

Complex treatment of pancreatic tumours

PhD Thesis

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1. Introduction

The prevalence of pancreatic cancer shows an increasing tendency. The resulting mortality ranks fourth, fifth among mortalities due to tumours. Aggressive character and poor clinical manifestation due to relatively late recognition can be detected in the background, therefore the ratio of resectability is merely around 10% , and the survival rate of patients having undergone radical surgeries is not favourable either, it amounts to ca. 20%. Nevertheless, the only chance for a long-term survival involves a curative resection combined with adjuvant chemotherapy in patients with pancreatic cancer.

Ductal adenocarcinoma originating from the exocrine substance is histologically the most common among the tumours of the pancreas amounting to about 90% of all cases. Tumours of cystic, acinary, and endocrine origin are significantly less prevalent.

Adenocarcinoma is most frequently confined to the head of the pancreas. Diagnostics begins with an abdominal ultrasound, though computed tomography (CT) overtakes the leading role in precise detection, staging and decision making whether the patient is operable. Endoscopic retrograde cholangiopancreatography (ERCP) and biliary stenting are often carried out, though the recommendation of these examinations is a matter of debate.

In tumours of proximal location pancreatoduodenectomy (PPPD, Traverso-Longmire procedure) preserving the pylorus, if needed, -in case the tumour is situated near the pylorus- the traditional Whipple procedure involving the removal of the antrum of the stomach, together with a regional lymphadenectomy are to be carried out (Figure 1-3).

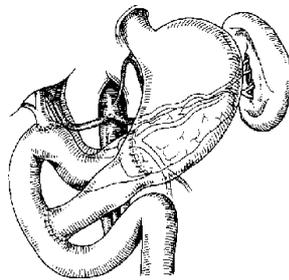


Figure 1. Pylorus-preserving pancreatoduodenectomy with pancreatojejunostomy

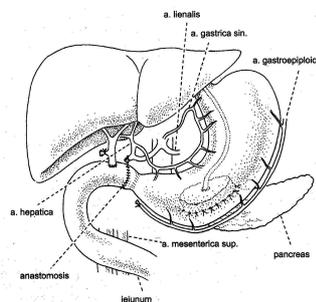


Figure 2. Pylorus-preserving pancreatoduodenectomy with pancreatogastrostomy

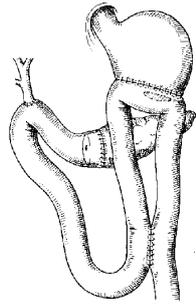


Figure 3. Classical Whipple procedure

Pancreatodigestive reconstruction can be prepared both with the jejunum and the stomach. According to a meta-analysis both methods provide the same results, the modality chosen may depend on the preference of the surgeon. Biliodigestive anastomosis (hepaticojejunostomy) is performed in all cases with the jejunum in an end-to-side fashion. The reconstruction of the continuity of the alimentary tract may (duodenojejunostomy in the course PPPD or gastrojejunostomy by traditional Whipple procedure) be performed in various fashions (interrupted, running sutures, antecolic or retrocolic gastro- or duodenojejunostomy respectively).

In distal tumours a left-sided resection with splenectomy combined with lymphadenectomy should be carried out (Figure 4).

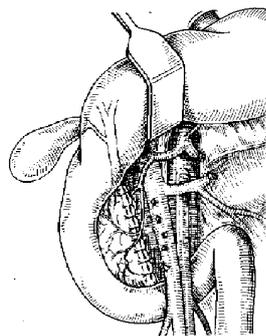


Figure 4. Distal pancreatic resection with splenectomy

Total pancreatectomy is indicated if the tumour is multicentric. This surgery has been favoured lately, since it eliminates the risk for developing a pancreatic fistula, at the same time the internal-medicine treatment of apancreatic conditions has developed significantly.

Though the operative mortality in specialized centres has dropped under 5 %, the postoperative morbidity is about 30-40%, indicating that pancreatic resections may be associated with several risk factors. The most frequent postoperative complications include delayed gastric emptying (19-23%), postoperative pancreatic fistula (9-18%), intraabdominal abscess (9-10%), gastrointestinal or intra-abdominal bleeding (1-8%). Based on the definition of the International Study Group on Pancreatic Fistula (ISGPF) set in 2005 any measurable quantity of discharge through an abdominal drain on the third postoperative day or after is indicative of a fistula, if its amylase content is at least three times higher than that of the normal serum amylase value. Based on the definition of the International Study Group of Pancreatic Surgery set in 2007 gastric emptying is considered delayed if the usage of a nasogastric probe is required even after the third day following the operation or if the probe needs to be reinserted due to persistent emesis after the third postoperative day. Also, gastric emptying is delayed if

the patient cannot tolerate solid food until the 7th postoperative day. The causes of DGE are not fully known, though local complications, innervation- or blood supply disorders and the resulting pyloric spasm are assumed as triggers. The dysfunction usually resolves on its own.

If the tumour is locally irresectable, patients need to undergo an oncological treatment (primary chemoradiotherapy) that may result in a regression that enables the surgeon to remove the tumour. Pancreatic tumours are inoperable if they evoke distant metastases (liver, lungs), carcinosis and/or involve the visceral arteries. At the same time, vena resection has become popular. Radical surgeries should be cancelled if the general condition of the patient does not allow for a resection. If the expected survival is approximately 6 months, nonoperative measures are considered, such as biliary stenting in jaundice, or if it cannot be carried out technically, percutaneous transhepatic drainage (PTD) is to be done. If the patient's condition is better, a surgical double bypass (biliodigestive anastomosis + GEA) is recommended in tumours of proximal location (Figure 5).

