate radicality is essential for pancreatic surgery, organ / tissue sparing techniques are only in proven not malignant cases acceptable.

Operations of the pancreas can be divided according to their indications:

### 1. Drainage operations

#### a. pseudocyst decompression

- cystojejunostomy
- anterior cystogastrostomy (Jurasz)
- posterior cystogastrostomy
- cystoduodenostomy

#### b. Bypassing of common bile duct, pancreatic duct, or duodenal stenosis

- Wirsungo-jejunostomy (Partington-Rochelle)
- Wirsungo-gastrostomy
- Hepatico-jejunostomy
- GEA (gastroenteroanastomosis)
- duodeno-jejunostomy with Roux-en-Y loop
- double bypass (hepatico-jejunostomy and GEA)

2. Conventional resections or organ sparing operations
  - a. proximal (right side) resection
    - Conventional Kausch-Whipple operation
    - pylorus preserving pancreatoduodenectomy (PPPD)
      - with pancreatojejunostomy (Traverso-Longmire)
      - with pancreatogastrostomy (Flautner)
  - b. distal (left side) resection
    - resection of the pancreatic tail
    - resection of pancreas body and tail
    - subtotal resection (near total, Fry-Child resection)
  - c. enucleation
  - d. ampullectomy
  - e. total pancreatectomy
  - f. organ preserving operations
    - duodenum preserving pancreas head resection (Beger)
    - pancreas head resection without transecting pancreas neck, anastomosis after longitudinal incision of pancreatic duct (Frey operation)
    - pancreas head resection without transection of pancreas neck and longitudinal incision of pancreatic duct (Büchler-Farkas operation)
    - distal pancreas resection with preservation of the spleen
    - central (segmental) pancreatectomy
3. Other, rarely used operations
  - necrectomy
  - fistulojejunostomy.

Due to the fragile tissue structure and high sensitivity of the pancreas to external impacts, the risk of complications following pancreatic surgery may also be high. The higher rates of morbidity, mortality and complications during surgery can also be connected with frequent onset of malabsorption, together with several co-morbidities, often with alcohol abuse, liver function disorders and cardiovascular diseases. These factors may increase the risk of complications and the length of recovery. The risk of complications increase moreover because of the complexity of pancreatic surgery, involving different types of anastomoses (bile duct, pancreas, and gastrointestinal tract). Although in the recent period the mortality of

pancreatoduodenectomies in specialized centers has decreased to less than 5%, the morbidity after pancreatic resection is around 50% (18–54%). The most common early complications are delayed gastric emptying (DGE) and postoperative pancreatic fistula (POPF), less common is bile duct fistula and postoperative bleeding. Later, anastomotic stenosis, biliar gastritis, intestinal adhesions, and ulcer formation may occur.

Exact definition and development of DGE have long been debated, DGE means based on definition if large amount of gastric contents appears through the nasogastric (NG) tube for more than 7th postoperative day and prokinetic drug administration or parenteral diet are needed before the resolution of DGE.

The definition of POPF determined by the University Centers in Heidelberg and Johns Hopkins means the secretion of high amylase-containing secretions through the abdominal drain on the tenth or subsequent day after surgery. Soft, fragile pancreatic tissue and a narrow pancreatic duct have a risk-enhancing effect on the formation of POPF. Consensus on the definition was worked-out by the ISGPF (International Study Group on Pancreatic Fistula) in 2005 and 2016, POPF severity was divided in three groups based on increase of serum amylase, length of drainage, and need for intervention, reoperation or intensive care (POPF A/B/C).

Recently, risk assessment scores have been developed to reduce fistula formation, examining the soft or fibrotic nature of the pancreas, the histopathological quality of the lesion, the diameter of the pancreatic duct, and blood loss during surgery. General surgical and pancreas anastomosis technique, possible use of (external or internal) stents, use of abdominal drain, and administration of Octreotide can affect risk for fistula developing. The risk-reducing effect of Octreotide on POPF has been studied several times. With Octreotide administration, some authors describe decrease in POPF incidence, while others found no directly associated risk-reducing effect. Drainage of digestive enzymes from the pancreas can help anastomosis healing. External stent (anastomosis relieved by a stent directed to the body surface, which must be removed later) or internal stent (the stent is in the lumen of the intestine and pancreatic duct, which later passes through the intestinal tract) can be used. The use of a stent can support a potential easier healing of the anastomosis, as drainage of pancreatic fluid is more effective in such cases, however, there is a potential risk for stent-related complications, increased digestive enzyme loss, and risk for consequent maldigestion and malabsorption. Untill now no study has proven that the use of stent significantly reduce the incidence of POPF.

