EARLY MANAGEMENT OF ACUTE PANCREATITIS

Ph.D. Thesis

Doctoral School of Pharmacological and Pharmaceutical Sciences

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University of Pécs Medical School

Pécs

2019

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Pécs

2019

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I. Publications

I.1. Publications related to the subject of the thesis

1) **Márta K**, Lazarescu AM, Farkas N, Mátrai P, Cazacu I, Erőss B, Vincze Á, Veres G, Czakó L, Sarlós P, Rakonczay Z, Hegyi P. Aging and Comorbidities in Acute Pancreatitis I: A meta-analysis and systematic review based on 194 702 patients. **Front Physiol.** 2019 DOI: 10.3389/fphys.2019.00328

   IF: 3.394, Q1, original publication[1]

2) **Márta K**, Hegyi P. Uncommon appearance of concurrent liver cirrhosis and chronic pancreatitis: The alcohol metabolism theory. **Dig Liver Dis**. 2019 Jan 11. pii: S1590-8658(19)30004-0. doi: 10.1016/j.dld.2018.12.023.PMID:30691775

   IF: 3.287, Q1, knowledge publication[2]

3) Szakács Z, Gede N, Pécsi D, Izbéki F, Kovács G, Fehér E, Dobszai D, Kui B, **Márta K**, Kónya K, Szabó I, Török I, Gajdán L, Takács T, Sarlós P, Gódi S, Varga M, Hamvas J, Vincze Á, Szentesi A, Párniczky A, Hegyi P. Aging and Comorbidities in Acute Pancreatitis II.: A Cohort-analysis of 1203 Prospectively Collected Cases. **Front Physiol**. 2018 DOI:10.3389/fphys.2018.01776

   IF: 3.394, Q1, original publication[3]

4) **Márta K**, Farkas N, Szabó I, Illés A, Vincze Á, Pár G, Sarlós P, Bajor J, Szűcs Á, Czimmer J, Mosztbacher D, Párniczky A, Szemes K, Pécsi D, Hegyi P. Meta-Analysis of Early Nutrition: The Benefits of Enteral Feeding Compared to a Nil Per Os Diet Not Only in Severe, but Also in Mild and Moderate Acute Pancreatitis. **Int J Mol Sci**. 2016 Oct 20;17(10). pii: E1691.PMID:27775609

   IF: 3.226, Q1, original publication[4]

5) **Márta K**, Szabó AN, Pécsi D, Varjú P, Bajor J, Gódi S, Sarlós P, Mikó A, Szemes K, Papp M, Tornai T, Vincze Á, Márton Z, Vincze PA, Lankó E, Szentesi A, Molnár T, Hágendorn R, Faluhelyi N, Battyáni I, Kelemen D, Papp R, Miseta A, Verzár Z, Lerch MM, Neoptolemos JP, Sahin-Tóth M, Petersen OH, Hegyi P; Hungarian Pancreatic Study Group. High versus low energy administration in the early phase of acute pancreatitis (GOULASH

trial): protocol of a multicentre randomised double-blind clinical trial. **BMJ Open**. 2017 Sep 14;7(9):e015874. doi: 10.1136/bmjopen-2017-015874. PubMed PMID: 28912191

IF: 2.413, Q1, pre-study protocol publication[5]

I.2 Publications not related to the subject of the thesis

6) Szakács Zs, Csiszár B, Kenyeres P, Sarlós P, Erőss B, Hussain A, Nagy Á, Kőszegi B, Veczák I, Farkas N, Bódis E, **Márta K**, Szentesi A, Tőkés-Füzesi M, Berki T, Vincze Á, Tóth K, Hegyi P, Bajor J. Hemorheological and hemostatic alterations in celiac disease and inflammatory bowel disease in comparison with non-celiac, non-IBD subjects (HERMES): A case-control study protocol. **BMJ Open.** 2019 Mar 23;9(3):e026315. doi: 10.1136/bmjopen-2018-026315.

IF: 2.413, Q1, pre-study protocol publication

7) Halász A, Pécsi D, Farkas N, Izbéki F, Gajdán L, Fejes R, Hamvas J, Takács T, Szepes Z, Czakó L, Vincze Á, Gódi S, Szentesi A, Párniczky A, Illés D, Kui B, Varjú P, **Márta K**, Varga M, Novák J, Szepes A, Bod B, Ihász M, Hegyi P, Hritz I and Erőss B. Outcomes and timing of endoscopic retrograde cholangiopancreatography for acute biliary pancreatitis. **Digestive and Liver Disease** 2019, DLD-18-1331R1, accepted on 25.03.2019

IF: 3.287, Q1, original publication

8) Nagy A, Mátrai P, Hegyi P, Alizadeh H, Bajor J, Czopf L, Gyöngyi Z, Kiss Z, **Márta K,** Simon M, Szilágyi ÁL, Veres G, Mosdósi B. The effects of TNF-alpha inhibitor therapy on the incidence of infection in JIA children: a meta-analysis. **Pediatr Rheumatol** Online J. 2019 Jan 18;17(1):4. doi: 10.1186/s12969-019-0305-x. Review. PMID: 30658717

IF: 2.543, Q1, original publication

9) Dobszai D, Mátrai P, Gyöngyi Z, Csupor D, Bajor J, Erőss B, Mikó A, Szakó L, Meczker Á, Hágendorn R, **Márta K**, Szentesi A, Hegyi P, Hungarian Pancreatic Study Group. Body-mass index correlates with severity and mortality in acute pancreatitis: A meta-analysis. **World J Gastroenterol**. 2019 Feb 14;25(6):729-743. doi: 10.3748/wjg.v25.i6.729. PMID: 30783376

IF: 3.3, Q1, original publication

10) Rumbus Z, Toth E, Poto L, Vincze A, Veres G, Czako L, Olah E, **Marta K**, Miko A, Rakonczay Z Jr, Balla Z, Kaszaki J, Foldesi I, Maleth J, Hegyi P, Garami A. Bidirectional Relationship Between Reduced Blood pH and Acute Pancreatitis: A Translational Study of Their Noxious Combination. **Front Physiol**. 2018 Oct 1;9:1360. doi: 10.3389/fphys.2018.01360. eCollection 2018. PMID: 30327613

IF: 3.394, Q1, original publication

11) Demcsák A, Lantos T, Bálint ER, Hartmann P, Vincze Á, Bajor J, Czopf L, Alizadeh H, Gyöngyi Z, **Márta K**, Mikó A, Szakács Z, Pécsi D, Hegyi P, Szabó IL. PPIs Are Not Responsible for Elevating Cardiovascular Risk in Patients on Clopidogrel—A Systematic Review and Meta-Analysis. **Front Physiol**. 2018 Nov 19;9:1550. doi: 10.3389/fphys.2018.01550. eCollection 2018. PMID: 30510515

IF: 3.394, Q1, original publication

12) Gódi S, Erőss B, Gyömbér Z, Szentesi A, Farkas N, Párniczky A, Sarlós P, Bajor J, Czimmer J, Mikó A, **Márta K**, Hágendorn R, Márton Z, Verzár Z, Czakó L, Szepes Z, Vincze Á, Hegyi P. Centralized care for acute pancreatitis significantly improves outcomes. **J Gastrointestin Liver Dis**. 2018 Jun;27(2):151-157. doi: 10.15403/jgld.2014.1121.272.pan. PubMed PMID: 29922760.

IF: 1.964, Q2, original publication

13) Mikó A, Farkas N, Garami A, Szabó I, Vincze Á, Veres G, Bajor J, Alizadeh H, Rakonczay Z Jr, Vigh É, **Márta K**, Kiss Z, Hegyi P, Czakó L. Preexisting Diabetes Elevates Risk of Local and Systemic Complications in Acute Pancreatitis: Systematic Review and Meta-analysis. **Pancreas**. 2018 Sep;47(8):917-923. doi: 10.1097/MPA.0000000000001122. PubMed PMID: 30113426.

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14) Szapáry L, Tinusz B, Farkas N, **Márta K**, Szakó L, Meczker Á, Hágendorn R, Bajor J, Vincze Á, Gyöngyi Z, Mikó A, Csupor D, Hegyi P, Erőss B. Intralesional steroid is beneficial in benign refractory esophageal strictures: A meta-analysis. **World J Gastroenterol**. 2018 Jun 7;24(21):2311-2319. doi:10.3748/wjg.v24.i21.2311. PubMed PMID: 29881240; PubMed Central PMCID: PMC5989245.

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15) Kiss Z, Tél B, Farkas N, Garami A, Vincze Á, Bajor J, Sarlós P, **Márta K**, Erős A, Mikó A, Szakács Z, Pécsi D, Mátrai P, Hegyi P, Veres G. Eosinophil Counts in the Small Intestine and Colon of Children Without Apparent Gastrointestinal Disease: A Meta-analysis. **J Pediatr Gastroenterol Nutr**. 2018 Jul;67(1):6-12. doi:10.1097/MPG.0000000000001904. PubMed PMID:29394213

IF: 2.752, Q1, original publication

16) Szakács Z, Mátrai P, Hegyi P, Szabó I, Vincze Á, Balaskó M, Mosdósi B, Sarlós P, Simon M, **Márta K**, Mikó A, Pécsi D, Demcsák A, Bajor J. Younger age at diagnosis predisposes to mucosal recovery in celiac disease on a gluten-free diet: A meta-analysis. **PLoS One**. 2017 Nov 2;12(11):e0187526. doi:10.1371/journal.pone.0187526. eCollection 2017. PubMed PMID: 29095937

IF: 2.766, Q1, original publication

17) Szabó IL, Mátics R, Hegyi P, Garami A, Illés A, Sarlós P, Bajor J, Szűcs A, Mosztbacher D, **Márta K**, Szemes K, Csekő K, Kővári B, Rumbus Z, Vincze Á. PPIs Prevent Aspirin-Induced Gastrointestinal Bleeding Better than H2RAs. A Systematic Review and Meta-analysis. **J Gastrointestin Liver Dis**. 2017 Dec;26(4):395-402. doi: 10.15403/jgld.2014.1121.264.hra. Review. PubMed PMID: 29253055.

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18) Mosztbacher D, Farkas N, Solymár M, Pár G, Bajor J, Szűcs Á, Czimmer J, **Márta K**, Mikó A, Rumbus Z, Varjú P, Hegyi P, Párniczky A. Restoration of energy level in the early phase of acute pediatric pancreatitis. **World J Gastroenterol.** 2017 Feb 14;23(6):957-963. doi: 10.3748/wjg.v23.i6.957. Review. PMID:28246469

IF: 3.3, Q1, knowledge publication

19) Rumbus Z, Matics R, Hegyi P, Zsiboras C, Szabo I, Illes A, Petervari E, Balasko M, **Marta K**, Miko A, Parniczky A, Tenk J, Rostas I, Solymar M, Garami A. Fever Is Associated with Reduced, Hypothermia with Increased Mortality in Septic Patients: A Meta-Analysis of Clinical Trials. **PLoS One**. 2017 Jan 12; 12(1): e0170152. doi: 10.1371/journal.pone.0170152. eCollection 2017 PMID:28081244

IF: 2.766, Q1, original publication

20) Huszár O, Kokas B, Mátrai P, Hegyi P, Pétervári E, Vincze Á, Pár G, Sarlós P, Bajor J, Czimmer J, Mosztbacher D, **Márta K**, Zsiborás C, Varjú P, Szücs Á. Meta-Analysis of the Long Term Success Rate of Different Interventions in Benign Biliary Strictures. **PLoS One**. 2017 Jan 11;12(1):e0169618. doi: 10.1371/journal.pone.0169618. eCollection 2017. PMID:28076371

IF: 2.766, Q1, original publication

21) Tenk J, Mátrai P, Hegyi P, Rostás I, Garami A, Szabó I, Solymár M, Pétervári E, Czimmer J, **Márta K**, Mikó A, Füredi N, Párniczky A, Zsiborás C, Balaskó M. In Obesity, HPA Axis Activity Does Not Increase with BMI, but Declines with Aging: A Meta-Analysis of Clinical Studies. **PLoS One**. 2016 Nov 21;11(11):e0166842. doi: 10.1371/journal.pone.0166842. eCollection 2016. PMID: 27870910

IF: 2.806, Q1, original publication

22) Szentesi A, Tóth E, Bálint E, Fanczal J, Madácsy T, Laczkó D, Ignáth I, Balázs A, Pallagi P, Maléth J, Rakonczay Z Jr, Kui B, Illés D, **Márta K**, Blaskó Á, Demcsák A, Párniczky A, Pár G, Gódi S, Mosztbacher D, Szücs Á, Halász A, Izbéki F, Farkas N, Hegyi P; Hungarian Pancreatic Study Group. Analysis of Research Activity in Gastroenterology: Pancreatitis Is in Real Danger. **PLoS One**. 2016 Oct 24;11(10):e0165244. doi: 10.1371/journal.pone.0165244. eCollection 2016. PMID:27776171

IF: 2.806, Q1, original publication

I.3 Scientific metrics

Number of publications **related to the subject** of the thesis:	5	(4 first author)
Cumulative impact factor of publications related to the thesis:	15.714	(12.32 first author)
Q1: 5, Q2: 0, Q3: 0, Q4: 0		
Number of **total accepted/published** articles:	22	(4 first author)
Cumulative impact factor of the published articles:	64.066	(12.32 first author)
Q1: 20, Q2: 2, Q3: 0, Q4: 0		
Number of total citation by **Google Scholar**	76	
https://scholar.google.hu/citations?hl=en&user=ajPL8rgAAAAJ		
Hirsch Index	5	
Number of total citation by **MTM2**	56	
https://m2.mtmt.hu/frontend/#view/Publication/SmartQuery/1127/		
Hirsch Index	4	

II. List of abbreviations

A70 – above 70 years
ABP – acute biliary pancreatitis
AE – adverse event
AP – acute pancreatitis
APACHE – Acute Physiology and Chronic Health Evaluation
BALI – BUN, Age, LDH, IL-6
BISAP – Bedside Index for Severity in Acute Pancreatitis
BMI – body mass index
CCI – Charlson Comorbidity Index
CI – confidence interval
CRF – case report file
CRP – C-reactive Protein
DCP – data cleaning plan
DMP – data management plan
DQF – data query form
eCRF – electronic clinical report form
EN – enteral nutrition
ES – effect sizes
GOULASH – the name of the study: general utilization of early energy administration in acute pancreatitis.
HPSG – Hungarian Pancreatic Study Group
ICU – intensive care unit
IDMB – Independent data management and biostatistics provider company
IQR – interquartile range
ITAB – International Translational Advisory Board
ITT – Intention to Treat
JNP– Japanese Severity Score
LOH – length of hospital stay/hospitalization
MAP – mild and moderate AP
MOF – multi-organ failure
NG – nasogastric
NJ – nasojejunal
OR – odd's ratio
PCT – procalcitonin
PN – parenteral nutrition
PPS – Per Protocol Set
PRISMA – preferred reporting items for systematic review and meta-analysis statement
SAE – severe adverse event
SAP – severe AP
SAPS II – Simplified Acute Physiology Score
SAS – Safety Analysis Set
SC – Steering Committee
SD – standard deviation
TPN – total parenteral nutrition
U20 – under 20 years
WBC – white blood cell count

III. Preface and General Introduction

III.1 Physiology of the pancreas and pathophysiology of acute pancreatitis

The pancreas is an organ which plays a crucial role in the digestive and endocrine systems. The exocrine part of the pancreas (digestive system) secrets approximately 1.5 liters of bicarbonate and enzyme-rich fluid into the duodenum where they break down carbohydrates, lipids, and proteins. The endocrine part of the pancreas secrets hormones including insulin and glucagon into the blood vessels to regulate the glucose homeostasis of the body.

Of course, derangement of either part of the organ can lead several diseases including pancreatitis, diabetes or pancreatic cancer. In this thesis, we decided to focus on the sudden inflammation of the pancreas namely acute pancreatitis (AP). We must state at the beginning that AP is one of the most challenging gastrointestinal disorders for several reasons:

(1) its development is not fully understood[6];

(2) it has no specific therapy[7];

(3) its incidence rate is continuously increasing[8]; and

(4) it has an unacceptably high mortality[9].

Our current understanding of pancreatitis is that toxic factors including bile acids, fatty acids, and alcohol damage the function of both acinar and ductal cells leading to intrapancreatic enzyme activation, cell death, and autodigestion of the organ. In recent years, our knowledge of the cellular mechanisms that play a crucial role in the development of the disease has improved. Until now, the following mechanisms have been proved in disease development:

(1) impaired autophagy[6-7]

(2) trypsinogen activation[6-7]

(3) excessive Ca^{2+} influx[6-7]

(4) calcineurin activation[6-7]

(5) mitochondrial dysfunction[6-7]

(6) cystic fibrosis transmembrane conductance regulator (CFTR) inhibition[6-7]

Experimental data showed that direct administration of ATP into the cells restored their functions including CFTR activity and prevented cell death. All in all, the results in basic science have demonstrated the crucial role of energy breakdown in the early phase of AP.