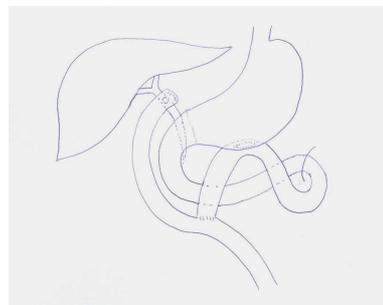


Figure 5. Palliative double bypass.

In left-sided localisation the obstruction due to tumour can be expected in the duodenojejunal transition, therefore it is more feasible to perform a duodenojejunostomy instead of a GEA.

Gemcitabin (Gemzar) monotherapy is the usual first-line cytostatic therapy in the adjuvant oncological treatment of pancreatic cancer, earlier 5-fluorouracil (5-FU) was administered. In case of good general condition the two drugs can be combined with each other, complemented with cisplatin respectively. Gemcitabin monotherapy is the first-line cytostatic therapy also in the treatment of locally advanced pancreatic tumours. Radiotherapy is used in the adjuvant management of pancreatic cancer following resection, and also in the management of locally advanced tumours. However, in the vast majority of adjuvant treatments chemotherapy is applied. In locally advanced tumours radio-chemotherapy does not ensure longer survival, than chemotherapy alone, moreover, its toxicity is higher.

Whilst radicality is of importance in malignomata (complemented even with vena resection), the so called organ-preserving procedures have become widespread in rare benign processes and tumours of low malignancy (enucleation, resection of the head of the pancreas preserving the duodenum, distal resection preserving the spleen, central pancreatectomy, papillectomy) (Figure 6-8).

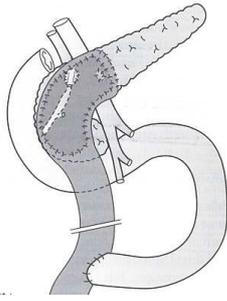


Figure 6. Duodenum-preserving pancreatic head resection

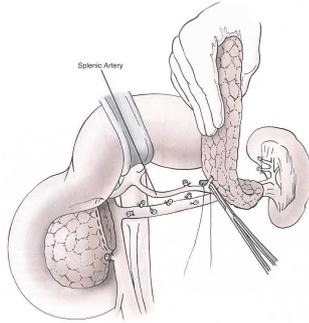


Figure 7. Spleen-preserving distal pancreatectomy

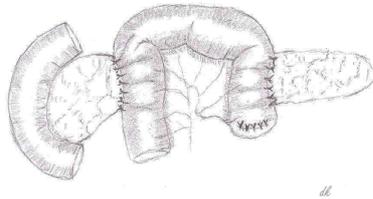


Figure 8. Central pancreatectomy

2. Objectives

At the Department of Surgery, Medical faculty of Pécs University – from January 1998 until January 2011 – we were seeking answers to the following questions raised regarding the retrospective analysis of pancreatic and periampullary tumours:

1. Are there any symptoms that might indicate operability or inoperability?
2. We are going to analyze the achieved results of implantational pancreatojejunostomy developed at our department, regarding the full period of 13 years, compared with other techniques.
3. We wish to analyze the impact of antecolic duodeno- or gastrojejunostomy performed during pancreatoduodenectomy on postoperative gastric emptying, compared with the retrocolic method performed earlier.
4. What surgical techniques are the most beneficial in the treatment of rare tumours

and tumours of low malignancy, i. e. do patients have a benefit of organ-preserving resections?

5. To what extent does adjuvant therapy improve long-term survival rates?
6. To what extent may neoadjuvant therapy result in downstaging or operability?
7. What are the mostly recommended methods of surgical palliation in irresectable cases?
8. A less invasive screening method enabling the early diagnosis of pancreatic cancer playing a role in also in the therapy would be desirable and of major importance. Can K-ras or micro- RNAs serve as biomarkers?

3. Reviewing own clinical results

3.1 Patients and Methods

3.1.1 Resectable tumours

Between January 1998 and January 2011 251 radical operations were performed due to pancreatic or periampullary malignant tumours at the Department of Surgery, Medical Faculty of Pécs University. Table I illustrates the characteristic preoperative data of 251 patients.

Table I. Preoperative data of radically operated patients

	male: 122	female: 129	mean age: 60 years (9-80)
origin of the tumor:	pancreatic head (common bile duct)		175 (70 %)
	body-tail		24 (10%)
	papilla Vateri		44 (17%)
	duodenum		8 (3%)
clinical symptoms:	jaundice		158 (63%)
	pain		139 (55%)
	weight loss		91 (36%)
	vomiting		15 (6%)

The type of intervention is shown in Table II.

Table II. Type of curative procedure

type of resection:		
	pylorus-preserving	
	pancreatoduodenectomy	186
	classical Whipple procedure	31
	distal resection	26
	total pancreatectomy	8
additional procedures:		
	portal vein resection	8
	liver metastasectomy	5
	large bowel resection	2
	gastric and small bowel resection	1-1
	adrenalectomy	1
	nephrectomy	1

Most frequently pylorus-preserving pancreatoduodenectomy was performed. We opted for a traditional Whipple procedure when the tumour was near the pylorus. We performed total pancreatectomy when a tumour was detected in the resection margin even after repeated resections. In malignant processes also regional lymphadenectomy was performed, and splenectomy was carried out in distal pancreatic resections.

At pancreatoduodenectomies the remnants of the pancreas were anastomosed mostly with the jejunum, in 183 cases, of which 143 were performed in one-layer, 40 were performed in two-layer technique. Implantation into stomach was performed in 34 patients. Since 2003 we have carried out end-to-side pancreatojejunal anastomosis with interrupted sutures, one-layer implantation technique (4/0 polydioxanone (PDS), Ethicon, Scotland). Two-layered pancreatojejunostomy was performed with 4/0 PDS, pancreatogastrostomy was carried out with 3/0 Ethibond (Ethicon, Scotland), using interrupted sutures. The parenchyma of the pancreas was soft in 43%, to lower the risks of postoperative complications octreotid (Sandostatin, Novartis Hungary Ltd, Budapest) was administered subcutaneously, 3x0.1 mg for five days.

