Small caliber pancreatic stents in the management of acute biliary pancreatitis and the prevention of post-ERCP pancreatitis

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TABLE OF CONTENTS

1. LIST OF PUBLICATIONS 4
1.1. First author papers related to this thesis 4
1.2. Non-first author papers related to this thesis 4
1.3. Full papers not related to this thesis 5
2. ABBREVIATIONS 8
3. INTRODUCTION 11
3.1. Diagnostic criteria of acute pancreatitis 12
3.2. Morhological types and complications of acute pancreatitis, phases of inflammation 12
3.3. Severity classification of acute pancreatitis and prognostic indices 14
4. PATHOPHYSIOLOGY OF ACUTE PANCREATITIS 17
4.1. Animal models of acute pancreatitis 18
4.2. Important pathophysiological molecular events 22
4.3. Characteristics and differences in molecular mechanisms in acute pancreatitis of different etiologies 29
5. CLINICAL ASPECTS OF POST-ERCP AND ACUTE BILIARY PANCREATITIS 33
5.1. Acute pancreatitis following ERCP (post-ERCP pancreatitis (PEP)) 33
5.1.1. Definition and severity classification of PEP 34
5.1.2. Risk factors of PEP 34
5.1.3. Prophylactic methods of PEP 35
5.2. Acute biliary pancreatitis (ABP) 38
5.2.1. History, role and results of ERCP in ABP 38
6. RELEVANT RESULTS PRECEDING THE PRESENT RESEARCH TOPIC 41
6.1. Results described in PEP patients 41
6.2. Lowering the intrapancreatic duct pressure in ABP 42
6.3. Previous knowledge related to stent types 42
7. NULL HYPOTHESIS 43
8. AIMS 43
8.1. Aims in PEP-prevention 43
8.2. Aims in ABP 44
9. METHODS 44
9.1. Our research in PEP-prevention 44

9.1.1. PEP occurring despite using PPS, complications related to stents, and different stents types 44
9.1.2. Comparing PPSs and NSAID suppositories 45
9.2. Our research in ABP 46
9.2.1. Non-randomized study 46
9.2.2. Randomized study 47
10. RESULTS 48
10.1. Our research in PEP-prevention 48
10.1.1. PEP despite prevention with PPS, complications related to PPS, and different stent types 48
10.1.2. Comparison of PPSs and NSAID suppositories 55
10.2. Our research in ABP 58
10.2.1. Non-randomized study 58
10.2.2. Randomized study 61
11. DISCUSSION 65
11.1. Our research in PEP-prevention 65
11.2. Our research in ABP 68
12. CONCLUSIONS 71
13. SUMMARY / NEW RESULTS 71
14. REFERENCES 73
15. ACKNOWLEDGEMENT 83

1. LIST OF PUBLICATIONS

1.1. First author papers related to this thesis

(n=7, cumulative impact factor: 16.956, cumulative citation: 60)

I. **Dubravcsik Z**, Gyökeres T, Novák P, Budai A, Mohácsi S, Velkei T, Madácsy L. Az endoszkópos retrográd cholangiopancreatographia szövődményei. [Complications of endoscopic retrograde cholangiopancreatography] Orv Hetil. 2022; 163: 911-919. **(Q3, H index: 21, IF: 0.41, citated: 0)**

II. **Dubravcsik Z**, Hritz I, Szepes A, Madácsy L. Risk factors of post-ERCP pancreatitis in high-risk patients despite prevention with prophylactic pancreatic stents. Scand J Gastroenterol. 2020; 55: 95-99. **(Q2, H index: 105, IF: 2.218, citated: 3)**

III. **Dubravcsik Z**, Hritz I, Szepes A, Madácsy L. Profilaktikus sztentek alkalmazása az endoszkópos retrográd cholangiopancreatographiát követő pancreatitis megelőzésében. [Prophylactic stents in the prevention of pancreatitis following endoscopic retrograde cholangiopancreatography]. Orv Hetil. 2021; 162: 31-38. [Hungarian] **(Q3, H index: 21, IF: 0.41, citated: 0)**

IV. **Dubravcsik Z**, Hritz I, Keczer B, Novák P, Lovász BD, Madácsy L. Network meta-analysis of prophylactic pancreatic stents and non-steroidal anti-inflammatory drugs in the prevention of moderate-to-severe post-ERCP pancreatitis. Pancreatology. 2021; 21: 704-713. **(Q1, H index: 74, IF: 3.996, citated: 1)**

V. **Dubravcsik Z**, Szepes A, Hritz I, Madácsy L. Small-caliber rescue pancreatic stenting for severe post-ERCP pancreatitis: a useful tool to pull the pancreas out of the fire. Endoscopy 2015; 47: 467–468. **(Q1, H index: 143, IF: 4.749, citated: 1)**

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VII. **Dubravcsik Z**, Madácsy L, Gyökeres T, Vincze Á, Szepes Z, Hegyi P, Hritz I, Szepes A. Hungarian Pancreatic Study Group. Preventive pancreatic stents in the management of acute biliary pancreatitis (PREPAST trial): pre-study protocol for a multicenter, prospective, randomized, interventional, controlled trial. Pancreatology. 2015; 15: 115-123. **(Q1, IF: 2.75, citated: 25)**

1.2. Non-first author papers related to this thesis

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I. Hritz I, **Dubravcsik Z**, Szepes A, Madácsy L. Does removal of prophylactic pancreatic stents induce acute pancreatitis? Gastrointest Endosc. 2011; 74: 1429–1430. (Q1, IF: 2.884, citated: 1)

II. Hritz I, Czakó L, **Dubravcsik Z**, Farkas G, Kelemen D, Lásztity N, Morvay Z, Oláh A, Pap Á, Párniczky A, Sahin-Tóth M, Szentkereszti Z, Szmola R, Szücs Á, Takács T, Tiszlavicz L, Hegyi P; Hungarian Pancreatic Study Group. Akut pancreatitis. A Magyar Hasnyálmirigy Munkacsoport bizonyítékon alapuló kezelési irányelvei. [Acute

pancreatitis. Evidence based management guidelines of the Hungarian Panceatic Study Group]. Orv. Hetil., 2015; 156: 244–261. [Hungarian] (Q3, H index: 21, IF: 0.28, citated: 59)

III. Pécsi D, Gódi S, Hegyi P, Hanák L, Szentesi A, Altorjay I, Bakucz T, Czakó L, Kovács G, Orbán-Szilágyi Á, Pakodi F, Patai Á, Szepes Z, Gyökeres T, Fejes R, **Dubravcsik Z**, Vincze Á; Hungarian Endoscopy Study Group. ERCP is more challenging in cases of acute biliary pancreatitis than in acute cholangitis - Analysis of the Hungarian ERCP registry data. Pancreatology 2021; 21: 59-63. (Q1, H index: 74, IF: 3.996, citated: 1)

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(n=23)

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II. Czakó L, **Dubravcsik Z**, Gyökeres T et al. Az endoszkópos retrográd cholangiopancreatographia oktatásának hazai helyzete az Európai Emésztőszervi Endoszkópos Társaság (ESGE) oktatási irányelve tükrében. Central European Journal of Gastroenterology and Hepatology. 2022; 8: 2-10.

III. **Dubravcsik Z**, Gyökeres T, Hritz I, et al. Az endoszkópos leletezés standard nyelve. A nemzetközileg érvényes klasszifikációk gyűjteménye. Central European Journal of Gastroenterology and Hepatology. 2021; 7: 157-163.

IV. Gyökeres T, Bor R,….. **Dubravcsik Z**, et al. Quality expectations in endoscopy – Hungarian guideline [Az endoszkópia minőségi követelményei. Magyar szakmai irányelv.] Magy Seb. 2021; 74: 75-103. [Hungarian]

V. Hegyi PJ, Soós A, ….. **Dubravcsik Z**, et al. Evidence for diagnosis of early chronic pancreatitis after three episodes of acute pancreatitis: a cross-sectional multicentre international study with experimental animal model. Sci Rep. 2021; 11: 1367.

VI. Gonczi L, Szanto K, ….. **Dubravcsik Z**, et al. Clinical efficacy, drug sustainability and serum drug levels in Crohn's disease patients treated with ustekinumab - A prospective, multicenter cohort from Hungary. Dig Liver Dis. 2021: S1590-8658(21)00377-7.

VII. Bor R, Szántó KJ, ….. **Dubravcsik Z**, et al, Hungarian GI Endoscopy COVID-19 Study Group. Effect of COVID-19 pandemic on workflows and infection prevention strategies of endoscopy units in Hungary: a cross-sectional survey. BMC Gastroenterol. 2021; 21: 98.

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IX. Lovász BD, Szalai M,….**Dubravcsik Z,** et al. Improved adenoma detection with linked color imaging technology compared to white-light colonoscopy. Scand J Gastroenterol. 2020; 55: 877-883.

X. **Dubravcsik Z**, Farkas G, Hegyi P, et al, on behalf of the Hungarian Pancreatic Study Group. Autoimmun pancreatitis. A Magyar Hasnyálmirigy Munkacsoport bizonyítékon alapuló kezelési irányelvei. [Autoimmune pancreatitis. Evidence based management guidelines of the Hungarian Pancreatic Study Group]. Orv Hetil. 2015; 156: 292-307. [Hungarian]

XI. Takács T, Czakó L, **Dubravcsik Z**, et al, on behalf of the Hungarian Pancreatic Study Group. Krónikus pancreatitis. A Magyar Hasnyálmirigy Munkacsoport bizonyítékon alapuló kezelési irányelvei. [Chronic pancreatitis. Evidence based management guidelines of the Hungarian Pancreatic Study Group]. Orv Hetil. 2015; 156: 262-288. [Hungarian]

XII. Párniczky A, Czakó L, **Dubravcsik Z**, et al, on behalf of the Hungarian Pancreatic Study Group. Gyermekkori pancreatitis. A Magyar Hasnyálmirigy Munkacsoport bizonyítékon alapuló kezelési irányelvei. [Pediatric pancreatitis. Evidence based management guidelines of the Hungarian Pancreatic Study Group]. Orv Hetil. 2015; 156: 308-325. [Hungarian]

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XIV. Balázs A, Ruffert C, …… **Dubravcsik Z**, et al, Hungarian Pancreatic Study Group. Genetic analysis of the bicarbonate secreting anion exchanger SLC26A6 in chronic pancreatitis. Pancreatology. 2015; 15: 508-513.

XV. Madácsy L, **Dubravcsik Z**, Szepes A. Postcholecystectomy syndrome: From pathophysiology to differential diagnosis - a critical review. Pancreat Disord Ther 2015; 5: 162.

XVI. **Dubravcsik Z**, Hritz I, Fejes R, et al. Endoscopic therapy of refractory post-papillotomy bleeding with electrocautery forceps coagulation method combined with prophylactic pancreatic stenting. Video Journal and Encyclopedia of GI Endoscopy 2014; 1: 628-631.

XVII. Czakó L, **Dubravcsik Z**, Gasztonyi B, et al. Az endoszkópos ultrahang alkalmazása a gastrointestinalis betegségek diagnosztikájában és terápiájában. [The role of endoscopic ultrasound in the diagnosis and therapy of gastrointestinal disorders]. Orv Hetil. 2014; 155: 526-540. [Hungarian]

XVIII. **Dubravcsik Z**, Serényi P, Madácsy L, et al. Endoszkópos ultrahang vezérelte finomtű-aspirációs citológia a mediastinumban. [Endoscopic ultrasound-guided fine needle aspiration cytology in the mediastinum]. Orv Hetil. 2013; 154: 338-344. [Hungarian]

XIX. Szepes A, **Dubravcsik Z**, Madácsy L. Endoszkópos sphincterotomia hatása az epehólyag és az Oddi-sphincter motilitására, valamint az epeürülés dinamikájára

acalculosus biliaris fájdalom szindrómás betegekben. [The effect of endoscopic sphincterotomy on the motility of the gallbladder and of the sphincter of Oddi in patients with acalculous biliary pain syndrome]. Orv Hetil. 2013; 154: 306-313. [Hungarian]

XX. Balogh G Jr, **Dubravcsik Z**, Szepes A, et al. Endoszkópos submucosus disszekció saját gyakorlatunkban - új lehetőség a tápcsatorna korai neoplasztikus elváltozásainak endoszkópos kezelésében. [Endoscopic submucosal dissection in our practice - new possibilities in the endoscopic treatment of neoplastic changes in the alimentary canal]. Orv Hetil. 2012; 153: 824-833. [Hungarian]

XXI. Czakó L, Gyökeres T, …. **Dubravcsik Z**, et al. Autoimmune pancreatitis in Hungary: a multicenter nationwide study. Pancreatology. 2011; 11: 261-267.

XXII. Czakó L, Gyökeres T, …. **Dubravcsik Z**, et al. Autoimmun pancreatitis Magyarországon: országos multicentrikus tanulmány. Lege Artis Medicinae, 2011; 21: 37-44.

XXIII. Mihaly A, Szente M, **Dubravcsik Z**, et al. Parvalbumin- and calbindin-containing neurons express c-fos protein in primary and secondary (mirror) epileptic foci of the rat neocortex., Brain Res. 1997; 761: 135-145.

2. ABBREVIATIONS

ABP = acute biliary pancreatitis,

AI=artificial intelligence,

ALAT = alanine aminotransferase,

ALP = alkaline phosphatase,

AMY=amylase,

AP = acute pancreatitis,

ASAT = aspartate aminotransferase,

ATF6 = activating transcription factor 6,

ATG = autophagy dependent proteins,

ATP = adenosine-triphosphate,

BE = base excess,

BG=blood glucose,

BP=blood pressure,

BUN=urea / carbamide nitrogen,

Ca^{2+} = calcium,

CARS = compensatory anti-inflammatory response syndrome,

CBD = common bile duct,

CCK = cholecystokinin,

CFTR = cystic fibrosis transmembrane conductance regulator,

CHOP = CEBP homologous protein,

CI = confidence interval,

CP = chronic pancreatitis,

creat = creatinine,

CRF = case report form,

CRP = C-reactive protein,

CT = computer tomography,

CXCL1 = CXC chemokine ligand 1,

CypD = cyclophylin D,

DAMP = damage-associated molecular pattern,

DPSG = Dutch Pancreatitis Study Group,

ER=endoplasmic reticulum,

ERCP = endoscopic retrograde cholangiopancreatography,

ESGE = European Society of Gastrointestinal Endoscopy,

EST = papillotomy (endoscopic sphincterotomy),

EUS-FNA = endoscopic ultrasound-guided fine-needle aspiration,

FAEE = fatty acid ethyl ester,

FiO_2 = fraction of inspired oxigen,

GCS = Glasgow Coma Scale,

GGT = gamma-glutamyl transferase,

Gpbar = G-protein-coupled bile acid receptor,

HCO_3^- = bicarbonate,

Hgb = hemoglobin,

HPSG = Hungarian Pancreatic Study Group,

HR = heart rate,

IAP/APA = International Association of Pancreatology / American Pancreatic Association,

ICU = Intensive Care Unit,

IL = interleukine,

IP_3R = inositol-triphosphate receptor,

IPMN = intraductal papillary mucinous neoplasm,

IRE1 = inositol-requiring enzyme 1,

ISRCTN = International Standard Randomized Controlled Trial Number,

K = potassium,

LDH = lactate dehidrogenase,

MCP1 = monocyte chemoattractant protein 1,

MIP2α = macrophage inflammatory protein 2α,

MLKL = mixed lineage kinase domain-like,

MPTP = mitochondrial permeability transition pore,

Na = sodium,

NA = no data / non-applicable,

NaTC = sodium-taurocholate,

NCX = sodium-calcium pump,

NFAT = nuclear factor of activated T-cells,

NF-κB = nuclear factor-κB,

NNT = number needed-to-treat,

NSAID = non-steroidal anti-inflammatory drug,

MODS = multi-organ dysfunction syndrome,

OR = Odds ratio,

ORAI1 = Ca^{2+}-release activated Ca^{2+} channel protein 1,

OSD = Oddi-sphincter dyskinesis,

p = significance,

PaO_2 = partial pressure of oxigen,

PD = pancreatic duct,

PEP = post-ERCP pancreatitis,

PERK = protein kinase RNS-llike endoplasmic reticulum kinase,

PLT = platelet count,

PMCA = plasma membrane Ca^{2+} channel,

PPS = prophylactic/preventive pancreatic stent,

PRISMA = Preferred Reporting Items for Systematic Reviews and Meta-Analyses,

Proc risk = procedure-related risk factors,

Pt risk = patient-related risk factors,

RCT = randomized controlled trial,

RIP = receptor interacting protein kinase,

ROS = reactive oxigen species,

RR = relative risk,

RespR = respiratory rate,

RyR = ryanodin-receptor,

Saraf = store-operated Ca^{2+} entry regulator factor,

sebi = serum bilirubin,

SERCA = sarcoplasmic reticulum Ca^{2+} channel,

SIRS = systemic inflammatory response syndrome,

SOCE = store operated Ca^{2+} entry,

STIM1 = stromal interactions molecule 1,

SOD = sprinter of Oddi dyskinesis,

SUCRA = surface under cumulative ranking,

Sum of risk = sum of all risks,

sXBP1 = spliced X-box binding protein 1,

TAJ = hungarian social security number,

TGFβ = transforming growth factor beta,

TRMP2 = transient receptor ptential melastatin 2,

TRPV4 = tranziens receptor potential vanilloid 4,

UPR = unfolded protein response,

USS = ultrasound scan,

WBC = white blood cell count,

3. INTRODUCTION

Acute pancreatitis (AP) is an acute inflammatory disease of the pancreas, which, in severe cases, can lead to multi-organ dysfunction, complications, or even death. Treatment costs of gastroenterological diseases are the highest in healthcare, and within that, acute pancreatitis is the third most common cause of hospitalization (1). Gallstones and alcohol consumption are the two most common etiological factors comprising 2/3 of all cases, but there are other known causes, including endoscopic retrograde cholangiopancreatography (ERCP) (Table 1.) (2, 3).

Table 1: Etiological factors of acute pancreatitis (3)

Etiológy	**Examples**
Gallstones	NA
Alcohol	NA
Trauma	ERCP EUS-FNA Aortic surgery Pancreas resection
Premalignant and malignant conditions	IPMN Ductal adenocarcinoma
Metabolic	Hypertrigliceridaemia Hypercalcaemia
Genetic	*PRSS1, SPINK1, CFTR, CASR, CTRC*
Autoimmune pancreatitis	NA
Drugsa	Azathioprine, mesalamine, furosemide, losartan
Infections	Viral, bacterial, parasitic
Idiopathic	NA

NA=not applicable, IPMN=intraductal papillary mucinous neoplasm, EUS-FNA=endoscopic ultrasound-guided fine-needle aspiration, ERCP=endoscopic retrograde cholangiopancreatography,

Gallstones are the etiological factors in 16-68% of cases in different parts of the world, however in Europe it is detected in 41% of all AP cases in average (2).

The average mortality of AP is 5%. In mild pancreatitis it is only 3%, but in severe (necrotizing) forms it is much higher, 17%, and in cases of infected necrosis it can reach 30%. (4).

The definition and severity classification of AP and the definition of complications were first standardized in 1992 (5). The so-called Atlanta classification was adopted

worldwide over the next 20 years. AP was defined as an acute inflammatory disease of the pancreas with variable involvement of other regional and distant organs. In terms of severity, a distinction was made between mild and severe AP, the latter involved organ failure and/or local complications. Complications were defined as acute fluid collections, pancreatic necrosis, acute pseudocysts and pancreatic abscesses. However, new insights into the pathophysiology, in particular the pathophysiology of organ failure, a better understanding of the course of the disease (especially the course of necrotizing form), and advances in diagnostic methods and imaging techniques have led to criticisms of the classification since the early 2000s and revision has become inevitable (6).

3.1. Diagnostic criteria of acute pancreatitis

The revised Atlanta Classification, which is still in use today, was published online at the end of 2012 and in print in 2013 (7).

Based on this, the diagnostic criteria for AP follows the "two out of three" concept, i.e. the presence of two out of the following three is required: 1 - abdominal pain characteristic of pancreatitis (severe, persistent epigastric pain, often belt-like and radiating to the back), 2 - elevation of serum amylase and/or lipase at least three times the upper limit of normal, 3 - abnormalities characteristic of AP detected on imaging studies (mainly contrast enhanced CT, possibly MRI or ultrasound scan (USS)). The onset of the inflammatory process is defined as the onset of abdominal pain.

3.2. Morhological types and complications of acute pancreatitis, phases of inflammation

Morphologically, two AP types are defined. The first is interstitial edematous pancreatitis, which is characterized by enlargement of the pancreas, moderate haziness and stranding of the surrounding retroperitoneal fat and only minimal peripancreatic free fluid at most. The vast majority of cases present in this form and recover in a few days. The other type is necrotizing pancreatitis, which involves necrosis of the pancreas, or peripancreatic tissue, or both. It accounts for 5-10% of cases. It takes a few days for necrosis to develop and be detected by imaging, which is why it is recommended to wait 72-96 hours for contrast enhanced CT scan. The course of the disease can be variable, the necrosis can remain solid but can also be liquefied, can remain sterile but can also become infected, or can spontaneously resolve or even can persist.