Therefore, targeting mitochondria and energy homeostasis may lead to the first specific therapy in AP[6-7].

III.2 Clinical features of acute pancreatitis

III.2.1 Diagnosis

Concerning the clinical points, all patients with AP should be admitted to hospital. The definition of AP is based on the 2/3 rules. At least two of the following criteria must be present to diagnose AP[8-9]:

(1) clinical feature (upper abdominal pain),

(2) laboratory measurement (serum amylase or lipase >3x upper limit of normal)

(3) imaging (CT, MRI, ultrasonography) alterations such as edema or intraabdominal fluid

III.2.2 Risk assessment

During the length of hospitalization continuous risk assessment is needed and when indicated the patients must be transferred to an intensive care unit. The outcome of AP is largely influenced by the comorbidities of the host, including but not restricted to metabolic syndrome, liver, and cardiac diseases. Metabolic syndrome is characterized by the clustering of abdominal obesity, hypertrigliceridemia, low levels of high-density lipoprotein, elevations in blood pressure and fasting glucose, or diabetes. These comorbidities are associated with an increased risk of development of severe AP and death from cardiovascular disease and chronic kidney disease during AP suggesting that risk assessment must be included in the general investigations at admission.

III.2.3 Therapy and outcome

Concerning the therapy of AP, the situation is not optimal, since currently, we have no specific drugs in pancreatitis. The general treatments are mainly supportive including fluid replacement, pain management, enteral feeding, antibiotic treatment and when indicated endoscopic, radiological or surgical interventions.

The hardest endpoint of AP has a variable severity ranging from mild and self-limited to severe and fatal. The mortality of the disease ranges approximately from 2 to 5% and depends on the development of organ failures and local complications, which are summarized in the revised Atlanta classification.

III.3 Motivation of this Ph.D. thesis

Unfortunately, gastrointestinal scientists are devoting ever less attention to AP[10]. In the last decades, it's turned out that most of the deteriorating events happen in the first 24h, which largely determine the outcome of the disease [11,12]. Therefore, we must accept the fact that AP is a "door to the needle" disease such as stroke or myocardial infarction. It is almost needless to say that based on the literature data we must

(1) predict the severity of the disease on admission; and importantly

(2) start the treatment of the patients as early as we can.

Therefore, when I joined to Professor Hegyi's workgroup in January 2016 and we decided to focus on the above mentioned clinical challenges. During my Ph.D. period we not only could make important discoveries, but I had a unique chance to learn the basics of Translational Medicine including the modern clinical methodology. In Chapter I, we concentrated on severity prediction, whereas in Chapter II we focused on early management.

IV. Chapter I

IV.1 Introduction

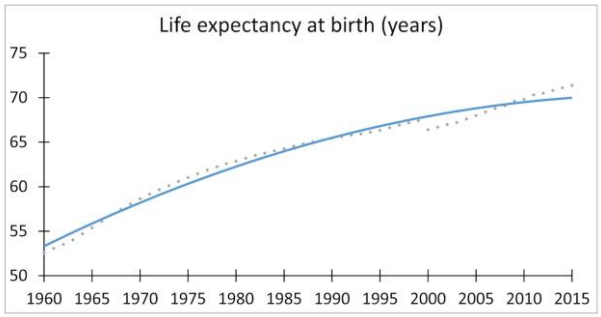


Figure 1 Life expectancy at birth. There is a steadily rising average life expectancy at birth. It has dramatically risen by 16 years (from 55.4 to 71.4y) in the last half-century. Data sources: between 1960 and 1999, World Bank; between 2000–2015, WHO.

Acute pancreatitis is an inflammatory disease mostly caused by alcohol consumption or biliary obstruction. Genetic alterations can also influence the disease development, therefore, not surprisingly, the disease is often called "*multiple hits on multiple targets*" disease.

The annual incidence of acute pancreatitis (AP) ranges from 10 to 100 cases per 100,000 persons 11, showing an increasing tendency throughout the past decades [12]. Multiple theories have been proposed to explain the increment: better diagnostics (e.g., general access to the measurement of pancreatic enzymes) [13], lifestyle factors (e.g., obesity, alcohol consumption, and tobacco use) [14,15] as well as ageing of the population [16] have been implicated.

Life expectancy has dramatically risen by 16 years (from 55.4 yrs to 71.4 yrs) in the last half-century, causing a number of changes and challenges to economies and healthcare systems (*Figure 1*). Needless to say, healthcare professionals should focus more intensively on the effects of ageing on the course and outcome of diseases.

Age is used as a predictive marker in different scoring systems for AP (*Table 1*). These scoring systems show a great variety in the age group: in the (i) Bedside Index for Severity in Acute Pancreatitis score (BISAP) [17], the topmost risk of age is above 60; (ii) in BALI (BUN, Age, LDH, IL-6), it is over 65 [18]; (iii) in the Simplified Acute Physiology Score (SAPS II), it

Score system	Publ. (year)	Outcome	Time at measurement	Age cutoff	Patient enrolment	LEB	Age	
							Med.	Mean
Ranson	1974	severity	48h	55	1971-1975	60.12		42 50
APACHE II	1982	severity	24h	45	1979-1981	62.9	–	
SASP II	1993	mortality	last 24h	40	1991	65.6		57.2
JPN	2002	severity	–	70	1995-1998	66.75	–	
BALI	2006	mortality	48h	65	–	–		61 +-16
BISAP	2008	morality	24h	60	2000-2001	66.55	53	

Table 1. Characteristics of the scoring systems. There is a slight elevation in the age of enrolled patients and cut-off values. (LEB: Life expectancy at birth). Ranson [20]; APACHEII–Acute physiology and chronic health evaluation [21]; SAPS II–Simplified Acute Physiology Score [19]; JNP–Japanese Severity Score [22]; BALI–BUN, Age, LDH, IL-6 [18]; BISAP–Bedside Index for Severity in Acute Pancreatitis [17].

is >40 [19]; (iv) in Ranson score, it is above 55 [20]; (v) in Acute Physiology and Chronic Health Evaluation (APACHE II), it is over 45 [21]; and (vi) in the Japanese Severity Score (JNP), it is >70 [22]. The wide range of age limits suggests that a low number of patients, a selection bias or a mathematical inaccuracy could have occurred. In addition, we can not exclude the possibility that hidden factors associated with ageing such as comorbidities play an important role.

It has been shown that the risk of morbidities increases with age [23]. Since the average age of AP onset is around 55-70 years [12,24,] most AP patients are exposed to the burden of comorbidities [25]. Sporadic studies reported on how comorbidities affect the outcomes of AP: they increase mortality [25-29] and the length of hospital stay, as well [25,27,30]. However, the predictive role of comorbidities is underutilized regarding AP severity and the development of complications.

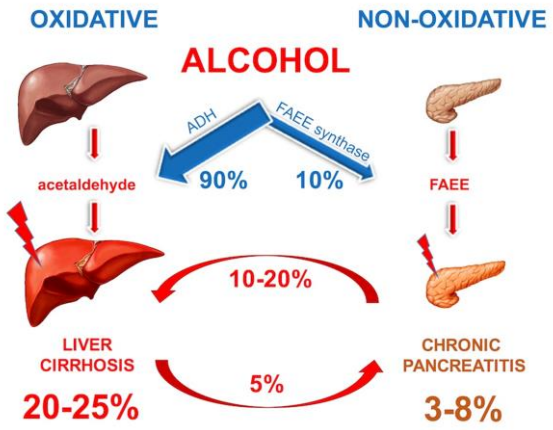


Figure 2. Comorbidities in pancreatitis. Pancreatic and liver diseases are frequently associated with each other. Alcohol is 90% metabolized via the oxidative pathway; liver cirrhosis is, therefore, more frequent in alcoholics than pancreatitis (20-25% versus 3-8%, respectively). In patients in which LC develops first, pancreatitis is

It is also well reported that some of the diseases which develop based on the same etiological background (for example alcohol) are more frequent in AP. National cohort analysis showed variable rates of liver cirrhosis (LC) in alcoholic pancreatitis. The Spanish cohort showed 2% [31], the Czech one 16.7% [32], the Indian one 8.4% [33] and the Italian one 12.5% [34] (*Figure 2*) [2].

Generally, age is included in all, whereas comorbidities are in none of the scoring systems. However, based on the summary described above this strong decision is more than questionable.

IV.2 Aims

We aimed to investigate (1) the effects of ageing and (2) comorbidities on the outcome of AP. Moreover, we wished to understand which factors predict mortality or severity better.

IV.3 Methods

IV.3.1 Methods to answer Aim IV.2.1

We choose the most appropriate clinical methodologies to answer each question. To answer Aim IV.1.1 we needed a preliminary sample size calculation. The event rate of mortality in AP is very low: 3/100. Therefore, it is not surprising that 10-50 thousand of patients would be necessary to answer Aim IV.2.1 precisely. The only possible methodology which is feasible to collect such a high amount of patients is meta-analyses. In this part of the study, we systematically reviewed the literature and performed a detailed meta-analysis.

IV.3.1.1 Study design, participants, interventions, comparators

The meta-analysis was performed using the preferred reporting items for systematic review and meta-analysis statement (PRISMA) [35]. We used the classical PICO format to form a question applicable for search in databases: P: acute pancreatitis; I and C: different age categories (under 20 (U20), 20–29, 30–39, 40–49, 50–59, 60–69 and above 70 (A70)); O: mortality and severity. In order to provide the highest level of quality, the meta-analysis was registered with the PROSPERO registry (CRD42017079253).

IV.3.1.2 Search strategy

A search was performed in three databases (E mbase, PubMed and Cochrane) in January 2017 using the following terms: PubMed: (acute[All Fields] AND ("pancreatitis"[MeSH Terms] OR "pancreatitis"[All Fields])) AND (cohort[All Fields] OR ("clinical trial"[Publication Type] OR "clinical trials as topic"[MeSH Terms] OR "clinical trial"[All Fields])) AND ("Age"[Journal] OR "age"[All Fields] OR "Age (Omaha)"[Journal] OR "age"[All Fields] OR "Age (Dordr)"[Journal] OR "age"[All Fields] OR "Adv Genet Eng"[Journal] OR "age"[All Fields]) Embase: acute pancreatitis and (cohort or clinical trial)

and age; and Cochrane: acute AND pancreatitis AND (cohort OR clinical) AND trial AND age.

IV.3.1.3 Data sources, study selection, and data extraction

Two independent authors read the articles for eligibility (age data from cohort and pilot studies). The flow diagram recommended by the PRISMA guidelines shows the article selection procedure (*Figure 3*) [35]. When conflicts arose, a third participant made the decision. Two authors collected data in an Excel file (Microsoft Corporation, Redmond, WA98052, USA) according to age (mean, median, range, standard deviation (SD) and interquartile range (IQR), where possible), study type, severity, mortality, and notes.

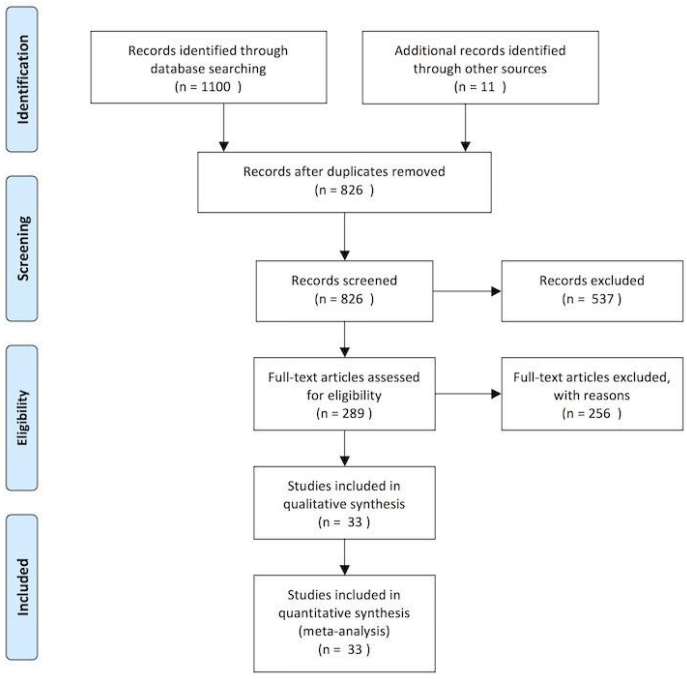


Figure 3. PRISMA flow diagram. The diagram for the study selection for this meta-analysis is based on the PRISMA-recommended flow chart [35]

IV.3.1.4 Data analysis

All meta-analytic calculations were performed with STATA software Version 11 (Stata Corporation, College Station, TX, USA). In our meta-analysis, the pooled effect sizes (ES) were the event rates with a 95% confidence interval (CI) for all outcomes. The random effect model by DerSimonian and Laird was used in all cases [36]. Heterogeneity was tested using Cochrane's Q and the I^2 statistics. I^2 statistics represent the percentage of effect size heterogeneity, which cannot be explained by random chance, but by other factors. I^2 values of 25, 50 and 75% corresponded to low, moderate and high degrees of heterogeneity, based on the Cochrane handbook [37]. If the Q test is significant, it implies that the heterogeneity among effect sizes reported in the observed studies is greater than could be explained only by random error. We considered the Q test significant if $p<0.1$. The forest plot was evaluated to represent the data. Publication bias was examined by visual inspection as asymmetry in the funnel plot and Egger's test [38]. A significant test result ($p<0.1$) indicates the presence of bias.

A meta-regression was used to consider the effect of ageing on mortality and severity. In both cases, we tested the hypothesis that all coefficients are zero. The results are provided as regression coefficients, 95% CIs, p-values and the explained variances of the models (R^2 analogs).

A conventional regression analysis was also performed to confirm the results of the meta-regression. In this case, we used the pooled event rates from the subgroup analyses and the middle of the age subgroups as independent variables. We used the IBM SPSS Statistics software for these calculations (IBM Corporation, Armonk, New York, USA, Version 24).

IV.3.1.5 Quality assessment

The quality of the articles was assessed by 3 main categories recommended by the modified Newcastle-Ottawa scale (*Table 2*).

IV.3.2 Methods to answer Aim IV.2.2

Study	Sample Size	Severe Case	Mortality	Study type	modified Newcastle-Ottawa Quality Assessment Scale									
					Selection				Comparability	Outcome				Sum
					S1	S2	S3	S4	C1	O1.1	O1.2	O2	O3	
Abou-Assi et al 2002	156	5	14	Prospective	1	1	0	1	1	0	1	1	1	7
Albulushi et al 2014	174	14	0	Retrospective	1	1	0	0	1	0	1	1	1	6
Beltrán et al 2013	24	1	0	Retrospective	1	1	1	1	1	0	1	1	1	8
de-Madaria et al 2014	403	28	17	Prospective	1	1	1	1	0	1	0	1	1	7
Dombernowsky et al 2016	359	nd	13	Retrospective	1	1	1	1	1	0	1	1	1	8
Gompertz 2012	128	nd	2	Retrospective	1	1	1	1	0	0	0	1	1	6
Gompertz 2013	1367	nd	115	Retrospective	1	1	1	1	0	1	0	1	1	7
González-González 2012	605	nd	30	Prospective	1	1	1	1	1	1	1	1	1	9
Gornik et al 2013	1058	210	41	Prospective	1	1	0	1	0	0	1	1	1	6
Gürleyiek et al 2005	55	13	1	Prospective	1	1	1	1	1	1	1	1	1	9
Karpavicius et al 2016	102	20	5	Prospective	1	1	1	1	0	1	0	1	1	7
Knoeplifi et al 2006	310	63	8	Prospective	1	1	1	1	1	1	0	1	1	8
Lautz et al 2011	211	nd	0	Retrospective	1	1	1	1	0	1	1	1	1	8
Milherio et al 1994	91	nd	10	Retrospective	1	1	0	1	0	0	0	1	1	5
Mole at el 2016	2053	390	102	Retrospective	1	1	0	1	0	0	1	1	1	6
Muller et al 2006	109	66	8	Prospective	1	1	1	1	1	1	1	1	1	9
Nijmeijer et al 2013	622	119	20	Prospective	1	1	0	1	0	0	0	1	1	5
Ocampo et al 2015	854	140	nd	Prospective	1	1	0	1	1	0	1	1	1	7
Pant et al 2014	55012	nd	509	Retrospective	1	1	0	0	0	0	0	1	1	4
Parniczky et al 2016	600	53	17	Prospective	1	1	1	1	1	1	1	1	1	9
Radenkovic et al 2009	91	24	8	Prospective	1	1	1	1	0	1	0	1	1	7
Rashidi et al 2016	670	43	37	Prosp and Retrosp	1	1	1	1	1	1	0	1	1	8
Spanier et al 2013	78257	nd	9515	Retrospective	1	1	0	1	1	0	1	1	1	7
Uomo et al 2007	1173	167	36	Prospective	1	1	1	1	0	1	1	1	1	8
Waele et al 2007	40	14	6	Retrospective	1	1	1	1	0	1	0	1	1	7
Wang et al 2015	120	31	13	Retrospective	1	1	0	1	0	0	0	1	1	5
Wei Ho et al 2015	12284	765	nd	Retrospective	0	1	0	1	0	0	0	1	1	4
Weitz et al 2016	346	21	12	Retrospective	1	1	1	1	0	1	1	1	1	8
Wu et al 2008	36178	nd	569	Retrospective	1	1	0	0	0	0	0	1	1	4
Yeung et al 1995	43	nd	1	Retrospective	1	1	0	0	1	0	1	1	1	6
Yue et al 2015	169	68	nd	Prospective	1	1	0	1	0	0	0	1	1	5
Zhang et al 2016	974	223	58	Retrospective	1	1	1	1	0	1	1	1	1	8
Zuidema et al 2014	64	11	3	Prospective	1	1	0	1	0	0	0	1	1	5

Table 2. The modified Newcastle–Ottawa Quality Assessment Scale. Ranks in three categories (green-1: low risk; red-0: high risk; yellow-0: unclear risk) are shown. S1: non-selected etiology AP; S2: all participants have an AP diagnosis; S3: AP diagnosis is confirmed using the latest guidelines; S4: non-selected severity cases. C1: comparability defined by exact age ranges in years. O1.1: severity assigned according to the latest guidelines; O1.2: described mortality (in-hospital and pancreas-related); O2–O3: adequate follow-up for outcome occurrence morality and severity.