Pancreatojejunostomy and hepaticojejunostomy were performed with a jejunum brought up retrocolically. In case the biliary duct was dilated due to cholestasis, biliodigestive anastomoses were stitched in one-layer with running sutures (4/0 PDS) just like in duodeno- and gastrojejunostomies (3/0 PDS). The latter sutures have been performed antecolically and not retrocolically since 2008, complemented with Braun anastomosis to further lower the risk of stagnation in the afferent limb. If the biliary duct was not dilated, biliodigestive anastomosis was performed with interrupted sutures.

In some cases multivisceral resection was performed, solitary hepatic metastasis was removed in five patients. Segmental or tangential resection of the vena portae was performed due to the tumorous infiltration of the vessel.

3.1.2. Central pancreatectomy

In 7 cases central pancreatectomy (middle segmental pancreatic resection, MSPR) was performed at the Department of Surgery, Medical faculty of Pécs University between 2002 and 2009, due to benign and low malignant pancreatic tumours (in 2 cases insulinoma, in 5 cases due to suspected cystic or endocrine tumour), since it was situated in the area of the neck and body of the pancreas. The mean size of the lesion was 30mm (10-80). Routine intraoperative frozen section was carried out to prove the tumour free resection margin.

With the distal pancreatic resection surface a pancreatojejunal anastomosis was prepared using the Roux-limb, this intervention has been carried out with a one-layer end-to-side implantation technique in six patients since 2003 (4/0 PDS). At the first operation we stitched the anastomosis in two layers. In one case the proximal resection surface was stapled closed. In the other six cases the separate closure of the Wirsungian duct freely probable toward the papilla of Vater was performed and then the manual suturing of the margins of the fish-mouthed cut surface took place (4/0 PDS). In the last four cases the proximal suture line was covered also with the Roux-limb brought up.

3.1.3. Irresecable tumours

Radical surgery could not be performed in 188 patients between January 1998 and January 2008, in their cases palliative surgical measures were taken. The fact of incurability turned out either during the surgery or was already known prior to the operation (e.g. distant metastases, local vessel invasion), but the nonoperative biliary drainage could not be performed, or rather surgical palliation appeared to be of more benefit due to the relatively good general condition of the patient.

Preoperative data regarding irresecable tumours are summarized in Table III. The most frequently performed palliative intervention was double bypass, the details are illustrated in Table IV.

Table III. Preoperative data regarding irresecable tumours

	male: 109	female: 79	mean age: 63 years (28-84)
origin of the tumor:	pancreatic head (common bile duct)		138 (73,4%)
	body-tail		40 (21,3%)
	papilla Vateri		8 (4,2%)
	duodenum		2 (1,1%)
clinical symptoms:	jaundice		113 (60%)
	pain		104 (55%)
	weight loss		100 (53%)
	vomiting		37 (19%)

Table IV. Type of palliative procedure

double bypass	131
biliodigestive anastomosis	21
duodenojejunosomy	13
GEA	11
exploration	12

At double bypass we performed hepatico (choledocho-) jejunostomy with the Roux-limb as a biliodigestive anastomosis and simultaneously a retrocolic posterior gastrojejunostomy (GEA) with the jejunum from the ligament of Treitz. The double bypass that we have performed since 1998 was first published by Büchler et al. in 2002. Usually, we did not have any positive histological findings, therefore we sampled the tumour intraoperatively with a Travenol needle, possibly transduodenally to prevent the formation of a pancreatic fistula.

3.1.4. Oncological treatment

Oncological consilium took place after both radical and palliative operations in order to decide if oncological therapy was needed, and if yes, what kind. Regarding the fact that the possibilities have changed over the 13 years, anti-tumour therapeutic measures have become diversified, even by the type of the tumour. Besides, we hardly had any information about the patients living outside County Baranya, since they received chemotherapy outside Pécs. Furthermore, there were patients who did not receive chemotherapy either because of their persistent complication, or because they did not sign the consent, or because the oncologist did not consider chemotherapy necessary due to the histological results (R0 resection) or chemotherapy was not considered feasible due to the patient's condition. We could not collect enough data on

everybody involved when determining survival, this fact can account for the smaller number of patients.

Before 2000, patients were administered 5-fluorouracil (5-FU) (425 mg/m² iv. over five days, every 28 day) as an adjuvant therapy, after this gemcitabin (1000 mg/m² iv. for 3 or 5 weeks every week, followed by one week intermission). When the patient's condition was good, gemcitabin was complemented with 5-FU, and if tumour recurrency evoked, it was complemented with cisplatin. In many cases only observation was performed in R0 resection.

In locally advanced tumours or as a palliative measure in case of massive pain and compression symptoms, also in R1/R2 resections radio-chemotherapy was considered with 1.8 Gy fractions until the total dose of 45 Gy. During this time 500 mg/m² 5-FU was administered to the patient weekly over the period of five weeks. In case of metastases palliative chemotherapy was applied.

The follow-up of patients involved clinical assessments, CT and lab tests on a regular basis.

3.2. Results

3.2.1. Results of surgical and oncological treatments in case of resectable tumours

On average, at radical surgeries 2 U (0-26) RBC transfusion was needed. Mean time of the days spent in the ICU was 2 days (0-29), postoperative day care amounted to 13 days (6-55) on average. In most cases an adenocarcinoma was histologically proved. In 232 cases the resection included an R0, in 14 patients an R1 and in 5 cases an R2.

In 39 % complications have developed following radical surgery, these complications are illustrated in Table V in the sequence of their frequency.