Currently three forms of **complications** are defined, namely organ failure, local and systemic complications (7).

When evaluating organ failure, the cardiovascular, respiratory and renal organ systems are assessed using the modified Marshall scoring system (see Table 2) (8). Organ failure is defined as a score of ≥2 for at least one of the 3 organ systems. Organ failure should be monitored continuously, as it can be transient (≤48 hours) or persistent (>48 hours) and has a role in the assessment of AP severity.

Table 2: Modified Marshall scoring system for organ failure (8)

	Score				
Organ system	**0**	**1**	**2**	**3**	**4**
Respiratory (PaO_2/FiO2)	> 400	301-400	201-300	101-200	≤ 100
Renal (serum creatinine, µmol/l)*	≤ 134	135-169	170-310	311-439	> 439
Cardiovascular (systolic blood pressure, mmHg)**	> 90	< 90, Fluid responsive	< 90, Not fluid responsive	< 90, pH < 7.3	< 90, pH < 7.2

For non-ventilated patients, the FiO_2 can be estimated from below:		***Comments:***
Supplemental oxigen (l/min)	**FiO_2**	**A score for patients with pre-existing chronic renal failure depends on the extent of further deterioration of baseline renal function. No formal correction exists for a baseline serum creatinine ≥134 mmol/l or ≥1.4 mg/dl*
Room air	21%	
2 - 3	25%	
4 - 5	30%	***Off inotropic support. For patients with the need for inotropic catecholamine support, a Marshall score of 2 is appointed for cardiovascular system and dependent on the pH a score of 3 or 4 can be appointed*
6 - 8	40%	
9 - 10	50%	

Local complications include acute peripancreatic fluid collection (APFC), pancreatic pseudocyst (PP), acute necrotic collection (ANC) and walled-off necrosis (WOPN) (see Table 3) (7). In addition, gastric outlet dysfunction, lienal and portal vein thrombosis, and large bowel necrosis have also been reported.

Systemic complications include recent onset organ failure, as discussed above, although this is mentioned separately in the revised Atlanta classification. It also includes progression of preexisting comorbidities due to AP (e.g. coronary artery disease or worsening of chronic pulmonary disease).

Table 3: Pancreatic and peripancreatic fluid collections (7)

	Edematous pancreatitis	Necrotizing pancreatitis
<4 weeks	**Acut peripancreatic fluid collection (APFC)** • Fluid density, homogeneous • No fully definable wall • Confined by normal fascial planes • Adjacent to pancreas	**Acut necrotic collection (ANC)** • Heterogeneous, variig density • Contains non-liquid component • No fully definable wall • Intra- and/or extrapancreatic
>4 weeks	**Pancreatic pseudocyst (PP)** • Well described • Fluid density, homogeneous • No non-liquid component • Well defined wall	**Walled-off necrosis (WOPN)** • Heterogeneous, liquid and non-liquid components • Well described, well defined wall • Intra- and/or extrapancreatic

Inflammation progresses in two, partly overlapping **phases**, and mortality has two peaks correspondingly. The early phase is usually detected in the first week, although it may extend into the second week. During this phase, activation of the cytokine cascade is present, which is clinically characterized by systemic inflammatory response syndrome (SIRS), and may lead to persistent organ failure, as indicated by persistent SIRS (7, 9). SIRS is present if at least two of the following four conditions are present: 1 - heart rate >90/min, 2 - core temperature <36 ^{O}C or >38 ^{O}C, 3 - white blood cell count <4000 or >12000/mm^3, 4 - respiratory rate >20/min or pCO_2 <32 mmHg. The second, late phase lasts for weeks, sometimes months. By definition, it is characterised by the persistence of inflammatory signs or by local complications, therefore it is only seen in moderate to severe AP. In this case SIRS is followed by a compensatory anti-inflammatory response syndrome (CARS), which is responsible for the higher infectious complications during this phase (7, 10).

3.3. Severity classification of acute pancreatitis and prognostic indices

The currently approved classification distinguishes three **severity** forms, namely mild, moderate and severe AP (7). AP is considered mild if there is no organ failure or local or systemic complications. It resolves in a few days without any complications, and mortality is extremely rare. Pancreatitis is moderate if organ failure is transient (<48 hours) or local or systemic complications occur without persistent organ failure. This form has a better prognosis than severe AP and may resolve spontaneously, but requires a longer time course than mild cases. In severe cases, organ failure is persistent (>48 hours), which may involve only a single organ system or may be multi-organ failure (MOF), with a markedly high mortality of 36-50% (7, 11, 12).

Predicting the severity and thus predicting the likely course of the disease has been a long-standing topic of interest for at least 40 years. Various prognostic indices have been developed over the years. Although we have seen before that the most commonly accepted, international consensus-based recommendation is the presence of SIRS and organ failure, we have briefly summarized the other main severity indices in Table 4 below. It should be noted, however, that none of these systems is good enough to predict severity, as they generally have a medium sensitivity and low positive predictive value (13). In the last few years, artificial intelligence, machine learning algorithms, have been used to estimate and predict severity, mortality, complications and disease progression (14). In a recently published review article, 24 publications with a total of 8327 patients and using 47 artificial intelligence-based (AI) prediction models were analyzed. Among these, 10 publications were involved in predicting severity, 10 in predicting complications and 3 in predicting mortality. Both, two and two articles focused on relapse and the timing of surgery. The AI-based systems all performed significantly better than traditional prognostic indices, but have not been widely adopted in the clinical setting as they only have been shared by a few authors and largely lack external validation. Under the auspices of the Hungarian Pancreatic Working Group (HPSG), admission data (first 12 hours) of 1148 AP patients from 28 institutions in 15 countries, collected in a prospective database, were analyzed to identify the most important parameters determining severity (15). Using 22 parameters from 4 groups, a prediction model has been developed using the XGBoost machine learning algorithm, which currently predicts the onset of severe AP with an accuracy of around 90%. The six most important parameters are respiratory rate, body temperature, abdominal muscle defense, age, sex and blood glucose. The validation cohort consisted of 3543 patients. A web based application (Easy-App) was developed, that is accessible and can be used online by everyone, which calculates the probability of developing severe AP and the confidence interval, and also provides an explanation showing which parameters are most likely to lead to a severe course. A common feature of AI-based algorithms is that their accuracy increases with the amount of input data, so the prediction accuracy of Easy-App is expected to increase even further.

Table 4: Severity prognostic indices of acute pancreatitis

Index	Year	Cutoff	Parameters	Pros	Cons
Acute Physiology and Chronic Health Examination (APACHE-II) (16)	1981	≥8	12 physiologic and other parameters (temp, BP, HR, RespR, oxigenization, arterial pH, Na, K, creat, Htk, WBC, GCS, age, chronic disorder)	American Gastroenterology Association advices	Difficult to use Poor predictive value in first 24 hours
Modified Glasgow (Imrie score) (17)	1984	≥3	9 parameters (age, WBC, BG, BUN, albumine, PaO_2, Ca^{2+}, ASAT/ALAT, LDH)		Requires 48 hours
Ranson (18, 19)	1974	≥3	11 parameters (on admission: 5 - age, WBC, BG, ASAT, LDH; at 48 hours: 6 - Htk decrease, BUN increase, Ca^{2+}, PaO_2, BE, fluid sequestration)		Not good at predicting prognosis, Requires 48 hours
Harmless acute pancreatitis score (HAPS) (20)	2009	>2	3 parameters (abdominal tenderness and/or guarding, Htk, creat)	Can be used within 48 hours	Inferior sensitivity and specificity to APACHE-II
Bedside Index of Severity (BISAP) (21)	2008	≥3	5 parameters (BUN, impaired mental status, SIRS, age, pleural effusion)	Useful in prediction of mortality in first 24 hours	Cannot differentiate transient to permanent organ failure at 24 hours
Sepsis-related Organ Failure Assessment (SOFA) (22)	1996	≥2	6 parameters (respiratory - PaO_2/FiO_2, cardiovascular - BP, renal - creat, central nervous system - GCS, coagulation - PLT, liver - sebi)	Can predict MOF	Originally it was developed for sepsis patients
Japanese Prognostic Severity Score (23)	2009	≥3	9 parameters (BE, PaO_2, BUN, LDH, PLT, Ca^{2+}, CRP, SIRS, age, CT grade)	Predicts mortality	
Chinese Simple Scoring System (CSSS) (24)	2019	≥4: severe AP, ≥6: morta-lite	6 parameters (creat, BG, LDH, CRP, HR, pancreas necrosis)		
Balthazar CT index (25)	1985	Grade A-E	Grade A-E: normal pancreas - ≥2 fluid collection	Correlates with local complications	Cannot detect necrosis

Index	Year	Cutoff	Parameters	Pros	Cons
CT Severity Index (CTSI) (26)	1990	0-3: mild, 4-6: mode-rate, 7-10: severe	Balthazar grades + degree of necrosis		Moderate interobserver agreement. Poorly correlates with organ failure. Similar outcome for >30% and >50% necrosis.
Modified CTSI (27)	2004	0-2: mild, 4-6: mode-rate, 8-10: severe	Pancreas inflammatory signs, necrosis and extrapancreatic complications		Moderate interobserver agreement. Poor correlation with organ failure
EASY-APP (15)	2022	NA	22 parameters in 4 groups (personal data - age, gender, BMI; medical history - alcohol consumption, smoking, abdominal pain in hours; vital signs - RR, HR, temp, RespR, abdominal tenderness, guarding; blood results - AMY, AST, BG, BUN, creat, CRP, Na, K, Ca^{2+}, WBC)	Online avaliable application. Works with data available at admission (<12 hours)	Cannot distinguish between mild and moderate AP. Probability is presented with a number between 0 and 1, instead of %.

ALAT = alanine aminotransferase, AMY = amylase, ASAT = aspartate aminotransferase, BE = base excess, BG = blood glucose, BMI = body mass index, BP = blood pressure, BUN = urea nitrogen, Ca^{2+} = calcium, creat = creatinine, CRP = C-reactive protein, CT = computer tomography, FiO_2 = fraction of inspired oxigen, GCS = Glasgow Coma Scale, HR = heart rate, Htk = hematocrit, K = potassium, LDH = lactate dehidrogenase, MOF = multi organ failure, Na = sodium, PaO_2 = partial pressure of oxigen, PLT = platelet count, sebi = serum bilirubine, SIRS = systemic inflammatory response syndrome, RespR = respiratory rate, temp = body temperature, WBC = white blood cell count,

4. PATHOPHYSIOLOGY OF ACUTE PANCREATITIS

The exocrine pancreas produces 2 liters of digestive enzyme- and bicarbonate rich ($HCO3^-$) fluid per day, which is crucial for normal digestion (28). The acinar cells produce the low volume but digestive enzyme-rich fluid, while the ductal cells produce the high volume and $HCO3^-$-rich fluid. The food coming from the stomach is acidic, which is neutralized by this alkaline, $HCO3^-$-rich fluid, and digestion of the food begins by activation of the digestive enzymes (28).

In exocrine pancreatic cells, intracellular Ca^{2+} levels play an important role. Its increase stimulates enzyme secretion in acinar cells, while fluid and electrolyte secretion in ductal cells.

Abnormal, unregulated elevation of Ca^{2+} levels, on the other hand, leads to acute pancreatitis (29).

The various known etiological factors all trigger the disease through a sustained abnormal increase in intracellular Ca^{2+} levels, causing premature activation of trypsinogen in acinar cells and impaired fluid and $HCO3^{-}$ secretion in ductal cells. Other pathophysiological events also occur, such as mitochondrial failure, endoplasmic reticulum (ER) stress, abnormal unfolded protein response (UPR), or increased production of reactive oxygen species (ROS). These ultimately lead to acinar cell necrosis and local and systemic inflammatory responses (30).

As the disease can lead to serious health impairment or even death, and no specific medical treatment is available, better understanding of the underlying molecular mechanisms has been the subject of intensive research.

The pathophysiological processes that have been described so far have been established in animal models, mostly in rodents. It is known, that pancreatitis in rodents and humans differs, however, the mechanisms revealed in animal models have been demonstrated in ex vivo experiments in human pancreas cells.

4.1. Animal models of acute pancreatitis

A better understanding of the etiology and pathomechanism of AP and searching for new therapeutic targets would be optimal in human pancreas, but the difficulty in predicting the course of the disease, the heterogeneity of the clinical setting and the limited access to human pancreatic tissue make research in human tissues difficult.

Traditionally, animal models, mainly rodents (rats, mice), have been used in basic research for more than 100 years. An ideal animal model is one, that reflects the etiology, pathomechanism, histological changes, clinical course and outcome of AP. This is impossible to achieve in practice, but rodent models are readily available, relatively inexpensive, easy to handle and can be used to study moderate to severe pancreatic injuries (31).

Experimental models can be divided into *in vivo* and *in vitro* models, and the former can be further subdivided into non-invasive and invasive subgroups.

1. In vivo models:

A. Non-invasive subgroup:

- <u>Cerulein-induced model</u>:

This is the most widely used animal model, which is easily reproducible and cost-effective (works both in mice and rats).

Cerulein is a cholecystokinin (CCK) analogous hormone, which induces digestive enzyme secretion in the pancreas, like CCK itself (32). CCK receptors respond to these hormones in a biphasic manner. They induce exocytosis of zymogen granules from acinar cells via the high-affinity receptors, i.e. they enhance digestive enzyme secretion, and then, after saturation of these receptors, they bind to the low-affinity receptors, whereby enzyme secretion is inhibited (32). When acinar cells are exposed to excessive CCK stimuli, exocytosis of zymogen granules is immediately inhibited and digestive enzymes accumulate in the acinar cells (33).

In the cerulein-induced animal model, this phenomenon is used to induce pancreatitis by overdosing the hormone (5 μg/kg/h in rats and 50 μg/kg/h in mice, given intravenously or intraperitoneally) (32). In this model, hyperamylasaemia, infiltration of the pancreas with inflammatory cells, edema, vacuolization of the acinar cells and appearance of activated digestive enzymes within the gland are observed, similar to human AP. Ductal and endocrine cells are not damaged.

• Amino-acid induced model:

- ***L-arginine***:

In rats, intraperitoneal injection of a single excessive dose (500 mg/100 g body weight) of L-arginine induces severe necrotizing pancreatitis, which reaches its maximum after 72 h (34). Within seven days, acinar cells begin to regenerate and become nearly normal after 14 days.

The disadvantage is that the pathogenetic mechanism is not fully understood

- ***L-ornithine***:

Hungarian authors have published this simple model, that is even more effective than L-arginine, in which AP can be induced by intraperitoneal injection of 3 g/kg L-ornithine in rats. In mice, a dose of 2x4 g/kg causes lethal outcome within a few hours (35).

- ***L-lysine:***

Also published by Hungarian authors. Early mitochondrial damage is observed, which is detectable before trypsinogen activation (36).

- ***L-histidine***:

Intraperitoneal administration of 2x4 g/kg L-histidine 1 hour apart induces AP in mice. In rats, a dose of 3 g/kg has no effect, but because of its relatively low solubility, it is assumed that higher doses would be required (37).

• Alcohol and free fatty acid model:

Although alcohol is a major etiologic factor in AP, there is no widely accepted animal model of alcoholic pancreatitis (31). In early animal models, alcohol administration by various routes has been shown to increase pancreatic duct permeability, reduce pancreatic blood flow and microcirculation, reduce glandular oxygen utilization and induce oxidative stress, yet does not cause acute pancreatitis in itself, but sensitizes the organ to other harmful factors (e.g. cerulein, bile). In the human body, alcohol is metabolized by oxidative and non-oxidative pathways. In the former, alcohol dehydrogenase and the enzyme cytochrome P450 2E1 play a major role and finally acetaldehyde and reactive oxygen radicals are generated and mitochondrial permeability increases, leading to mitochondrial failure. In the non-oxidative pathway, a group of enzymes called fatty acid ethyl ester (FAEE) synthases produce FAEEs from ethanol by the addition of free fatty acids, which induce changes characteristic of AP (31).

In mice, two intraperitoneal administrations of fatty acids and non-toxic amounts of alcohol (150 mg/kg palmitoleic acid, 1.35 g/kg ethanol) can induce pancreatitis (pancreatic edema, neutrophil cell infiltration, necrosis, and thickening of the alveolar membrane and inflammatory cell infiltration of the lung, but minimal effects on the kidney, heart and liver) (38).

B. Invasive subgroup:

• Bile-acid induced model:

Bile reflux through the pancreatic ducts into the pancreas is thought to be the most common cause of human acute pancreatitis. This animal model is intended to reproduce this mechanism (39).

A cannula is introduced into the rat or mouse pancreatic duct through the orifice in the duodenum and 0.2-0.3 ml of 3-5% sodium taurocholate (NaTC) is infused retrogradely. The bile duct is temporarily clamped at the level of the liver hilus. After the infusion, the cannula is removed. The exact mechanism by which NaTC induces AP is not known, but severe hemorrhagic inflammation is observed 6-12 hours after the infusion and therefore this is a representative model of severe pancreatitis. NaTC is the most commonly used bile acid, but others may be used (e.g. sodium glycodeoxycholic acid, taurodeoxycholate, taurolithocholic acid-3-sulphate).

Its advantage is that it can induce severe hemorrhagic necrosis in a relatively short time, but its disadvantage is that it is invasive and the insertion of the cannula requires considerable experience.

- Pancreatic duct ligation model:

If the pancreatic duct of a rat is ligated, the tissue changes characteristic of AP (edema, inflammatory cell infiltration, hyperamylasemia) occur in the early phase, 1-5 hours after the intervention, and the best time to examine these changes is after 24-48 hours. Over time, changes more characteristic of chronic pancreatitis (e.g. atrophy, acinar cell necrosis, fibrosis) occur.

Ligation of the bile duct and pancreatic duct of an American opossum causes acute necrotizing pancreatitis, but this animal is very difficult to find (40).

More recently, this model has been adopted for mice. Here, AP can be induced by ligation of the pancreatic duct, which is associated with a systemic inflammatory response and multi-organ failure.

- Post-ERCP pancreatitis (PEP) model:

In the rat, AP was first induced by retrograde biliopancreatic administration of a sugar-derived alcohol, meglumine, in various concentrations at a pressure of 20-50 mmHg for 30 seconds (edema, leukocytosis, necrosis and hemorrhage were observed after 4 days), which was used to simulate PEP (31). Many factors, such as mechanical, thermal, chemical, hydrostatic, enzymatic and microbiological insults are also assumed to play a role in the pathogenesis of PEP, therefore other agents can be used in this model, such as contrast agents of different concentrations and different pH values. In addition, the pressure at which the agent is administered can be varied, which can induce disruption of the pancreatic ductal system and the acinar lobules. More recently, this model has been implemented in mice (the solutions used were iohexol, iopamidol and physiological saline).

- Genetic models:

Genetics has made significant progress in recent years. The identification of genetic mutations in trypsin-dependent pathways is a milestone in AP research (31). Various techniques (transgenic, knock out, knock in, or knock down) can be used to provide evidence for premature intrapancreatic enzyme activation (31).

C. Other experimental models:

There are many other pancreatitis animal models available in the literature, but these have now been neglected or have not been successful. Examples include the choline-deficient,

ethionine-rich diet model, the immune-induced model, the hemolysis-induced model, the dibutyltine dichloride-induced model, and the Coxsackievirus-induced model (4).

2. *In vitro* models:

Pancreas acinar cells, isolated pancreatic lobules and ductal system models are included here.

Methods of isolating different pancreas cells have been known for decades. These can be used to study the effect of CCK in vitro, among other factors. The main limitation of this model is the short lifetime of the cells, making it unsuitable for long experiments or cell culture.

These limitations can be overcome with organoids. An organoid is a stem cell-derived cell culture that retains a 3-dimensional structure and exhibits self-organization (41). Organoids are derived from either pluripotent stem cells or tissue-derived stem cells. They can be made from ductal cells, for example, which preserve cell polarity and can be used to study membrane transporters (42).

The isolated pancreatic lobule is a more physiological platform for studying signaling pathways and interactions between different cells. Lobules as small as 1 mm^3 can be manually excised from mouse pancreas injected with saline, or even much smaller lobules can be excised by collagenase digestion.

The role of pancreatic ductal cells in the pathogenesis of acute pancreatitis is not negligible. Pancreatic ductal epithelial cells produce bicarbonate-rich fluid, thus contributing to the maintenance of the appropriate pH of the pancreatic juice. Altered pH due to reduced bicarbonate secretion can trigger premature enzyme activation and consequently AP (43). In some conditions, for example, reduced bicarbonate secretion may result in mucin plugs in the ductal system (44). Ductal obstruction can affect the signaling mechanisms of the acini, and pancreatitis-inducing bile acids can decrease the pH of the ductal cells.

By introducing low melting point agarose into the pancreatic ducts post-mortem and simultaneously cooling the organ, the 3-dimensional structure of the ductal system can be preserved and the ductal system can be studied using vibratome sectioning, or it can be embedded in paraffin and sectioned as such, or frozen in cryomatrix and sectioned by cryostat (31, 45).