The technique of pancreatic anastomosis can also affect the risk of complications (especially POPF). The two most commonly used techniques for pancreatic anastomosis are pancreatogastrostomy (PG) and pancreatojejunostomy (PJ). Both techniques are widely used,

no significant difference in the development of POPF was described, but multicentre studies in PG found higher rate of postpancreatectomy bleeding. Different techniques were worked-out to form PJ: end-to-side intussusception, duct-to-mucosa technique, binding anastomosis technique, Blumgart anastomosis, pancreatojejunostomy with an isolated jejunum loop, and a tobacco bag suture similar to the binding technique. There is no consensus on the risk of POPF: some authors found the intussusception technique to be more favorable, while others found no significant difference between the conventional (duct-to-mucosa) versus binding techniques for POPF, DGE, and other complications risk. In the case of soft pancreatic tissue, a lower incidence of POPF was reported when PJ was made with a tobacco bag stitch, while others experienced less frequent pancreatic fluid leakage using longer jejunum loop and duct-to-mucosa PJ formation. However, the International Conference of ISGPS found no clear evidence which technique is significantly better than the other one.

Following abdominal surgeries, operator very often leaves a drain tube to detect early complications (bleeding, anastomosis insufficiency) as soon as possible. Several studies and meta-analyzes have shown that the use of abdominal drain for routine abdominal surgeries (appendectomy, hepatectomy, colectomy) does not reduce the number and frequency of postoperative complications, on the other hand, need for routine abdominal drainage after pancreatoduodenectomy cannot be rejected because of the risk for abdominal abscess formation and increased mortality. Some authors did not recommend routine prophylactic abdominal drainage based on the incidence of POPF and associated complications, while other authors noticed increase in the incidence and severity of postoperative DGE, abdominal abscess, and elongated hospitalization time after omitting the abdominal drain. Based on this, use of abdominals drain is questionable and debatable. Further question is the postoperative drainage length and the optimal time of drain removal. In case of patients with low POPF risk (drain amylase level is less than 5 000 U/l on the first day), it was shown that the abdominal drain can be safely removed on day 3 after pancreatic resection (early drainage removal), but if it is done on the 5th postoperative day, POPF rate increases significantly. Similarly, for amylase levels of 5 000 U/l in the first day after surgery, early drain removal is recommended, but its negative predictive value is low. If the drainage amylase level does not exceed 350 U/l on the 3rd postoperative day, early drain removal seem to be safe.



## PACAP

PACAP (Pituitary Adenylate-Cyclase Activating Polypeptide) is a 38 amino acid neuropeptide (PACAP38), member of the secretin/glucagon/VIP family (N-terminal portion is 68% homologous to VIP). Its extremely important physiological role is also demonstrated by the fact that the active N-terminal region is highly conserved during phylogenesis, and PACAP-containing neurons can be detected in both mammalian and non-mammalian vertebrates: birds, fishes, and amphibians. Although the distribution of PACAP is observed throughout the body, its highest concentrations are found in the central nervous system and endocrine glands. It has important effect in many places in the body, from the cardiovascular system, through the gastrointestinal tract and the genitourinary tract, to the endocrine glands.

It exerts an important regulatory effect in the gastrointestinal tract in both physiological and pathological conditions: stimulation of the secretion of digestive enzymes, smooth muscle contraction, and circulation of organs. In addition, it may play a role in the reduction of inflammatory lesions in the intestinal tract (ileitis, colitis), and decreased PACAP immunoreactivity may be observed in some colon tumor cell lines.

PACAP acts through its G-protein-coupled receptors (PAC1, VPAC1, and VPAC2 receptors). The PACAP binding of the PAC1 receptor is specific, VPAC1 and VPAC2 receptors are binding PACAP and VIP with equal affinity on their surface. Activation of the PAC1 receptor induces activation of adenylate cyclase (AC), phospholipase C (PLC), and protein kinase C (PKC), VPAC1 and VPAC2 receptors increase the levels of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). The distribution of receptors in the body is varied: the PAC1 receptor is found in different parts of the cerebrum and cerebellum, the VPAC1 receptor is found in several areas of the central nervous system (cortex, hippocampus, thalamus, hypothalamus), while higher concentration of VPAC2 receptor is found in the thalamus, amygdala and pons. PACAP receptors can also be detected outside the central nervous system: PAC1 is found in the retina, kidney, liver and pancreas, VPAC1 receptor in the lung, liver, thymus and gastrointestinal tract, VPAC2 receptor mRNA was described in the testis, stomach, pancreatic  $\beta$ -cells and adrenal gland.

PACAP has a wide range of effects, its cytoprotective effects have been described in many organs. Its cardio-, neuro-, and renoprotective effects have been demonstrated in numerous studies. PACAP plays a role in the pathophysiology of Parkinson's disease, and recent research is investigating the provocative effects of PACAP in migraine and cluster headaches. It also plays an important role in the cardiovascular system and the gastrointestinal tract, with a proven

protective effect in intestinal ischemia. It also has a protective endotoxin-induced inflammation in the airways. In the absence of PACAP, spermatogenesis is disrupted and plays a role in many areas of the aging process.