In order to understand the effects of comorbidities on the outcome of AP detailed clinical data are necessary. We have performed a preliminary literature search which revealed that unfortunately such clinical data are not provided in the articles. Therefore, performing a meta-analysis is not feasible. To answer Aim II.1.2 we needed to get access to a high-quality AP cohort. Since one of the biggest international AP registries run by the Hungarian Pancreatic Study Group, we had no difficulties to access the necessary clinical data.

IV.3.2.1 Population of the cohort analysis

As mentioned above, we extracted data from the International AP Registry established in 2011 by the Hungarian Pancreatic Study Group in order to advance clinical care and research in Pancreatology [7]. AP Registry contains data on consecutive cases of AP attending several Hungarian centers between 2011 and 2017. Accuracy of data recorded is secured by a four-level quality check system involving both medical administrative personnel and gastroenterologist specialists.

IV.3.2.2 Comorbidities

Registry forms of AP cases involve an admission form (A form) and follow-up forms (B forms) covering the entire hospital stay, as well as the de-identified electronic discharge files. All files were carefully reviewed by an author with a medical degree to aggregate Charlson Comorbidity Index (CCI) [39] with the International Classification of Diseases 9/10 coding algorithm [40]. No search engines were used when reviewing charts. CCI items were dedicated to rating common chronic pre-existing diseases along 19 health-related (groups of) conditions. Every CCI item has a weight according to the severity of comorbidities covered [39]. CCI of each case was calculated by compiling the weighted items. Earlier studies proved that CCI is an effective predictor of hard outcomes in several acute and chronic conditions [41-43].

IV.3.2.3 Eligibility criteria

To be included in analysis, the following criteria should be met; 1) diagnosis of AP ('Two out of three') [44] i) abdominal pain, ii) serum amylase and/or lipase greater than three times the upper normal limit and iii) characteristic findings on abdominal cross-sectional imaging, 2) age ≥ 18 years and 3) available history for CCI [39].

IV.3.2.4 Outcomes

Our AP-related outcomes included in-hospital mortality, severity, length of hospitalization (LOH), local complications (including peripancreatic fluid collections, pseudocysts, and pancreatic necrosis), and organ failure (including respiratory, renal, and cardiac failure).

IV.3.2.5 Ethical approval

AP Registry has been approved by the Scientific and Research Ethics Committee of the Medical Research Council (22254-1/2012/EKU).

IV.3.2.6 Statistical analysis

An expert biostatistician carried out the analysis with SPSS 19.0.0 (IBM Analytics, US). Case numbers and percentages were calculated for categorical variables, medians with 25% and 75% quartiles (Q_1 and Q_3, respectively) and ranges were computed for numerical variables in descriptive analysis (due to non-normal distribution of data indicated by the Kolmogorov–

Smirnov test). In all analysis, a probability (p) <0.05 indicated a significant difference, whereas a p-value between 0.05 and 0.10 indicated borderline significance.

Representativeness of the study population was tested by binomial, one sample median, and Goodness-of-fit $\chi 2$ tests.

In univariate analysis, Spearmann's rho was calculated to explore correlations between age, CCI, and LOH. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated from 2x2 tables. If OR was not calculable, association were investigated with chi^2- or Fisher's tests.

In multivariate analysis, binary logistic and multinominal regressions were used to investigate the joint effect of age categories and CCI categories or that of age categories and individual comorbidities. We used a three-level age-stratification (young-aged between 18 and 34 years of age, middle-aged between 35 and 64 years of age, and old-aged ≥65 years of age) and a four-level comorbidity stratification (none if CCI=0, mild if CCI=1, moderate if CCI=2, and severe if CCI≥3).

IV.4 Results

Our systematic search yielded 1100 articles (704, 379 and 17 in Embase, PubMed, and Cochrane, respectively) (*Figure 3*). Eleven additional articles were found with potential data eligibility for the meta-analysis in the references of the primarily selected articles. After excluding duplicates and irrelevant articles, a total of 33 articles involving 194 702 patients met the inclusion criteria (*Table 2*).

IV.4.1 The effects of ageing on the severity of AP

Age	Severe AP	Patient no.	%
U20	1	24	4.2
20-29	0	36	0.0
30-39	5	75	6.7
40-49	726	7882	9.2
50-59	1352	11933	11.3
60-69	390	2344	16.6
A70	15	157	9.6
SUM	2489	22451	11.1

Table 3. Data of patient's number and severe cases in age groups. There was only one severe AP in patients under 30; however, the incidence of severe AP rose continuously between ages 30 and 70.

A total of 23 studies with 22451 patients were suitable for analyzing severity (*Table 3*) [45-67]. Two thousand four hundred eighty-nine severe cases were found divided into seven age groups with a low severity rate under 30 years. There was a low incidence severe AP rate in patients under 30 and rose continuously between ages 30 and 70 (*Table 3*).

Firstly, a meta-regression was performed to investigate the relationship between age and severity (*Figure 4*). The number of patients in each age group category was extremely diverse (between 24 and 11 933); however, a significant relationship was detected (coefficient: 0.035 CI: 0.019–0.052, p<0.001; adjusted r^2: 31.6%). A conventional regression analysis was also performed showing a **linear increase** (0.193%/year) from ages U20 to A70 (*Figure 5*).

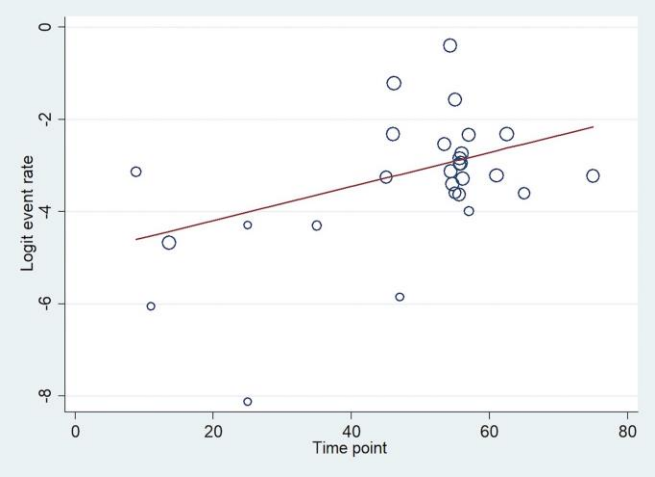


Figure 4. Meta-regression of severity. The figure shows 29 data from 23 reports where x= age (mean), y=logit event rate: ln(p/(1-p)), and circle diameters show the weight of each study based on the random effect model. The meta-regression shows a significant (p<0.001) relationship between age and severity (r^2=31.6), therefore the risk for developing severe cases is elevated by ageing.

This continuous elevation was also confirmed by forest plot (*Figure 6*). There was 1 severe AP U20: 4.2% (1/24; pooled event rate: 0.042 CI: -0.077–0.161); 20–29: 0% (0/36; pooled event rate: 0.014 CI: 0.077–0.104); 30–39: 6.7% (5/75; pooled event rate: 0.067 CI: -0.005–0.128); 40–49: 9.2% (726/7882; pooled event rate: 0.109 CI: 0.046–0.172); 50–59: 11.3% (1352/11 933; pooled event rate: 0.201 CI: 0.158–0.245); 60–69: 16.6% (390/2344; pooled event rate: 0.157 CI: 0.110–0.203); A70: 9.6% (15/157; pooled event rate: 0.096 CI: 0.049–0.143). In sum, 11.1% (2489/22 451).

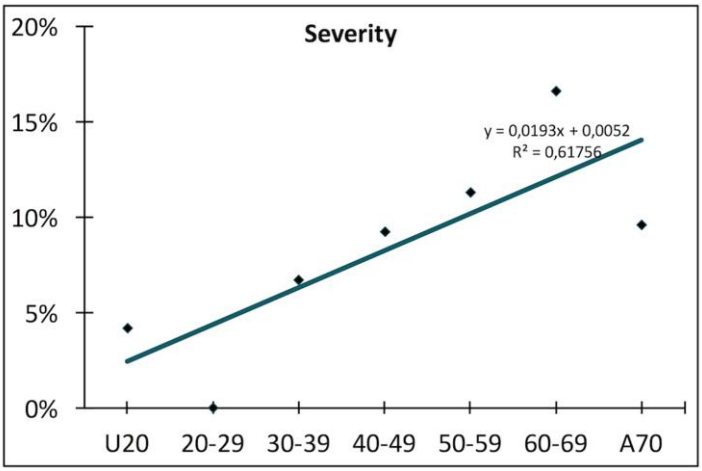


Figure 5. Conventional regression of severity. The conventional regression, which is independent of distortion from diverse numbers of patients, shows a linear rise (0.193%/year) in severity from young to old age.

Publication bias was tested by inspection of funnel plot and Egger's test (CI: 1.961–6.728; p=0.001). The visible asymmetry (plots are mostly concentrated on the right side) is most probably due to the fact that authors mostly present data with high volume examinations (*Figure 7*).

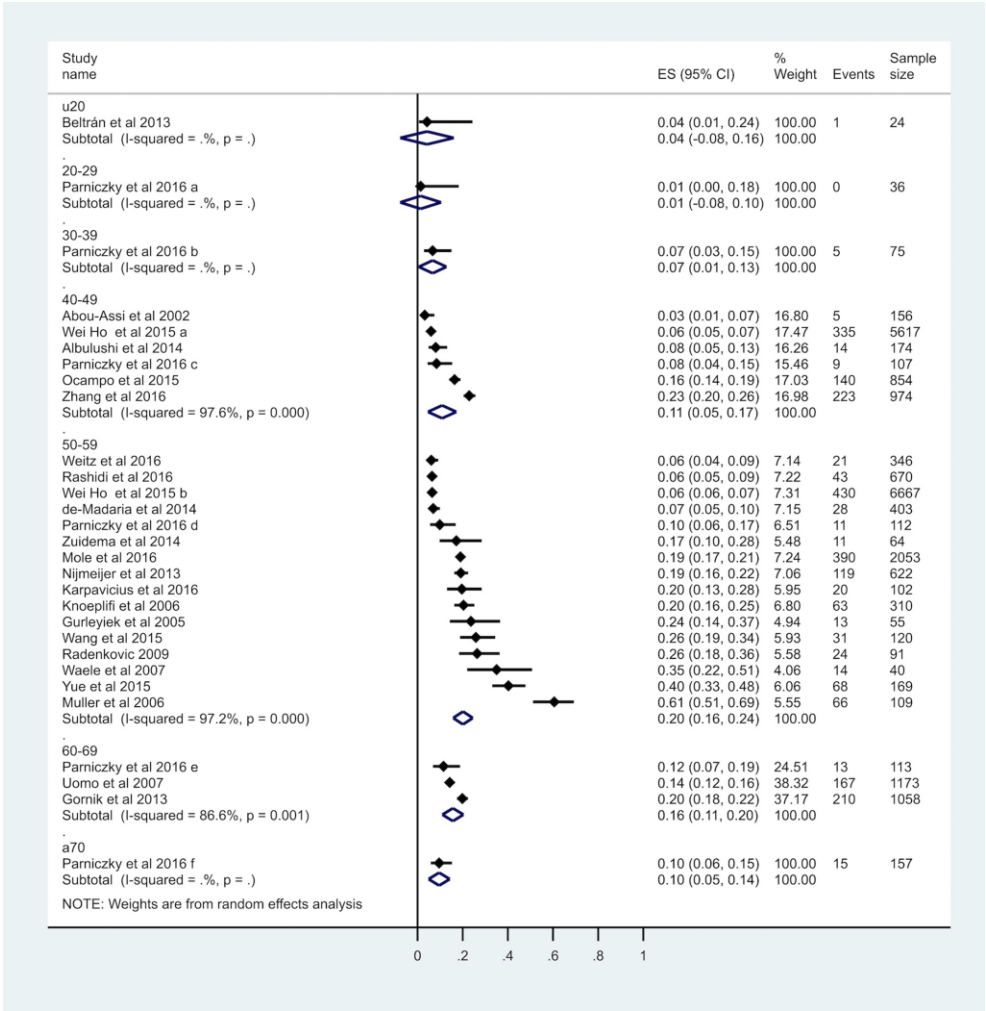


Figure 6. Forest plot results for cut-off values for severity. Summary table of pooled effect with CI and significance levels to detect cut off value.

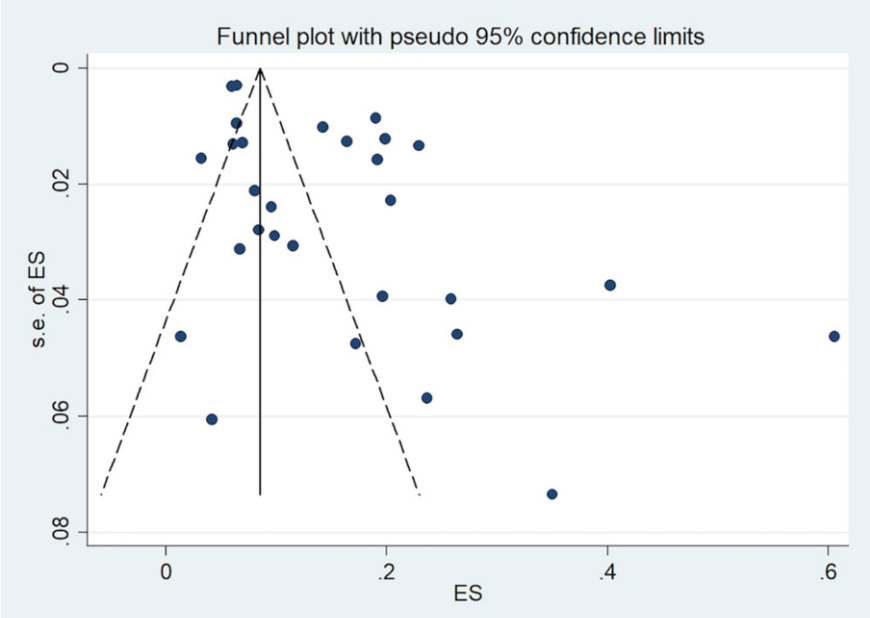


Figure 7. Funnel plot of severity in terms of publication bias. Funnel plots represent the standard error (SE) plotted against event rates (ES) for each study. The dotted line shows the 95% confidence limits. Plots are mostly on the right side showing that publication bias might present (CI: 1.961–6.728; p=0.001)

The cut-off values in sorting articles to U20 and A20, U30 and A30, U40 and A40, U50 and A50, U60 and A60, and U70 and A70 (*Figure 8-9*) resulted in significant differences considering three comparison, respectively (U30 vs. A30 p=0.036; U40 vs. A40 p=0.009; U50 vs. A50 p=0.021) (*Table 4*).