Table V. Complications after radical operation (98 cases)

pancreatic fistula	22
wound infection	21
delayed gastric emptying	18
intraabdominal abscess	7
biliary fistula	6
respiratory insufficiency	5
lymphatic leak	4
GI bleeding, intraabdominal bleeding	3-3
cardiac insufficiency, renal insufficiency, liver abscess, wound disruption	2-2
small bowel perforation	1

Most frequently a pancreatic fistula has developed, this complication will be analyzed in depth in Table VI.

Table VI. Occurance of pancreatic fistula /22 cases (8,7%)/

pancreatogastrostomy: 34, fistula: 6 (17%), reoperation: 3, death: 2
single layer pancreatojejunostomy: 143, fistula: 9 (6,2%), conservative th.
double layer pancreatojejunostomy: 40, fistula: 5 (12,5%), conservative th.
distal resection: 26, fistula: 2 (7,7%), conservative th.

On evaluating the results we performed statistical calculations.

Using the Fisher exact test we found that at pancreatojejunostomies performed in one-layer significantly less fistulae developed compared to pancreatogastrostomies ($p=0.044$). No significant deviation was detected between the development of fistula in pancreatojejunostomies (prevalence 6.2%) performed in one-layer and pancreatojejunostomies performed in two-layers (prevalence 12.5%), but the percentage ratios are noteworthy.

The conservative treatment – proper drainage, administration of somatostatin analogue octreoid- resulted in recovery in every case of pancreatic fistula, reoperation was not needed. However, the insufficiency of pancreatogastrostomy was associated with substantially more severe clinical signs, at times with septic conditions. In half of the cases reoperation was required, one third of the patients died. At the repeated operation breakdown of anastomosis and the removal of the pancreatic stump were performed.

225 patients underwent a pancreatoduodenectomy, 18 patients presented with delayed gastric emptying, accounting for an 8.0% of prevalence. Before 2008 this complication occurred in 16 cases following 156 radical surgeries (10.2%), while after 2008 it presented only in two cases following 69 radical resections (2.9%). In both cases duodeno- or gastrojejunal anastomosis was performed antecolically. In antecolic reconstructions the prevalence of DGE was significantly lower ($p=0.079$, alpha (criterion level)=0.1) with a Chi-square test compared with retrocolic reconstructions.

Reoperation was needed in 5.9 % of the cases. In 15 cases, reoperation was performed due to the insufficiency of pancreatogastrostomy, respectively due to intraabdominal hemorrhage in 3-3 patients, due to abdominal abscess, disruption in the abdominal wall, hepatic abscess or insufficiency of the biliodigestive anastomosis in 2-2 cases and in one case the operation was repeated due to small bowel perforation. Early postoperative mortality amounted to 3.9 % (10 cases) resulting from cardiopulmonary insufficiency in 6 patients, renal failure in one patient and surgical causes in three patients.

In three patients out of 251 having undergone a radical surgery, a total pancreatoduodenectomy, a Whipple operation and a pylorus preserving pancreatoduodenectomy we found a rare complication, a bleeding originating from the pseudoaneurysm of the common hepatic artery into the hepaticojejunal anastomosis 2-3 months after resection. Endoscopic examinations did not reveal the source of bleeding in any of the cases. Angiography was performed in two patients, making the diagnosis possible. Angiographic intervention was carried out in one case, without any results. All the three patients underwent a surgery, during which the pseudoaneurysm was removed and the direct suture of the common hepatic artery took place with 5/0 polypropylene (Prolene, Ethicon, Scotland). Reparation of biliodigestive anastomosis was necessitated in all the three cases. Rebleeding or a biliary fistula did not develop in any of the cases. One patient died of multiorgan failure associated with sepsis, the other two patients were discharged.

Patients received an adjuvant oncological treatment mostly at the Institute of Oncotherapy, Medical faculty of Pécs University. We can report on the treatment results of 52 patients with pancreatic adenocarcinoma receiving adjuvant Gemzar-5-FU therapy. On average the disease-free survival was 18.6 months, the median was 17 months. The duration of total survival (until the last appearance or death) was 26.5 months on average, median was 24 months.

Pancreatic adenocarcinoma was the most common among operable tumours (176 patients) and as it has been mentioned the modality of adjuvant therapy was

diversified, many times the therapy took place at another oncological unit according to the place of residence, or the therapy lagged behind. Based on the data of the 13 years we can determine survival in 98 patients having undergone a curative surgery due to pancreatic adenocarcinoma, on average it means 35.1 months, median is 22 months, and the five-year cumulative survival amounted to 15%. These data are illustrated with the Kaplan-Meier curve in figure 9.

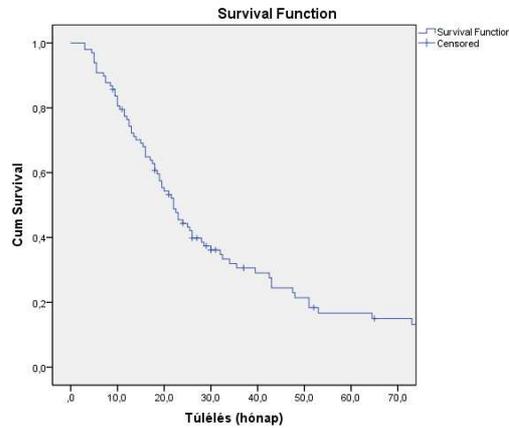


Figure 9. Kaplan-Meier survival curve

3.2.2. Results of central pancreatectomies

At central pancreatectomies the intraoperative transfusion need was on average 1 U (0-2), postoperative hospitalization 15 days (7-51), duration of treatment at the ICU 2 days (0-8).

Histological results are shown in Table VII.