PACAP and its receptors can be detected throughout the body, in almost every gland. It is involved in the regulation of both endocrine and exocrine glands in numerous places. In the present dissertation, the pancreas, which also functions as an exocrine gland, is the subject of the study, so the research of other externally secreted glands, such as the lacrimal gland, sweat gland, salivary glands and mammary gland, can give a good comparison. The lacrimal gland is innervated by a rich PACAP-erg plexus, with PAC1 receptors responsible for activating tear secretion. PACAP-erg nerves also network the salivary glands and mammary glands, enhancing protein production through inhibition of PACAP secretion and calcium channels. PACAP also enhances the function of the sweat glands. PACAP can also be detected in the milk of several mammalian species. The effect of PACAP on the exocrine pancreas is analogous to that described in the salivary glands, resulting increased lipase secretion in acinar cells. In addition, the polypeptide plays a regulatory role in insulin and glucagon secretion and  $\beta$ -cell proliferation. PACAP and its receptors also play an essential role in cell proliferation and differentiation under physiological and pathological conditions (inflammation, malignant transformation). PACAP and its receptors act as growth factors in some tumor lesions and as inhibitors in others. PACAP enhances cell proliferation in the AR4-2J rat PC cell line via the G-protein-coupled VPAC1 receptor in vitro, PACAP gene-bound proliferation and stress response have been described in PC, but the effect of direct growth enhancement on PC cells is still not clear. PACAP and PAC1 expression are altered in a number of tumors, such as thyroid papillary carcinoma and testicular cancer, and PAC1, VPAC1, and VPAC2 mRNA have also been detected in  $\beta$ - and insulinoma cells.

## **Aims of the study**

- 1) The role of PACAP and its receptors has been extensively studied in a number of tumor types, and the results have been shown to enhance and inhibit tumor progression in various tumors. In addition to its role in tumorigenesis, PACAP, or its receptors, also play a role in the regulation of almost all exocrine and endocrine glands.

Despite detailed and thorough research, it is not clear how the expression of PACAP and its receptors is altered in endocrine and exocrine pancreatic tumors.

In the first part of my PhD work, I aimed to investigate the expression of PACAP and PAC1 receptor in histological samples from patients operated for PDAC, insulinoma, and CP.

- 2) Pancreas operations are still belasting for the patient and often involve serious professional challenges for the operator. Although mortality is on a declining trend, it is still high at 30-50%. Despite keeping minimally invasive principles in mind, the rates of postoperative complications and morbidity in pancreatic surgery do not show as declining trend as in other areas of surgery.

In the second part of my PhD work, I aimed to analyze how to reduce the incidence of postoperative complications (especially POPF) in large pancreatic centers.

## **Examination of PACAP and PAC1 receptor expression in histological samples from patients undergoing operation because of pancreatic ductal adenocarcinoma, insulinoma or chronic pancreatitis**

### **Materials and methods**

In our study, we collected patients from a six-year period (September 2012 to August 2018). Histological samples of surgical specimens from patients operated for PDAC, insulinoma proven by fine-needle aspiration biopsy (FTAB) and CP (suspected malignancy based on preoperative examinations), and patient data were collected (permission number for statistical analysis and patient data collection: „PTE/83069/2018”). Histological specimens from 55 patients who underwent pancreatoduodenectomy or enucleation surgery were examined during the six-year study period.

When collecting patient data, we also analyzed the type of surgery, the results of routine histological examination, the degree of the tumor, and the assessment of resection.

New sections were made from the histological specimen removed during surgery and used for immunohistochemistry (IHC) of PACAP and PAC1 receptor expression. 2 µm thick sections were prepared and fixed in 4% buffered formalin.

During the preparation for the IHC assay, new sections were prepared from the previous surgical preparation, followed by standard 1: 200 dilutions of human anti-PACAP antibodies (Peninsula CA, USA) and 1: 200 dilutions of human PAC1 receptor antibodies (Sigma-Aldrich, Budapest, Hungary). The IHC study was performed with an EnVision FLEX visualization system, DAKO IHC automaton (Dako, Denmark). Liquid fast-red substrate (Abcam, UK) was used for staining.

The results of the IHC assay were analyzed semi-quantitatively with the assistance of an experienced pathologist as follows: no staining, weak, moderate, or strong staining. A negative (non-staining) control was generated using the primary anti-serum. Eligible elements (island cells, nerve elements) served as positive controls. The staining strength of the tumorous and tumor-free part of the same pancreatic tissue was analyzed by the semi-quantitative method described above.

## Results

Of the 55 patients, 50 had surgery for CP, pancreatic cyst or histologically confirmed PDAC, 5 had surgery for insulinoma.

In cases of the patients operated on for PDAC, 14 of the 50 patients' histological sampling (FTAB or brush cytology) demonstrated malignancy preoperatively (one patient had also neoadjuvant chemotherapy), 36 of 50 patients underwent operation because of cystic pancreatic lesions, or undifferentiable pancreatic mass (CP vs. tumor). In case of these patients, the removed surgical specimen showed malignant degeneration in 6 of the 36 cases. Adenocarcinoma was confirmed in 20 of the 50 patients.