4.2. Important pathophysiological molecular events

A. Role of calcium

In acinar cells, pathological elevation of intracellular Ca^{2+} levels is a crucial event in AP, which, among other things, prematurely activates trypsinogen to trypsin, activates nuclear factor-κB (NF-κB), also results in mitochondrial dysfunction and eventually cell death and activation of the inflammatory cascade occurs (29).

Under physiological conditions, Ca^{2+} released from the ER initiates the exocytosis of zymogen granules and enhances mitochondrial adenosine-triphosphate (ATP) production. In response to physiological stimuli that activate acinar cells, the ER "rushes" into the apical part of the cell. This is the part where zymogen granules (or the mitochondria necessary to maintain homeostasis) are located. Ca^{2+} release here occurs through activation of inositol-triphosphate and ryanodine receptors (IP_3R and RyR). However, this increase in Ca^{2+} levels is only transient as the cell empties it. This involves a low affinity, but high capacity sodium-Ca^{2+} pump (NCX) in the plasma membrane and two ATP-dependent Ca^{2+} channels. One of the latter is the sarcoplasmic reticulum Ca^{2+} channel (SERCA), which pumps Ca^{2+} back into the ER, and the other is the plasma membrane Ca^{2+} channel (PMCA), which pumps Ca^{2+} out of the cell. However, decreasing Ca^{2+} levels in the ER also activate an extracellular Ca^{2+} influx to ensure replenishment, which is called store operated Ca^{2+} entry (SOCE). The stromal interaction molecule 1 (STIM1) located in the ER and functioning as a Ca^{2+} sensor, and the Ca^{2+} release-activated Ca^{2+} channel protein 1 (ORAI1) located in the plasma membrane play an important role in this process. The release of Ca^{2+} from the ER upon acinar cell activation causes a conformational change of STIM1, previously distributed in the ER, to the vicinity of the plasma membrane, where it binds and activates ORAI1 to release extracellular Ca^{2+} into the ER. However, it has also been demonstrated that Ca^{2+} also enters the cell through the basal cell membrane and reaches the apical region through the ER, recharging the store (this process is known as Ca^{2+} tunneling) (29). The store-operated Ca^{2+} entry regulator factor (Saraf) protein, that regulates the function of the ORAI1 channel, also plays an important role in the development of pathological Ca^{2+} pathways (29). Its levels are reduced in AP (the absence of Saraf causes more severe AP in animal models).

Various pathological stimuli (e.g. alcohol, bile acids, intraductal pressure increase) can disrupt this smoothly functioning system and excessive amount of Ca^{2+} accumulates in the cell (30). The overload of intracellular Ca^{2+} levels causes mitochondrial dysfunction (mitochondrial permeability transition pores (MPTP) open, resulting in elimination of the membrane potential necessary for ATP production). Due to ATP depletion, counter-regulatory mechanisms (ATP-dependent SERCA and PMCA are unable to remove pathological amounts of Ca^{2+} from the cell), and other ATP-dependent cytoprotective mechanisms (such as autophagy and UPR) are also impaired. This eventually leads to acinar cell necrosis. Other

cytotoxic pathways such as early trypsinogen activation and NF-κB activation are also activated (see Figure 1) (29, 30, 46).

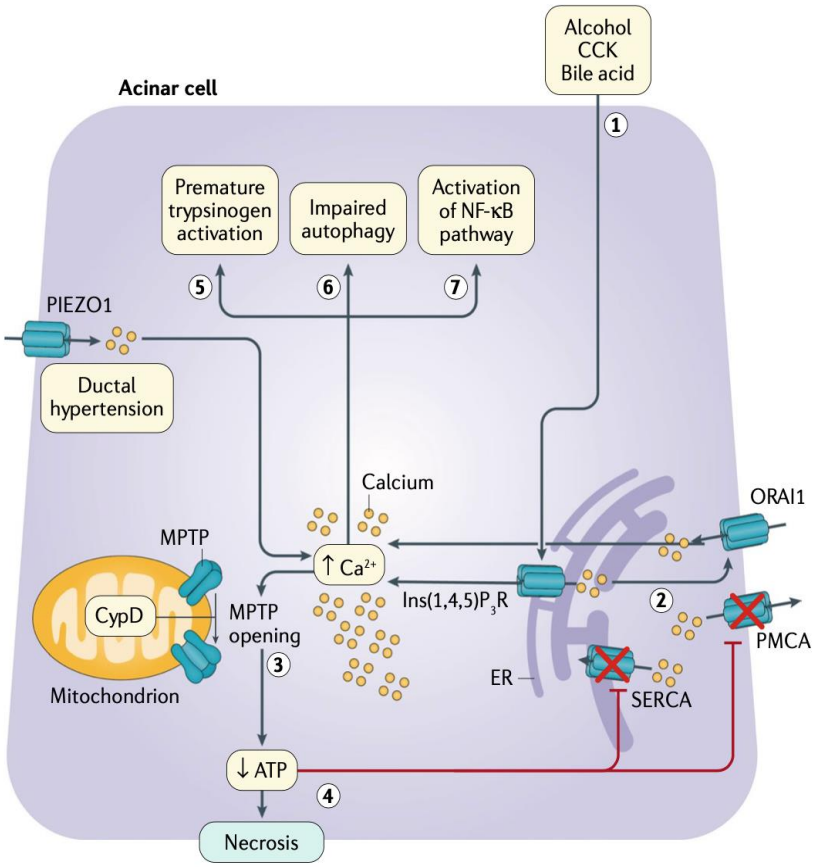

Figure 1: Role of calcium in acute pancreatitis

In response to alcohol, CCK, bile acids, Ca^{2+} is released from the endoplasmic reticulum (ER) of acinar cells via IP_3 receptors (Ins(1,4,5)P_3R) (1). Reduced Ca^{2+} levels in the ER activate ORAI1, resulting in Ca^{2+} influx from the extracellular space into the ER and the cell (2). The PIEZO1 receptor, which is activated by an increase in intraductal pressure in the Wirsung's duct, can also trigger the process. The increase in intracellular Ca^{2+} levels causes the MPTP found in mitochondria to open, thereby disrupting their function by eliminating the membrane potential essential for ATP production (3). The decrease in ATP levels also impairs the function of ATP-dependent SERCA and PMCA, resulting in the inability of the cell to excrete the accumulated Ca^{2+} (4). Pathological Ca^{2+} levels have other cytotoxic consequences, such as premature trypsinogen activation (5), impaired autophagy (6), and the production of proinflammatory cytokines via the NF-κB pathway (7). All of these steps cause acinar cell necrosis and an amplification of the inflammatory process.

Abbreviations: ATP=adenosine triphosphate, Ca^{2+}=calcium, CCK=cholecystokinin, CypD=cyclophillin D, ER=endoplasmic reticulum, Ins(1,4,5)P_3R=inositol-triphosphate receptors (IP_3R), MPTP=mitochondrial permeability transition pores, NF-κB=nuclear factor-κB, ORAI1=Ca^{2+}-release activated Ca^{2+} channel protein 1, PMCA=plasma membrane Ca^{2+} channel, SERCA=sarcoplasmic reticulum Ca^{2+} channel,

(After Lee PJ, Papachristou GI. New insights into acute pancreatitis. Nat Rev Gastroenterol Hepatol. 2019; 16: 479-496. (30))

B. Premature trypsinogen activation

Various stimuli (e.g. pancreatic trauma, duct obstruction, alcohol) increase the synthesis of lysosomes and digestive enzymes (30). Apical exocytosis of zymogen granules is impaired by microtubule dysfunction in the cell, and fusion of lysosomes and zymogen granules is induced. The lysosomal enzyme cathepsin B activates the trypsinogen, generating trypsin. The acinar cell and pericellular space self-digest. The released cathepsin B also activates the receptor-interacting protein kinase (RIP), which activates the mixed lineage kinase domain-like (MLKL) pathway. MLKL oligomerizes and translocates in this form to the plasma membrane, causing cell membrane perforation, rupture and ultimately cell necrosis (see Figure 2) (30).

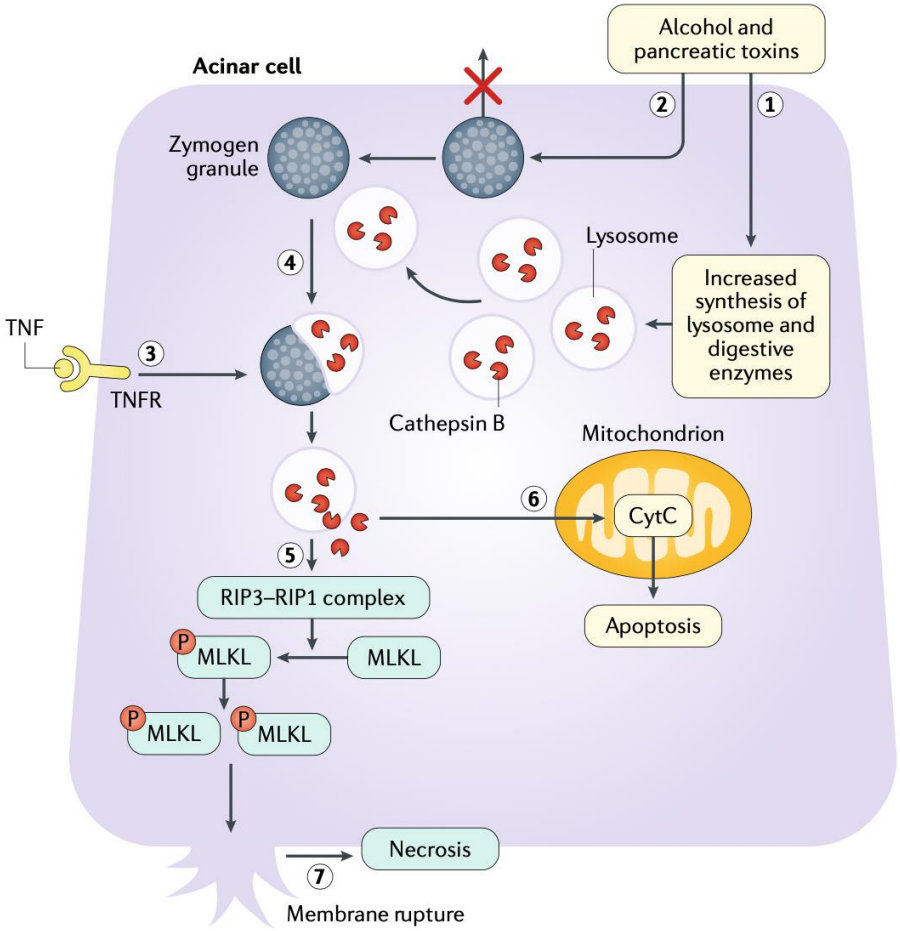

Figure 2: Premature trypsinogen activation in acute pancreatitis

Alcohol and other pancreatic toxins induce the synthesis of lysosomes and digestive enzymes in the acinar cells (1). Apical exocytosis of the zymogen granules is impaired (due to microtubular dysfunction) (2). TNF released during inflammation activates TNF receptors (3), which also leads to the activation of the fusion of lysosomes and zymogen granules (4). The lysosomal enzyme cathepsin B causes trypsinogen to be converted into trypsin, and the cell and its surroundings are self-digested. The released cathepsin B also activates RIP (5) and initiates

apoptosis (6). MLKL is phosphorylated and oligomerized by RIP and translocates to the plasma membrane, causing its perforation, rupture and cell necrosis (7).

Abbreviations: MLKL= mixed lineage kinase domain-like, RIP= receptor-interacting protein kinase, TNF= tumor necrosis factor

(After Lee PJ, Papachristou GI. New insights into acute pancreatitis. Nat Rev Gastroenterol Hepatol. 2019; 16: 479-496. (30))

C. Autophagy, endoplasmic reticulum stress, unfolded protein response (UPR)

Autophagy is a cytoprotective mechanism that removes outdated, damaged, or abnormal cellular components and proteins from the cell (30). Since acinar cells produce a large amount of proteins, a well-functioning autophagy is important for the maintenance of these cells. The process starts with autophagosome formation. A double membrane originating from the ER, Golgi apparatus and plasma membrane surrounds the damaged component, fuse with the lysosome and forms the autolysosome, in which hydrolases degrades the incorporated components. The process is mediated by autophagy-dependent proteins (ATG). Impaired autophagy is associated with trypsinogen activation, ER stress and mitochondrial dysfunction, leaving the acinar cell (among other things) more vulnerable to other insults.

ER stress refers to the accumulation of poorly packaged or unpackaged proteins in the ER. It occurs when the amount of proteins to be eliminated exceeds the capacity of the ER. ER stress that exceeds protective cellular mechanisms leads to apoptosis.

During ER stress, the UPR is activated in acinar cells to maintain cell homeostasis. The cell defends itself against ER stress with this mechanism. The UPR alleviates ER stress by increasing the capacity and efficiency of the protein removal ability of ER. There are three important pathways: inositol-requiring enzyme 1 (IRE1), activating transcription factor 6 (ATF6) and protein kinase RNA-like ER kinase (PERK). The IRE1 and ATF6 pathways generate transcription factors (such as spliced X-box binding protein 1 (sXBP1) and cleaved ATF6 (cATF6)) that enhance the generation of substrates required for ER expansion and degradation of unwanted proteins. When these mechanisms fail to maintain the cell homeostasis, the apoptotic PERK pathway is activated, where the final transcription factor is the CEBP homologous protein (CHOP). During prolonged ER stress, CHOP induces cell death and inflammation (see Figure 3) (30).

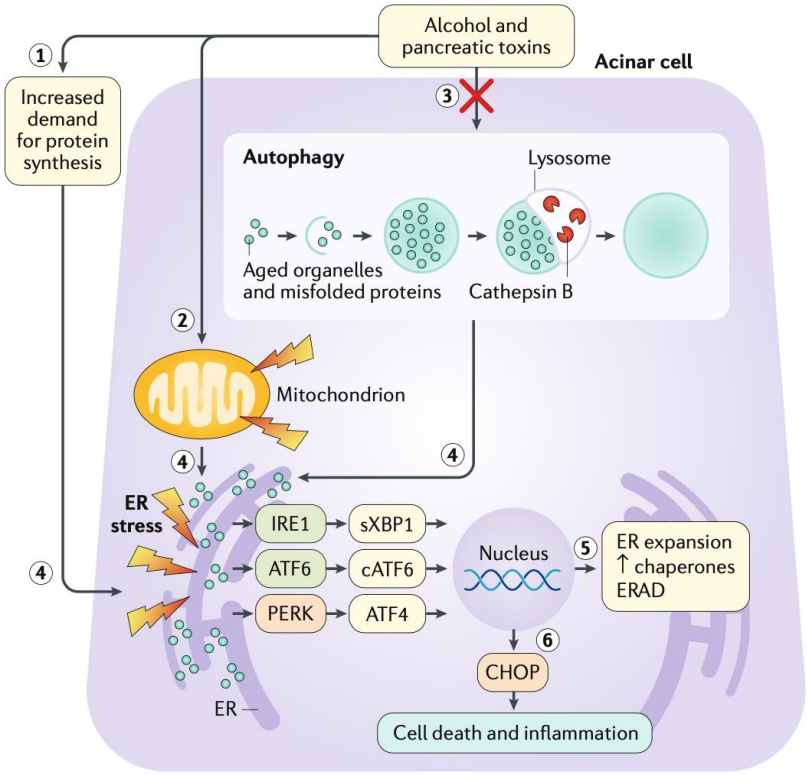

Figure 3: Endoplasmic reticulum stress, the unfolded protein response and autophagy in acute pancreatitis

In acinar cells, alcohol and other pancreatic toxins increase the demand for protein synthesis (1), also result in mitochondrial dysfunction (2) and autophagy (3). All of these lead to ER stress (4). The ER is unable to remove the produced proteins from the cell. Transmembrane proteins such as IRE1, ATF6 and PERK sense misfolded proteins. The sXBP1 and cATF6 are transcription factors produced through IRE1 and ATF6 pathways that can induce ER expansion and enhance ER-associated degradation (5) to meet increased ER demands. In the presence of extensive ER stress, the PERK pathway leads to apoptosis and inflammation mediated by CHOP, or necrosis in prolonged cases (6).

Abbreviations: ATF6=activating transcription factor 6, cATF6=cleaved ATF6, CHOP=CEBP homologous protein, ER=endoplasmic reticulum, IRE1=inositol-requiring enzyme 1, PERK=protein kinase RNA-like ER kinase, sXBP1=spliced X-box binding protein 1

(After Lee PJ, Papachristou GI. New insights into acute pancreatitis. Nat Rev Gastroenterol Hepatol. 2019; 16: 479-496. (30)).

D. Intraductal events and ductal cell dysfunction

The secretion of HCO_3^- by ductal cells is essential for the production and composition of a normal pancreatic juice. Under physiological conditions, the HCO_3^--rich secretion of ductal cells neutralizes the protons secreted by acinar cells with digestive enzymes, and also washes out the proenzymes into the duodenum, furthermore the alkaline pH inhibits trypsinogen activation. Transmembrane water channels (e.g. aquaporin 1) and the cystic

fibrosis transmembrane conductance regulator (CFTR) channel also play an important role. As an example, alcohol impairs CFTR and bile acids impair aquaporins. As a consequence, intraductal pH increases, acidification occurs in the ducts, leading to intraductal stasis and premature enzyme activation. The various intraductal events (increase in intraductal pressure, acidification, ductal cell exposure to bile acids) can cause acinar cell damage and death (30).

E. Role of the immune system

Chemokines, cytokines and various adhesion molecules are released from damaged acinar cells and attract immune cells to the site of inflammation (30). Monocyte chemoattractant protein 1 (MCP1) attracts monocytes, macrophage inflammatory protein 2α (MIP2α) and CXC chemokine ligand 1 (CXCL1) attract neutrophils, which are activated by dead and damaged cells. Neutrophil NADP oxidase causes oxidative stress and increased intraacinar trypsinogen activation. The proteins, DNA and histones released from neutrophils form a sticky web, an extracellular trap (NET), which occludes the ducts and leads to early trypsinogen activation. Damage-associated molecular patterns (DAMPs) released from damaged acinar cells are important mediators of monocyte activation. Activated monocytes augment systemic inflammation and tissue damage. DAMPs are able to bind to various receptors on immune cells (e.g. HMGB1, HSP70, TLR, P2X7). The NF-κB and STAT3 (signal transducer and activator of transcription 3) pathways are also activated. Ultimately, the production of pro-inflammatory cytokines such as tumor necrosis factor (TNF) and certain interleukins (IL) such as IL-1, IL-6, IL-18 is increased. Monocytes and macrophages, that are activated in distant organs, aggravate systemic inflammation and damage distant organs. The pro-inflammatory phase is followed by a compensatory anti-inflammatory response. This is characterised by the predominance of anti-inflammatory cytokines such as transforming growth factor beta (TGFβ), IL-4 and IL-10. In this stage the chance of developing infections of pancreatic necrosis is increased (30).

F. The role of free fatty acids

Previously, we have seen that in acute pancreatitis the clearance of zymogen granules on the apical surface of acinar cells is impaired, and instead secretion on the basolateral surface is more characteristic (30). The released lipase degrades triglycerides stored in peri- and intrapancreatic adipocytes, generating unsaturated free fatty acids, which are cytotoxic by damaging mitochondria and enhancing inflammation by increasing the release of TNF and other cytokines.

G. Reactive oxigen species (ROS)

ROS are also found in cells under normal conditions, and are generated during mitochondrial activity, and originate from complexes I and III of the mitochondrial electron transport system (30). A balance of oxidant and antioxidant mechanisms maintains their physiological equilibrium. They play a role in intracellular signalling, but their abnormal accumulation damages lipid membranes, proteins and nucleic acids, and may contribute to the development of many diseases. Bile acids induce ROS formation in the mitochondria of pancreatic acinar cells, resulting in an increase in mitochondrial Ca^{2+} levels and acinar cell necrosis.

In cells, a Ca^{2+}-permeable non-selective cation channel, the transient receptor potential melastatin-like protein 2 (TRMP2), serves as a redox sensor, which is activated by hydrogen peroxide (H_2O_2) through an indirect mechanism. It is found on the basolateral membrane of acinar cells, but apically in ductal cells. These receptors are responsible, for example, for about 22% of the Ca^{2+} influx observed in acinar cells in biliary pancreatitis. TRMP2-mediated Ca^{2+} influx appears to play a central role in oxidative stress-induced mitochondrial damage, but bile acids also cause Ca^{2+}-independent direct damage (29).