Table VII. Histology

insulinoma	2
cystadenoma	3
solid pseudopapillary tumour	1
low malignant endocrin neoplasia	1

In three cases out of seven a complication has developed. Pneumonia developed in one patient, pneumothorax in another patient following the insertion of the central venous catheter and a peripancreatic abscess due to a pancreatic fistula in the third patient. Percutaneous drainage was performed due to the latter surgical complication (12%). Reoperation or death did not supervene. The average duration of the follow-up period was 28 months (1-84), during which both the endocrine and the exocrine pancreatic functions remained normal. All the patients were satisfied with the results, no tumour recurrency supervened.

3.2.3. Results of surgical and oncological treatment in case of irresectable tumours

Regarding the clinical signs, we can ascertain that with the chi-square test weight loss and emesis occurred significantly more frequently in irresectable patients ($p < 0.001$).

Complication developed in 36 inoperable patients, accounting for 19.1%. The distribution of complications was identical with that of double bypass and other palliative surgeries providing biliary or duodenal passage.

The ratio of reoperation was 1.6%, involving three patients. The need of reoperation resulted from a gastrointestinal bleeding, from an abdominal abscess and from a duodenal perforation. Early postoperative mortality supervened in 13 cases, amounting to 6.9% resulting from 11 cases of cardiopulmonary insufficiency and 2 cases due to surgical causes. The intraoperative transfusion need was low, on average 1 U (0-8). Postoperative hospitalization lasted for 9 days (1-34) on average.

34 patients with locally advanced tumours received mono-Gemzar chemotherapy or Gemzar-5FU therapy, simultaneously complemented with radiotherapy. Treatment results involve: duration of time until progression was 8 months on average, median 7 months, however the total survival amounted to 12 months, median was 10 months. None of the tumours proved resectable, except one case, an irresectable squamous cell carcinoma of the head of the pancreas, in which systemic chemotherapy (mitomycin, epirubicin, carboplatin), respectively selective chemotherapy with the these same drugs were administered through the celiac trunk. Following chemotherapy radiotherapy was started in 1.6 Gy fractions until the total dose of 40 Gy, after this gemcitabin monotherapy was commenced. Based on the abdominal CT following the neoadjuvant treatment regression was suspected, therefore 14 months after having made the diagnosis reexploration and pylorus-preserving pancreatoduodenectomy were performed at the Department of Surgery, Medical faculty of Pécs University. The postoperative period was eventless. Histological investigation detected full tumour regression, chronic pancreatitis and reactive lymph nodes.

3.3. Conclusion

3.3.1. Conclusions drawn from surgical and oncological treatment of resectable tumours

In our cases the tumour was most frequently confined to the head of the pancreas causing icterus and pain, histologically adenocarcinoma was proved and mostly pylorus-preserving pancreatoduodenectomies were performed.

Following radical interventions early postoperative mortality amounted to 3.9%, morbidity accounted for 39%, these ratios correspond with the requirements set by the pancreatic centres.

We have gained several experiences over the 13 years, i.e. at proximal resections we have rather switched from pancreatogastrostomy over to pancreatojejunal anastomosis, from 2003 we have adopted its one-layer modification. With this we have reached better results and the prevalence of a pancreatic fistula significantly dropped to 6.2%.

Regional lymphadenectomy has become a routine intervention and in some cases we have performed multivisceral resections. Vascular resection is rather considered in the tumorous infiltration of the vena portae in selected cases, if by this we can reach R0 resection.

According to recommendations duodeno- and gastrojejunal anastomoses have been lately performed antecolically and not retrocolically, we complement this surgery with Braun anastomosis to further reduce the risk of stagnation in the afferent limb. Based on our results antecolic reconstructions are associated with significantly less cases of delayed gastric emptying compared with retrocolic reconstructions. No

studies comparing the effect of ante- and retrocolic reconstruction have been published yet in Hungarian reviews.

With reference to the three massive gastrointestinal bleedings into the biliodigestive anastomosis, originating from the pseudoaneurysm of the common hepatic artery we could ascertain that CT angiography proved to be of most benefit in the detection of the source of bleeding. Surgical management was necessary in all the three cases, both the direct sutures and the reconstruction of the biliodigestive anastomosis were successful. This rare complication should be considered if late gastrointestinal bleeding presents.

Usually, curative surgery was followed by oncological treatment and care. Over the years more modern drugs beside 5-FU have appeared in the adjuvant therapy of pancreatic cancer, such as gemcitabin and cisplatin. On account of several factors introduced in chapter "Patients and Methods" even in the case of the most common type of cancer, pancreatic adenocarcinoma, we can report only on a small group of patients receiving chemotherapy. It is interesting to note that the average survival of the 52 patients having undergone a curative resection and an adjuvant therapy was shorter (26.5 months) than that of those other 98 patients having undergone a curative surgery, whose group involves 46 patients beside the formerly mentioned 52 patients, at whom we could only detect survival and not the fact, whether they have received an oncological treatment. With regard to the median survival the 24 months in 52 patients are somewhat more favourable to the survival of 22 months in the 98 patients. These and our 5-year-survival results are similar to those of the literature. We can agree with the observations in the literary reviews, i.e. the improvement of long-term results can be expected from more efficient adjuvant therapies.

3.3.2. Conclusions of experiences with central pancreatectomies

Parenchyma-saving segmental pancreatic resection carried out due to proper indication and with an adequate surgical technique can be performed with an acceptable mortality ratio. Main indications involve tumours of benign and low-malignancy nature in the middle part of the pancreas, where the limited resection does not jeopardize the oncological radicality. The unnecessary sacrifice of healthy organs (duodenum, distal biliary duct, head and tail of the pancreas, spleen) can be avoided, and the endocrine and exocrine functions of the gland do not get damaged.