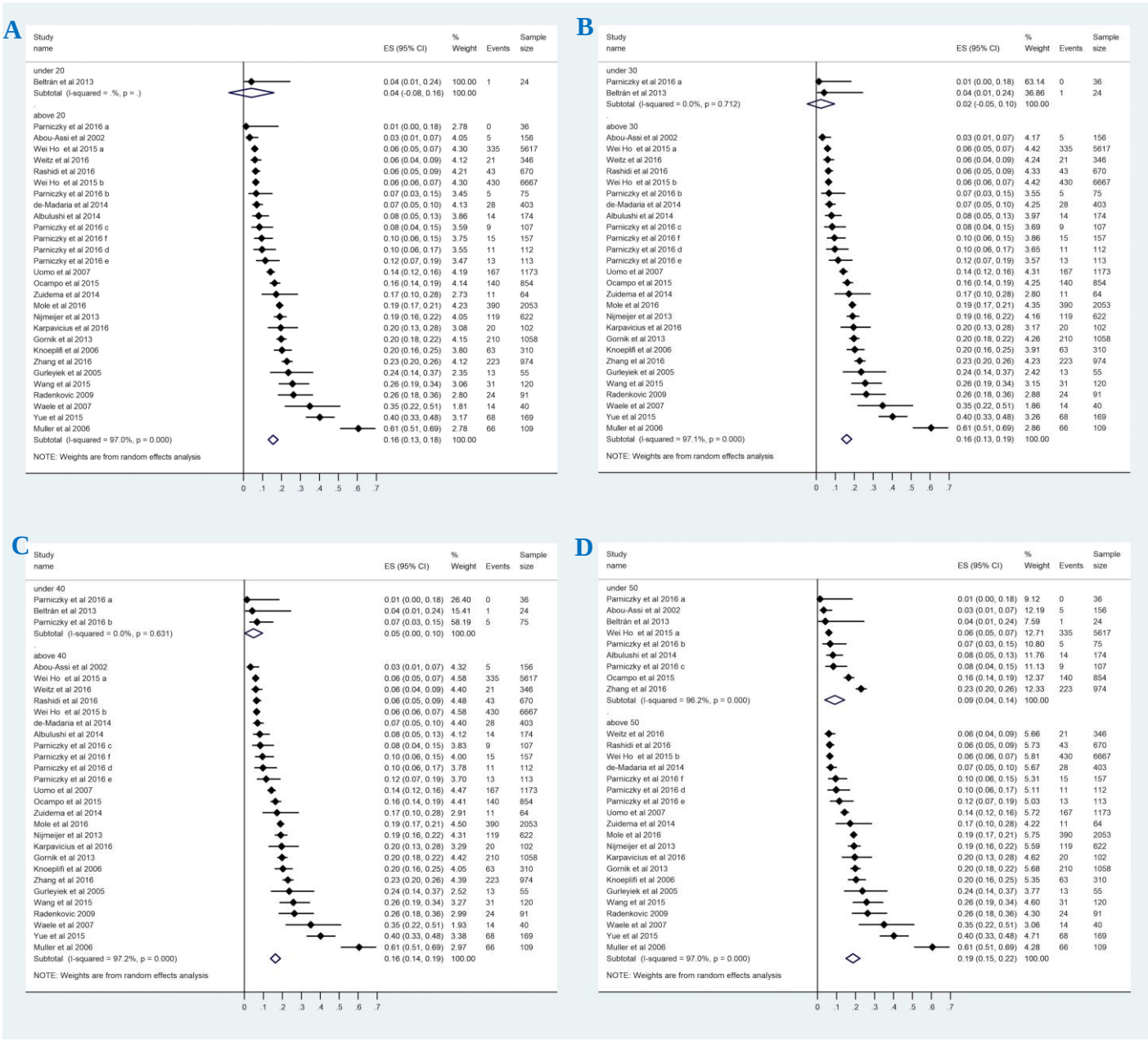


Figure 8. Forest plot of studies evaluating severity at A: age U20 compared to A20. Full diamonds show the weighted event rates for studies respectively, the line represents the 95% confidence interval (CI), and empty diamonds show the pooled results of severe cases. A non-significant difference can be observed in severity under 20 and above 20 (p=0.188). **B: age U30 compared to A30.** Full diamonds show the weighted event rates for studies respectively, the line represents the 95% confidence interval (CI), and empty diamonds show the pooled results of severe cases. A non-significant difference can be observed in severity under 30 and above 30 (p=0.036). **C: age U40 compared to A40.** Full diamonds show the weighted event rates for studies respectively, the line represents the 95% confidence interval (CI), and empty diamonds show the pooled results of severe cases A significant difference can be observed in severity under 40 and above 40 (p=0.009). **D: age U50 compared to A50.** Full diamonds show the weighted event rates for studies respectively, the line represents the 95% confidence interval (CI), and empty diamonds show the pooled results of severe cases. A significant difference can be observed in severity under 50 and above 50 (p=0.021).

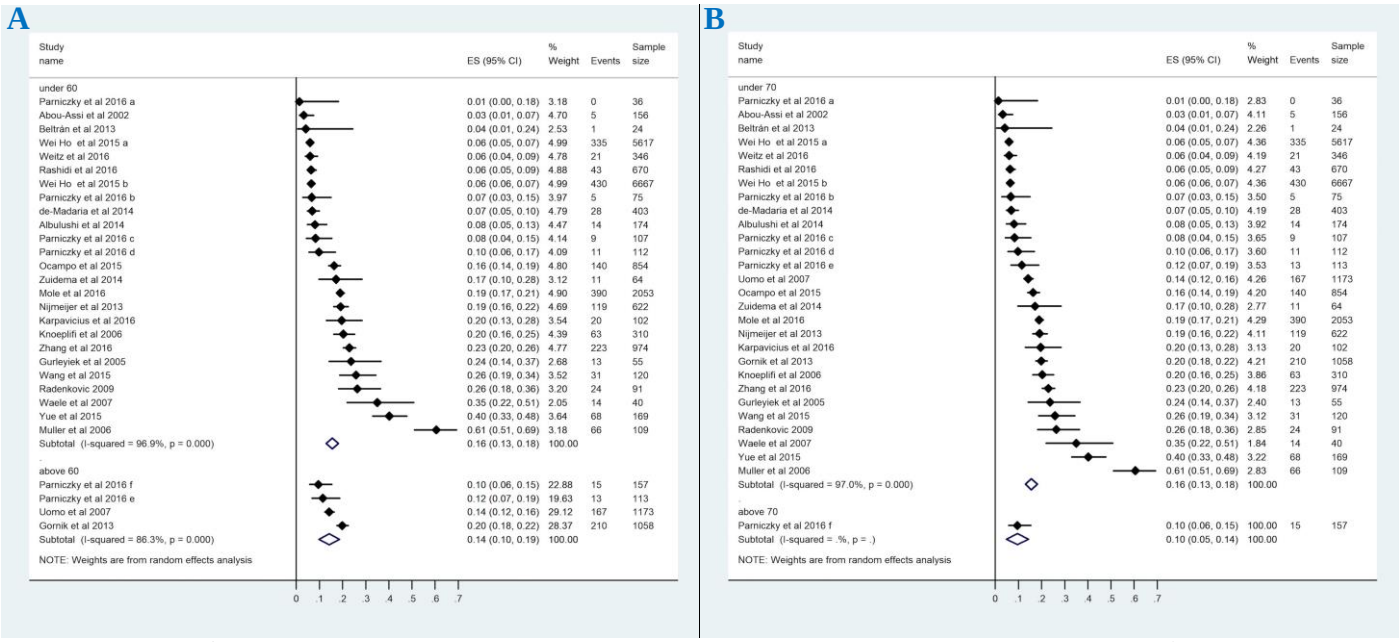


Figure 9. Forest plot of studies evaluating severity at A: age U60 compared to A60. Full diamonds show the weighted event rates for studies respectively, the line represents the 95% confidence interval (CI), and empty diamonds show the pooled results of severe cases. A non-significant difference can be observed in severity under 60 and above 60 (p=0.994). **B: age U70 compared to A70.** Full diamonds show the weighted event rates for studies respectively, the line represents the 95% confidence interval (CI), and empty diamonds show the pooled results of severe cases. A non-significant difference can be observed in severity under 70 and above 70 (p=0.133).

Age	Fatal event	Patient no.	%
U20	510	55290	0.9
20-29	5	1912	0.26
30-39	139	11527	1.2
40-49	202	3002	6.7
50-59	838	41790	2.0
60-69	2157	25496	8.5
A70	7319	42378	17.3
SUM	11170	181395	6.2

Table 4. Forest plot results for cut-off values for severity. Summary table of pooled effect with CI and significance levels to detect cut off value. Concerning mortality, all comparisons were significant, however examining severity only 3. An explanation might be that in young ages there is a low event rate, in middle age groups there is a higher proportion, therefore, the difference is equalized leading to a non-significant difference. The same occurs in the aged versus middle-aged groups.

In addition, we performed several sub-group analysis in order to decrease the heterogeneity in our study. Firstly, we used articles only where severity was assessed by the Atlanta or the revised Atlanta classification. This additional analysis could largely decrease the heterogeneity (I^2= 40-49: 0%, 50-59:96.9%, 60-69:86.6% (*data are shown in supplementary figure 9 in article No.1*[1]). Secondly, we excluded the low quality (NOS 4 and 5) studies from the analysis. This analysis also could improve the heterogeneity (I^2= 40-49: 96.3%, 50-59:96.5%, 60-69:86.6% (*data are shown in supplementary figure 10 in article No.1*[1])). And finally, we excluded studies from the analysis where age ranges might overlap between the groups because of given age ranges. We could also successfully decrease the heterogeneity ((I^2= 40-49: 98%, 50-59:97.1%, 60-69:86.6% (*data are shown in supplementary figure 11 in article No.1*[1]). Importantly, none of them modified the outcome of the study which decreases the overall limitations of our results.

Age	Fatal event	Patient no.	%
U20	510	55290	0.9
20-29	5	1912	0.26
30-39	139	11527	1.2
40-49	202	3002	6.7
50-59	838	41790	2.0
60-69	2157	25496	8.5
A70	7319	42378	17.3
SUM	11170	181395	6.2

Table 5. Data of patient's number and deceased cases in age groups. The incidence of severe AP rose continuously between ages 30 and 70.

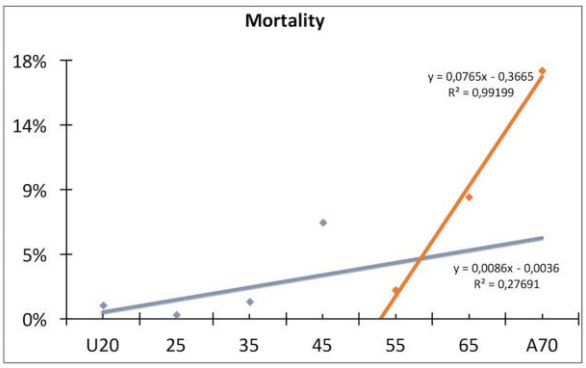


Figure 10. Conventional regression of mortality. The conventional regression shows a linear elevation until 59, however from this age the mortality rate started elevating with 9 times higher rate until the age of 70.

IV.4.2 The effects of ageing on the mortality in AP

30 studies involving 181,395 subjects contained data on mortality (Table 2, 5) [16,45-57,60,62-76]. 11 170 deceased cases were found in the seven age groups with the highest rates in groups 40–49 and A60 (*Table 5*). The mortality rate was 0.9% in patients under 20 and demonstrated a continuous, linear elevation until 59, however from this age the mortality rate started elevating with 9 times higher rate until the age of 70 (*Figure 10*). The mortality rate grew 0.086%/year between ages 20 and 59 and 0.765%/year between 59 and 70 (*Figure 10*). Overall, patients above 70 had a mortality rate 19 times higher than those under 20 (*Table 5*). The mortality rate rising with age was also confirmed by forest plot, showing a clear elevation from pediatric to elderly patients: U20: 0.9% (510/55 290; pooled event rate: 0.009 CI: 0.008–0.010); 20–29: 2.6% (5/1912; pooled event rate: 0.009 CI: -0.011–0.029); 30–39: 1.2% (139/11 527; pooled event rate: 0.012 CI: 0.010–0.014); 40–49: 6.7% (202/3002; pooled event rate: 0.052 CI: 0.025–0.079); 50–59: 2% (838/41 634; pooled event rate: 0.045 CI: 0.032–0.057); 60–69: 8.5% (2153/25 452; pooled event rate: 0.052 CI: 0.015–0.088); and A70: 17.3% (7312/42 322; pooled event rate: 0.112 CI: 0.007–0.217) (*Figure 11*). In summary, 6.2% (11 170/181 395).

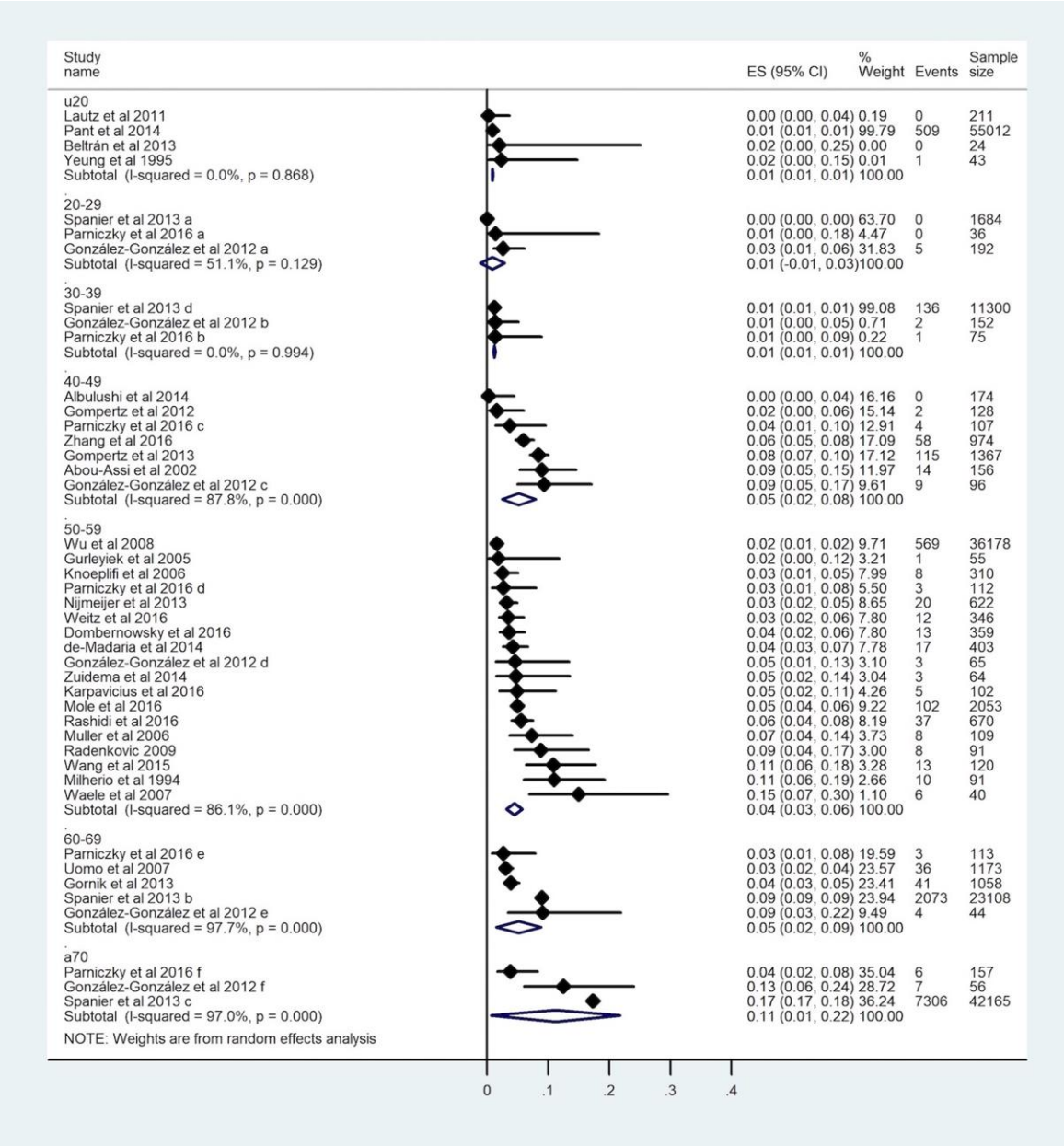


Figure 11. Forest plot of studies evaluating mortality in acute pancreatitis. Full diamonds show the weighted event rates for studies respectively, the line represents the 95% confidence interval (CI), and empty diamonds show the pooled results of mortality with a steadily rising frequency from young to an older age.

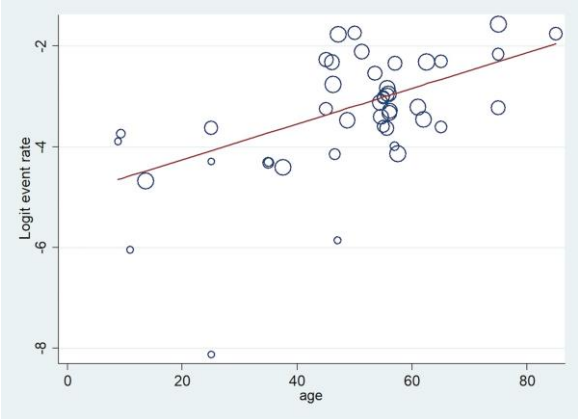


Figure 12. Meta-regression of mortality. The figure shows 43 data from 30 reports where x= age (mean), y=logit event rate: ln(p/(1-p)), and circle diameters show the random size of each study. The meta-regression shows a significant relationship (p=0.022) between age and mortality.