4.3. Characteristics and differences in molecular mechanisms in acute pancreatitis of different etiologies

A. Bile acid induced pancreatitis

According to Opie's "common channel theory", bile acids that enter the pancreatic duct are responsible for acute biliary pancreatitis (29). Bile acids increase intracellular Ca^{2+} levels in both acinar and ductal cells in a dose-dependent manner. On the one hand, they induce Ca^{2+} release from intracellular stores by activating IP_3R and RyR, inhibit SERCA and thus Ca^{2+}-reuptake into the ER, and increase extracellular Ca^{2+}-influx by activating ORAI1. In addition, they also activate the G protein-coupled bile acid receptor (Gpbar) on the apical cell membrane in the acinar cell, which also contributes to the sustained Ca^{2+} elevation. In ductal cells, it leads to a marked decrease in HCO_3^- production. In both cell types, ATP production is reduced, but this occurs in a partially Ca^{2+} level independent manner. The high Ca^{2+} level damages mitochondria and also causes activation of calcineurin, a serine-threonine phosphatase, that also contributes to the systemic inflammatory response through the activation of nuclear factor of activated T-cells (NFAT) and NF-κB (see Figure 4) (29).

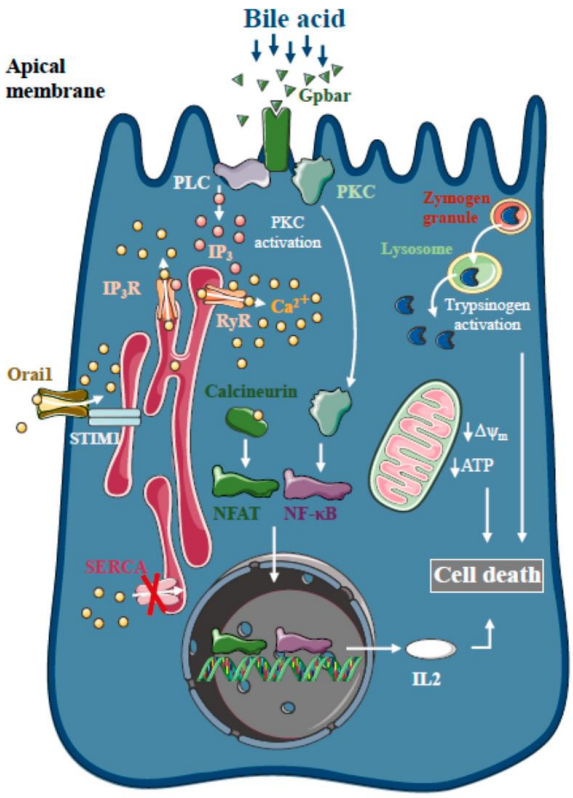

Figure 4: Intracellular mechanisms in bile acid-induced acute pancreatitis

Bile acids on one hand activate IP_3R and RyR, which release Ca^{2+} from intracellular stores. This causes further Ca^{2+} influx through activation of ORAI1. On the other hand, they can also release Ca^{2+} through activation of Gpbar. Mitochondrial function is impaired, ATP production is reduced, therefore SERCA is inhibited, which also impairs Ca^{2+} reuptake into the ER. Furthermore, bile acids activate calcineurin in a Ca^{2+}-dependent pathway, leading to premature enzyme activation and NF-κB activation.

Abbreviations: Ca^{2+}=calcium, Gpbar=G-protein-coupled cell surface bile acid receptor, IP_3R=inositol triphosphate receptor, NF-κB=nuclear factor-κB, ORAI1=Ca^{2+}-release activated Ca^{2+}-channel protein 1, RyR=ryanodine receptor,

(After Pallagi P, Madácsy T, Varga Á, Maléth J. Intracellular Ca^{2+} signalling in the pathogenesis of acute pancreatitis: recent advances and translational perspectives. Int J Mol Sci. 2020; 21: 4005. (29))

B. Alcohol induced pancreatitis

Alcohol is the second most common etiological factor, but as AP develops in only a small percentage of people who drink significant amounts of alcohol, other factors (e.g. genetic predisposition) are also thought to play a role. Acinar and ductal cells are damaged by ethanol and its non-oxidative metabolites. In the pancreas, non-oxidative degradation is the main metabolic pathway of alcohol, during which FAEE-synthase enzymes produce FAEE from the conjugation of ethanol and fatty acids. These accumulate in the mitochondria, causing its dysfunction, consequent ATP depletion, elevation of intracellular Ca^{2+} levels, and ultimately

cell death. They also activate IP_3R, which also contributes to the rise in intracellular Ca^{2+} levels. In ductal cells, CFTR expression and function are impaired, and so are water and HCO_3^- secretion. CFTR expression is impaired due to increased plasma membrane turnover and impaired membrane stability of CFTR. In AP, alcohol and its metabolites, FAEEs, increase protein production (trypsinogen, chymotrypsinogen, lipase, cathepsin B) and may induce ER stress and impair autophagy (see Figure 5) (29).

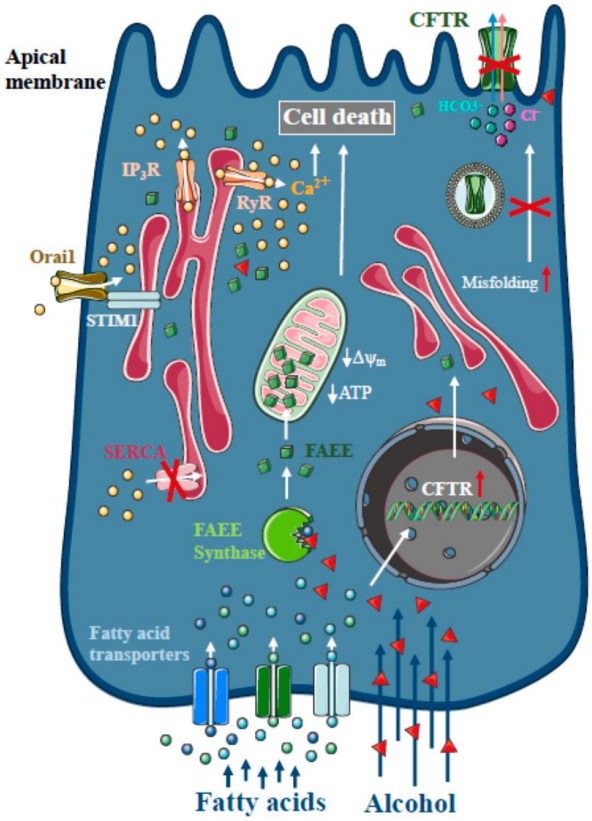

Figure 5: Molecular mechanisms in alcohol induced pancreatitis

FAEE-synthase produces FAEE from alcohol and fatty acids, which accumulates in the mitochondria. FAEEs, like bile acids, lead to cell necrosis by increasing Ca^{2+} levels and decreasing ATP levels. Moreover, in ductal epithelial cells, alcohol and fatty acids inhibit water and $HCO3^-$ secretion through impaired CFTR expression and function.

Abbreviations: CFTR=cystic fibrosis transmembrane regulator, FAEE=fatty acid ethyl ester,

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C. Intraductal pressure increase induced pancreatitis

It was assumed even decades ago, that an increase in intraductal pressure of the Wirsung's duct would lead to AP. A typical example is PEP or acute biliary pancreatitis (ABP)

induced by a stone impacted in the papilla. Using an American opossum model, it was demonstrated that ligation of the pancreatic duct without the presence of bile acids can induce necrotizing AP. Human studies have also demonstrated that transient obstruction of the pancreatic duct can lead to AP. Abnormal intracellular Ca^{2+} levels are also a central feature in these cases. The increase in intraductal pressure activates the so-called PIEZO1. This is a mechanoreceptor with cationic channel properties that, although non-selective, prefers Ca^{2+}. It seems that it can produce Ca^{2+} elevations that lead to premature enzyme activation and mitochondrial failure not by itself, but in cooperation with another Ca^{2+} channel. This other channel is the transient receptor potential vanilloid 4 (TRPV4), which is activated by PIEZO1 via phospholipase A_2 and has a significantly higher ion permeability than PIEZO1, but is inactivated more slowly. It can also activate calcineurin, a potent activator of T cells via NFAT, thus contributing to the systemic inflammatory response.

Elevated intraductal pressure inhibits ductal cell secretion by activating 5-HT_3 receptors through a serotonin-mediated pathway, leading to acidification. Intraductal acidification activates transient receptor potential vanilloid 1 (TRPV1) and leads to pancreatitis.

The increased pressure can cause loosening of the tight junctions between acinar cells, thus the cells move away from each other, and enzymes released from the disintegrating cells can enter the intercellular spaces, exacerbating the self-digestion.

PIEZO1, calcineurin and TRPV1 mediated mechanisms are highly characteristic of both post-ERCP and biliary pancreatitis (29).

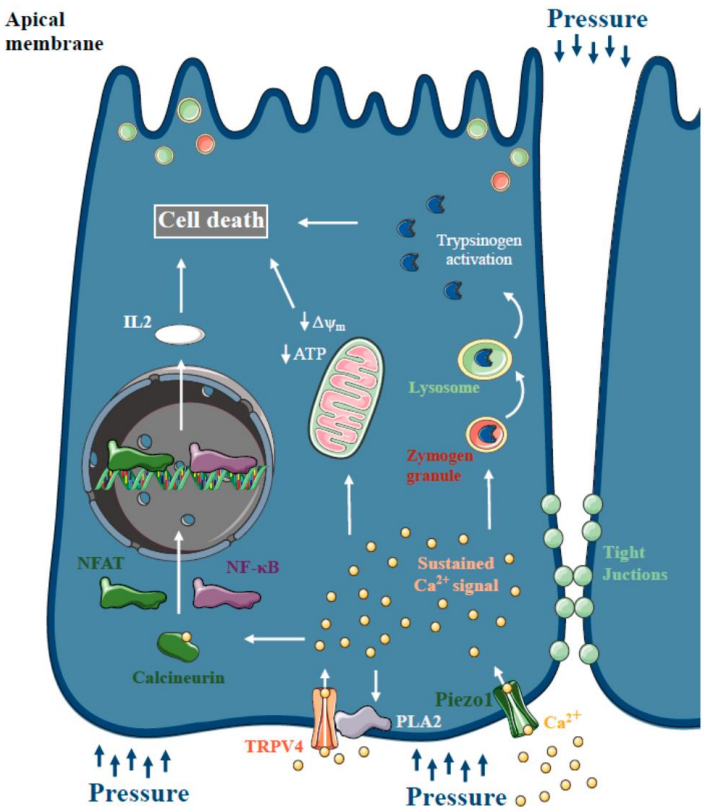

Figure 6: Molecular mechanisms in pressure-induced pancreatitis

The increase in intracellular Ca^{2+} levels and mitochondrial damage is similar to that in pancreatitis of other origin. However, the process is initiated by a recently discovered mechanoreceptor, PIEZO1, which is activated by an increase in intraductal pressure. PIEZO1 activates TRPV4 via PLA_2, which is an important step in sustained Ca^{2+} influx. High pressure can also directly damage tight junction structures between cells.

Abbreviations: PLA_2 = phospholipase A_2, TRP4 = transient receptor potential vanilloid 4

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5. CLINICAL ASPECTS OF POST-ERCP AND ACUTE BILIARY PANCREATITIS

5.1. Acute pancreatitis following ERCP (post-ERCP pancreatitis (PEP))

ERCP is one of the well known etiological factors of AP, and PEP is one of the most frequent and potentially most severe complication of ERCP. Its incidence is 9.7%, and mortality rate is 0.7% in general, but in high-risk patients these indicators are 14.7% and 0.2% respectively (47).

The mechanism of its evolution is not fully understood, but several factors are thought to play a role in its pathogenesis. Mechanical stress on the Vater papilla and sometimes on the pancreatic duct (PD) during the cannulation maneuver, chemical and hydrostatic effects during contrast media filling, thermal effects due to papillotomy (EST), enzymatic and even

microbiological insults are also suspected. In animal studies, it has been demonstrated that cannulation of the Vater papilla itself or filling of the PD with physiological saline can induce PEP, furthermore, injection of physiological saline or contrast media into the PD causes more severe histological damage than papillary cannulation alone (48). All these data suggest that an important element of the presumably complex pathomechanism is the increase in intraductal pressure in the PD.

5.1.1. Definition and severity classification of PEP

Diagnosis of PEP is defined by the Cotton consensus criteria as a new or worsened abdominal pain, with serum amylase or lipase levels at least three times the upper limit of normal at more than 24 hours after the ERCP, requiring hospital admission or a prolongation of a planned admission (49). These criteria classify PEP into 3 subgroups. Mild forms require hospitalization for 2-3 days, while PEP is moderate, when hospitalization lasts for 4-10 days, and severe when the patient is hospitalized for >10 days or develops complications (hemorrhagic pancreatitis, phlegmone or pseudocyst), or requires intervention or surgery. Although the 2020 European Society of Gastrointestinal Endoscopy (ESGE) guideline suggests using the modified Atlanta criteria for classifying PEP severity, we used the Cotton criteria in our scientific work as it was the most commonly used and accepted classification system over the past 30 years (50, 7).

5.1.2. Risk factors of PEP

As PEP has always been of great interest, a huge body of knowledge on the risk factors and preventive measures has accumulated. ESGE issued a specific, regularly updated recommendation about it (51, 52, 50). We summarized the PEP risk factors based on the 2010 ESGE guideline, as this was the actual recommendation in use during the time of our data collection (Table 2) (51). The patient is at high-risk of developing PEP if there is at least 1 definite or 2 likely risk factors present.

Table 2: Risk factors of post-ERCP pancreatitis (51)

Risk factors	Odds Ratio (OR)
Patient-related risk factors	
Definite risk factors	
- suspected sphincter of Oddi dyskinesis	4.09
- female gender	2.23
- previous pancreatitis	2.46
Likely risk factors	
- younger age	1.09-2.87
- non-dilated extrahepatic bile ducts	NA
- abscence of chronic pancreatitis	1.87
- normal serum bilirubin	1.89
Procedure-related risk factors	
Definite risk factors	
- precut sphincterotomy	2.71
- pancreatic injection	2.2
Likely risk factors	
- high number (>5) of cannulation attempts	2.40 - 3.41
- pancreatic sphincterotomy	3.07
- balloon dilation of biliary sphincter	4.51
- failure to clear bile duct stones	3.35

NA=no data, OR=Odds ratio

5.1.3. Prophylactic methods of PEP

Prophylactic measures range from the correct choice of adequate ERCP indication, through using low risk cannulation techniques, to active prophylaxis.

Examination based on the appropriate indication is essential to prevent complications (53). Patients who are at the highest risk of developing PEP are those who require ERCP the least (54). The American Society of Gastrointestinal Endoscopy (ASGE) also highlights the importance of appropriate patient selection in its recommendations (55).

When cannulating the Vater papilla, the guidewire technique is recommended over the contrast-assisted method because it can reduce the risk of developing PEP (56). In a summary publication of data from 4426 patients in 15 randomized controlled trials (RCTs), the Odds ratio (OR) for developing PEP was 0.51 for the guidewire technique.

However, if you have difficulties entering the common bile duct (CBD) and your cannulation attempts become complicated and prolonged, we refer it to difficult cannulation (>5 contacts with the papilla, or >5 minutes spent on cannulation attempts, or >1 Wirsung's duct cannulation and/or filling) (57). Biliary cannulation with conventional techniques is unsuccessful in 20% of cases, even in experienced hands (58). In such cases, so-called advanced approaches should be used, which can increase the success rate to 90-95%. However, as prolonged cannulation attempts increase the risk of PEP, alternative methods should be used instead of continuous attempts (59).

When we cannot access either of the ducts, or can only insert our instruments into the common segment of the sphincter of Oddi, an early precut, also known as a precut papillotomy, performed by a skilled endoscopist, can help. In this case, the OR is 0.47 for the development of PEP based on a meta-analysis of data from 966 patients in 6 RCTs (60). However, when we introduce our guidewire into the Wirsung's duct (PD) instead of the CBD, we have several options. One is to insert a preventive pancreatic stent (PPS) into the PD in order to stabilize the papilla and such to try introducing the guidewire into the bile duct, or to perform a precut papillotomy alongside the PPS and cannulate this way. Another option is to leave the wire in the PD (and stabilize the papilla this way) and try to get into the CBD through the reintroduced papillotome with another guide wire. This is the so-called double-wire technique (61). Based on a meta-analysis of available data (7 RCTs, 577 patients), it does not have a higher cannulation rate (RR=risk ratio 1.04) compared to other techniques, but it increases the risk of PEP (RR 1.98) (62). Therefore, the ESGE recommends the use of PPS implantation when using a double-wire technique (63). A third option is the so-called transpancreatic sphincterotomy, where the EST is performed from the PD in the direction of the CBD, by transecting the septum between the two ducts within the Vater papilla (64). In a recently published meta-analysis, the success rate of CBD cannulation was also higher compared to needle-knife papillotomy (OR 2.32) and the double-wire technique (OR 2.72) (65). The incidence of complications was also examined. The risk of developing PEP was not significantly higher compared to the double-wire technique or needle-knife papillotomy, but subgroup analysis of the latter method showed that the risk of PEP was higher when it was compared to needle-knife fistulotomy (cutting at the top of the papilla and not touching the orifice) (OR 4.62).

In active prophylaxis, the most important options are rectal non-steroidal anti-inflammatory drug (NASID) suppositories and PPS, although more recently high-volume hydration and sublingual nitrate have been incorporated into the recommendations (50). In the lack of contraindications, all patients should receive a 100 mg indomethacin or diclofenac

NSAID suppository half an hour before or immediately after ERCP (the most recent recommendation suggests to use it before the examination) (14). It reduces the risk of PEP to 60% compared with placebo (OR 0.60) and the number needed-to-treat (NNT) is 20, based on a meta-analysis of data from 4741 patients in 17 RCTs (66). The ESGE recommendation gave an OR of 0.24-0.63, a NNT of 8-21, and a NNT of 33-39 for moderate to severe PEP (50). The use of NSAID suppositories is contraindicated in patients with hypersensitivity to the drug, pregnancy more than 30 weeks, Stevens-Johnson or Lyell syndrome in the patient's history or in a first-degree relative, and increased caution is recommended in chronic renal failure. In patients in whom NSAID suppositories cannot be used, high-volume hydration is indicated, primarily by infusion of Ringer's lactate (3 ml/kg/hour during ERCP, followed by 20 ml/kg bolus, then 3 ml/kg/hour for 8 hours, a total of 35-45 ml/kg over 8-10 hours). It has been shown to be more effective than standard volume hydration (1.5 ml/kg/hour during ERCP and then for another 1.5-8 hours) in studies with a meta-analysis showing an OR of 0.47 (7 RCTs, 1047 patients) (67). In elderly, cardiac or renal disease patients, increased attention is required due to the risk of overload. If both of the two prophylaxis just mentioned are contraindicated, 5 mg sublingual nitrate may be given. It was not included in the older recommendations, but it does in the most recent one, as a meta-analysis of 12 RCTs with data from 2649 patients demonstrated an OR of 0.67 compared with placebo (68). It should be noted that it does not prevent moderate to severe PEP. However, in high-risk patients, PPS can reduce the risk of pancreatitis and is therefore recommended. Compared with placebo, it significantly reduces the risk of both mild-to-moderate and severe PEP (RR 0.45 and 0.26, respectively) and this effect applies to both the high-risk and unselected patient groups (RR 0.41 and 0.23, respectively), the NNT being 8 (69). In the most recent ESGE recommendation, the OR was 0.22-0.39, the NNT 7-8, and for severe PEP the OR was 0.22-0.26 (50). Relatively little data is available on the combination of these methods. A multicenter RCT in 886 patients demonstrated the efficacy (RR 0.59) of the prophylaxis with the combination of NSAID suppository and sublingual nitrate compared to prevention with a standard NSAID suppository (70). NSAID suppository and hydration in combination was also more effective than any single methods in a network meta-analysis of 7559 patients from 24 RCTs (71).

The data clearly show the efficacy of prevention, but it is not always used in clinical practice, only in approximately 1/3 of cases (72). This certainly underlines the importance of further education of gastrointestinal endoscopists.

5.2. Acute biliary pancreatitis (ABP)

The evidence of gallstones as etiological factors in AP was first described by Opie in 1901 (73). He found a gallstone impacted in the Vater papilla in a patient dying from acute hemorrhagic pancreatitis. This finding led him to the “common channel hypothesis”. The essence of this is that a gallstone when impacted in the Vater papilla occludes both the CBD and the PD, which then causes reflux of the bile into the pancreas leading to early, intraductal activation of the pancreatic enzymes and AP.

It has been proven since decades that gallstone impaction is mostly temporary (74). In experimental models it was demonstrated that simultaneous ligation of both the CBD and PD or ligation of the PD alone can cause necrotizing pancreatitis similar to each other (75). It is generally accepted, that PD obstruction, increased intrapancreatic duct pressure, PD disruption and acinar hyperstimulation all play an important role in the pathogenesis (76). On the other hand, individual vulnerability of the pancreas, individual difference of inflammatory responses, and edema or prolonged spasm of the pancreatic segment of the sphincter of Oddi can explain why ABP develops in some patients when a gallstone passes through the papilla, however, only a transient biliary colic and/or elevation of the liver function tests can be observed in others (77).