The technique of a central pancreatectomy, preferred by our team involves a distal implantational pancreatojejunostomy with a Roux-limb, followed by the separate suturing of the Wirsungian duct proximally, then the manual suturing of the proximal resection surface and the serosal covering of the surface with the Roux-limb brought up.

3.3.3. Conclusions drawn from surgical and oncological treatment of irresectable tumours

The prevalence of weight loss and emesis was significantly higher in irresectable tumours. In the vast majority of irresectable cases a double bypass was performed.

Following the neoadjuvant therapy of locally advanced tumours- except the case of the reported pancreatic squamous cell carcinoma- we did not find any regression of that extent that could have enabled the radical operation. In distant metastases, in selected cases palliative chemotherapy can be indicated.

In advanced tumours, when the expected survival is approx. 6 months, or due

to the poor general condition only nonoperative modalities are considered, such as biliary stenting in jaundice, or if it can not be managed technically percutaneous transhepatic drainage (PTD) can be performed. Our main objective is to improve the quality of life as far as possible and to achieve this adequate analgesia is of decisive importance.

4. K-ras mutations in peripheral blood samples and tumour tissues of patients with pancreatic cancer

4.1. Introduction

A less invasive screening method, such as peripheral blood or stool samples, enabling the early diagnosis of pancreatic cancer could be preferable and of major importance.

K-ras proto-oncogene located at the locus p13 of chromosome 12 is the most frequently mutated gene in the carcinogenesis of the pancreas. K-ras proto-oncogene encodes a cell membrane bound protein called guanosine triphosphatase, weighing 21 kDa and binding guanine nucleotide. In mutations this enzyme binds GTP constantly. The task of Ras-proteins is the transmission of extracellular signals stimulating growth to the intracellular signal pathways ensuring cell division and differentiation. The constant activity of these proteins results in uncontrolled cell division playing a major role in carcinogenesis. The prevalence of K-ras mutations is associated with smoking, eating habits, alcohol abuse, exposition to certain chemical substances and the presence of chronic pancreatitis. Several investigations have successfully detected mutations of the K-ras gene in the blood plasma and serum of patients with pancreatic cancer, however there have been some examinations with negative results. The investigations have not confirmed unanimously that the detectable K-ras mutations circulating in the DNA were available biological markers of pancreatic tumours.

4.2. Patients and Method

The examination of K-ras mutations was performed involving 28 patients with pancreatic cancer (17 male and 11 female patients, aged 37-76, average age 57.71) having undergone a surgery, with the assistance of the Department of Public Health, Medical Faculty of Pécs University.

The participants were asked to fill in a questionnaire including questions associated with their living conditions, residence, physical activity, regular medical check-ups and the known risk factors of pancreatic tumours (smoking, eating habits), these questionnaires were filled in on their own, without any interlocutors.

DNA and cellular RNA isolation took place from blood samples taken right before the surgery and from intraoperative tumour samples. We examined the presence of K-ras mutations in codons 12, 13 and 61 with a mutant allele-specific PCR, while the expression of codons 12, 13 and 61 of the K-ras gene was investigated with quantitative real-time PCR. Altogether 8 specimens were accessible for a RT-PCR investigation. Possible correlations between different risk factors, the presence of K-ras mutations and K-ras expressions were studied with logistic regression.

4.3. Results

4.3.1. Risk factors

Based on the questionnaires of the 28 examined patients in point of the risk factors we have found the followings: 16 of them live in contaminated conditions in their judgement, 17 have obtained a secondary or tertiary-level degree, 17 present to regular check-ups, 13 do regular physical activity, 12 are smokers, 12 are passive smokers, 10 drink alcohol on a regular basis, 12 drink coffee on a regular basis, 4 eat hot and spicy food daily and 16 regularly eat animal fat.

4.3.2. Mutant allele-specific PCR

With a mutant allele-specific polymerase chain reaction K-ras mutations have been detected in 20 tumour samples (71.5%) and in 21 blood samples (75%) collected from 28 patients with pancreatic cancer.

15 samples out of the 20 tumour samples harbouring mutation carried the mutation of codon 61. In the associated blood samples K-ras mutation was detected in only 13 of them. K-ras codon 61 mutation was present in 2 of all blood samples without the presence of the mutation in the tumour itself.

Codon 12 mutation was proven in 9 malignant histological samples. In 7 of the associated blood samples K-ras codon 12 mutation was detected. In 3 of all blood samples K-ras codon 12 mutation was detected without the presence of the mutation in the tumour itself.

We have not detected K-ras codon 13 mutation either in any of the tumours or blood samples.

However, three histological and blood samples harboured both codon 61 and 12 mutations.

4.3.3. Quantitative RT-PCR

2 of the 8 examined histological samples collected from patients with pancreatic cancer have shown a K-ras gene overexpression with a quantitative real-time PCR. K-ras codon 61 mutation was found in one of them, and K-ras codon 12 mutation was revealed in the other one.

Likewise 2 out of the 8 peripheral blood samples have shown a K-ras gene overexpression. In one of them K-ras codon 12, in the other both K-ras codon 12 and codon 61 mutations have been detected.

4.3.4. Statistical analysis

During the course of statistical analysis significantly less K-ras gene mutation has been detected in any codons of the gene in patients living in an uncontaminated environment (OR=0.15, CI: 0.03-0.77).

The likelihood of K-ras codon 61 mutation has been significantly increased by alcohol consumption (OR=28.38, CI: 1.04-772.55), however eating meat proved to be a protecting factor in point of K-ras codon 61 mutation (OR=0.01, CI: 0.0002-0.99).

The prevalence of K-ras codon 12 mutation was significantly lower in patients drinking less coffee (OR=0.1, CI: 0.01-0.97).

No significant correlation has been found between the presence of K-ras gene mutations and the extent of K-ras gene expression.