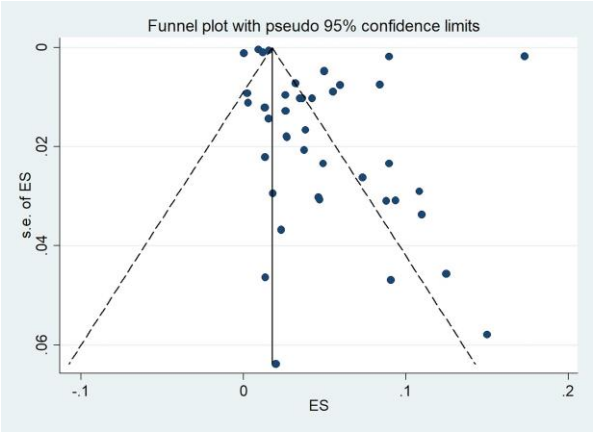


Figure 13. Forest plot results for cut-off values for mortality. Forest plot results from studies evaluating the cut-off values for mortality in acute pancreatitis with significant results in each of four groups. All comparisons showed a significant difference.

A meta-regression analysis on mortality showed a significant difference (coefficient: 0.037 CI: 0.006–0.068, p=0.022; adjusted r^2: 13.8%, *Figure 12*). Publication bias was tested by funnel plot and Egger's test (CI: -0.901–9.234; p=0.104) and showed mild asymmetry, but based on Egger's test publication bias was unlikely (*Figure 13*). Forest plot analyses comparing U20 to A20, U30 to A30, U40 to A40 and U50 vs A50 showed significant differences, respectively (U20 vs. A20 p<0.001; U30 vs. A30 p=0.001; U40 vs. A40 p<0.001; U50 vs. A50 p=0.018; U60 vs. A60 p=0.028, and U70 vs A70 p=0.038) (*Figure 14 and 15*). Forest plot results are summarized in *Table 6*. We excluded the low quality (NOS 4 and 5) studies from the analysis to lower the heterogeneity (I^2= 40-49: 96.3%, 50-59:96.5%, 60-69:86.6% (*data are shown in supplementary figure 19 in article No.1*[1])).

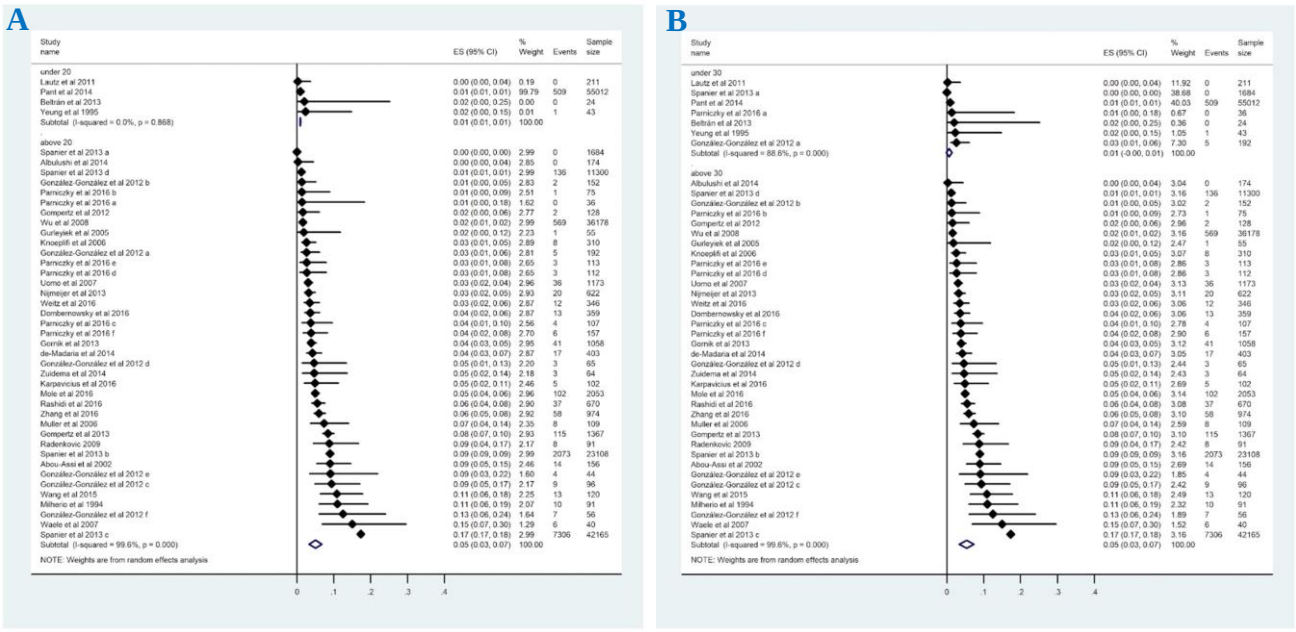


Figure 14. Forest plot of studies evaluating mortality at A: age U20 compared to A20. Full diamonds show the weighted event rates (ER) for studies respectively, the line represents the 95% confidence interval (CI), and empty diamonds show the pooled results of severe cases. A significant difference can be observed in mortality under and above 20 (p<0.001). **B: age U30 compared to A30.** Full diamonds show the weighted ER for studies respectively, the line represents the 95% confidence interval (CI), and empty diamonds show the pooled results of severe cases. A significant difference can be observed in mortality under and above 30 (p=0.001).

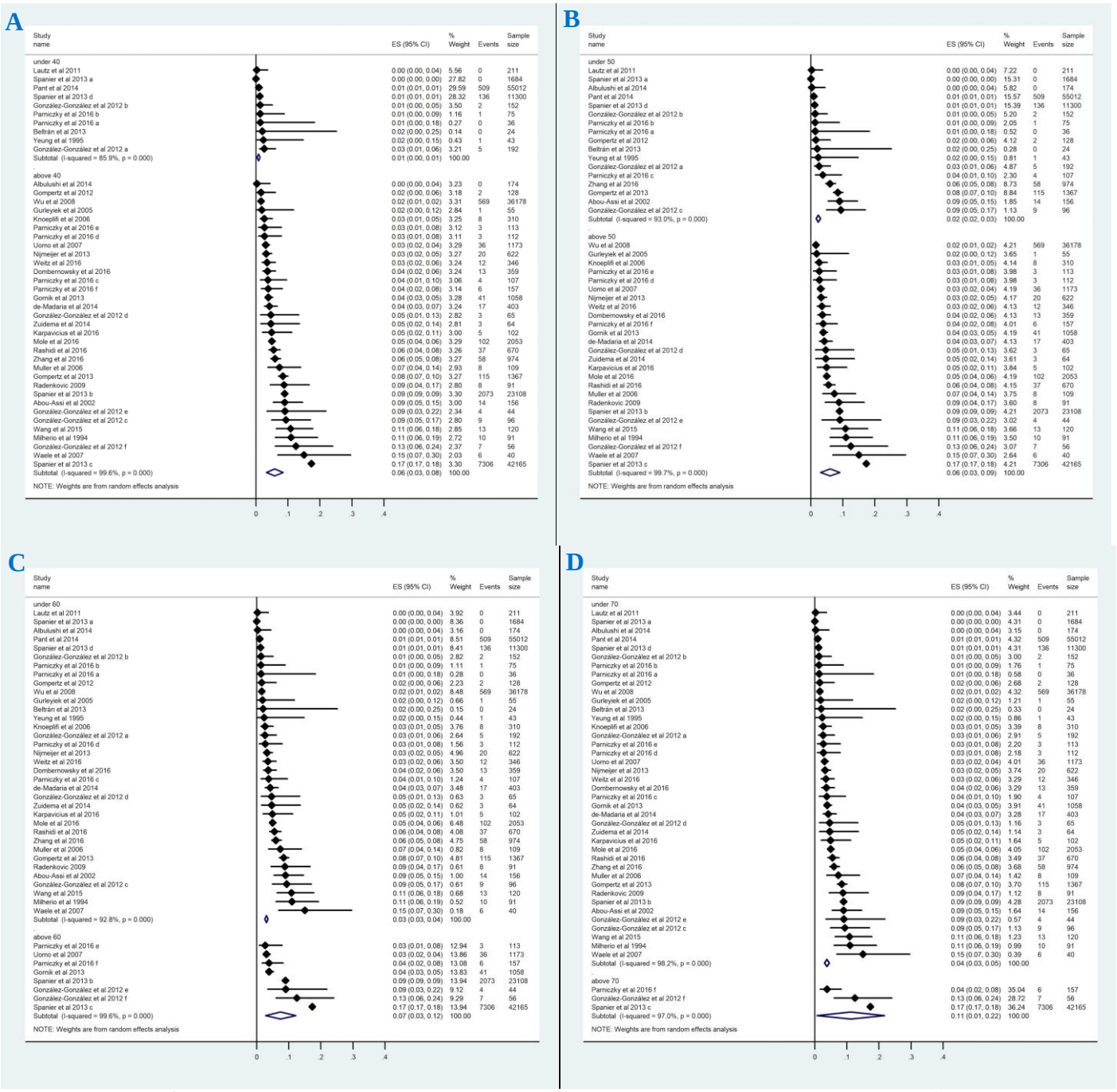


Figure 15 Forest plot of studies evaluating mortality at A: age U40 compared to A40. Full diamonds show the weighted event rates for studies respectively, the line represents the 95% confidence interval (CI), and empty diamonds show the pooled results of severe cases. A significant difference can be observed in mortality under 40 and above 40 (p<0.001). **B: age U50 compared to A50.** Full diamonds show the weighted event rates for studies respectively, the line represents the 95% confidence interval (CI), and empty diamonds show the pooled results of severe cases. A significant difference can be observed in mortality under 50 and above 50 (p=0.018). **C: age U60 compared to A60.** Full diamonds show the weighted event rates for studies respectively, theline represents the 95% confidence interval (CI), and empty diamonds show the pooled results of severe cases. A significant difference can be observed in mortality under 60 and above 60 (p=0.028). **D: age U70 compared to A70.** Full diamonds show the weighted event rates for studies respectively, the line represents the 95% confidence interval (CI), and empty diamonds show the pooled results of severe cases. A significant difference can be observed in mortality under 70 and above 70 (p=0.038).

Age groups	Mortality					Significancy
	ES	95% CI		ES	95% CI	
U20 vs A20	0.01	0.01-0.01		0.05	0.03-0.07	P<0.001
U30 vs A30	0.01	–0.00-0.01		0.05	0.03-0.07	P=0.001
U40 vs A40	0.01	0.00-0.01	vs	0.06	0.03-0.09	P<0.001
U50 vs A50	0.02	0.02-0.03		0.06	0.03-0.09	P=0.018
U60 vs A60	0.02	0.01-0.02		0.07	0.02-0.12	P=0.028
U70 vs A70	0.04	0.03-0.05		0.11	0.01-0.22	P=0.038

Table 6. Forest plot results for cut-off values for mortality. Forest plot results from studies evaluating the cut-off values for mortality in acute pancreatitis with significant results in each of four groups. All comparisons showed a significant difference.

IV.4.3 Risk of bias and quality assessment of papers used in the meta-analysis

The risk of bias was examined by funnel plot and Egger's test. The quality of the included articles was assessed by using the modified Newcastle–Ottawa scale as described earlier [77-79].

Two independent investigators have evaluated the articles and classified using clear guidance described in *Table 2*. The following three main categories were applied: (i) selection of study groups (including four subgroups: S1: non-selected etiology AP; S2: all participants have an AP diagnosis; S3: AP diagnosis is confirmed using the latest guidelines; S4: non-selected severity cases); (ii) comparability of the groups (C1: comparability defined by exact age ranges in years); and (iii) outcome of interest (including four subgroups: O1.1: severity assigned by the latest guidelines; O1.2 described mortality (in-hospital and pancreas-related); and O2–O3: adequate follow-up for outcome occurrence, morality, and severity). Each item was marked: green-1: low risk; red-0: high risk and yellow-0: unclear risk of bias. A total of 9 points was the maximum that could be assigned (*Table 2*) [16,45-58,60-71,73-76,80,81]. Whenever different points were given by the investigators a third member of the team made the final decision.

Age, median (Q_1-Q_3)	58 (44-70)
Sex, n_{male} ($\%_{male}$)	670 (55.7)
Etiology (pure)	
Biliary, n (%)	528 (43.9)
Alcoholic, n (%)	269 (22.4)
Hypertriglyceridemic, n (%)	69 (5.7)
Mortality, n (%)	28 (2.3)
Severity of pancreatitis	
Mild, n (%)	825 (68.6)
Moderate, n (%)	313 (26.0)
Severe, n (%)	65 (5.4)
Length of hospitalization, median (Q_1-Q_3)	9 (7-14)
Local complications, n (%)	358 (29.8)
Fluid collection, n (%)	303 (25.2)
Pseudocyst, n (%)	120 (10.0)
Necrosis, n (%)	111 (9.2)
Systemic complications, n (%)	92 (7.7)
Respiratory failure, n (%)	55 (4.6)
Heart failure, n (%)	19 (1.6)
Renal failure, n (%)	33 (2.7)
Charlson Comorbidity Index, median (Q_1-Q_3)	2 (0-2)
Severity of comorbidities	
No comorbidities, n (%)	444 (36.9)
Mild comorbidities, n (%)	345 (28.7)
Moderate comorbidities, n (%)	190 (15.8)
Severe comorbidities, n (%)	224 (18.6)

Table 7. Demography of study population including a total of 1203 cases of acute pancreatitis.

IV.4.4 Demography of the AP cohort

In order to understand the relationship between ageing, comorbidity, severity, and mortality we used the high-quality International AP Registry run by the HPSG. It contained 1241 cases, of them 1203 (96.9%) from 18 centers were eligible for inclusion. Demography of the study population and that of AP Registry are presented in *Table 7, Figure 16*. Distribution of sites of recruitment is presented in *Figure 17*. Study population proved to be representative to that of AP Registry regarding

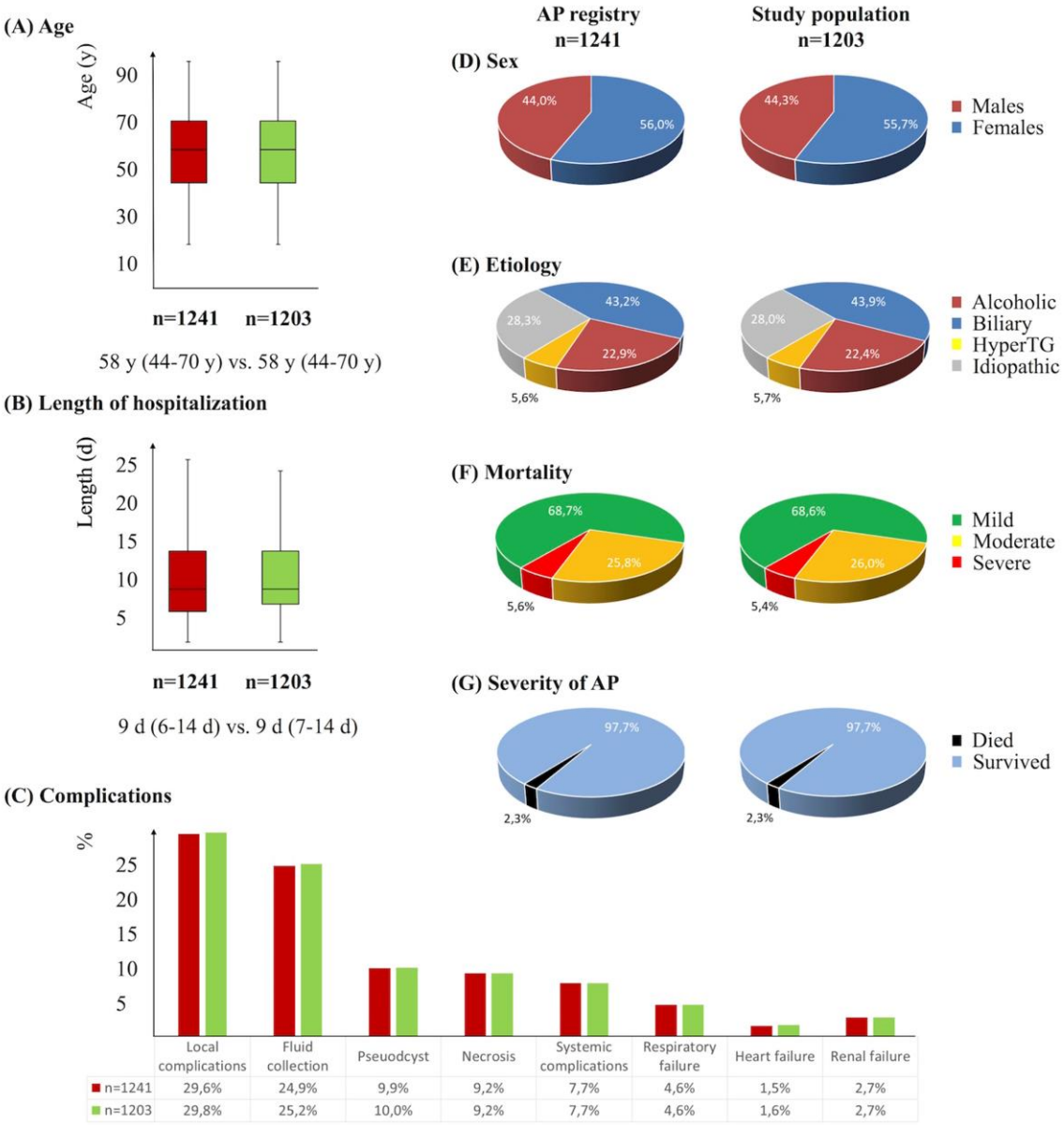


Figure 16. Demography and representativeness of the study population. Analysis of representativity showed no difference between the features of the population in AP Registry (n=1241) and that included in Study Population (n=1203), p≥0.05 for all comparisons. Representativeness of the included population was tested by binomial (sex, etiology, mortality, and complications), one sample median (age and length of hospitalization), and Goodness-of-fitχ2 tests (severity of AP).

demography and disease outcomes (p>0.05 for all variables analyzed) (*Table 7*). Data quality for all variables was >99% in the study population (*Table 8*).