5.2.1. History, role and results of ERCP in ABP

Introduction of ERCP into clinical practice, the development and improvement of its therapeutical methods opened up new horizons in minimal invasive treatment of pancreatobiliary diseases (78-84). The first ERCP procedures and Vater papilla cannulations were performed in the late 1960s, followed by the first papillotomies published simultaneously in 1974 by a Japanese and a German working group, and the first biliary stenting procedures in the late 1970s (78, 79, 80, 81).

The first period of ERCP usage in ABP ranges from the early 1980s to the early 2000s. The treatment strategy used until then, namely early surgical solution of biliary obstruction causing acute pancreatitis, was associated with disappointing results and high mortality (85). However, in the setting of a persistent CBD stone, even if the initial ABP regresses, more than half of patients have recurrent pancreatitis attacks until gallbladder removal (86). On the other hand, animal and human data have also supported the finding that the longer the biliary obstruction persists, the more likely is to have a severe AP course (87, 88). In cases of obstruction persisting beyond 48 hours, severe necrotizing course has been detected in nearly 85% of patients (88). So the less invasive endoscopic solution has been of great interest.

Sáfrány and his colleagues published their results as early as 1980 (89). Fifteen patients with known gallstones, who developed ABP underwent ERCP, EST, and biliary stone extraction. The serum amylase values of all patients normalized within 48 hours. Since the end of the 1980s, randomized trials have also appeared. The first randomized trials demonstrated the superiority of early ERCP (completed within 72 hours from the onset of pain) over conservative treatment in predicted severe ABP in reducing complications and hospital stay (90-92). In the English RCT, patients with predicted severe ABP had fewer complications with ERCP performed within 72 hours compared to conservative treatment (24% vs. 61%, $p<0.01$). Severity was determined using the modified Glasgow criteria. The difference was detectable even if the data of acute cholangitis patients were not included in the analysis (15% vs. 60%, $p=0.003$) (90). The Hong Kong working group published similar results a few years later (91). In their study, only 2/3 of the patients had AP of biliary origin, but for them, ERCP performed within 24 hours after admission resulted in fewer complications compared to conservative treatment (13% vs. 54%, $p=0.002$). Of note, half of the patients in the conservative group ultimately required ERCP. This study has been criticized on several reasons. On one hand, severity was predicted based on serum urea and blood glucose levels, which is a controversial predicting method, and on the other hand, patients with acute cholangitis were not excluded. Although the results of the Polish working group also showed the advantage of endoscopic intervention (complication: 16% vs. 36%, mortality: 2% vs. 12%), this was only published in abstract form (92).

A German study investigated whether ERCP is still beneficial if there is no persistent biliary obstruction, therefore patients with jaundice (sebi >85 μmol/l) were excluded (93). Twenty-two centers participated in this study, 238 patients were included over 5 years and the ERCP was performed within 72 hours. The endoscopic intervention did not result in fewer complications (46% vs. 51%), in fact, the rate of respiratory failure was higher in the intervention group, so the study was stopped during the interim analysis, earlier than originally planned. The study was later criticized by many, as the appropriate endoscopic practice necessary for the treatment of ABP patients was questioned, because only 1 patient/center was enrolled in many centers.

An Argentinian working group investigated whether ERCP performed within 72 hours or conservative treatment results in a better outcome in cases of AP without acute cholangitis but with biliary obstruction (94). In terms of complications, there was no difference between the two groups (6% for both). During the ERCP, choledocholithiasis was found in 72% of the cases, but even in the conservative treatment group the rate of gallstones was still 40% later, at the time of cholecystectomy.

Seeing these RCTs that resulted in different conclusions, the demand to summarize the results and to perform meta-analyses arose around the millennium. Based on the summary and analysis of the results of 834 patients from the first 4 RCTs, an American working group concluded that early ERCP, EST results in a better outcome compared to conservative treatment (complications: 25% vs. 38%, $p<0.001$; mortality: 5% vs. 9%, $p<0.05$) (95). The NNT was 7.6 for complications and 25.6 for mortality. Later, a meta-analysis by Petrov et al (3 RCTs - Neoptolemos, Fölsch, Oria, 450 patients) showed that in cases of ABP without associated acute cholangitis, regardless of AP severity, ERCP does not result in a better outcome (96). Regarding complications, the OR was 0.76 in favor of endoscopic intervention, but it was not significant.

Later on, the Dutch Pancreatitis Study Group (DPSG) conducted intensive research on the subject. Their patients, included in a multicenter probiotic RCT, were also analyzed in a separate publication, based on their endoscopic interventions (97). The severe ABP patient group (Apache-II ≥8, or Imrie-Glasgow score ≥3, or CRP >150 mg/l) was classified into subgroups based on the presence or absence of cholestasis. In those with cholestasis but no cholangitis (CBD >8 mm, sebi >40 μmol/l, body temperature <38.5 ^{O}C), early ERCP had a better outcome compared to conservative treatment (complications: 25% vs. 52 %, $p=0.02$; mortality: 6% vs. 15%, $p=0.21$), but the outcome was similar in patients without cholestasis (complication: 45% vs. 41%, $p=0.81$). The DPSG recently published the results of the multicenter RCT, the APEC study, where in a similar patient population (severe ABP without cholangitis but with cholestasis), unlike the previous study, no difference was found between early ERCP and systematic EST (performed within 24 hours after admission) and conservative treatment in terms of major complications and mortality (98).

Based on the recommendations prepared by international consensus in the early 2010s which is still valid, ERCP is “clearly indicated” in ABP if co-existing acute cholangitis is present and “probably indicated” if common bile duct obstruction is present. This recommendation is accepted by the joint guidelines of the International and American Pancreas Associations (IAP/APA), as well as the consensus recommendation of the Hungarian Pancreatic Working Group (HPSG) (99, 100).

Despite all, contradictory publications still appear. According to a recent meta-analysis, early ERCP in ABP without cholangitis does not reduce either the complication rate or mortality compared to conservative treatment (101). Almost at the same time, an American study was published which, based on the analysis of the data of more than 150,000 ABP patients without cholangitis, confirmed a significant reduction of mortality by 43% in the ERCP group (102).

The debate of ERCP in ABP can be explained by several factors, for example the lack of uniform definitions and criteria (eg. definition of biliary origin), or the early studies focused only on the predicted severity and not on CBD obstruction, or the timing of ERCP is not uniform, or complications of ERCP and their prevention methods were less known before, moreover, endoscopic intervention concentrates only on solving biliary obstruction, but does not affect PD obstruction and the increased intrapancreatic duct pressure.

6. RELEVANT RESULTS PRECEDING THE PRESENT RESEARCH TOPIC

6.1. Results described in PEP patients

We have previously shown that the literature recommends the usage of PPSs in high-risk patients for PEP prophylaxis. In Hungary, Madácsy et al reported a new way of using PPSs in patients at high-risk of PEP (103). Sphincter of Oddi dyskinesis (SOD) patients, in whom biliary cannulation was difficult and the guidewire entered the PD several times, PPS implantation was performed as a first step, instead of further forcing the standard cannulation methods, and then a needle-knife precut fistulotomy was done followed by the completion of the biliary therapy. The data of 22 patients treated this way were compared to the data of 35 SOD patients treated with the standard cannulation methods, and it was shown that there was no PEP in the PPS group (0 vs 43%, $p<0.001$), while in the standard group in addition of 3 mild PEP, there were 10 moderate and 2 severe PEP cases.

They published another pioneer concept with PPS usage (104). Out of 1225 average PEP risk patients who underwent ERCP in whom PPS implantation was originally not applied, severe PEP was anticipated in 6 cases based on the laboratory markers taken 6-8 hours post-procedure and the severe abdominal pain. During a second ERCP session a PPS was inserted into the PD of them 8-20 hours after the first procedure. Pancreatic pain was promptly reduced and no AP complications occurred, all PEP remained mild. The method was named “rescue ERCP”. A few years later, American authors also described that this method is also beneficial in cases where a PPS is implanted during the original ERCP, but early dislodgment or blockage of PPS occured (105). PPS applied in the early phase of the pancreatitis induced by ERCP in these patients, and by reducing the intraductal pressure in the PD, the further progression of the inflammation process could be prevented.

Even that time, there was a demand to learn more about the complications associated with the usage of PPS, the predisposing factors for these, and the differences between specific stent types, and possibly the most optimal stent (106).

6.2. Lowering the intrapancreatic duct pressure in ABP

The results obtained in the basic research show that although PEP and ABP are diseases with complex pathomechanisms, the increase in intraductal pressure and the mechanisms mediated by PIEZO1, calcineurin and TRPV1 play an important role in both. We have seen that the application of PPS is very effective in the prevention of PEP, and in the case of early, incipient PEP it was also able to prevent further progression, so based on the pathogenetic steps showing many similarities, the idea may arise that PPS application can also be effective in ABP.

This was first reported by Hungarian authors in their case series (107). The data of a total of 87 ABP patients were analyzed. ERCP, EST and biliary stone extraction were performed in 60 cases, but in 27 patients PPS implantation was only performed as a "bridging solution", because biliary cannulation was technically unsuccessful or contraindicated. In the latter cases, the biliary obstruction was resolved during a second session. In the PPS group, there were significantly fewer complications (PPS vs. EST = 7.4 vs. 25%, p=0.021), and mortality was also more favorable (PPS vs. EST = 0 vs. 6.7%, not significant).

6.3. Previous knowledge related to stent types

PPS is inserted into an intact Wirsung’s duct in cases of PEP prophylaxis and of ABP. In these cases, the so-called small diameter, 3-5 Fr stents are used. Based on a meta-analysis of available data in the literature (6 RCTs, 561 patients), it can be established, that the 5 Fr stent is better than the 3 Fr, as its insertion is technically easier and more successful (108). Different practice can be encountered regarding the length of the PPS. A Japanese working group conducted an RCT comparing 3 and 5 cm long PPSs (109). In their study, 200 patients were examined. The incidence of PEP was significantly less frequent using the short stent (3 cm vs. 5 cm = 2% vs. 8.8%, p=0.035). Regarding the duration of stenting, only expert opinion is available (50, 110). Based on this, it can be said that they should be in place for a minimum of 12-24 hours, but a maximum of 5-10 days. An American working group investigated needle-knife precut papillotomy in their RCT (111). A total of 151 patients were included, of whom PPS was not used in 58 patients, in 47 it was inserted only for the duration of the precut for guidance of cutting, but it was removed after EST, and finally in 46 patients the PPS remained in place at least until the next day. The incidence of PEP was highest (21.3%) in those who only had PPS for the duration of the precut, and the difference was significant compared to the unstented (13.8%) and stented (4.3%) groups. Furthermore, the rate of moderate and severe PEP was significantly higher in this group compared to the stented group (12.8% vs. 0%, p=0.026).

Different stent designs are available among the small diameter, short stents, such as straight stents, with an external flange on the duodenal end, that can prevent migration into the PD, but on the inner end these stents either have a flange or not. The inner flange prevents early, premature dislodgement. Stents without an inner flange is frequently used, as the PPS dislodges spontaneously and thus removal does not require another endoscopic procedure. In the case of certain stents, the outer flange is also omitted, the inner migration is prevented by the pigtail-like design of the outer end (this is the so-called Freeman type stent).

7. NULLHYPOTHESIS

It is well known, that the use of PPS significantly reduces the risk of PEP, but some patients still develop this complication, so we investigated how PPS affects some of the already known risk factors.

It is also known, that small-diameter, short PPSs without an inner flange are the most often used stents for PEP prophylaxis, however, there is no uniform recommendation regarding the stent types, so we examined stents of different designs in terms of efficacy and complications.

It is a well-known fact, that NSAID suppositories and PPSs are the most commonly used methods for active PEP prophylaxis, but we do not know if there is a difference between them in the prevention of moderate-severe PEP which is associated with severe health consequences, so we also examined this.

It is well known, that there are several common steps in the pathomechanisms of PEP and ABP, with an increase in intraductal pressure of the PD that activates PIEZO1, causing an increase in intracellular Ca^{2+} levels and early enzyme activation in addition to mitochondrial insufficiency. Considering that PPS promptly abolishes the elevated intraductal pressure, we investigated whether PPSs are effective not only for PEP but also for ABP.

8. AIMS

8.1. Aims in PEP-prevention

To analyze the data of our prospectively collected database of high-risk patients who underwent PPS implantation to prevent PEP, 1) firstly, targeting pancreatitis that develops despite PPS usage, 2) secondly, analysing stent types and stent-related complications, 3) thirdly, comparing the preventive effects of PPSs and NSAID suppositories in moderate-to-severe PEP.

8.2. Aims in ABP

To examine the PPS usage in ABP, 1) firstly, in a non-randomized study, where ERCP and biliary cannulation are difficult and procedure-related PEP risk factors are present, 2) secondly, later in a randomized fashion with developing a clinical study in this topic.

9. METHODS

9.1. Our research in PEP-prevention

9.1.1. PEP occurring despite using PPS, complications related to stents, and different stents types

A prospective database was initiated at the end of 2009 of high-risk patients (≥3 risk factors present) with native papillas without previous EST treated with PPS implantation for PEP prophylaxis. The predefined collected parameters were: patient identification number; demographic data (sex, date of birth, date of ERCP, age at time of ERCP); each risk factor based on the 2010 ESGE guideline (present or absent); cumulative, patient and procedure related risk factors (arithmetic sum of the above parameters); PPS type used (length, diameter, type); attempted, but unsuccessful PPS placement; date of PPS removal; PEP (present or absent, and severity); post-procedure abdominal pain and serum amylase levels (6-8 hours and 16-24 hours after ERCP) and any other complications that might have occurred (112, 113). PEP was defined and categorized according to the Cotton consensus criteria (49).

The database contains data from patients collected between 2009 and 2014 in high volume endoscopy units of two large university teaching hospitals in Hungary. All ERCPs were performed by four experienced endoscopists (>200 ERCPs annually and ≥10 years ERCP experience). Indications of the ERCPs were obstructive jaundice, CBD stones on imaging studies, SOD type 1, acute cholangitis, known or suspected benign or malignant biliary stricture. All patients with acute biliary pancreatitis were excluded. For PPS placement small caliber short (5 Fr, 3–5cm) stents were used. The choice of stents (flanged/unflanged/Freeman-type) was at the discretion of the endoscopist. All stents were removed endoscopically in less than a week with polypectomy snare or foreign body forceps, unless dislodged spontaneously and the patient attended their follow up procedure. All patients were observed in hospital for at least overnight according to our national routine practice. They received similar treatment of nil by mouth for 8-12 hours and intravenous fluids (2000–3000 ml lactated Ringer′s solution). NSAID suppositories were not used. Analgetics and spasmolytics were provided when required. Serum hemoglobin and amylase levels were tested at 6-8 hours after ERCP (the same evening) and the following morning (16–24 h post-procedure). Symptom free patients were discharged next morning, while PEP patients only after AP resolved and all

complicated cases were followed up in 3 months. PEP was treated according to actual guidelines.

All stent related complications were registered, specifically paying attention to those described in the literature (early dislodgement, proximal migration, PD perforation, ductal and parenchymal changes, stent fragmentation in PD, AP induced by PPS removal). Data of the stented patients were compared to a historical cohort of similarly high-risk patients from 2000-2004, where PPSs were not used, as it was not part of the ERCP practice then.

Usual statistical methods (mean, standard deviation, Mann–Whitney U-test, independent samples t-test, Chi-square test, Fischer's exact test), data mining methods and random forest analysis were used. A $p<0,05$ considered as significant. Data mining and random forest analysis were used to examine which predictor had a greater impact on PEP. The analyses were carried out by an independent statistician with SPSS 19.0.0 (SPSS Inc., Chicago, IL) software.

9.1.2. Comparing PPSs and NSAID suppositories

In clinical practice, severe PEP can have a significant negative impact on the patient's health on the long term, therefore its prevention or shifting to a milder form is important. We compared the two most commonly used prophylactic methods in the form of a network meta-analysis (114). Two reviewers independently searched the well known databases (PubMed, EMBASE, Cochrane Central Library) from initiation of the methods through 2nd January 2021. Reference lists of relevant studies, guidelines and meta-analyses were additionally searched for any other potential RCT. Only placebo controlled, rectal NSAID and PPS studies performed on adult population (age >18 years) for prevention of PEP, published in full text were included. Placebo was the common comparator. The outcome measure was moderate to severe PEP defined according to the Cotton consensus criteria. This classification was intentionally chosen, because this was the most widely used, accepted, consensus based classification system over the past three decades, designed specifically for post-ERCP complications. The predefined protocol was registered in PROSPERO (CRD42020183641). The analysis was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement and the PRISMA Extension Statement for Reporting of Systematic Reviews Incorporating Network Meta-analyses of Health Care Interventions (115, 116). Search terms included: "NSAID", "non-steroidal anti-inflammatory drug", "indomethacin", "diclofenac", "prophylactic pancreatic stent", "preventive pancreatic stent", "PPS" and "post-ERCP pancreatitis". Any differences between the two reviewers were

resolved by mutual agreement and in consultation with a third reviewer. RCTs with imcomplete or missing data were excluded, unpublished data were not requested from authors.

The following data were abstracted from eligible RCTs into a predefined database: first author, year of publication, publishing journal, country of origin, single or multi-center nature, study population (including sample size, percentage of female gender, mean age in certain study population), low- or high-risk patients, PEP definition, PEP severity criteria, sample size, the incidence of PEP (all, and in certain severity groups). For NSAID studies, we registered the type, dose, route and timing of the used medication; and for PPS studies the diameter, length, type of PPS used and the time of extraction.

The patient groups were classified as high-, low-, average-, or mixed-risk in the included studies. High-risk patients were analyzed separately, while low-, average- and mixed-risk patients were analyzed together classified as average-risk group.

Analysis was performed by a biostatistician with random effect model using Monte-Carlo methods, and interventions were ranked by their posterior probability via calculating the surface under cumulative ranking (SUCRA) curve values. Network estimates (pooled direct and indirect data) of each intervention compared to placebo and other interventions were presented in forest plots, summarized in a league table. All computations were performed using the R (V. 3.5.2) package gemtc (V. 0.8e2) along with the Markov Chain Monte Carlo engine JAGS (V. 3.4.0), package netmeta (V. 1.1e0), and STATA 16.0 (StataCorp LLC).

9.2. Our research in ABP

9.2.1. Non-randomized study

In 2009 a prospective, non-randomized trial was initiated in 2 large, Hungarian teaching hospitals (117). ABP patients in whom ERCP was indicated were enrolled.

Inclusion criteria were as follows: 1) abdominal pain consistent with pancreatitis, 2) serum amylase levels at least three times the upper normal limit, 3) objective demonstration of gallbladder stones and/or sludge, or dilated CBD, or CBD stone on abdominal USS, or a past history of cholecystectomy, 4) elevated liver function tests suggesting cholestasis (Goodman′s criteria: serum bilirubin, alanine-aminotransferase (ALAT) and alkaline phosphatase (ALP) levels more than two times the upper normal limit) and a predicted severe course of ABP (Glasgow score ≥3), 5) possibility of performing ERCP within 72 hours calculated from the onset of pain.

Exclusion criteria were: age <18 or >85 years, pregnancy, results suggestive for alcoholic pancreatitis, pain onset >72 hours before admission, past history of gastric resection or pancreatobiliary surgery (except cholecystectomy), liver cirrhosis Child-Pough score A–C,

INR >1.8, pancreatic cyst or abscess on initial abdominal USS or CT scan, failure to insert PD stent, failed or contraindicated biliary cannulation / EST / complete CBD clearance.

The severity of AP was assessed at admission within 48 hours using the Glasgow prognostic index (a score of ≥3 is regarded as severe pancreatitis) (118). All patients were hospitalized. All ERCP procedures was performed within 72 hours from the onset of pain. Indication for ERCP was ABP with concomitant clinical signs and laboratory and/or radiological signs of biliary obstruction and cholangitis. During the endoscopy procedure EST and CBD stone extraction was carried out for every patient, furthermore a PPS was inserted into the PD in a subgroup of patients. Indications for PD stenting were severe papillary edema due to impacted gallstone, repeated PD cannulation with a guidewire or contrast filling (>5), difficult biliary cannulation (>5 separate unsuccessful cannulation attempts during >10 minutes) or needle-knife precut papillotomy. All PPSs were removed during a gastroscopy within 10 days.

All patients received similar medical treatment irrespective of the study group, including aggressive fluid replacement, analgetics, spasmolytics and nasojejunal feeding when indicated. Antibiotic treatment was not started routinely for prophylaxis, only for therapeutic purposes. An abdominal contrast-enhanced CT scan was carried out on day 3–5 to detect any pancreatitis-related complications. Patients who responded very well and quickly were discharged at the end of the first week. All patients were scheduled then for an out-patient follow-up within 4 weeks and monthly thereafter for those with complications for a minimum of 3 months. A laparoscopic cholecystectomy was scheduled at 6 weeks after ABP.

At the final outcome-analysis the mortality rate within 90 days and the overall complication rate were exclusively analyzed, but the following complications were also addressed: intensive care unit (ICU) transfer due to multi-organ dysfunction syndrome (MODS), septic shock or infected pancreatic necrosis (phlegmon), or pancreatic abscess, surgical interventions (such as drainage or necrosectomy), large (diameter >6 cm) pancreatic pseudocyst formation.