The mean age of patients operated on for CP/PDAC at the time of surgery was 57 years (25-79 years), 19 female and 31 male patients underwent surgery. Separating tumor and non-tumor patients based on the final histological result, female predominance (12 females, 8 males) was observed in cancer patients, and significant male predominance (7 females, 23 males) was in CP cases. Of the 20 tumor patients, 15 had icterus at the time of surgery. In the tumor group, Kausch-Whipple surgery was performed in 5 cases and PPPD in 15 cases. The majority of 30 CP patients underwent Frey surgery (20 cases), 3 patients underwent Kausch-Whipple surgery, 3 patients pancreato-cysto-jejunostomy, 1 patient underwent Wirsungojejunostomy, 1 patient distal pancreatic resection, and 2 patients underwent Beger operation. The preoperative examinations evaluated Grade 2 adenocarcinoma in 7 patients and Grade 3 adenocarcinoma in 13 patients. Pathological examination of the removed surgical specimen in these patients confirmed Grade 2 adenocarcinoma in 11 patients, Grade 3 adenocarcinoma in 7 patients, and the final histological finding was mucinous adenocarcinoma in case of 1 patient. In all cases, the tumor stage was described as pT3, pN0 in 5 cases, and pN1 in 15 cases. Analyzing the resection surface, the pathologist described perineural invasion in case of 1 sample, 1 sample showed tumorous infiltration of the venous wall, 9 cases described complete R0 resection, and in the other cases the resection surface was considered narrow (0.25-1 mm). Histological specimen from 1 patient showed R2 resection, in this case the tumor involved hepatic artery and vena portae. In the other 30 patients, histological examination demonstrated CP with extensive, severely advanced scarring.

In case of the 5 patients underwent surgery for insulinoma, 1 patient had histological evidence for insulinoma, the case of the other 4 patients the radiological findings (EUS, CT, MR, MR cholangiography) and clinical signs were suspicious for insulinoma: loss of consciousness and hypoglycemia causing vegetative symptoms. The mean age of the 5 patients was 38 years (23-

48 years) at the time of surgical care, 2 female patients and 3 male patients underwent surgery. Enucleation was performed in 3 cases, in 1 case distal pancreatic resection and another 1 case Kausch-Whipple surgery was performed. In 3 cases the lesion was localized to the head of the pancreas, in 1 case the tail, another one the uncinate process was affected. The final histological examination confirmed Grade 1 insulinoma in 4 cases, Grade 2 insulinoma was confirmed in a histological specimen of 1 patient. 3 cases were described in pT1, pT2 in 2 cases. The sample of 2 Whipple operated patients showed no lymph nodes affection (pN0), in cases of enucleation the sample did not contained any lymph nodes due to the type of surgery. The resection edges of 3 histological specimens were not affected (R0 resection), in 2 specimens the edge was affected (R1 resection), but neither perineural nor perivascular invasion was observed.

In case of the PDAC patients, PAC1 receptor expression was observed in both exocrine and endocrine pancreatic sections during IHC staining, the staining intensity was also strong at the area of the Langerhans Islands. Receptor staining of the sections with pancreatic adenocarcinoma was significantly weaker compared to non-tumor tissues. Difference between strength of PAC1 receptor staining formed a visible border between normal and tumorous pancreatic tissue. Nerve elements with no PAC1 positivity were also found in the sections.

In histological specimens from insulinoma patients, IHC staining of the PAC1 receptor was similarly strong in the Langerhans Islands, as in samples from PDAC patients, the healthy exocrine sections showed significant staining. Although the PAC1 receptor positivity of insulinoma cells was well observed, it was weaker compared to normal islet cells.

In the samples of CP patients, advanced fibrosis and chronic inflammation, decrease in exocrine parts as well as islet cell structures was noticeable, with strong PAC1 staining in the islet cell parts in the chronically inflamed areas.

Examination of PACAP IHC showed poor staining in exocrine PDAC sample sections, whereas endocrine sections were very positive. PACAP staining of tumor-containing areas was weak, and staining of nerve elements was strong. A similarly strong PACAP positivity was seen in the nerve elements of the duodenal fragments (this area is also removed during pancreatoduodenectomy because of the surgery). The intensity of PACAP staining in the myenteric and submucosal plexus was marked.

In sections from insulinoma, PACAP expression was well observed but weaker compared to normal islet cells.

Strong PACAP IHC staining was seen in CP samples, with signs of fibrosis and chronic inflammation previously described, preserved islet cell structures, and lost exocrine formulas.