4.4. Conclusion

Based on our investigations, we have found a significant correlation between contaminated environment in the judgement of the patients and the presence of K-ras mutations in any of the examined codons, between regular alcohol consumption and the presence of K-ras codon 61 mutations, furthermore between regular coffee consumption and the presence of K-ras codon 12 mutations. These results correlate with those having been published in the literature so far.

Large amounts of meat and fat increase the risk of pancreatic cancers. However, regular meat consumption proved to be a protective factor regarding the K-ras codon 61 mutations. It may result from the fact that in the course of preparation and conservation of meat products (barbeque, smoking and roasting) the resulting N-nitroso compounds similarly to those found in tobacco smoke, lead to mutations in codon 12.

In 71.4 % of the examined samples the K-ras mutational profiles of the histological and blood samples belonging to one patient were consistent.

Regarding the K-ras gene expression in the course of real-time PCR analysis no conclusions can be drawn due to the low number of samples, however we need to note that no significant correlation was detected between the presence of K-ras gene mutation and the K-ras gene expression.

We can ascertain that the K-ras mutations found in the samples of tumour tissue collected from patients with pancreatic cancer can often be detected in the peripheral blood samples of patients with pancreatic cancer, however further investigations are needed to establish the clinical importance of this manifestation and its adaptability as serum biomarker.

5. Micro- RNA examination in tumour tissue samples of patients with pancreatic cancer

5.1. Introduction

Following the transcription from the double-stranded DNA in the nucleus, the information on the single-stranded messenger RNA can be blocked even before protein-synthesis (translation) takes place in the ribosome. This process is termed posttranscriptional gene silencing through the messenger RNA. A form of this machinery is RNA interference, during which short RNA molecules (micro-RNA, miRNA) inhibit the function of messenger RNAs. Craig C. Mello, American researcher along with Andrew Z. Fire was awarded the 2006 Nobel Prize for the discovery of RNA interference. Their discovery was published in 1998.

With reference to the genes being regulated by micro-RNAs, micro- RNAs can function as oncogenes (onkomir) or as tumour suppressive genes. Micro-RNAs play a role not only in the development of tumours but also in the progression of the disease and metastases. Since 2008, micro-RNAs have been known to be detected also in bodily discharges. If we managed to adopt micro-RNAs as non-invasive tumour-biomarkers in symptom-free early tumours, the prospects of patients would improve significantly. By influencing the micro-RNA expression and therefore altering the phenotype of the tumour a new molecular biological opportunity opens up in the

treatment of tumours. Since K-ras is the most frequently mutated gene in pancreatic adenocarcinoma (involved in more than 90% of pancreatic carcinogenesis), the regulation of K-ras through micro-RNAs is of major importance in the matter of pancreatic cancer.

In the United States, based on the recommendation and guidelines of the College of American Pathologists, with the cooperation of Asuragen Clinical Services Laboratory a micro-RNA diagnostic panel, miRInform Pancreas is in use that dependably identifies - based on the expression ratio of miR-196a and miR-217- ductal pancreatic adenocarcinoma compared with the micro-RNA expression ratio characteristic of chronic pancreatitis and benign pancreas. Micro-RNA expression is investigated with a quantitative real-time PCR following the processing of pancreatic specimen.

5.2. Patients and Method

With the assistance of the Department of Public Health, Medical Faculty of Pécs University we have performed micro-RNA investigations using a quantitative real-time PCR in histological samples collected from patients with pancreatic cancer. Our objective was to determine the micro-RNA expression profile of patients (8 patients), carrying a K-ras codon 61 mutation, having undergone a curative surgery due to pancreatic cancer (the investigation involved the expression of 10 micro-RNAs). We have sampled normal pancreas as control tissue. We have performed micro-RNA isolation and a complementary DNA quantitative RT-PCR on specimens obtained from 8 pancreatic carcinomas and a normal tissue following a reverse transcription. PCR products have been detected with DNA-binding fluorophores. The amount of developed PCR products was indicated by the intensity of fluorescence. We calculated and illustrated the expression results from the intensity results with the help of the associated LightCycler 480 software- with reference to the program's standard concentration curve- followed by the expression ratio of U6 snRNA (small nuclear RNA, expressed both in tumour and normal tissues, and part of the complex having a role in the nuclear maturing of the messenger RNA). We have not done any statistical calculations due to the low number of samples available.

5.3. Results

A significant deviation has been detected in the micro-RNA expression of pancreatic adenocarcinomas in each of the 10 investigated micro-RNAs compared to the normal pancreatic tissue. (Figure 10).

	1	2	3	4	5	6	7	8	9
miR21	104	500	500	500	167	34	162	3,42	2,24
miR27a	31,2	500	298	500	61,2	93	43	1,54	1,2
miR34a	92,3	137	385	135	73,4	110	202	39,2	16,4
miR93	500	138	366	500	53,5	127	191	95,5	134
miR143	60	121	500	500	97,1	96,3	196	16	243
miR155	136	194	125	204	93	249	500	156	28,2
miR196a	0,65	215	7,07	24,2	0,4	0,9	2,4	0,4	43,6
miR203	8,26	149	19,7	72,9	8,57	14,9	19,8	13,2	9,2
miR205	32	92,7	232	498	36,8	53,4	69,5	47,5	18,4
miR221	45,6	106	50,8	365	27,5	60,7	95	71,6	26,5

Figure 10. Micro-RNA expression values characteristic of 8 pancreas adenocarcinomas and a normal pancreatic tissue (sample 9) in relation to U6 snRNA, as a unit

Figure 11 compares the mean absolute values of tumorous micro-RNA expressions with the micro-RNA expression of a normal tissue.