Our study was carried out in accordance with the ethical standards of the Helsinki Declaration of 1975, as revised in 1983.

Statistical analysis was performed using the Mann–Whitney U test and the Fisher exact test with comparison of the individual groups.

9.2.2. Randomized study

Based on the results of our non-randomized study, organizing and conducting a multicenter, prospective, randomized, controlled, interventional trial was needed. HPSG was

also involved in the process. The leader of HPSG and those national interventional endoscopists, who are well known ERCP experts and familiar with PPS implantation were invited for planning this study. The basics of the study was discussed and accepted on 7th October 2013 and all involved specialists signed a letter of intent to participate.

The aim of the trial is to compare the standard endoscopic treatment (EST and CBD stone extraction) with PPS implantation added to standard treatment in ABP cases where ERCP is indicated, irrespective of the predicted severity. The protocol was accepted by all participants and then submitted to the Hungarian National Ethical Committee, who approved it on 13th October 2014 (ETT-TUKEB ref.: 030174/2014/OTIG). The trial protocol was registered at the International Standard Randomized Controlled Trial Number (ISRCTN) Register (trial ID: ISRCTN13517695), and published in an international peer-reviewed journal (41). This was the first randomized, controlled trial of HPSG. The trial summary, protocol, ethical approval, letter of intent to join, patient informed consent and other documents were uploaded to the HPSG website (https://tm-centre.org/hu/vizsgalatok/prepast-hu/, https://pancreas.hu/en/studies/prepast). An intent to join is evaluated and approved by the organizing and steering committee of this study and the decision is made upon endoscopic experties in PPS implantation. The case report form (CRF) and the online electronic version (eCRF) have been developed (http://opr2.pancreas.hu/opr/forms/PREPAST). All the investigators receive an individual access codes to the eCRF.

An independent statistical company prepared the sample size calculation and the randomization lists. Finally, the study was started in 2017 with 4 participating centers.

10. RESULTS

10.1. Our research in PEP-prevention

10.1.1. PEP despite prevention with PPS, complications related to PPS, and different stent types

PEP incidence in different treatment groups:

Our database includes data of 317 high-risk patients, because PPS implantation was attempted in these cases out of 2462 ERCPs (12.9%). There were 209 females (65.9%) and the mean age was 61.2 ± 16.5 years (112).

PPS placement was successful in 288 cases (90.9%, “successful stent” group), but it was unsuccessful in 29 cases (9.1%, “unsuccessful stent” group). Our retrospective cohort from previous years comprised 121 similarly high-rik patients without attempted PPS placement (“no stent” group). There were no significant differences among the groups in terms of PEP risk (Table 6) (113).

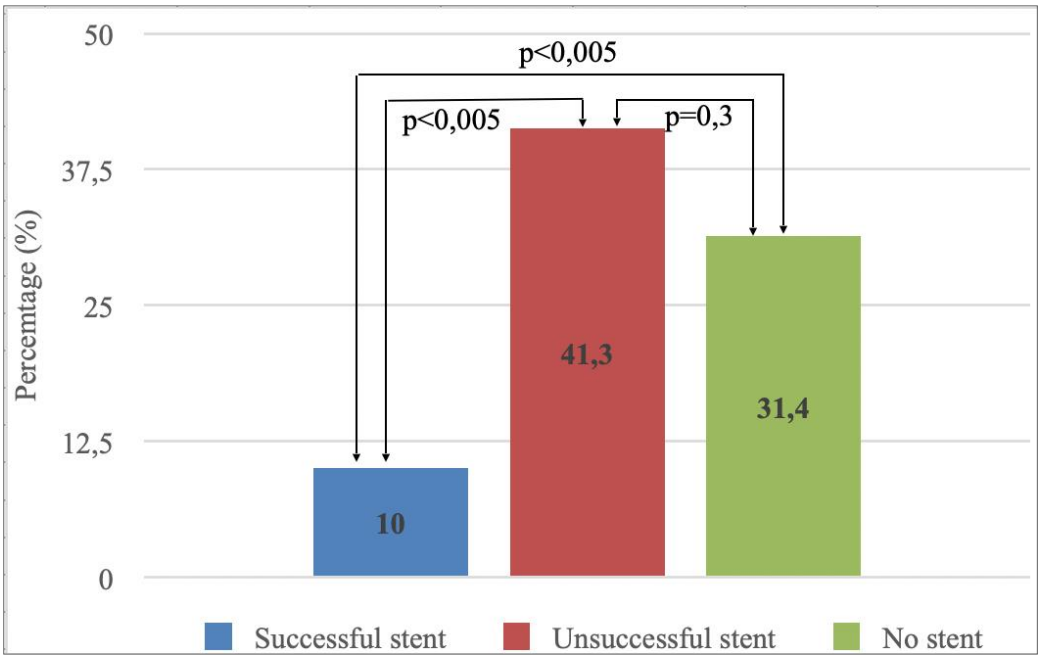
Table 6: PEP risks and the significance levels between groups (113)

	Successful stent	**Unsuccessful stent**	**No stent**
Age (years)	60.97 ± 16.51	64.21 ± 16.89	60.86 ± 11.73
Sum of all risk fators	4.86 ± 1.47	5.38 ± 1.14	5.13 ± 1.25
Sum of patient-related risk factors	3.22 ± 1.27	3.34 ± 1.23	3.45 ± 0.99
Sum of procedure-related risk factors	1.64 ± 0.97	2.03 ± 0.82	1.68 ± 1.11
Significance (*p*=)	**Successful vs. unsuccessful**	**Successful vs. no stent**	**Unsuccessful vs. no stent**
Age (years)	0.32	0.95	0.21
Sum of all risk fators	0.06	0.07	0.33
Sum of patient-related risk factors	0.61	0.07	0.61
Sum of procedure-related risk factors	0.03	0.72	0.11

Data represent the mean and standard deviation in each groups (mean ± SD)

PEP occured in 29 patients (10%) in the „successful stent", 12 patients (41.3%) in the „unsuccessful stent" and 38 patients (31.4%) in the „no stent" group. There were significantly less PEP in the „successful stent" group, then in the other two groups (p<0.005), while the difference was not significant between these latter two (p=0.3) (Figure 7).

Figure 7: Incidence of post-ERCP-pancreatitis in the study groups (113)



Concerning PEP severity, all incidences in the subgroups of different severity forms of PEP were lower in the "successful stent" group, than in the other two, because the overall incidence was lower also (Figure 8, Table 7).

Figure 8: Incidence of different severity forms of PEP (113)

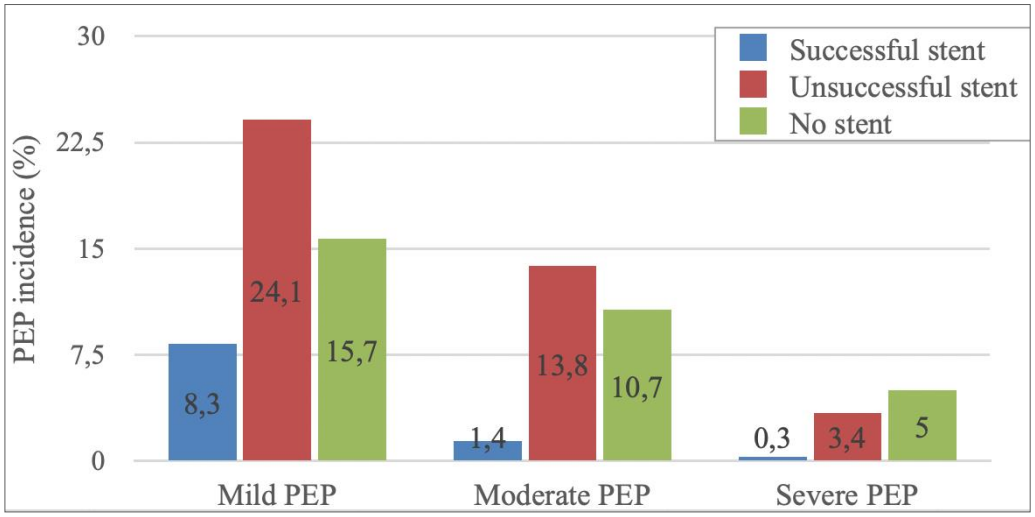


Table 7: Absolute numbers and distribution of each PEP severity in the study groups and the significance levels (113)

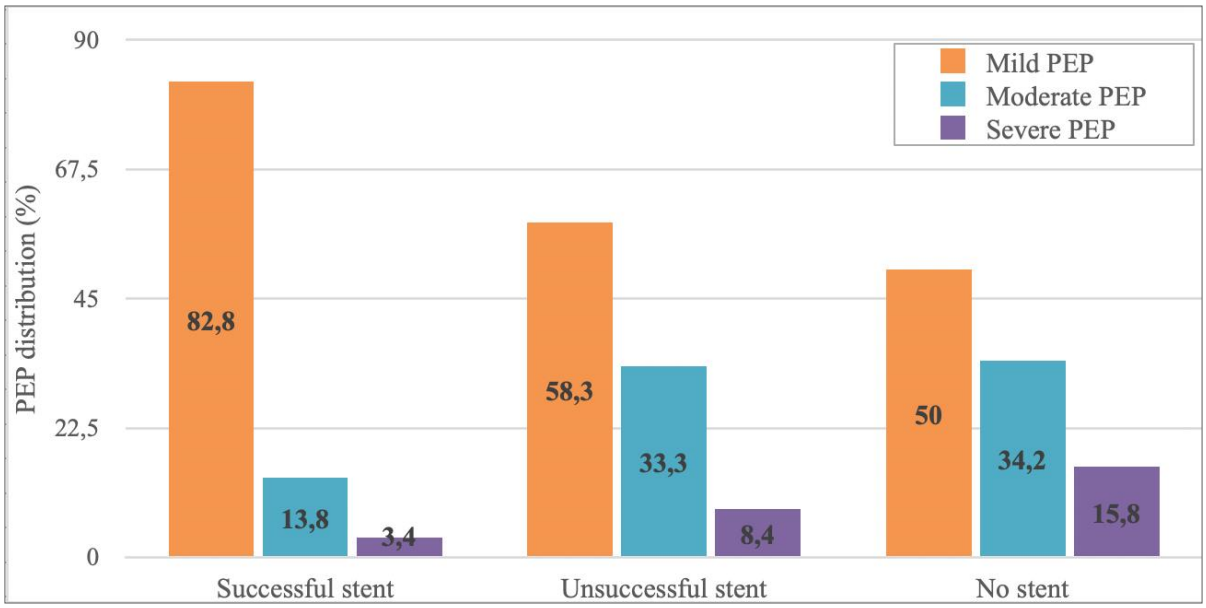
Study groups (n)	**Mild PEP**	**Moderate PEP**	**Severe PEP**
Successful stent (n=288)	24	4	1
Unsuccessful stent (n=29)	7	4	1
No stent (n=121)	19	13	6
Distribution of PEP in each group (%)			
Successful stent (PEP=29, 10.03%)	82.8	13.8	3.4
Unsuccessful stent (PEP=12, 41.3%)	58.3	33.3	8.4
No stent (PEP=38, 31.4%)	50.0	34.2	15.8
Significance (*p*)			
Successful vs. unsuccessful	***0.006***	***<0.005***	***0.044***
Successful vs. no stent	***0.026***	***<0.005***	***<0.005***
Unsuccessful vs. no stent	0.284	0.644	0.731

PEP=post-ERCP pancreatitis; Note: significant differences are presented in bold, italic

When demonstrating different severity in proportion to all PEP in the treatment groups, it is apparent, that the rate of moderate and severe PEP is significantly lower in the "successful

stent" group (p<0,05 in every case) compared to the other two (Figure 9). The differences between PEPs of different severity in the latter two groups are not significant (see table 7).

Figure 9: Distribution rates of PEP in the study groups (113)



PEP despite PD stenting:

Data of the "successful stent" group were further analyzed to detect any differences between the subgroups where PEP occurred ("PEP present") versus PEP did not developed ("PEP absent"). In the "PEP present" subgroup significantly more patient-related risk factor was present (3.76 vs. 3.16) (Table 8) (112).

Table 8: The mean of the sum of risk factors in the "successful stent" group (112)

Successful stent group (n=288)	**PEP present (n=29)**	**PEP absent (n=259)**	***p***
Age (years)	57.5 ± 17.2	61.4 ± 16.4	0.23
Sum of all risk fators	5.62 ± 1.47	4.77 ± 1.44	**0.0029**
Sum of patient-related risk factors	3.76 ± 1.43	3.16 ± 1.23	**0.015**
Sum of procedure-related risk factors	1.86 ± 0.88	1.61 ± 0.98	0.19

Data represent the mean and standard deviation in each groups (mean ±SD), significant *p* values are written in bold

Distribution of individual risk factors was compared in the two subgroups of "successful stent" group. In other terms the incidence rates of individual risk factors in the

"PEP present" and "PEP absent" groups were examined. There were no significant differences except for SOD (the ballon dilation of intact papilla showed significant difference even, but the case number was negligible, therefore it could not be commented) (Table 9).

Table 9: Individual risk factors in the "successful stent" group (112)

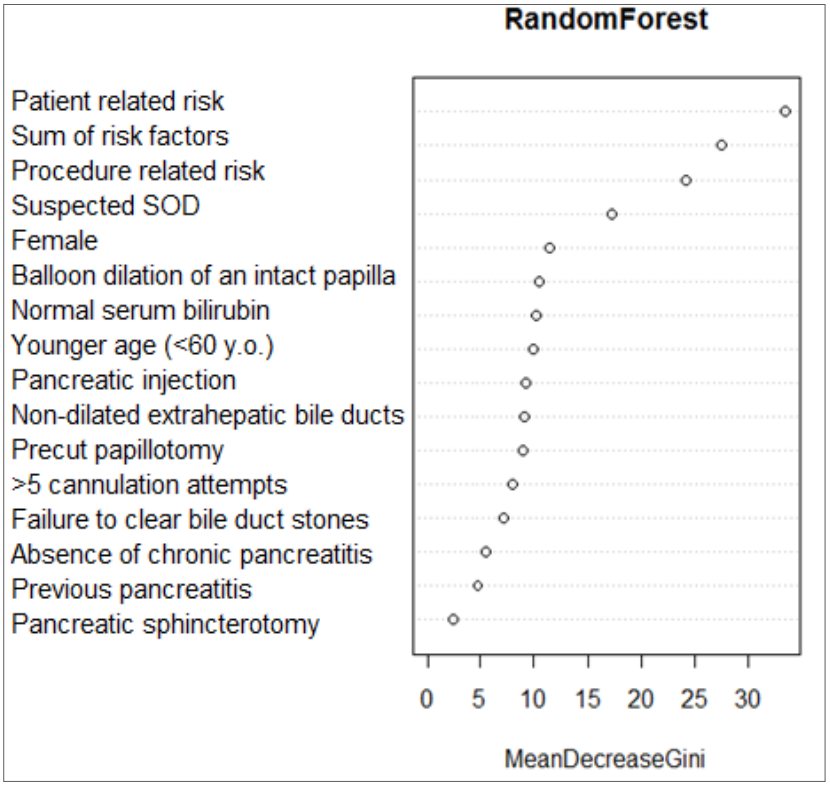
Risk factors	**PEP present (n=29)**	**PEP absent (n=259)**	**OR**	**95% CI**	***p***
Sphimcter of Oddi dyskinesis	11 (37.9%)	39 (15.1%)	3.45	1.51-7.86	**0.003**
Female gender	21 (72.4%)	169 (65.3%)	1.39	0.59-3.28	0.442
Previous PEP	4 (13.7%)	31 (11.9%)	1.17	0.38-3.61	0.776
Younger age (<60 years)	13 (44.8%)	104 (40.2%)	1.21	0.56-2.62	0.627
Non-dilated CBD	15 (51.7%)	115 (44.4%)	1.34	0.62-2.89	0.454
Normal serum bilirubin	16 (65.5%)	123 (47.5%)	1.36	0.63-2.94	0.434
Absence of chronic pancreatitis	29 (100%)	237 (91.5%)	5.59	0.33-94.57	0.233
Precut sphincterotomia	11 (37.9%)	69 (26.6%)	1.68	0.76-3.74	0.203
PD filling	21 (72.4%)	169 (65.3%)	1.39	0.59-3.28	0.442
Difficult cannulation	16 (65.5%)	132 (50.9%)	1.18	0.55-2.56	0.668
Pancreatic EST	0 (0%)	6 (2.31%)	0.66	0.04-12.03	0.779
Failed CBD clearance	4 (13.7%)	42 (16.2%)	0.82	0.27-2.49	0.736
Ballon dilation of intact papilla	2 (6.9%)	0 (0%)	47.2	2.21-10.08	**0.014**

CI=confidence interval, EST=endoscopic sphincterotomy, OR=Odds ratio, p=significance PEP=post-ERCP pancreatitis, PD=pancreatic duct, CBD=common bile duct, EST=endoscopic sphincterotomy

Note: significant *p* values are written in bold

Every individual risk factor was then analyzed with forest analyses using data mining methods to determine which factor has the most impact on developing PEP. The most important predictor of PEP when PPSs were used was the sum of "patient related risk factors", and SOD from individual risk factors (Figure 10).

Figure 10: The importance of different predictors (112)



SOD=sphincter of Oddi dyskinesis,

Factors predisposing to unsuccessful stenting:

Data of "unsuccessful stent" group were reviewed and further analyzed. We found, that significantly more patient-related risk factors were present in this group compared to the "successful stent" group (2.03 vs. 1.64) (Table 10).

Table 10: The mean of the sum of risk factors in the study groups (112)

High-risk study patients (n=317)	**Successful stent (n=288)**	**Unsuccessful stent (n=29)**	***p***
Age (years)	60.9 ± 16.5	64.2 ± 16.9	0.32
Sum of all risk fators	4.86 ± 1.47	5.38 ± 1.15	0.06
Sum of patient-related risk factors	3.22 ± 1.27	3.34 ± 1.23	0.61
Sum of procedure-related risk factors	1.64 ± 097	2.03 ± 0.80	**0.035**

Data represent the mean and standard deviation (mean ±SD), significant *p* values are written in bold

Complications of PD stenting:

We observed early dislodgement and proximal migration during PPS usage, but other complications mentioned in the literature were not present in our study.

It is generally accepted that in high-risk patients PPS should be in place for a minimum of 24 hours, but not longer than 1 week to have its beneficial effect. Out of the 288 successfully stented patients in our database, early stent dislodgment was observed in 5 (1.74%) which caused late onset PEP. All patients were symptom free for 8-12 hours after ERCP and the first serum amylase control was normal, however developed PEP later on with typical abdominal pain and abnormally high serum amylase levels measured next morning. We checked the position of the PPS with fluoroscopy or gastroscopy/duodenoscopy, but it was not in place. Two of the 5 dislodged stents were straight stents without an inner flange, but the other 3 were Freeman-type stents (single inner flange and outer pigtail end). One of the latter 3 was dislodged by a post-papillotomy bleeding, while in the other 2 cases ballon dilation of the intact papilla may have played a role in early dislodgment. PEP were mild in all cases.

In cases of proximal migration the stent slides into the PD and the outer end is not visible in the papillary orifice. Three such cases were detected in our database (1.04%). All patients were stented with a straight PPS with double inner and outer flange, furthermore 2 patients did not attend their follow up appointment for stent extraction, therefore their PPSs remained in place for longer than 1 month. The third patient had SOD. Although her stent was in place for only 6 days, but literature data suggest that retrograde sphincter of Oddi phasic contractions are frequent in such cases which is a risk factors of this complication (50). Two of the 3 were successfully removed after pancreatic EST, and a properly paced PPS prevented PEP after extraction. Extraction of the third stent was unsuccessful after multiple attempts, finally the patient underwent a distal pancreatectomy.

Comparison of different stent types showed no significant differences in efficacy, but early stent dislodgment was observed with PPSs without inner flange, while proximal migration occurred with double inner flanged stents, although the number of complications was low, therefore statistical analysis could not be performed (Figure 11, Table 11).

The recently published complication of AP after removal of a PPS was not observed in our study, but we removed all PPSs in 1 week, while those stents published in the literature were in place for more than 2 weeks (121, 122).