## Discussion

In our study we analyzed the expression of PACAP and its receptor (PAC1 receptor) in histological samples containing normal pancreatic tissue, PDAC, CP, and insulinoma. Histological samples of 55 patients were examined using IHC analysis of the staining pattern of the various lesions and the intensity of the staining. Because the removal of the tumor (suspected tumor) was performed with a tumor-free resection margin, the staining of the abnormal and intact tissue could be examined in the same section, which allowed for even more accurate comparability of the pattern.

Comparing the samples, it was well evident that regardless of the stage of tumor lesion, the staining intensity of both PACAP and PAC1 receptor was much weaker compared to PDAC with normal pancreatic tissue.

In the role of tumorigenesis numerous growth factors were demonstrated. In case of FGF (fibroblast growth factor), there is strong relationship with the control of the growth and differentiation of pancreatic tumor cells, which strongly influence tumor stage and curability. The involvement of TGF- $\beta$  (Transforming Growth Factor beta) in tumor growth of the pancreas is proven, stronger expression of NGF (Nerve Growth Factor) may indicate poorer prognosis, stronger inflammation, and pain. The same poorer prognosis can be shown in case of PDAC with higher EGF (Epidermal Growth Factor) expression level, and there is strong correlation between IGF (Insulin-like Growth Factor) and increased PC risk also. PACAP can also act as a growth factor, but the expression and inhibitory or stimulatory effects are very diverse. In addition to the effect of PACAP, its receptors also play an important role in the development of many tumor types (breast, prostate, lung, liver). VIP receptor scintigraphy in PDAC patients showed overexpression. The results of in vivo studies are contradictory in some respects, with the exception of the latter only in vitro studies are available, which are not yet sufficient to develop a possible (sufficiently sensitive and specific) tumor marker, and in vitro overexpression of VPAC1 has not been demonstrated.

Former studies did not show FGF expression in pancreatic ductal cells, while very high expression was observed in islet cells. In our studies, PACAP IHC staining of our histological specimens containing PDAC and insulinoma yielded similar results: tumor ducts were virtually unstained, endocrine areas showed strong positivity, and staining of normal exocrine parts was weak. Examination of tumor-containing samples revealed significantly decreased PACAP and PAC1 receptor expression. Although our studies to date have not revealed whether decreased PACAP and PAC1 receptor expression is due to tumorigenesis or reduced PACAP/PAC1

receptor signaling is tumor-generating, the results so far support the role of decreased/absent PACAP/PAC1 signaling in tumor growth and differentiation.

Using the results of the first examinations, we analyzed the staining of the histological sections of patients operated on for insulinoma and CP.

The diverse role of PACAP in tumor growth and differentiation is known (it may enhance growth through its antiapoptotic effect, it may inhibit tumor cell migration). However, PACAP and its receptors' effect is little known on benign tumor diseases and endocrine tumors, PACAP expression is highly increased in endocrine organs. PACAP inhibits apoptosis in human pituitary adenoma cell lines, found in most human pheochromocytomas, whereas PACAP and PAC1 receptor expression are alternating in thyroid tumors. The different effect of growth factors can be well noticed in the endocrine part of the pancreas, the immunofluorescent labeling of NGF is weak, and after administration of EGF, vitronectin and fibronectin, increase in the JNK pathway can be observed in case of insulinoma cells.

The staining pattern in our samples was consistent with the results of previous studies: staining of normal exocrine pancreatic tissues was strong, PACAP/PAC1 receptor expression was significantly reduced or absent in insulinomas. In CP samples, PACAP staining was strong with missing PAC1 receptor staining. Comparing the staining strength of normal islet cell-containing and insulinoma sections, the staining of insulinoma sections was significantly weaker than healthy ones. These results also support our previous hypothesis (examined on PDAC samples), that the PACAP/PAC1 receptor signalling pathway may have influence on malignant transformation, tumorigenesis, and tumor progression.



## **Innovations in pancreatic anastomosis technique and postoperative follow-up of complications after pancreatic resections**

### **Materials and methods**

We analysed the data of 130 patients who underwent between January 2013 and March 2020 pancreatoduodenectomy operation at the Department Surgery University Pécs, mainly due to pancreatic, gallbladder, biliary and duodenal malignancies.

After radical removal of the lesion and regional lymphadenectomy, an end-to-side PJ was made using tobacco bag forming technique. During operation, the resected pancreatic stump was mobilized, and an appropriate sized hole was made on the anti-mesenteric side of the closed jejunum loop, 2-3 cm distal from the closed end of the sealed jejunum, around which a tobacco bag stitch was inserted with 2/0 monophilic non-absorbable thread. At the cranial and caudal corners of the pancreatic stump, a U-shaped stitch was inserted with 3/0 monophilic resorbable sutures to insert the pancreas into the intestinal lumen and then knotted. After this, the intestinal mucosa was inverted, and the tobacco bag stitch were also knotted. A soft silicone drain tube was placed in front of the anastomosis and covered with the major omentum.