City	Center	N° of patients
Budapest	Bajcsy-Zsilinszky Hospital	137
	Joint Saint Istvan and Saint Laszlo Hospitals	4
	Heim Pál National Institute of Pediatrics	1
Békéscsaba	Dr. Réthy Pál Hospital	53
Debrecen	Second Department of Medicine, University of Debrecen	76
	Institute of Surgery, University of Debrecen	7
Gyula	Pándy Kálmán Hospital of County Békés	26
Kecskemét	Bács-Kiskun County Hospital	11
Makó	Healthcare Center of County Csongrád	10
Miskolc	Borsod-Abaúj-Zemplén County Hospital and University Teaching Hospital	11
Pécs	First Department of Medicine, University of Pecs	360
Szeged	First Department of Medicine, University of Szeged	198
	Second Department of Medicine, University of Szeged	74
	Emergency Unit, University of Szeged	10
	Department of Surgery, University of Szeged	4
Szentes	Department of Gastroenterology, Dr. Bugyi István Hospital	10
Szombathely	Markusovszky University Teaching Hospital	8
Székesfehérvár	Szent György University Teaching Hospital of Fejér County	199
	Total N° of patients	1203

Figure 17 Distribution of centers recruiting the study population.

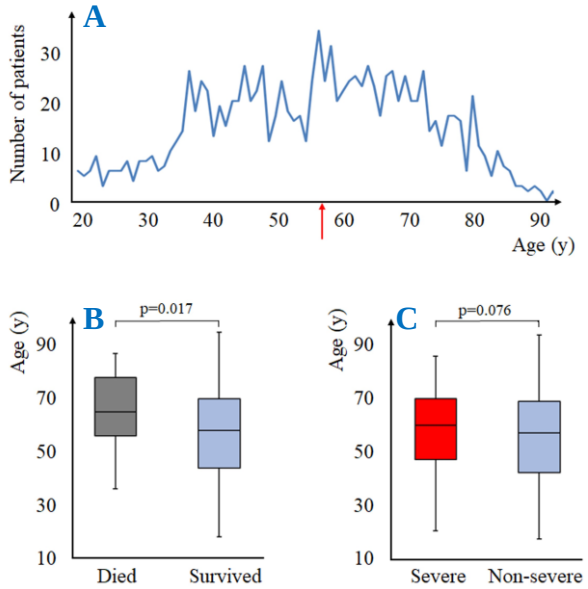


Figure 18. Ageing and acute pancreatitis. A: age-distribution of the study population, the red arrow indicates the median age of the population (that is, 58 years of age). **B:** mortality and age (Mann-Whitney test). **C:** severity and age (Mann-Whitney test).

Variable	Data quality (%)
Age at the time of admission	100
Sex	100
Etiology	100
Mortality	100
Severity of pancreatitis	100
Length of hospitalization	100
Charlson Comorbidity Index	100
Local complications	99.5
Fluid collections	99.5
Pseudocyst	99.6
Necrosis	99.6
Systemic complications	99.3
Respiratory failure	99.2
Heart failure	99.3
Renal failure	99.3
Overall data quality	**99.7**

Table 8. Data Quality. 99.7% overall data quality shows that the prospectively collected data is extremely good quality and suitable for analysis.

IV.4.5 Association between ageing and comorbidities in AP

The median age on admission was 58 y (Q_1-Q_3: 44-70 y, range: 18-95 y) (*Figure 18A*). Deceased were older than survivors (65 y [Q_1-Q_3: 56-78 y] vs. 58 y [Q_1-Q_3: 44-70 y], p=0.017, respectively) (*Figure 18B*). The age difference between severe and non-severe cases was of borderline significance (61 y [Q_1-Q_3: 48-71 y] vs. 58 y [Q_1-Q_3: 43-70 y], p=0.076) (*Figure 18C*), as well as the detected weak positive correlation between age and LOH (r=0.055, p=0.058) (*Figure 19*) Interestingly, patients developing local complications were younger than those not doing so (56 y [Q_1-Q_3: 43-68 y] vs. 59 y [Q_1-Q_3: 44-71 y], respectively, p=0.028). The association is true for necrosis (p=0.049) and fluid collections (p=0.095), unlike for pseudocysts (p=0.839) (*Figure 20*). On the contrary, patients developing systemic complications were older than those not doing so (62 y [Q_1-Q_3: 50.5-74 y] vs. 58 y [Q_1-Q_3: 43-70 y], respectively, p=0.008).

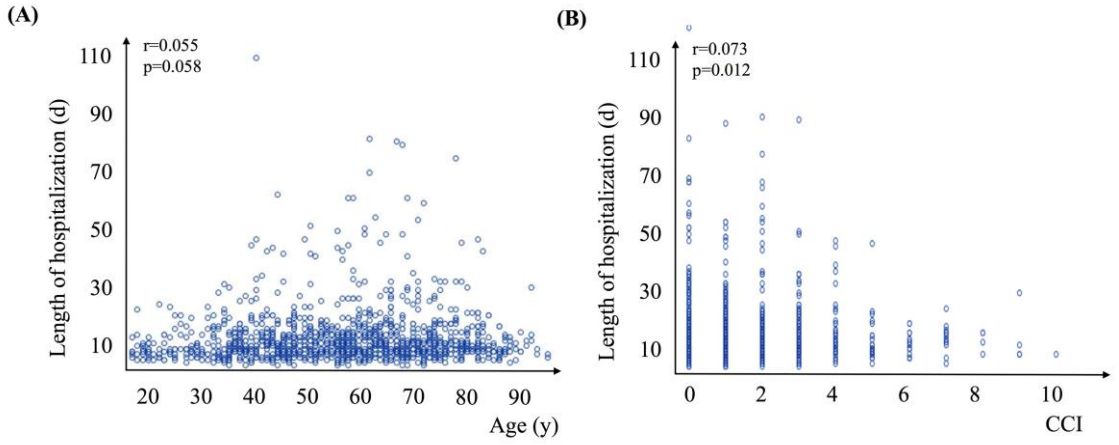


Figure 19. Correlation between age and **LOH** (A panel), and CCI and LOH (B panel)

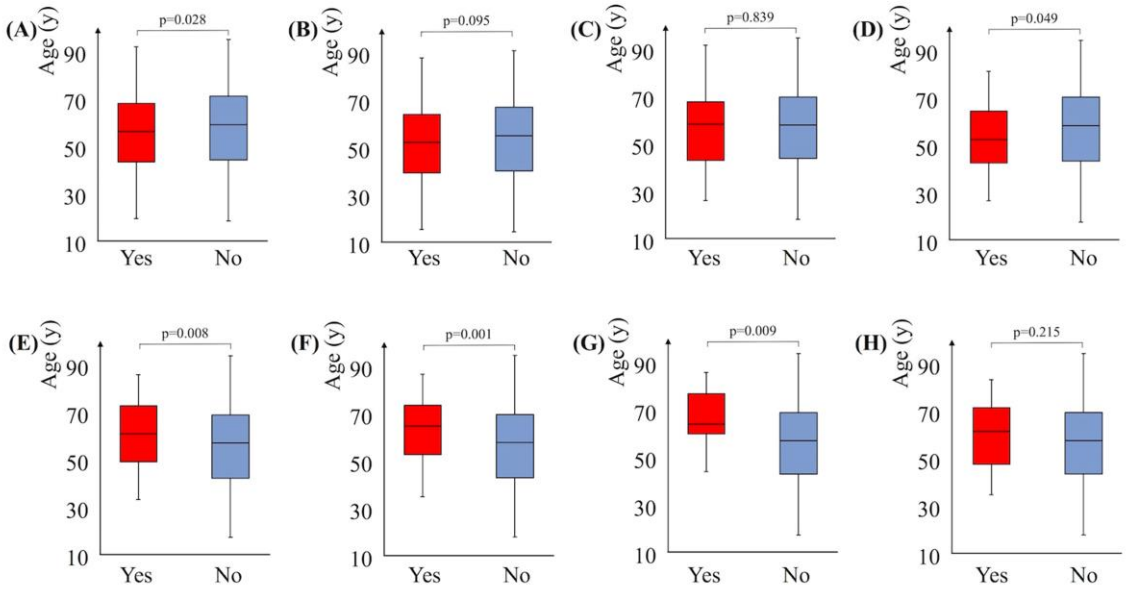


Table 1

		Total n, (%)	Age median (IQR)	p-value
Local complications *(Figure A)*	Yes	358 (29.9)	56 (43-68)	0.028*
	No	839 (70.1)	59 (44-71)	
Fluid collection *(Figure B)*	Yes	303 (25.3)	56 (43-68)	0.095
	No	894 (74.7)	59 (44-71)	
Pseudocyst *(Figure C)*	Yes	120 (10.0)	58.5 (43.5-68)	0.839
	No	1078 (90.0)	58 (44-70)	
Necrosis *(Figure D)*	Yes	111 (9.3)	53 (43-65)	0.049*
	No	1087 (90.7)	59 (44-71)	
Systemic complications *(Figure E)*	Yes	92 (7.7)	62 (50.5-74)	0.008*
	No	1103 (92.3)	58 (43-70)	
Respiratory failure *(Figure F)*	Yes	55 (4.6)	65 (53-74)	0.001*
	No	1139 (95.4)	58 (43-70)	
Heart failure *(Figure G)*	Yes	19 (1.6)	65 (61-78)	0.009*
	No	1176 (98.4)	58 (44-70)	
Renal failure *(Figure H)*	Yes	33 (2.8)	62 (48-72)	0.215
	No	1162 (97.2)	58 (44-70)	

Figure 20. Ageing and complications in acute pancreatitis. **A** any local complication. **B** pancreatic fluid collection. **C** pseudeocyst. **D** pancreatic necrosis. **E** any systemic complication. **F** respiratory failure. **G** heart failure. **H** renal failure. Groups were compared with the Mann-Whitney test. Table1 shows the data which the figures rely on.* represents a significant difference between groups.

Specifically, respiratory (p=0.001) and heart failure (p=0.009) were age-dependent (*Figure 20*). These data suggest that ageing strongly influences the outcomes of AP in univariate models.

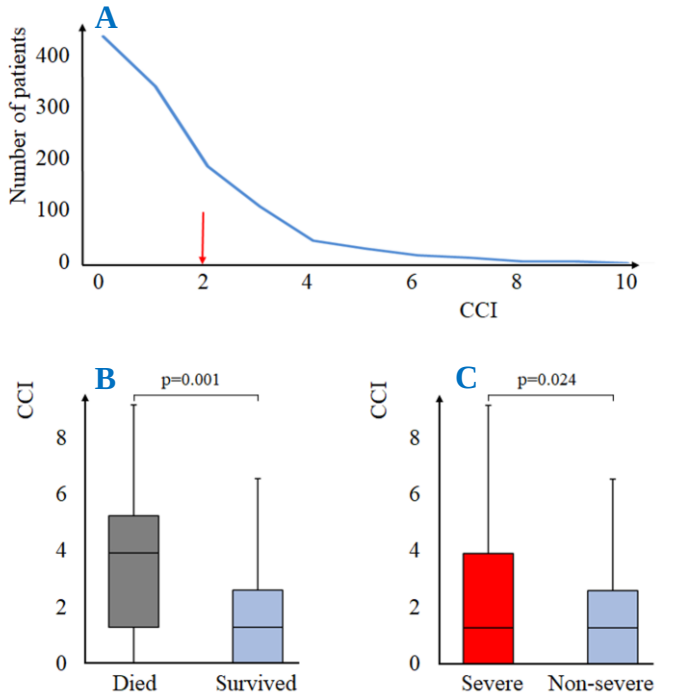
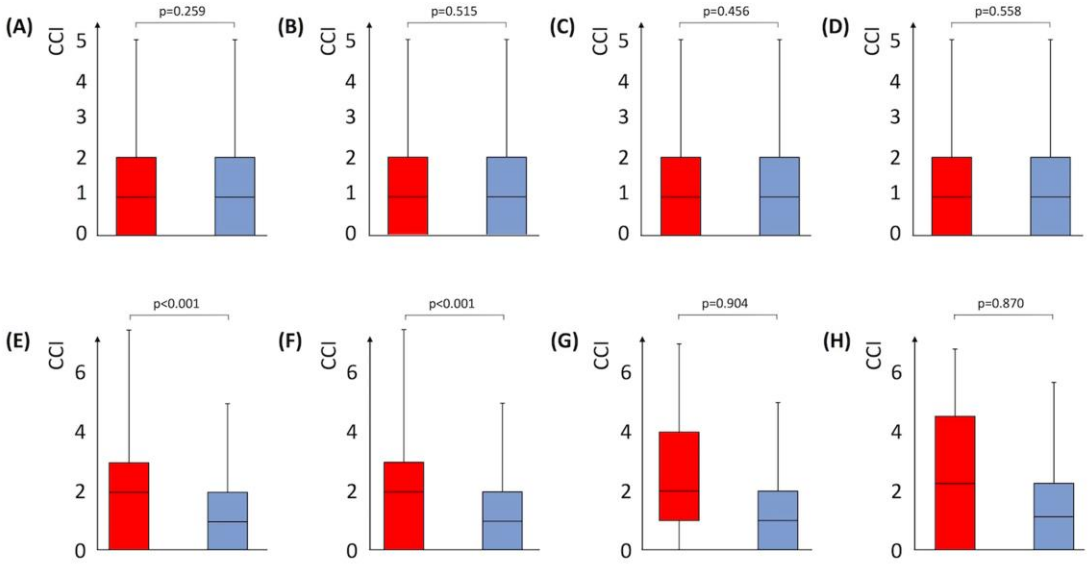


Figure 21. Charlson Comorbidity Score and acute pancreatitis. **A** distribution of CCI in the study population, the red arrow indicates the median CCI of the population. **B** mortality and CCI (Mann-Whitney test). **C** severity and CCI (Mann-Whitney test). CCI: Charlson Comorbidity Index.

Concerning comorbidity, Median CCI was 2 (Q_1-Q_3: 0-2, range: 0-10) (*Figure 21A*). Deceased had higher CCI than survivals (3 [Q_1-Q_3: 1-4] vs. 1 [Q_1-Q_3: 0-2], p=0.001, respectively), as well as those with severe AP (1 [Q_1-Q_3: 0-3] vs. 1 [Q_1-Q_3: 0-2], p=0.024) compared to those with non-severe AP, respectively (*Figure 21B-C*). A weak, significant, positive correlation was detected between age and CCI (r=0.073, p=0.012) (*Figure 19*). Local complications seemed independent of CCI (p=0.259), as were fluid collections (p=0.515), pseudocysts (p=0.456), and necrosis (p=0.558) (*Figure 22*). Systemic complications were associated with higher CCI (p<0.001). This association applies to respiratory failure (p<0.001), as well (*Figure 22*). These data suggest that CCI strongly influences the outcomes of AP in univariate models.

Furthermore, bivariate analysis of age and CCI revealed a moderate, positive correlation between the variables (r=0.334, p<0.001) (*Figure 23*). Importantly, patients with previous myocardial infarction, co-existing congestive heart failure, peripheral arterial disease, and cerebrovascular disease were significantly older than those without these conditions (p<0.001 for each). These associations applied to chronic pulmonary diseases and dementia (p<0.001 for both), as well as to peptic ulcers/erosions (p=0.015). Both diabetes with and without complications were associated with older age (p<0.001).

Patients with malignant tumors were older (p<0.001) but we failed to detect this association regarding metastatic tumors (p=0.112), probably due to low event rates. The latter may apply to autoimmune diseases (p=0.961).