Figure 11: PEP rate when using different stent types (113)

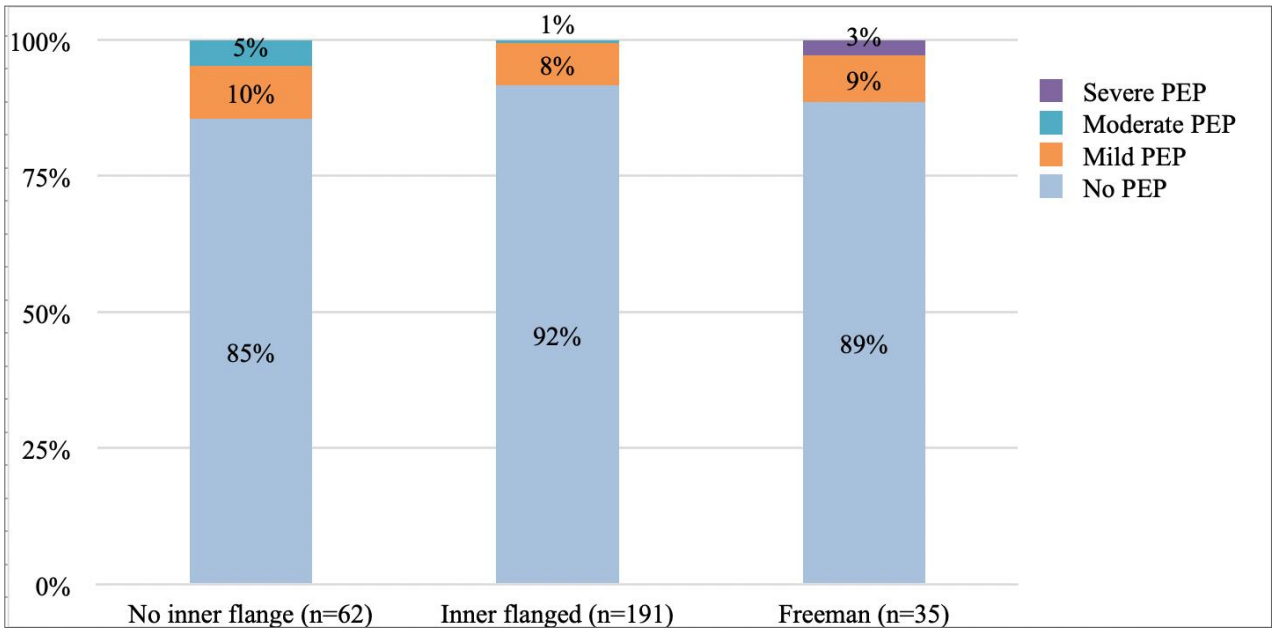


Table 11: Complications associated with different stent types (113)

No inner flange PPS	Inner flanged PPS	Freeman type PPS	
2	-	3*	**Early dislodgement**
-	3	-	**Proximal migration**

*: predisposing factor always present (1 post-EST bleeding, 2 large ballon dilations of the intact papilla)

10.1.2. Comparison of PPSs and NSAID suppositories

The effect of rectal NSAID and PPS in preventing moderate-to-severe PEP was compared in a network meta-analysis. 11 NSAID RCTs comprising 4296 patients and 10 PPS RCTs comprising 1239 patients defining and classifying PEP according to the Cotton criteria were included, separately analyzing the high- and average-risk population. The outcome of the included RCTs are summarized in Table 12 (114).

Table 12: Outcome of the trials in our network meta-analysis (114)

Author	Control (n=)	PEP in control group				Treatment (n=)	PEP in treatment group			
		All	Mild	Mod.	Severe		All	Mild	Mod.	Severe
Average-risk patients (NSAID studies)										
Sotoudehmanesh	221	15	10	-	-	221	7	7	0	0
Döbrönte 1	98	11	10	0	1	130	11	9	0	2
Otsuka	53	10	7	3	0	51	2	2	0	0
Katsinelos	260	27	19	6	2	255	12	10	2	0
Döbrönte 2	318	22	18	0	4	347	20	16	0	4
Patai	269	37	33	3	1	270	18	15	2	1
Ucar	50	7	3	4	0	50	1	1	0	0
SUM	***1269***	***129***	***100***	***16***	***8***	***1324***	***71***	***60***	***4***	***7***
High-risk patients (NSAID studies)										
Elmunzer	307	52	25	24	3	295	27	14	10	3
Katsinelos (subgroup)	203	25	18	5	2	188	11	9	2	0
Andrade-Dávila	84	17	14	3	0	82	4	3	1	0
Lua	75	4	4	0	0	69	7	4	3	0
Patil	200	23	14	5	4	200	6	6	0	0
SUM	***869***	***121***	***75***	***37***	***9***	***834***	***55***	***36***	***16***	***3***
NSAID SUM	**2138**	**250**	**175**	**53**	**17**	**2158**	**126**	**96**	**20**	**10**
Average-risk patients (PPS studies)										
Sofuni	103	14	8	6	0	98	3	2	1	0
Tsuchiya	32	4	2	1	1	32	1	1	0	0
SUM	***135***	***18***	***10***	***7***	***1***	***130***	***4***	***3***	***1***	***0***
High-risk patients (PPS studies)										
Tarnasky	39	10	5	5	0	41	1	0	1	0
Fazel	36	10	5	2	3	38	2	2	0	0
Harewood	8	3	3	0	0	10	0	0	0	0
Ito	35	8	8	0	0	35	1	1	0	0
Sofuni	204	31	22	8	1	203	16	12	4	0
Kawaguchi	60	8	8	0	0	60	1	1	0	0
Lee	51	15	12	2	1	50	6	5	1	0
Cha	58	8	3	2	3	46	2	2	0	0
SUM	***491***	***93***	***66***	***19***	***8***	***483***	***29***	***23***	***6***	***0***
PPS SUM	**626**	**111**	**76**	**26**	**9**	**613**	**33**	**26**	**7**	**0**

For NSAID studies, 7 RCTs with 2593 patients in the average-risk group and 5 RCTs with 1703 patients in the high-risk group were included.

For PPS studies, only 2 RCTs with 265 patients in the average-risk group, however 8 RCTs with 974 patients in the high-risk group were included. PPSs were placed intentionally with a success rate of 88-100%.

According to the results of our network meta-analysis both in average- and in high-risk patients only PPS reduces the risk of moderate to severe PEP significantly compared to placebo. Rectal NSAID compared to placebo and PPS compared to NSAID shows a clear trend, but the difference was not significant (Table 13).

Table 13: League tables comparing the preventive methods (114)

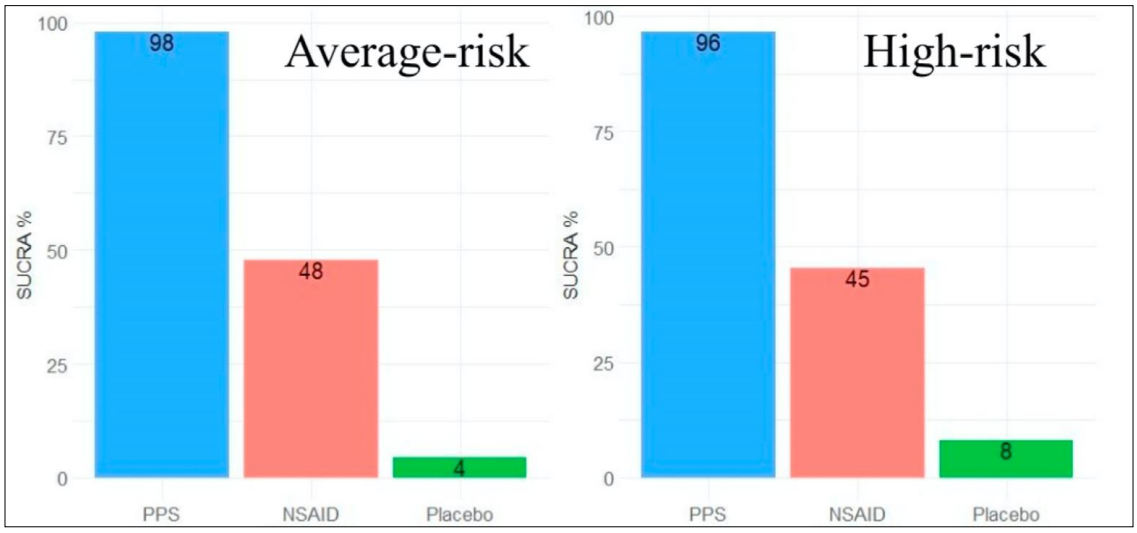
AVERAGE-RISK PATIENTS		
PPS		
RR: 0.12; [95% CI: 0.0033-1.2]	**NSAID**	
RR: 0.070; [95% CI: 0.0020-0.58]	RR: 0.58; [95% CI: 0.22-1.3]	**Placebo**
HIGH-RISK PATIENTS		
PPS		
RR: 0.32; [95% CI: 0.037-1.5]	**NSAID**	
RR: 0.19; [95% CI: 0.043-0.54]	RR: 0.57; [95% CI: 0.17-2.5]	**Placebo**

RR=relative risk, PPS=preventive pancreatic stent, NSAID=non-steroidal anti-inflammatory drug, CI=confidence interval

It is also remarkable, that not a single severe PEP case was reported in the PPS studies either in average- or in high-risk patients, but it was present in the NSAID studies (7/71 in average- and 3/55 in high-risk patients) (Table 12).

Ranking probabilities based on SUCRA indicated that PPS placement had the highest likelihood of being ranked as the best treatment method compared to rectal NSAID and placebo, both in average- and high-risk patient groups (average-risk: PPS 98%, NSAID 48%, placebo 4%; high-risk: PPS 96%, NSAID 45%, placebo 8%) as highlighted in Figure 12.

Figure 12: Ranking probabilities based on SUCRA of the examined preventive methods (114)



PPS=preventive pancreatic stent, NSAID=non-steroidal anti-inflammatory drug, SUCRA=surface under cumulative ranking,

10.2. Our research in ABP

10.2.1. Non-randomized study

A total of 187 ABP patients were referred for ERCP between 1st January 2009 and 1st July 2010 in the two participating centers (117). Forty six patients had to be excluded from our study as they did not meet the enrollment criteria: (3 patients were more than 85 years old, 8 had a past history of gastric or pancreatobiliary surgery, 2 had pancreatic abscess on initial CT scan, 10 had liver cirrhosis, 5 had an INR >1.8, 8 had passed the optimal time frame of 72 hours from the initial symptoms, in 4 patients biliary cannulation failed, 2 had incomplete CBD clearance, and 4 other patients were excluded due to an inability to insert the PD stent).

Finally, data of 141 patients were analyzed (100 were female and 41 were male; average age was 62.4 ± 15.1 years), from whom 71 had PPS insertion (PD stent group) and 70 had standard endoscopic therapy (control group).

There were no significant differences between the two groups in demography data (age, gender distribution), symptom-to-ERCP time, Glasgow score, length of hospital stay. They were also comparable regarding liver function tests, mean white blood cell counts, hemoglobin levels and peak serum amylase and CRP levels without any significant differences (Table 14).

Table 14: Characteristics of enrolled ABP patients in our non-randomized study (117)

Variable	**PD stent group (n=71)**	**Control group (n=70)**	***p***
Age (mean ± SD) /years/	60.6 ± 14.2	64.3 ± 16.0	0.14
Gender (male:female) /n/	20:51	21:49	
Symptom-to-ERCP time (mean ± SD) /hours/	34.4 ± 29.2	40.2 ± 35.2	0.28
Glasgow score (mean ± SD)	3.21 ± 1.1	3.27 ± 1.3	0.77
Serum bilirubin (mean) /µmol/l/	57.8	54.3	0.73
ASAT (mean) /U/l/	327.4	271.5	0.20
ALAT (mean) /U/l/	364.9	302.5	0.15
GGT (mean) /U/l/	594.1	490.1	0.35
ALP (mean) /U/l/	536.8	558.9	0.67
Serum amylase (mean) /U/l/	2446.9	2114.3	0.33
CRP (mean) /mg/l/	121.1	152.4	0.21
WBC (mean) /G/l/	12.8	13.5	0.36
Hgb (mean) /g/L/	140.8	138.3	0.41

ASAT=aspartate aminotransferase, ALAT=alanine aminotransferase, GGT=gamma-glutamyl transferase, ALP=alkaline phosphatase, CRP=C-reactive protein, ERCP=endoscopic retrograde cholangiopancreatography, WBC=white blood cell count, Hgb=hemoglobin,

CBD stones were found in 70% of all cases, the characteristics of these stones are summarized in Table 15.

PPS insertion was successful in 94.7% (71 of 75 cases). The patients in whom PD stent insertion was attempted but failed did not develop any serious complications throughout the course of their ABP. Minor post-papillotomy bleeding occurred in two patients (one in each groups), that were managed endoscopically.

Table 15: Characteristics of CBD stones in our non-randomized study (117)

Variable	**PD stent group (n=71)**	**Control group (n=70)**
Patients with CBD stones (n (%))	49 (69%)	50 (71.4%)
CBD stones >5 mm in diameter	*30*	*32*
→ stones impacted in papilla of Vater	*12 out of 30*	*13 out of 32*
CBD stones <5 mm in diameter or sludge	*19*	*18*
Patients without CBD stones (n (%))	22 (31%)	20 (28.6%)

Outcome of ABP:

Relevant ABP complication occurred in 9.86% in the PD stent group (7/71 cases), as follows: MODS requiring intensive care unit (ICU) transmission: 1 patient (severe pancreatitis with necrosis and pseudocyst formation could be treated conservatively), sepsis: 2 patients (treated with broad spectrum antibiotics), large (>6 cm in diameter) pseudocyst formation: 3 further patients, surgical necrosectomy: 1 patient. No mortality was observed in this group.

In the control group complication occurred in 31.43% (22/70 cases), as follows: MODS requiring ICU transmission: 4 patients, sepsis: 4 patients, large pseudocyst: 10 patients, surgical necrosectomy: 1 patient. Three patients died, two of them (54 and 65 years old females) in the early phase, because of severe, uncontrollable MODS; while the third patient (69-year-old male) died after 21 days of hospitalization because of septic shock. The mortality rate was 4.28% (3 of the 70 patients) in the control group.

The overall complication rate was significantly lower in the PD stent group (9.86 vs. 31.43%, $p<0.002$). There was a trend toward a higher mortality rate in the control group compared to the PD stent group (0 vs. 4.28%), but because of the low case number it did not reach the level of significance (Table 16).

Table 16: Outcome of ABP in our non-randomized study (117)

Variables	**PD stent group (n=71)**	**Control group (n=70)**	***p***
Hospital stay (mean ± SD) /days/	10.8 ± 5.6	12.8 ± 10.6	0.17
Hospital stay range /days/	4 – 37	3 – 65	
Mortality (n (%)) at 90 days	0 (0%)	3 (4.28%)	NS
Morbidity (n (%))	7 (9.86%)	22 (31.43%)	<0.002
- ICU transmission (MODS)	1	4	
- Septic shock or infected necrosis (phlegmon or abscess)	2	4	
- Pancreatic pseudocyst (>6 cm)	3	10	
- Surgical necrosectomy	1	1	

ICU=Intensive Care Unit, MODS=multi-organ dysfunction syndrome, SD=standard deviation

10.2.2. Randomized study

The outcome of the PPS group in our non-randomized study was significantly better, than in the control group. Based on these results, we hypothesized that PPS not only prevents the potentially harmful effects of papilla manipulation during ERCP resulting in worsening the pancreatitis, but it may reverse the process of ABP in the early phase. Therefore we developed a randomized controlled trial, which is called Prepast study (PREventive PAncreatic STents in the management of acute biliary pancreatitis) (119).

The primary endpoint of the study is the percentage of ABP patients with complicated courses in the different treatment groups, namely the standard endoscopic therapy group, meaning EST and CBD clearance (A1+B1), and the investigation group, meaning standard therapy with PPS implantation added to it (A2+B2). Definitions were specified according to the IAP/APA guidelines (23). The complicated course was described by a composite endpoint, which includes moderate and severe AP (temporary and persistent organ failure), any systemic (exacerbation of pre-existing co-morbidity) and/or local complications (acute peripancreatic fluid collection, pancreatic pseudocyst, acute necrotic collection, walled-off necrosis - as described in the revised Atlanta classification (30)) and mortality. Study-specific secondary endpoints were on one hand related to the outcome of ABP (multi-organ failure and mortality in each subgroup, pain score on admission, 24 and 72 hours after ERCP, new onset of sepsis, the proportion of patients with severe ABP, the proportion of patients with severe organ failure requiring organ support), on the other hand related to the endoscopic therapy (PPS insertion

success rate, consequences of attempted, but failed pancreatic stenting, endoscopist's experience on PPS insertion success rate and ABP outcome, the influence of the used endoscopic technique on the outcome of ABP, influence of patient and procedure related risk factors of PEP on outcome of ABP).

Inclusion criteria are: age > 18 years, diagnosis of ABP, signed informed consent, possibility of performing ERCP within 48 hours calculated from the onset of pain. Exclusion criteria are: pregnancy, AP due to alcohol, malignancy or PEP, pain onset > 48 hours, abscence of abdominal pain (the onset cannot be assessed), liver cirrhosis Child score C, pancreatic fluid collection or necrosis on initial imaging at presentation, INR > 1.6 and uncorrectable by the time of ERCP, previous EST.

Definitions used in this study are: Diagnosis of acute pancreatitis is based on the definition of IAP/APA guideline, following the „2 out of 3" principle, namely out of upper abdominal, pancreatic type pain; serum amylase or lipase > 3x upper limit of normal range (ULN); and characteristic findings on imaging studies (abdominal USS/CT/MRI) two should be present (however those patients without abdominal pain should be excluded). Biliary origin is based on the criteria suggested by the DPSG, namely one of the following three should be present: gallstones and/or sludge diagnosed on imaging; in the abscence of gallstones and/or sludge a dilated CBD on US (> 8 mm in patients 75 years old or > 10 mm in patients > 75 years old); ALAT > 2xULN with ALAT > aspartate aminotransferase (ASAT) (97). The presence of acute cholangitis has defined based on the Tokyo guidelines (TG13), with slight modification, as both items in group A, plus one item in both B and C should be present for „definite diagnosis" in our study (Table 17) (123). Criteria of cholestasis are the following: demonstration of gallbladder stones and/or sludge, or dilated CBD, or CBD stone on abdominal USS and elevated liver function tests (ALAT, ALP and bilirubin levels more than 1.5xULN).

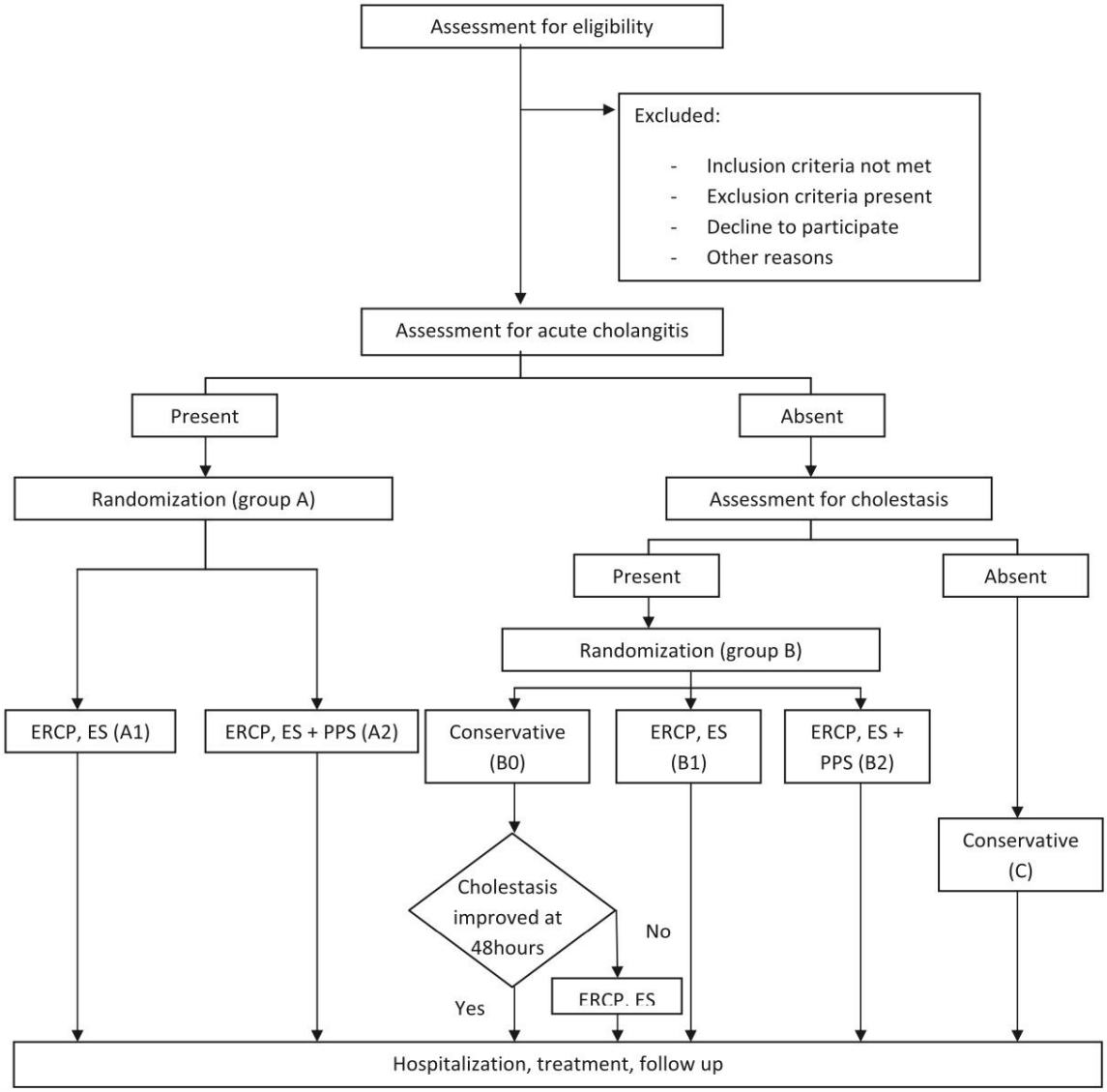
Table 17: Diagnostic criteria of acute cholangitis based on the Tokyo Guidelines (123)

A:	**Systemic inflammation**
A-1	Fever (> 38 ^{O}C) and/or shaking
A-2	Laboratory data: evidence of inflammatory response (WBC <4 or >10 x10^9/L, CRP ≥10 mg/l and other changes indicating inflammation)
B:	**Cholestasis**
B-1	Jaundice (serum bilirubin ≥34.2 μmol/l)
B-2	Laboratory data: abnormal liver function tests (ASAT, ALAT, GGT, ALP >1.5x ULN)
C:	**Imaging**
C-1	Biliary dilatation
C-2	Evidence of etiology on imaging (stricture, stone, stent, etc.)
Suspected diagnosis: one item in A + one item in either B or C *Definite diagoszis*: one item in both A, B and C	

WBC=white blood cell count, CRP=C-reactive protein, ASAT=aspartate aminotransferase, ALAT=alanine aminotransferase, GGT=gamma-glutamyl transferase, ALP=alkaline fosfatase,

Patients are categorized into two groups. When coexisting acute cholangitis is present besides ABP, they form the first group (A). These patients should undergo urgent ERCP according to the previously mentioned international guidelines. They are randomized into two therapeutical subgroups (standard treatment of EST and CBD stone extraction: A1, or standard treatment+PPS: A2) with an allocation ratio of 1:1. Patients without signs of cholangitis are assessed for evidence of cholestasis, because ERCP is also indicated for them ("probably indicated" category in the guideline). Those patients in whom signs of cholestasis are absent (C) are excluded from Prepast study and treated with conservative treatment. When cholestasis is present (B), these patients are randomized into 3 subgroups with an allocation ratio of 1:1:1; into the previously mentioned two subgroups (standard treatment of EST and CBD stone extraction: B1, or standard treatment+PPS: B2), plus a conservative treatment subgroup (B0), because published data suggests, that spontaneous resolution of biliary obstruction can occur in up to 70% in 48 hours (Figure 13) (124).