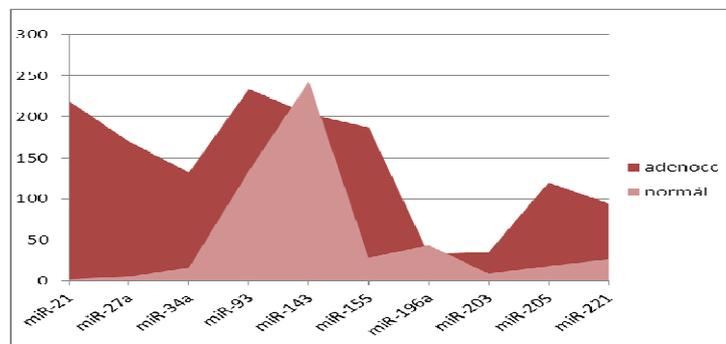


Figure 11. Micro-RNA expression in adenocarcinoma and in normal pancreas

Based on the figure a pronounced expression deviation is detectable between malignant and normal tissues in cases of miR-21, miR-27a, miR-34a, miR-155, miR-205 and miR-221.

5.4. Conclusions

Based on our results demonstrative deviations have been detected in micro-RNA expression of the investigated 8 pancreatic adenocarcinoma specimens compared with that of the control tissue. Its role is adaptable in molecular differential diagnostics of pancreatic carcinomas known from micro-RNA present on the micro-RNA panel used and drafted by us. These include miR-221, miR-155, miR-21, miR-143 and miR-93, revealing a specific and significant overexpression in the examination of pancreatic adenocarcinoma as compared to chronic pancreatitis investigated by Mark Bloomston et al on detecting micro-RNA expression in pancreatic adenocarcinoma, chronic pancreatitis and normal pancreatic tissue.

MiR-21, miR-27a, miR-34a, miR-155, miR-205 and miR-221 have been proven to be the most marked isolating micro-RNAs in distinguishing malignant tissues from normal tissue. We have detected miR-196a overexpression only in one specimen of all adenocarcinomas; this micro-RNA is routinely detected in the Asuragen Clinical Laboratory using the miRInform Pancreas diagnostic panel. However, on investigating pancreatic adenocarcinoma we have not investigated the other fix element of the panel- miR-217- we have demonstrated a significant overexpression of a similar microRNA, miR-27a, belonging to the same micro-RNA cluster. The result of the investigation performed only on a few specimens clearly

demonstrates molecular differences between malignant and normal entities, therefore our investigation is noteworthy.

We plan further investigations on the demonstrated micro-RNA panel involving samples collected from malignant and normal pancreatic tissues as well as from chronic pancreatitis to achieve statistical significance and to elucidate the clinical course of the diseases. To the best of our knowledge it has been the first micro-RNA investigation in Hungary with regard to pancreatic adenocarcinoma.

6. Summary, novel findings

1. The implantational, one-layer pancreatojejunal anastomosis developed at our clinic significantly reduced the prevalence of pancreatic fistula compared to pancreatogastrostomies and it also proved to be a better technique than pancreatojejunostomies performed in two layers.
2. The conservative treatment of a developed pancreatic fistula was successful in the course of all anastomoses of the remnant pancreas into the jejunal limb being excluded from the way of nourishment.
3. The prevalence of delayed gastric emptying was significantly lower in antecolic duodeno- and gastrojejunal anastomoses performed during pancreatoduodenectomies and complemented with a Braun anastomosis compared to retrocolic reconstructions. No studies investigating the effect of ante- and retrocolic reconstructions on gastric emptying have been published so far in Hungarian reviews.
4. Reflecting our results, the 5-year survival amounting to 15% following radical surgeries (due to also adjuvant therapy besides curative resection) along with the early morbidity and mortality rates can be considered good and meet the published data of other pancreas centres.
5. Our survival results are similar to those of other centres in point of adjuvant therapy. However, these results can give rise to a certain degree of optimism after the formerly published depressing data, we can agree with the declarations that confirm the need for improved long-term results due to more effective adjuvant therapies.
6. Parenchyma-preserving segmental pancreatectomy can be performed with an acceptable morbidity rate beside proper indication and surgical technique and at the same time the endocrine and exocrine functions of the gland do not get damaged. The technique of a central pancreatectomy preferred by our team involves a distal implantational pancreatojejunostomy with a Roux-limb followed by the proximal separate sutures of the Wirsungian duct and the manual suture of the proximal resection surface, followed by the serosal covering of the surface with the Roux-limb brought up.
7. Double bypass is recommended in irresectable proximal tumours as a palliative intervention, as it does not increase morbidity and mortality compared to palliative surgeries providing biliary passage merely. Further operation is avoidable, that could be necessary to be performed in a tumorous patient with impaired condition due to a later evolving duodenal stenosis. The double bypass method preferred by

our team was first published in 2002 in international medical reviews, however we did use this method routinely in 1998.

8. In contrast with the international data unfortunately we have not found any regression of that degree that could have enabled the resection of the tumour following neoadjuvant therapy in patients with locally advanced pancreatic adenocarcinoma.
9. The complete remission following the neoadjuvant therapy of the outlined pancreatic squamous cell carcinoma emphasizes the major role of oncological treatment.
10. K-ras mutations detected in the malignant tissue samples of patients with pancreatic cancer can often be found in the peripheral blood samples of patients with pancreatic cancer. Beside the encouraging results further investigations are needed to determine the clinical importance of this phenomenon and its adaptability as a biomarker.
11. Micro-RNAs playing a major role in differentiating malignant tissues from normal ones include miR-21, miR-27a, miR-34a, miR-155, miR-205 and miR-221 based on the micro-RNA investigation of tumour samples collected from patients with pancreatic cancer. Our investigation is of noteworthy importance, since it clearly demonstrates molecular differences between malignant and normal tissue entities even if the research was conducted on a small number of patients. To the best of our knowledge it has been the first micro-RNA expression investigation in Hungary regarding pancreatic adenocarcinoma.

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