		Total n, (%)	CCI median (IQR)	p-value
Local complications *(Figure A)*	Yes	358 (29.9)	1 (0-2)	0.259
	No	839 (70.1)	1 (0-2)	
Fluid collection *(Figure B)*	Yes	303 (25.3)	1 (0-2)	0.515
	No	894 (74.7)	1 (0-2)	
Pseudocyst *(Figure C)*	Yes	120 (10.0)	1 (0-2)	0.456
	No	1078 (90.0)	1 (0-2)	
Necrosis *(Figure D)*	Yes	111 (9.3)	1 (0-2)	0.558
	No	1087 (90.7)	1 (0-2)	
Systemic complications *(Figure E)*	Yes	92 (7.7)	2 (1-3)	<0.001*
	No	1103 (92.3)	1 (0-2)	
Respiratory failure *(Figure F)*	Yes	55 (4.6)	2 (1-3)	<0.001*
	No	1139 (95.4)	1 (0-2)	
Heart failure *(Figure G)*	Yes	19 (1.6)	2 (1-4)	0.904
	No	1176 (98.4)	1 (0-2)	
Renal failure *(Figure H)*	Yes	33 (2.8)	2 (0-4)	0.870
	No	1162 (97.2)	1 (0-2)	

shows the data which the figures rely on.* represents a significant difference between groups.

Figure 22. Ageing and complications in acute pancreatitis. **A** any local complication. **B** pancreatic fluid collection. **C** pseudeocyst. **D** pancreatic necrosis. **E** any systemic complication. **F** respiratory failure. **G** heart failure. **H** renal failure. Groups were compared with the Mann-Whitney test Table.

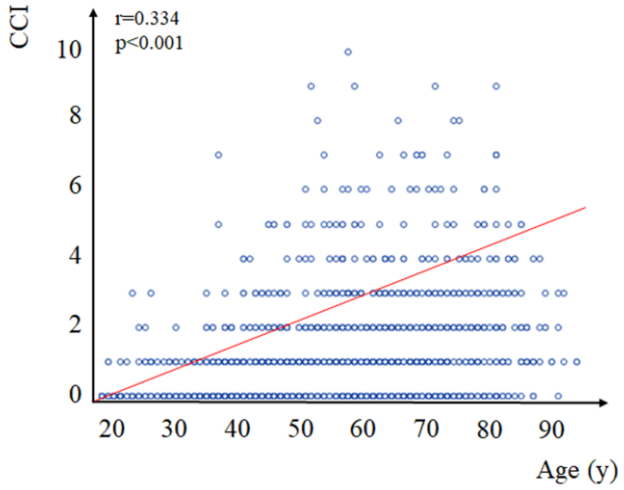


Figure 23. Correlation between age and Charlson Comorbidity Index. Spearman's correlation established a significant positive correlation of moderate strength (r=0.334, p<0.001) between age on admission and Charlson Comorbidity Index. CCI: Charlson Comorbidity Index.

Interestingly, patients with mild liver disease were younger than their healthy counterparts (p<0.001); however, this difference disappeared regarding moderate and severe liver diseases (p=0.555).

Summaries of multivariate analysis are presented in *Figure 22* and *Table 9*, raw data are presented in *Table 10*. The

Variables	Deceased vs. survivors			Severe vs. mild AP			LOH≤9 days vs. LOH>9 days		
	β	OR (95% CI)	p-value	β	OR (95% CI)	p-value	β	OR (95% CI)	p-value
Age categories									
18-34 y (young-aged)	NA[a]	NA[a]	0.961	0	1 (reference)		0	1 (reference)	
35-64 y (middle-aged)	0.76	0.76 (0.35-1.67)	0.493	2.00	7.40 (0.99-55.31)	0.051	0.62	1.86 (1.22-2.83)	0.004
> 65 y (old-aged)	0	1 (ref)		1.93	6.92 (0.91-52.70)	0.062	0.40	1.50 (0.96-2.33)	0.073
Comorbidity categories									
CCI=0 (none)	0	1 (reference)		0	1 (reference)		0	1 (reference)	
CCI=1 (mild)	0.11	1.12 (0.32-3.90)	0.863	0.04	1.04 (0.52-2.08)	0.911	0.00	1.00 (0.75-1.34)	0.983
CCI=2 (moderate)	0.09	1.10 (0.26-4.68)	0.900	-0.02	0.98 (0.45-2.24)	0.960	0.30	1.35 (0.95-1.92)	0.092
CCI>2 (severe)	1.50	4.48 (1.57-12.80)	0.005	0.74	2.10 (1.08-4.09)	0.029	0.15	1.16 (0.83-1.62)	0.387

Table 9. The joint effect of ageing and comorbidities on the **outcomes** of acute pancreatitis**.** Red highlights indicate p<0.05, orange highlights indicate p<0.10 but ≥0.05. AP: acute pancreatitis; Charlson Comorbidity Index; CI: confidence interval; LOH: length of hospitalization; NA: not applicable; OR: odds ratio. [a]analysis is impossible due to zero events.

	Local complications[a]			Fluid collection[a]			Pseudocyst[b]			Necrosis[b]		
	β	OR (95% CI)	p-value	β	OR (95% CI)	p-value	β	OR (95% CI)	p-value	β	OR (95% CI)	p-value
Age categories												
18-34 y (young adults)	0	1 (ref)		0	1 (ref)		0	1 (ref)		0	1 (ref)	
35-64 y (middle-aged adults)	0.75	2.127 (1.300-3.480)*	0.003*	0.63	1.874 (1.124-3.124)*	0.016*	1.20	3.331 (1.305-8.504)*	0.012*	1.17	3.209 (1.257-8.191)*	0.015*
> 65 y (old adults)	0.29	1.332 (0.790-2.244)	0.282	0.23	1.258 (0.731-2.164)	0.407	0.91	2.486 (0.938-6.590)	0.067#	0.53	1.707 (0.633-4.605)	0.291
Comorbidity categories												
CCI=0 (none)	0	1 (ref)		0	1 (ref)		0	1 (ref)		0	1 (ref)	
CCI=1 (mild)	0.20	1.226 (0.895-1.678)	0.204	0.03	1.033 (0.742-1.439)	0.847	-0.24	0.787 (0.489-1.267)	0.325	0.15	1.163 (0.722-1.875)	0.534
CCI=2 (moderate)	0.11	1.116 (0.758-1.644)	0.579	0.05	1.056 (0.705-1.582)	0.791	-0.11	0.894 (0.510-1.569)	0.696	-0.03	0.973 (0.531-1.785)	0.930
CCI>2 (severe)	0.24	1.267 (0.881-1.823)	0.202	0.16	1.169 (0.800-1.709)	0.420	-0.30	0.741 (0.423-1.298)	0.295	-0.20	0.819 (0.447-1.501)	0.519
	Systemic complications[c]			**Respiratory failure[d]**			**Heart failure[c]**			**Renal failure[c]**		
	β	OR (95% CI)	p-value	β	OR (95% CI)	p-value	β	OR (95% CI)	p-value	β	OR (95% CI)	p-value
Age categories												
18-34 y (young adults)	0	1 (ref)		NA[e]	NA[e]	0.964	NA[e]	NA[e]	0.967	NA[e]	NA[e]	0.972
35-64 y (middle-aged adults)	2.19	7.824 (1.059-57.788)*	0.033*	-0.37	0.688 (0.395-1.197)	0.186	-0.31	0.731 (0.289-1.847)	0.508	-0.09	0.919 (0.450-1.875)	0.815
> 65 y (old adults)	2.06	8.933 (1.195-66.794)*	0.044*	0	1 (ref)		0	1 (ref)		0	1 (ref)	
Comorbidity categories												
CCI=0 (none)	0	1 (ref)		0	1 (ref)		0	1 (ref)		0	1 (ref)	
CCI=1 (mild)	0.20	1.217 (0.664-2.232)	0.525	0.02	1.023 (0.444-2.354)	0.958	-0.18	0.835 (0.185-3.765)	0.814	0.88	0.877 (0.323-2.386)	0.797
CCI=2 (moderate)	0.37	1.452 (0.738-2.856)	0.280	0.63	1.870 (0.818-4.275)	0.138	0.32	1.378 (0.302-6.278)	0.679	0.42	0. 416 (0.089-1.957)	0.267
CCI>2 (severe)	0.92	2.505 (1.396-4.494)*	0.002*	0.98	2.658 (1.260-5.605)*	0.010*	1.26	3.522 (1.054-11.770)*	0.041*	2.78	2.779 (1.179-6.551)*	0.020*

*p<0.05, #p<0.10 but ≥0.05. CCI: Charlson Comorbidity Index, [e]not applicable due to zero event number, [a]n=1197, [b]n=1998, [c]n=1995, [d]n=1994, CI: confidence interval, OR: odds ratio.

Table 10. The joint effect of ageing and comorbidities on **local and systemic complications** of AP.

Variables	Mortality n (%)	Severe AP n (%)	LOH ≥9 d n (%)	Local complication n (%)	Fluid collection n (%)	Pseudocyst n (%)	Necrosis n (%)	Systemic complication n (%)	Respiratory failure n (%)	Heart failure n (%)	Renal failure n (%)
Age categories											
18-34 y (young adults)	0 (0.0)	1 (0.8)	40 (33.3)	23 (19.2)	21 (17.5)	5 (4.2)	5 (4.2)	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)
35-64 y (middle-aged adults)	13 (2.0)	36 (5.6)	320 (49.7)	223 (34.6)	186 (28.9)	76 (11.8)	77 (12.0)	49 (7.6)	26 (4.0)	9 (1.4)	18 (2.8)
> 65 y (old adults)	14 (3.2)	28 (6.4)	198 (45.1)	112 (25.5)	96 (21.9)	39 (8.9)	29 (6.6)	42 (9.6)	29 (6.6)	10 (2.3)	15 (3.4)
Comorbidity categories											
CCI=0 (none)	5 (1.1)	19 (4.3)	194 (43.7)	120 (27.0)	107 (24.1)	48 (10.8)	40 (9.0)	22 (5.0)	12 (2.7)	4 (0.9)	2 (0.5)
CCI=1 (mild)	5 (1.5)	16 (4.6)	155 (44.9)	110 (31.9)	87 (25.2)	32 (9.3)	37 (10.7)	23 (6.7)	11 (3.2)	3 (0.9)	2 (0.6)
CCI=2 (moderate)	3 (1.6)	9 (4.7)	100 (52.6)	56 (29.5)	48 (25.3)	20 (10.5)	17 (9.0)	16 (8.4)	12 (6.3)	3 (1.6)	2 (1.1)
CCI>2 (severe)	14 (6.3)	21 (9.4)	109 (48.7)	72 (32.1)	61 (27.2)	20 (8.9)	17 (7.6)	31 (13.8)	20 (8.9)	9 (4.0)	2 (0.9)

AP: acute pancreatitis, CCI: Charlson Comorbidity Index, LOH: length of hospitalization

Table 11. Data used in multivariate analysis

	Systemic complications			LOH		
	β	OR (95% CI)	p	β	OR (95% CI)	p
Age categories						
18-34 y (young adults)	0	1 (ref)		0	1 (ref)	
35-64 y (middle-aged adults)	2.15	8.55 (1.16-63.03)	0.035*	0.62	1.85 (1.22-2.81)	0.004*
> 65 y (old adults)	2.03	7.60 (1.02-56.93)	0.048*	0.43	1.53 (0.98-2.39)	0.061
Comorbidity categories						
Myocardial infarction	0.00	1.00 (0.40-2.51)	0.998	0.80	2.24 (1.22-4.10)	0.009*
Congestive heart failure	0.34	1.40 (0.61-3.21)	0.422	0.21	1.28 (0.69-2.21)	0.472
Peripheral vascular disease	0.39	1.48 (0.80-2.72)	0.214	0.02	1.02 (0.68-1.54)	0.926
Cerebrovascular disease	0.83	2.29 (1.29-4.07)	0.005*	0.09	1.10 (0.73-1.66)	0.662
Dementia		-		-0.78	0.46 (0.15-1.37)	0.162
Chronic pulmonary disease	0.35	1.42 (0.77-2.63)	0.265	-0.39	0.68 (0.45-1.01)	0.057#
Connective tissue disease		-		-0.77	0.47 (0.16-1.38)	0.168
Peptic ulcer/erosion		-		0.0	1.00 (0.66-1.51)	0.984
Mild liver disease		-		0.17	1.18 (0.92-1.53)	0.198
Diabetes without complication		-		0.18	1.20 (0.86-1.69)	0.287
Hemiplegia		-		-0.19	0.83 (0.13-5.13)	0.839
Moderate or severe renal disease	0.51	1.66 (0.65-4.27)	0.292	0.12	1.13 (0.57-2.26)	0.728
Diabetes with complication		-		0.15	1.16 (0.59-2.30)	0.666
Malignant tumor		-		0.09	1.09 (0.68-1.77)	0.714
Lymphoma		-		-12.32	NA	0.978
Leukemia		-		1.19	3.06 (0.31-30.04)	0.337
Moderate or severe liver disease		-		-0.12	0.89 (0.36-2.17)	0.795
Metastatic solid tumor		-		-0.62	0.54 (0.16-1.79)	0.312
Any tumors	0.40	1.49 (0.77-2.89)	0.240		-	
Liver disease at any stage	-0.33	0.72 (0.44-1.17)	0.180		-	
Diabetes with or without complications	0.20	1.22 (0.72-2.09)	0.470		-	

	Mortality	
	β	OR (95% CI)
Age categories		
18-34 y (young adults)	-12.51	NA
35-64 y (middle-aged adults)	-0.42	0.66 (0.30-1.46)
> 65 y (old adults)	0	1 (ref)
Comorbidity categories		
Moderate or severe renal disease	1.35	3.86 (1.07-13.88
Moderate or severe liver disease	2.09	8.12 (2.13-20.96
Metastatic tumors	1.93	6.90 (1.36-34.88

	Severity	
	β	OR (95% CI)
Age categories		
18-34 y (young adults)	0	1 (ref)
35-64 y (middle-aged adults)	-0.09	0.92 (0.53-1.59)
> 65 y (old adults)	1.82	6.20 (0.81-47.18
Comorbidity categories		
Malignant tumors	-0.68	0.51 (0.25-1.04)
Liver diseases	0.55	1.73 (0.96-3.12)
Diabetes	-0.30	0.74 (0.42-1.39)
Cardiovascular diseases	-0.27	0.77 (0.42-1.39)
Pulmonary diseases	-0.20	0.82 (0.39-1.74)

Table 12. Results of multivariate analysis on the effects of individual comorbidities on the outcomes of acute pancreatitis

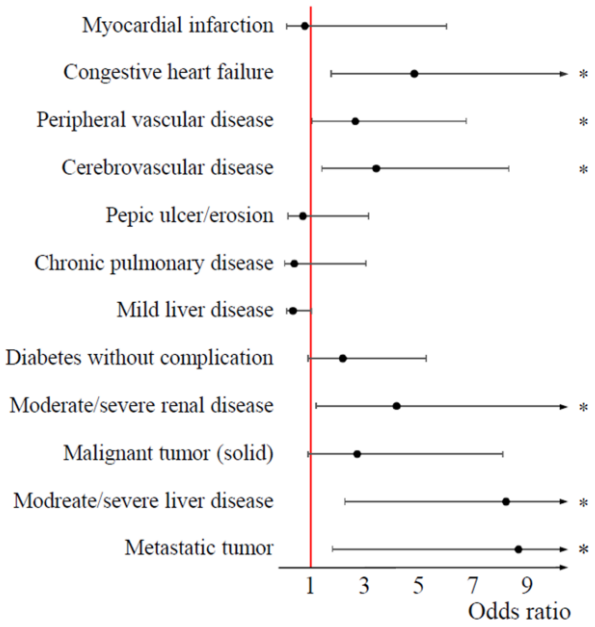


Figure 24. Forest plot on the effect of **individual comorbidities on mortality**. 95% confidence intervals did not cross the boundary of significance (red, vertical line at an odds ratio of 1) regarding six comorbid conditions: congestive heart failure, peripheral vascular disease, cerebrovascular disease, moderate/severe renal disease, moderate/severe liver disease, and metastatic tumor (asterisks indicate a p-value less than 0.05). These comorbidities were associated with higher mortalitv.

exclusive predictor of mortality was a CCI≥3 (ß=1.50; OR=4.48; CI: 1.57-12.80); in accordance, the main predictor of severe AP was a CCI≥3 (ß=0.74; OR=2.10, CI: 1.08-4.09), though the middle- and old-aged were exposed to a severe episode with a high OR of borderline significance. Unexpectedly, the middle-aged were more likely to spend ≥9 days in the hospital. Along with this, the only predictors of local complications (including pancreatic necrosis) was to be middle-aged (ß=1.17; OR=3.21, CI: 1.26-8.19). On the contrary, the middle- and old-aged were about eight times more likely to develop systemic complications than their younger counterparts (β=2.19, OR=7.82, CI:

1.06-57.79 and β=2.06, OR=8.93, CI: 1.20-66.79, respectively), though comorbidities were important determinants, as well.