Figure 13: Randomization folwchart in the Prepast study (119)



Patients are assigned an identification number consisting of 5 numbers and 1 letter. The first two numbers are the center identification number, then the letter is the group identifier, finally the last three numbers are the patient-specific randomization number (eg. 01-A-001). The eCRF not only shows the identification number at randomization, but also indicates the therapeutical subgroup for the clinician (eg. Randomizatinon number: 01-A-001/A1, Assignment: A1: acute cholangitis present; ERCP, ES treatment).

Basic treatment principles are defined for all participating patients, including initial goal directed intravenous fluid resuscitation with isotonic crystalloid solution (lactated Ringer is preferred), decision of oral refeeding or nasojejunal feeding on day two after hospitalization, and avoidance of preventive antibiotic therapy. Several parameters should be monitored in order to assess the clinical course and to identify for example MODS (based on the Marshall

scoring system), severity of ABP based on systemic inflammatory response syndrome (SIRS), Glasgow scale and BISAP score. Contrast-enhanced abdominal CT is required in 72-96 hours from the onset of pain in patients with a suspicion of severe or complicated course of ABP, to determine the presence or absence of pancreatic necrosis or fluid collection. Any further therapeutic decisions are left to the discretion of treating gastroenterologist. A patient is discharged from hospital if they become symptom free and tolerate oral feeding, and is scheduled for an outpatient follow-up in 3 months. Those, who are treated with nasosejunal feeding, but become stable, also discharged home, but scheduled for follow up in every 2 weeks until final decision for intervention (eg. drainage, surgery) has been made. A final follow up is scheduled three months after the intervention.

Sample size calculation is based on the results of our previous non-randomized study and with a 5% rate of lost to follow up, so 230 patients should be enrolled in this study (115 in both, PPS and control study arms). A safety interim analysis is scheduled at 50% enrollment status, which we have just achieved, as the centers have enrolled 141 patients so far, of whom 22 were assigned to the conservative (B0) arm, so 119 cases were assigned to one of the intervention subgroups planned to be analyzed (A1, A2, B1, B2).

11. DISCUSSION

11.1. Our research in PEP prevention

In the first part of our scientific work, we examined the effects of PPSs on PEP. The effect of these stents in the prevention of PEP has been examined since the mid-1990s, initially mostly in SOD patients underwent ERCP, but after recognition of risk factors more precisely in other high-risk patient populations as well. Every prospective trial shows a decrease of the PEP incidence in stented patients, furthermore trials have been conducted since 1999 described a significant difference compared to placebo (125). We also demonstrated the beneficial effect of PPSs in high-risk patients compared to non-stented and unsuccessfully stented patients. Not only the lower incidence of PEP could be observed, but significantly less moderate and severe pancreatitis developed in the stented group than in the other two, in other words PPS placement shifted this complication towards milder cases.

When analyzing risk factors, we were the first to describe in the literature, that in patients with preventive PD stenting, certain risk factors are as likely to be present in the subgroup that developed PEP as in those who did not, and only SOD was an exception. Based on these results, we concluded that PPS implantation (excluding SOD) prevents the effects of various risk factors. The American EPISOD study (similarly to our own results) confirms that the incidence of PEP is high in SOD patients despite the usage of PPSs (126).

We also found that patients who developed PEP despite PPS placement had significantly more patient-related risk factors than those without PEP, but there was no significant difference in the number of procedure-related risk factors. Based on our results, the risk carried by each patient appears to be more important, so we recommend that the risk of PEP should be assessed very accurately before the ERCP and that in high-risk patients every effort should be made to avoid this complication, including proper assessment of the indication, using less invasive/non-invasive testing methods if necessary, and active prophylaxis too with NSAID suppository, hydration, sublingual nitrate, PPS placement and with the combination of these methods.

However, if PPS insertion is attempted but remains unsuccessful, significantly more procedure-related risk factors were present in the subgroup of patients who developed PEP, than in the subgroup who did not, while the number of patient-related risk factors did not differ in the two groups. This can be interpreted as, that more complex, more difficult, more complicated procedures are more likely to end up in failure of PPS implantation. In our own practice, in cases of difficult cannulation, if the guidewire enters the PD, it is not removed but the ERCP is continued with either PPS implantation or a double-guidewire technique, especially if the patient is (also) at high-risk of PEP. Unsuccessful stenting has an especially high risk of PEP. Freemant and colleagues described this risk as 66.7%, although this conclusion was based only on data of 3 patients (127). In our database we have almost ten times more, altogether 29 unsuccessfully stented patients, and we found a PEP incidence of 41.3% after analyzing these data, which was not significantly different from the “no stent” group of high-risk patients (31.4%). We can say, that with appropriate routine, there is no need to be afraid of PPS insertion, but there is no literature data on the extent to which stenting can/should be enforced. We recommend common sense, and moderation.

We also examined the complications of PPS usage. In our own material, only early dislodgment and proximal migration were observed, but rarely, in roughly 1% of all cases. Late onset PEP draws attention to early stent dislodgment (or occlusion), that is, if the patient is symptom free for 6-8 hours after ERCP and becomes symptomatic and develops elevated serum amylase levels only after that, this complication should always be considered. Pancreatic stents without inner flange or an ERCP-complication (eg. post-papillotomy bleeding) are tend to cause early dislodgment. A repeated PPS implantation, the so called rescue ERCP can be beneficial for these patients. Proximal migration, although not more frequent complication, but more unfavorable for the patient, because removal of those stents can be challenging and can sometimes fail, causing symptoms and morphological signs typical of chronic pancreatitis as a consequence in long term. Predisposing factors based on the

literature are SOD, long (> 7 cm) stents and/or stents with double inner flanges, while the outer pigtail (or partial pigtail) end is preventive (128). In our own study, we only observed this complication with straight, double inner flange stents. We didn't found a single case when using Freeman-type stents, thus, in our experience, this, i.e., a PPS with a single inner flange and an outer pigtail end, appears to be the safest to use.

The weakness of this publication, that although the database is prospectively collected with predefined principles, data were collected from a few years earlier, but we intentionally selected that period, because at that time rectal NSAID was not routinely suggested for ERCP patients, therefore we could exclusively analyze the effect of PPSs compared to no intervention.

In the next part of our research, we were looking for an answer to the question of how effective is each of the two most widely used preventive methods in the prophylaxis of moderate-to-severe PEP. For a patient, any complication of any medical intervention is disadvantageous, however, those that recover completely after a short treatment have no long-term negative impact on their health. For this reason, concerning the ERCP procedure, a transient mild amylase elevation or mild PEP may be considered a less pronounced problem, however, if severe PEP develops, it can lead to severe complications or even death. In our network meta-analysis, we therefore intentionally chose severe PEP as a complication to be investigated as an outcome measure, but it was well known previously, that NSAID studies sometimes discuss them in the same category as moderate PEP, so we examined these two categories together. Based on our results, the incidence of moderate-to-severe PEP was significantly lower with PPS compared to placebo, but not to rectal NSAID suppositories. Also when comparing these two preventive methods, PPS is the more preferred prophylaxis based on SUCRA results. These results are valid in both average- and high-risk patient groups. A previous meta-analysis showed opposite result, but observational studies were also included in that analysis (129). However, our results are supported by a recent network meta-analysis, which came to a similar conclusion, but only our analysis focused exclusively on moderate-to-severe PEP (130). It is noteworthy, however, that none of the RCTs in our analysis showed severe PEP formation in the PPS group, whereas they did in the rectal NSAID group.

Our network meta-analysis has limitations. First, we have to mention the marked heterogeneity among studies, especially NSAID RCTs. Second, despite our efforts to homogenize the different RCTs, there could be conceptual heterogeneity in trial design, patient population, or intervention (e.g. timing of rectal NSAID, or variable routine of PPS placement). ERCP is a complex procedure, where several steps, sometimes only nuances can affect the outcome. These are usually not described in RCTs in details so that cannot be

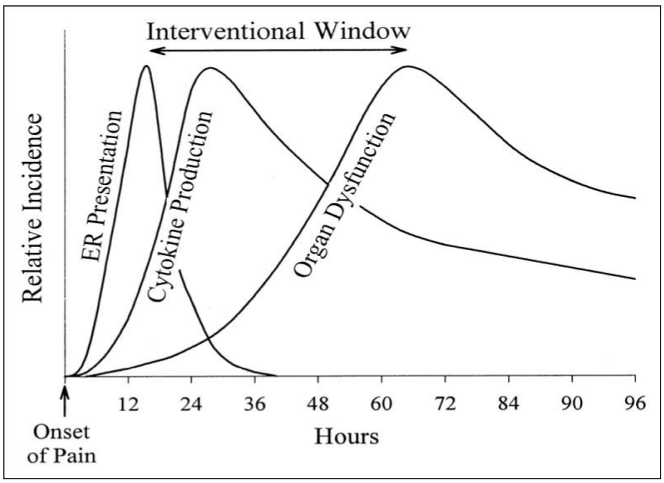
standardized. Transitivity is always at risk when we analyze such complex interventions like ERCP. Furthermore, not only the procedure, but the pre- and post-ERCP care and also the susceptibility of the patient population can be different.

Despite the limitations, our network meta-analysis has strengths as well. First, we used strict selection criteria. We selected those RCTs where the interventions and outcomes could be compared best (using Cotton criteria both for definition and classification of PEP, selecting full-text RCTs only, categorize patients into average- and high-risk groups, excluding non-rectal NSAID studies, or those with multiple preventive methods used at the same time or those RCTs with incomplete information). Second, we concentrated only on moderate and severe PEP, which on one hand has clinical significance, but on the other hand can be identified more precisely. The two debatable complications, namely mild PEP and hyperamylasemia, were not included. Third, we analyzed both, the average- and high-risk patient groups separately. The result for both patient groups was the same, PPS was ranking first, as the best preventive method.

11.2. Our research in ABP

There was a great debate over decades about the advantages and necessity of ERCP in ABP patients. Early studies in the 1980s categorized patients on the predicted severity of AP, and showed a favorable outcome with ERCP, EST and CBD stone extraction in severe ABP cases, meaning less AP complications and shorter hospital stay. Endoscopic therapy should be performed at the early phase, in 48-72 hours from the onset of pain, because the organ failure initiated by the inflammatory cytokines cannot be influenced afterwards (Figure 14) (131).

Figure 14: Inflammatory processes and therapeutic window in AP (131)



If we take into account, that it takes hours from the onset of complaints to hospitalization (it was 9-15 hours in average in a previously mentioned study (107)), then we can see, that there is a very narrow time interval for performing ERCP. It is also worth mentioning, that severity of AP cannot be predicted in the very early phase, because diagnostic elevation of parameters used in severity scores or appearance of radiology signs that are characteristic for severe AP takes 2-3 days. At the same time, at the end of the 1990s, a multicenter German study showed more respiratory insufficiencies, therefore worse outcome in the ERCP group compared to the conservative treatment group (93). Debate started about the necessity of ERCP and endoscopic treatment, which was further enhanced by the meta-analysis published at the end of 2000s, which showed that not the predicted severity but the presence of cholangitis indicates the need for ERCP (96). The problem with it is, that diagnostic criteria of acute cholangitis was not developed for AP patients, and yet in ABP laboratory markers caused by the pancreatitis itself can be similar to those described in Tokyo Guidelines for acute cholangitis (123). On the other hand DPSG demonstrated, that ERCP is beneficial for AP patients with bile duct obstruction (97). The confusion was further intensified, that definitions and diagnostic criteria were not uniform. These were unified by International and American experts at the beginning of 2010s and published their IAP/APA guidelines (99). Both the International and Hungarian guidelines and a Dutch RCT published since then emphasizes, that not the predicted severity, but the presence of bile duct obstruction and cholangitis indicates ERCP (99, 100, 98, 132).

When examining the effect of ERCP on ABP we should not neglect the fact, that the endoscopic procedure itself can cause AP or can worsen its severity. In that sense an ABP patient is considered high-risk (50). Furthermore, those ERCPs performed in ABP are technically more difficult, than in acute cholangitis for example (133). PEP research has confirmed, that PPS implantation reduces the risk of AP induced by ERCP itself (69). Pancreatic stents were first introduced in the late 1980s, mainly for treating PD abnormalities and acute recurrent pancreatitis was also among the indications, furthermore they were also used to prevent PEP early on (84, 134). Although patomechanism of ABP is not fully understood even nowadays, but PD obstruction, increased intrapancreatic duct pressure and early, intraductal enzime activation is among the proposed steps, therefore, theoretically, relieving the obstruction, lowering the intraductal pressure and drainage of pancreatic fluid can improve the outcome of inflammation.

In the first part of our research, PPS implantation was performed in those ABP patients in a non-randomized fashion, in whom the ERCP and bile duct cannulation, which are otherwise considered indicated by international recommendations, have proved difficult. The

endoscopy procedure, as mentioned before, theoretically can worsen the ongoing AP. We hypothesized, that PPS placement can compensate the negative effect of difficult cannulation similarly to prevention of PEP. The results of our study were more favorable, as the results of the PPS group were much better, with significantly less complication rate and although not significant, but clearly lower mortality, compared to EST and CBD stone extraction which is considered as standard therapy. Based on these results, we could not only demonstrate for the first time in the literature, that in ABP patients PPS implantation is safe, feasible and effective, but also it can prevent the unfavorable effect of difficult cannulation which can aggravate AP. Furthermore, as the results were even better than the standard endoscopic treatment, we hypothesized, that PPS placement not only can prevent the harmful effect of papilla manipulation during ERCP, but by lowering the intrapancratic duct pressure and maintaining the outflow of pancreatic juices, it can manipulate the inflammatory cascade and evolution of ABP into a more favorable direction. There are only a few publications in this topic, currently only a Chinese prospective trial with similar results available only in abstract form and an also Chinese, but retrospective study with similarly good results published in the literature (135, 136).

The shortcoming of our study is that this is a non-randomized design, therefore partly because of that, partly because of the favorable results (which suggested the reversal of the progression of ABP) a need for a similar study but in a randomized fashion arose. As a result, the Prepast study was born, and the development of its protocol forms the next part of the present work. Under the auspices of HPSG, this was the first randomized, controlled trial, which has since been followed by many others. The study examines the effects of ERCP performed in the early stages of ABP, ie within the therapeutic window, and compares the effects of standard endoscopic treatment (EST and CBD stone extraction) with PPS implantation in addition of the standard treatment on the outcome of acute pancreatitis. Based on the results of our study, hopefully we will be able to demonstrate the beneficial effect of PPS implantation in the ABP patient population, but at least the safety of the intervention. A Finnish study has been published recently, where they attempted pancreatic stent implantation in acute necrotizing pancreatitis, assuming that they can treat PD disruption often found in such patients. However, results showed significantly more infectious complications, overinfection of the pancreas necrosis in the stented group (137). PD stenting in the late phase, during the presence of pancreatic necrosis does not improve the course of the disease, only increases the chances of complications. Prepast study specifically concentrates PD stenting in the early phase. Our results are expected to provide additional result or clarify the timing of PPS implantation.

12. CONCLUSIONS

Our research on PPS can be divided into two main parts, on the one hand, on the role of PPS in PEP prevention, and on the other hand, on the use of PPS in ABP.

In the first part of our work, we demonstrated, that the use of PPSs reduces the incidence of PEP in the high-risk group, and in addition, if PEP does develop, it will be more favorable than no stenting. We have also shown, that PPS protects against the harmful effects of almost all known risk factors, except for SOD. Based on our results, development of PEP despite PD stenting should be expected if the individual patient has more patient-related risk factors, while in the case of more difficult ERCP procedure, i.e., more procedure-related risk factors, PPS implantation can be unsuccessful. Out of the used pancreatic stents, a PPS with a single inner flange and an outer pigtail end appears to be the safest to use. The results of our network meta-analysis show, that PPS is more likely to prevent the development of moderate-to-severe PEP in both moderate- and high-risk patients, than NSAID suppositories or placebo.

In the second part of our work, we demonstrated that PD stenting performed in the early phase of ABP, within the therapeutic window, within 72 hours of onset, is safe, can prevent the potentially harmful effect of ERCP, and even provides better outcomes than no stenting during ERCP. We hypothesize, that this beneficial effect can be demonstrated not only in cases of difficult cannulation, but also in a randomized manner. The study is ongoing and the results of the interim analysis are expected in the near future.

13. SUMMARY / NEW RESULTS

1. In an analysis of our prospectively collected database of high PEP risk patients undergoing PPS implantation for PEP prevention, we demonstrated that PPS application significantly reduces the risk of PEP carried by previously described and known risk factors. We are the first in the literature to show that PPS, with the exception of SOD, protects against the adverse effects of known PEP risk factors.
2. Our data show, that patients at high-risk of PEP are significantly less likely to develop PEP with PPS, and if they do develop PEP, it is milder than in patients whose stenting has failed, or have not been stented.
3. We showed, that although unsuccessfully stented high-risk patients develop significantly more PEP than successfully stented patients, this incidence is not significantly higher compared to the non-stented cases.
4. We have demonstrated, that patients who develope PEP despite PPS implantation have significantly more individual risk factors than those who did not develop PEP; and that

patients with failed PPS implantation have significantly more procedure-related risk factors (ie. more complex ERCP), than those who were successfully stented.

5. We have been published in the literature for the first time in a large case-series, that if PPS insertion was not performed during the first ERCP, and the patient develops severe PEP symptoms, PPS implantation during a “rescue ERCP” performed 8-20 hours after the first ERCP prevents severe PEP complications.
6. In a network meta-analysis we have compared the preventive effect of the two most commonly used PEP prophylactic methods, NSAID suppositories and PPS, for moderate and severe PEP, and found PPS to be a more effective preventive method in both the average- and high-risk groups.
7. Another new finding is that no severe PEP occurred in the stented group in either the average- or high-risk groups in the examined studies, but this was not the case for NSAID suppositories, where a few severe PEP cases have been published.
8. Based on our prospective database, the prophylactic efficacy of the different types of PPS we used did not differ, but the type with a single inner flange and an outer pigtail end seems to be the most optimal in terms of stent-related complications.
9. For the first time in the literature, we published the beneficial effect of PPSs on complications and disease progression in a patient population with ABP where endoscopic intervention (EST and biliary stone extraction) proved difficult.
10. Based on these results, we were able to demonstrate for the first time in the literature not only that PPS implantation in the ABP patient population is safe and effective, but also that it can prevent the adverse effects of difficult cannulation.
11. Based on these results, we have also developed the protocol for the Prepast RCT, to investigate the efficacy of PPS added to ERCP and EST in early ABP, and this is the first RCT in the international literature on this topic. The trial is ongoing and interim analysis results are expected in the near future.

14. REFERENCES

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15. ACKNOWLEDGEMENT

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