Amylase levels from drain secretions were measured on the days after surgery and immediately before drain removal. Drain removal was performed according to the principles of the Verona group, Octreotide was administered only in the case of detectable POPF. In our cases, the abdominal drain was removed in accordance with the amylase content of the drain secretion (limit was 5 000 U/l). Patients were divided into two groups: the first group had an average drain amylase level of 2 137 U/l on the first postoperative day, which decreased to an average of 264 U/l on the fourth day, the drain was removed, and no pancreatic fistula developed. In the other group of patients, the average level of drain amylase was much higher (19 550 U/l), here a longer drainage was chosen. Drain removal was performed after a corresponding reduction in secretory amylase levels after closure of the fistula. We examined perioperative mortality, the rate of reoperations, and the total morbidity after surgery, and separately analyzed the incidence of the two most common complications (DGE, POPF). Levels of amylase excreted in the abdominal drain inserted during surgery (if data were available) were compared to form two groups of patients (no fistula, clinically relevant pancreatic fistula - Grade B/C - fistula).

## Results

The gender distribution was nearly the same (67 female, 63 male), with a mean age of 60 (23-81) years at the time of surgery. 130 patients were operated, 81 of the cases because of PC, 24 tumor of the Vater papilla, 12 because of distal bileway malignancy, 5 of the 130 CP, 4 patients duodenal tumor, and 4 cases gallbladder tumor. Pylorus preserving pancreatoduodenectomy was performed in case of 56 patients, partial antrum resection in 74 patients.

During surgery, the remaining pancreas tissue was normal in consistence in 66 cases and fibrotic in 64 cases of patients.

Surgery was generally followed by 2-3 days of intensive care unit treatment, with a mean hospital stay of 13 days (7-75 days). Operative mortality was 0.7% (1 patient died, cause of death was not surgical complication), reoperation was required in 7 cases (5.3%) (2 patients developed abdominal wall disruption requiring surgery, 2 patients underwent abdominal abscesses, in case of 1 patient pancreatectomy had to be completed, 1 patient required further surgery due to stenosis of the hepaticojejunostomy and 1 patient had to be re-operated because of bleeding from the resecting surface of the pancreas). Non-surgical complications were those seen in other cases (pneumonia, arrhythmia, urinary tract infection, chest fluid formation). The overall morbidity rate was 43.8%, clinically relevant postoperative pancreatic fistula occurred in 6.9% (9 cases), DGE rate of 4% (5 cases) was observed.

In 84 patients, drain fluid amylase levels were determined on the first postoperative day. These patients were divided into two groups:

group 1 (75 patients): appeared no pancreatic fistula

group 2 (9 patients): clinically relevant postoperative pancreatic fistula  
(CR-POPF) appeared.

In group 1 (75 patients of 84 cases), in the majority of cases the level of drain secretion amylase remained below 5 000 U/l (average 2 737 U/l, range 6-46 000 U/l), except of 9 cases, with higher level, but rapid decrease. In this group, the drain was removed on average on the fourth (2-6) day after surgery. The amylase level decreased on average of 264 U/l (3-3 370 U/l). No CR-POPF was observed in case of the patients of group 1.

In case of group 2 (9 of the 84 cases), amylase levels were well above 5 000 U/l (mean 19 550 U/l, range 28-63 690 U/l). In this group one patient was noticed with amylase level below 5 000

U/I (later amylase level increased significantly). In this group, drain remained longer time. Patients in group 2 received Octreotide, two patients required repeated surgery after unsuccessful interventional (radiological) drainage due to an abdominal abscess.

## **Discussion**

Among the most common complications following pancreatic surgery, methods for reducing the risk of POPF were examined in our analysis of patient data.

Perioperative outcomes and incidence of postoperative complications in 130 patients over a seven-year-long study period were analyzed.

The gender and age distribution of our patients are congruent to internationally described trends, the majority of cases is pancreatic tumor, consistent with the incidence of tumors reported in the pancreas area. During the operation, soft and fibrotic pancreas stumps were detected in almost the same proportion (66 vs. 64 cases). Morbidity and mortality rates associated with surgery have been shown to be similar to those of large pancreatic centers in other parts of the world. A good way to reduce the incidence of reoperations is the ultrasound-guided drainage and aspiration of postoperative fluid collection, but its success also depends on the patient's body and the examiner's practice.

Based on our experience, consistent with the results of other authors, the incidence of DGE can be effectively reduced by antecolic gastro-entero anastomosis (combined with Braun entero-entero anastomosis).

Our anastomosis forming technique is a modification of method of former developed binding anastomosis or tobacco bag stitch PJ. The guiding principle was that no puncture duct may enter the abdominal cavity, along which a pancreatic fistula may develop. This will realize also the case with our tobacco bag stitching method. It is important inverting the mucosa of the jejunum into the intestinal lumen during knotting of the suture. It requires increased attention (the serosa layers are known to heal better with all anastomoses). The incidence of POPF in the study population was less than 14.5% reported in the literature (ISGPS data). The results so far show that this method is simple and quick to implement (only three stitches are required) and is sufficiently safe.

Till now, there is no clear consensus on abdominal drainage after pancreatic surgery. Some authors take an increasingly widespread view in many other areas of abdominal surgery: there is no need for abdominal drainage, and omitting them does not increase the rate of postoperative

complications, which is in pancreatic surgery often POPF. If drainage is done, a highly important question is the early or late removal of it. During our study, abdominal drain was left at the end of the operation, the time of removal was determined mainly on the basis of Verona criteria. Our results support the recommendation that drain amylase levels measured on the first postoperative day, but especially the trend observed in later days, well reflect the risk of possible POPF formation, providing a good basis for selecting the appropriate time for drain removal. The importance of the trend is evidenced by the exceptions observed in both groups (when amylase levels were above or below 5 000 U/l on the first day), where the high first daily value but rapid decrease in drain can be removed, while a gradual increase in low levels refers to pancreatic fistula and need for longer drainage. Based on this, it is recommended to determine the drain amylase level before drain removal and compare it with the result on the first postoperative day: if the first daily value is below 5 000 U/l and below 350 U/l on the third postoperative day, early drain removal appears safe.

Leaving a soft silicone drain near the pancreatic anastomosis (not in contact with the pancreatojejunostomy) does not present a risk for complication based on our experience, but at the same time provides an opportunity to measure drain amylase levels, which helps reduce drainage as soon as possible.

## Summary of new findings

### *1. Investigation of PACAP and PAC1 receptor expression in histological samples from human patients undergoing surgery for PDAC.*

In our IHC studies, we showed that in the tumor type (the majority of pancreatic tumors is ductal adenocarcinoma) both PACAP and its specifically binding receptor, PAC1, showed a significantly weaker staining pattern compared to intact exocrine cells surrounding tumor cells. It was also conspicuous that the observed staining strength was completely independent of the oncological stage of the removed tumor.

This pattern suggests a reduced PACAP/PAC1 receptor stimulating malignant transformation and promoting tumor cell survival. The staining strength independent of the oncology stage further strengthens our hypothesis.

### *2. PACAP and PAC1 receptor expression in histological specimens from patients operated for insulinoma or CP.*

Histological specimens from patients with histologically non-malignant but often very severe clinical pancreatic disease (who underwent surgery for this purpose) were also examined. Benign tumors are represented by insulinoma, a non-tumorous condition represented by chronic inflammation of the pancreas.

Expression of PACAP and PAC1 receptor was also significantly weaker in samples from patients operated on for insulinoma compared to surrounding exocrine pancreatic tissues. Compared with the staining pattern of islets with normal function, the IHC staining of the insulinoma parts was much weaker in these cases as well. For CP samples, strong PACAP IHC staining was found, accompanied by missing PAC1 receptor staining.

Our results further confirmed the role of the PACAP/PAC1 receptor line in malignant transformation. Based on a comparison of normally functioning and tumor islet cells, the role of PACAP/PAC1 receptor signaling in tumor transformation is suspected. This seems to be supported analogously by the normal exocrine tissue-CP-PDAC comparison.

3. *Innovations in pancreatic anastomosis technique and postoperative follow-up of complications after pancreatic resections.*

Based on the collected retrospective data, it can be said that our clinic is characterized by a patient population, tumor type and surgical method comparable to the international literature, with same postoperative results and perioperative outcomes reported in other pancreatic surgery centers.

Based on our experience, the technique using as few stitches as possible, and the use of our modified tobacco bag stitch PJ, yielded favorable results. It is important that the puncture channel formed by the suture and yarn mustn't communicate with the abdominal cavity so that the pancreas fluid cannot leak towards the abdominal cavity, which can further reduce the risk of complications.

In our work, we preferred drainage after pancreatojejunostomy. This made it possible to monitor the level of drain secretion amylase in the postoperative period, based on which we developed a system for determining the time of drain removal. We found that not only the first daily value is important, but the trend is crucial in determining how long drainage is needed. We can find out when the drain can be removed early (leaving an unnecessarily long drain can be really harmful due to the risk of over-infection and local irritation). If, on the other hand, changes in amylase levels indicate the development of a pancreatic fistula, the condition can be treated by maintaining drainage. In the absence of drainage, we would be relying on UH-guided drainage, which is not always successful and the thickness of the inserted drain is not comparable to that of a surgical drain. It can be said that the above findings have proven to be useful and reliable in reducing the risk of POPF.

### Publications related to the thesis

1. **Ferencz S**, Toth D, Kaszas B, Bardosi S, Vicena V, Karadi O, Reglodi D, Kelemen D: PACAP and PAC1 Receptor Expression in Human Insulinomas. *Int J Pept Res Ther* 2021; 27:1719-1728. doi: 10.1007/s10989-021-10204-0. (IF: 1,931; Q3)
2. **Ferencz S**, Bíró Zs, Vereczkei A, Kelemen D: Innovations in pancreatic anastomosis technique during pancreatoduodenectomies. *Langenbecks Arch Surg* 2020; 405: 1039-1044. (IF: 3,445; Q1)
3. **Ferencz S**, Reglodi D, Kaszas B, Bardosi A, Toth D, Vekony Z, Vicena V, Karadi O, Kelemen D: PACAP and PAC1 receptor expression in pancreatic ductal carcinoma. *Oncol Lett* 2019; 18: 5725-5730. (IF: 2,311; Q3)
4. Kelemen D, Lőcsei Z, Papp R, **Ferencz S**, Vereczkei A: Appleby-műtét – lehetőség truncus coeliacust infiltráló pancreastest tumor sebészi kezelésére. *Magy Seb* 2018; 71: 16-20. (Q4)

*Impact factor of publications related to the thesis: 7,687*

### Publications not related to the thesis

1. Mangel L, Lukács M, Hajnal A, Sarkany H, Forgacs-Menyhert M, Varga Zs, Herendi E, Papp E, Jeglne Illes Zs, Szigeti N, Almasi R, **Ferencz S**, Kanizsai P, Sebestyen A, Csikos A: Az első tapasztalatok a palliatív onkoteamrendszer működésével kapcsolatban a Pécsi Tudományegyetemen. *Orv Hetil* 2020; 161: 1423-1430. (IF: 0,497; Q3)
2. Miklós Z, Kürthy M, Degrell P, Ranczinger E, Vida M, Lantos J, Arató E, Sínay L, Hardi P, Balatonyi B, **Ferencz S**, Jávör Sz, Kovács V, Borsiczky B, Wéber Gy, Róth E, Jancsó G: Ischaemic postconditioning reduces serum and tubular TNF- $\alpha$  expression in ischaemic-reperfused kidney in healthy rats. *Clin Hemorheol Microcirc* 2012; 50: 167-178. (Q2)
3. Takács I, Wegmann J, Horváth Sz, Ferencz A, **Ferencz S**, Jávör Sz, Odermatt E, Róth E, Weber Gy: Efficacy of different hemostatic devices for severe liver bleeding: a randomized controlled animal study. *Surg Innov* 2010; 17: 346-52. (IF: 2.255; Q2)
4. Fuchs K-H, Breithaupt W, Schulz T, **Ferencz S**, Varga G, Weber G: Transgastric small bowel resection and anastomosis: a survival study. *Surg Endosc* 2011; 25: 1791-1796. (IF: 4,013; Q1)
5. Ferencz A, Takács I, Horváth S, **Ferencz S**, Jávör S, Fekecs T, Shanava S, Balatonyi B, Wéber G: Examination of protective effect of ischemic postconditioning after small bowel autotransplantation. *Transplant Proc* 2010; 42: 2287-2289. (IF: 0,993; Q2)
6. Horváth Sz, Gál I, Rákóczi I, Jávör Sz, Balatonyi B, Takács I, Ferencz A, **Ferencz S**, Wéber Gy: Transvaginalis cholecystectomy állatmodellen – kezdeti tapasztalataink. *Magy Seb* 2009; 62: 120-124. (Q4)



7. **Ferencz S**, Mangold V, Dérczy K, Takács I, Balatonyi B, Horváth Sz, Jávör Sz, Bránemark R, Horváth O P, Roth E, Wéber Gy: Alsóvégtag-amputált érbetegek új protézis-lehetősége: kezdeti tapasztalataink az összeintegrációs technikával. *Magy Seb* 2009; 62: 293-297. (Q4)
8. Sínay L, Kürthy M, Horváth Sz, Arató E, Shafiei M, Lantos J, **Ferencz S**, Bátor A, Balatonyi B, Verzár Zs, Sütő B, Kollár L, Wéber Gy, Roth E, Jancsó G: Ischaemic postconditioning reduces peroxide formation, cytokine expression and leukocyte activation in reperfusion injury after abdominal aortic surgery in rat model. *Clin Hemorheol Microcirc* 2008; 40: 133-142. (IF: 1.814; Q2)
9. Arató E, Jancsó G, Sínay G, Kürthy M, Lantos J, **Ferencz S**, Horváth S, Shafiei M, Kasza G, Verzár Z, Kollár L, Roth E, Wéber G, Menyhei G: Reperfusion injury and inflammatory responses following acute lower limb revascularization surgery. *Clin Hemorheol Microcirc* 2008; 39: 79-85. (IF: 1.814; Q2)
10. Cserepes B, Jancsó G, Gasz B, Rác B, Ferencz A, Benkő L, Borsiczky B, Kürthy M, **Ferencz S**, Lantos J, Gál J, Arató E, Miseta A, Wéber Gy, Róth E: Cardioprotective action of urocortin in early pre- and postconditioning. *Ann N Y Acad Sci* 2007; 1095: 228-239. (IF: 1.731; Q1)

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