Summaries of univariate and multivariate statistics of individual comorbidities, together with raw data, are presented in *Table 11-12*. In univariate analysis, out of the six comorbidities associated with higher mortality, moderate/severe liver diseases and metastatic solid tumors proved to be the strongest predictors (OR=8.04, CI: 2.22-29.13 and OR=8.47, CI: 1.78-40.23, respectively) (*Figure 24*). Peripheral vascular diseases, cerebrovascular diseases, and diabetes without complications predicted severe AP. Patients with mild liver diseases were two times more likely to develop local complications, including necrotizing pancreatitis (OR=1.86, CI: 1.25-2.75). Congestive heart failure, peripheral vascular diseases, cerebrovascular diseases, chronic pulmonary diseases, and diabetes without complications were associated with a higher rate of systemic complications. Preexisting cardiovascular, renal, and pulmonary diseases predicted the development of respiratory, heart, and renal decompensation, respectively. Interestingly, pre-existing moderate/severe liver diseases and malignant tumors were strongly associated with cardiac decompensation (OR=7.16, CI: 1.55-33.21 and OR=4.09, CI: 1.32-12.64, respectively). The multivariate analysis only minimally changed the direction of the main associations.

IV.5. Discussion

Here we provide the first detailed meta-analysis on the effects of ageing on AP. Ageing has been demonstrated to play an important role in AP; however, due to the lack of detailed mathematical analysis, there is a great difference between the cut-off values used in predictive scoring systems [17-22].

With regard to severity, unfortunately, we only have two articles in which severity was one of the outcome parameters in youth. In one of these studies, Párniczky et al. found no severe cases in the 36 patients under 30 yrs of age [7]. Similarly, Beltrán et al found only a single severe case in a cohort of 24 patients suggesting a low incidence rate of severe AP in youth [53]. Our situation was far easier regards mortality as data from large nationwide cohorts were available. In a large epidemiology study involving 55 012 patients under 20 yrs in the USA, Pant et al. showed that mortality is only 0.92% [75]. Others have also described low mortality in smaller cohorts. Lautz et al. found 0% (0/211 patients) mortality under 20 yrs, while Yeung et al. reported 2.33% (1/43 patients) [71,82]. In contrast, no mortality was found among 1720 patients between the ages of 20 and 29 in a Hungarian and a Dutch cohort [7,16]. Middle-aged patients (30–59y) had a mortality rate more than two times higher [45-50,52,55-67].

Our second main observation was that up until 59 yrs (this cut-off value was mathematically calculated), both severity and mortality rise linearly (*Figure 5 and 10*). The rate of severity increases 0.193%/year, and mortality grows 0.086%/year. It has been documented that almost all death cases come from the severe AP group; therefore, we can assume that although the number of severe cases rises every year, the risk for mortality in severe AP remains constant at around 20% [7].

Thirdly, we found that above 59 yrs the mortality rate rapidly increases; meanwhile, the rate of severe pancreatitis follows the earlier, slightly elevated pattern (*Figure 5 and 10*). These data clearly suggest that additional factors which are lacking or rare below 59 yrs also affect mortality in AP. One of the best candidates responsible for the increased elevation of mortality in the elderly is definitely co-morbidity. It has been shown that the burden of co-morbidities increases with age [23,25]. In addition, it has been also reported that the outcome of AP is worsened by severe co-morbidities [27,83]. Therefore, we can hypothesize that the elevation of severity and mortality with age is attributed to co-morbidity rather than ageing.

The incidence of severe AP in patients, however, showed a continuous, linear rise between the ages of 20 and 70 (0.193%/year) of up to 16.6%. The mortality rate was 0.9% in patients under 20 and demonstrated a continuous increase until the age of 70. The mortality rate between 20 and 59 grew 0.086%/year and 0.765%/year between 59 and 70. Overall, patients above 70 had a mortality rate 19 times higher than patients under 20. The rise of mortality rate with age was thus also confirmed.

In adults, the severity of AP clearly increases with age. With regard to mortality, it follows a similar linear rise until 59 yrs; however, after that, a nine-fold change is observed in its steepness. This result completely confirms the observation of Ranson et al. that age is associated with a significantly increased risk of death over 55 yrs. [20,84]. Imrie et al. 85 modified the scoring system; however, they still considered age above 60 as a valuable parameter. Balmey et al. [20] evaluated a prospective study with 347 patients in a seven-year period to simplify the system and to improve its accuracy. With regard to age, they also found the cut-off point at 55 yrs.

The BISAP scoring system was established as the first population-based prognostic scoring system in order to evaluate the risk of in-hospital mortality prior to the onset of organ failure [17]. The CART analysis identified age above 60 years for prediction of in-hospital mortality based on parameters collected in 2000–2001 in the first 24 h from a patient population of 17 922 suffering from AP [17].

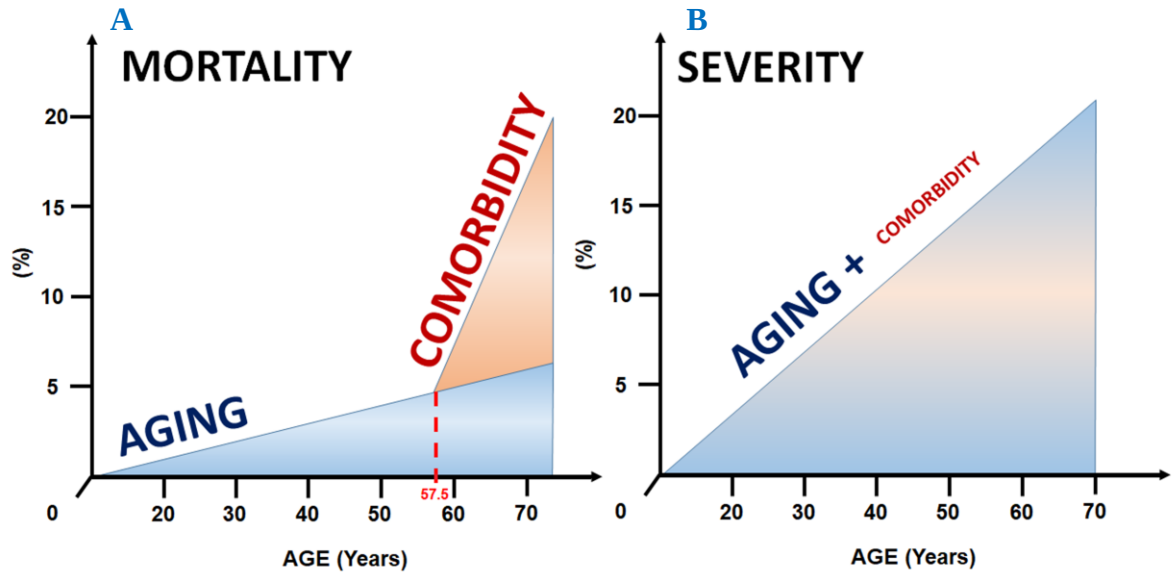


Figure 25. Model for the joint effect of **ageing and comorbidities on mortality and severity.** **A** The excess in mortality in the elderly is likely to be explained by the increment in comorbidities with ageing. **B** In contrast, age seems to be the strongest predictor of the severity of acute pancreatitis, whereas comorbidities have a less prominent effect.

In summary, the predictive scoring systems correspond with our results that mortality rises quickly above 59 years of age. These data suggest that other factors such as comorbidity may be associated with older age and can elevate the mortality in AP. Importantly, our analysis showed that severe comorbidities (CCI≥3) predict mortality (OR=4.48; CI: 1.57-12.80) much better than age, suggesting that comorbidity is an important additional predictor for mortality (*Figure 25*).

V. Chapter II

V.1 Introduction

Despite the extensive research in the field, no specific therapy is available to treat AP [46]. With regard to the pathomechanism of the disease, it is clear that mitochondrial injury and ATP depletion play key roles in the early phase of AP almost irrespectively of the etiology of the disease [88-90]. Bile acids, ethanol, and fatty acids were shown to be responsible for around 80% of the etiological factors initiating AP [91]. All of these factors were shown to induce a toxic calcium signal and severe mitochondrial damage in both acinar and ductal cells [12,90,92-95]. Importantly, direct administration of ATP (i.e., energy) into the cells restored their functions and prevented cell death [96,97]. Therefore, if we take a translational approach, it is more than likely that patient energy intake would be beneficial. Not surprisingly, enteral nutrition (EN) has almost been the only therapeutic change in recent decades to be highly beneficial and to be widely utilized in severe AP (SAP)[98]. However, in mild and moderate AP (MAP), the primary therapy is still the nil per os diet (NPO) [99]. Since the results in basic science have demonstrated the crucial role of energy breakdown in the early phase of AP, in this chapter we focused on providing evidence whether early enteral feeding is beneficial in AP.

V.2 Aim

The major aim of this chapter is to understand whether enteral feeding should be the primary therapy in the early phase of AP.

V.3 Materials and Methods

A randomized controlled trial (RCT) is the only type of clinical scientific methods which can reduce selection bias when testing a new treatment. However, before performing a time consuming, expensive RCT a meta-analysis is crucially important.

(i) If the meta-analysis is decisive, no RCT is needed. The intervention can be used in clinical practice directly.

(ii) If the meta-analysis suggests a significant difference but has several limitations, RCT should be performed.

In this chapter firstly we performed a meta-analysis and then we developed a prestudy protocol for an RCT.

V.3.1 Article Search for the meta-analysis

A meta-analysis was performed using the preferred reporting items for systematic review and meta-analysis (PRISMA)[37]. An article search was performed in the PubMed, EMBASE, and Cochrane databases in February 2016. The PICO process was used to frame and answer our clinical questions.

V.3.2 PICO (Problem, Intervention, Comparison, Outcome) for the meta-analysis

PICO was broken down as follows: P: nutrition in AP; I: enteral nutrition; C: nil per os diet; and O: outcome. We split our data into two groups: SAP and MAP. In SAP, only three primary endpoints were checked (mortality, multi-organ failure, and intervention), whereas in MAP, due to the low amount of data, 14 secondary endpoints were collected besides the primary endpoints: length of hospital stay (LOH), inflammatory parameters (C-reactive protein (CRP), white cell count (WCC), and presence of SIRS (systemic inflammatory response syndrome)), complications (necrosis, infection, hospital readmission, and progression of severity), intervention, necessity of antibiotic, pain relapse, visual analogue scale (VAS)-pain, opiate-free treatment, start of oral intake, and clinical symptoms (nausea and vomiting).

V.3.3 Database search for the meta-analysis

A search was made using the following terms: in PubMed: (acute (All Fields) and "pancreatitis" (MeSH Terms) or "pancreatitis" (All Fields)) and ("clinical trial" (Publication Type) or "clinical trials as topic" (MeSH Terms) or "clinical trials" (All Fields)) and ("loattrfull

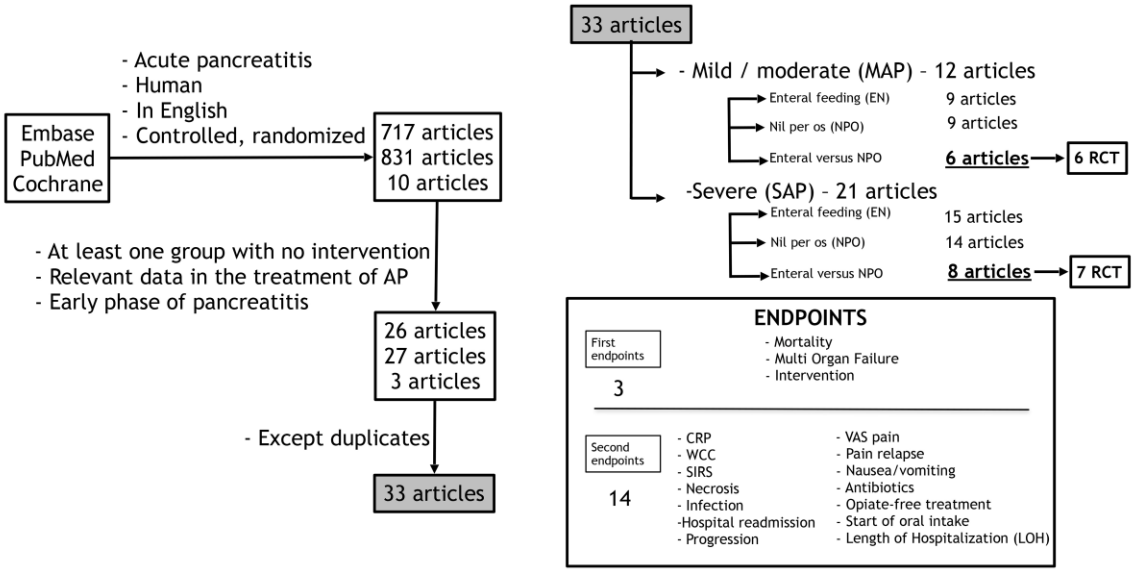


Figure 26. Organogram of article search in PubMed, EMBASE, and Cochrane databases. RCT, randomized and controlled trial; CRP, C-reactive protein; WCC, white cell count; SIRS, systemic inflammatory response syndrome; VAS, visual analog scale.

Article	MAP	SAP	EN	NPO	RTC
Abou-assi et al 2002	✓		✓	✓	✓
Eckerwall et al 2007	✓		✓	✓	✓
Li et al 2013	✓		✓		✓
Ma et al 2016	✓		✓	✓	✓
McClave et al 1997	✓		✓	✓	✓
Ockenga et al 2002	✓			✓	✓
Oláh et al 2002	✓		✓	✓	✓
Pandey et al 2004	✓		✓		✓
Petrov et al 2013	✓		✓	✓	✓
Pongratz et al 2013	✓			✓	✓
Sathiaraj et al 2008	✓		✓		✓
Wu et al 2011	✓			✓	✓
Andersson et al 2006		✓		✓	
Bakker OJ et al 2014		✓	✓		✓
Besselink et al 2008		✓	✓		✓
Doley et al 2009		✓	✓	✓	✓
Eatock et al 2005		✓	✓		✓
Eckerwall et al 2006		✓	✓	✓	✓
Kalfarentzos et al 1997		✓	✓	✓	✓
Karakan et al 2007		✓	✓		✓
Kumar et al 2006		✓	✓		✓
Kyhala et al. 2012		✓		✓	✓
Modena et al 2006		✓	✓	✓	
Pearce et al 2006		✓	✓		✓
Pettila et al 2010		✓		✓	✓
Singh et al 2012		✓	✓		✓
Sun et al 2004		✓	✓	✓	✓
Sun et al 2013		✓	✓	✓	✓
Vege et al. 2015		✓		✓	✓
Wang et al 2013		✓	✓	✓	✓
Wu et al 2010		✓	✓	✓	✓
Xian-li et al 2004		✓		✓	✓
Zhao et al 2013		✓		✓	✓

Table 13. Articles with data on the early phase of AP. SAP: severe acute pancreatitis; MAP: mild and moderate AP; EN: enteral nutrition; NPO: nil per os diet; RCT: randomized controlled clinical trial.

text" (sb) and "humans" (MeSH Terms) and English (lang)) in EMBASE: "acute pancreatitis" and (humans)/lim and (English)/lim and (abstracts)/lim and ((controlled clinical trial)/lim or (randomized controlled trial)/lim) and in Cochrane: "acute pancreatitis": ti,ab,kw and "human" and "English" in Trials (the search included various forms of the terms). "Acute pancreatitis" in Title, Abstract, and Keywords and "human" and "English" in Trials (the search included various forms of the terms). Altogether, 1634 articles (EMBASE: 717; PubMed: 831; Cochrane: 10) were found (*Figure 26*).

V.3.4 Inclusions and Exclusion criteria of the meta-analysis

A manual search was performed to find the relevant articles. Only articles in English and with relevant data in the early phase treatment of AP were included. Duplications were excluded. Thirty-three articles (21 articles containing patients suffering from SAP as well as 12 articles with MAP patients) were selected. They contained two non-randomized and 31 randomized controlled clinical trials (*Table 13*) [47,100-131]. Finally, statistical analyses were performed on data from articles where both EN and NPO groups were presented, the trial was randomized, and the relevant data were available. Altogether, seven SAP and six MAP articles met these criteria.

V.3.4 Statistical Analyses

In SAP, forest plots were used to illustrate the mortality, multi-organ failure, and intervention. In the case of mortality and multi-organ failure, the pooled estimates were

Points	Mortality (%)	Organ Failure (%)	Intervention (%)	CRP (mg/L)	WCC (109/L)	SIRS (%)
0	0–0.9	0–0.09	0–0.09	0–19.9	4000–9999.9	0–0.09
1	1–2.9	0.1–0.19	0.1–0.19	20–39.9	10,000–11,999	0.1–0.14
2	3–4.9	0.2–0.29	0.2–0.29	40–59.9	12,000–13,999	0.15–0.19
3	5–6.9	0.3–	0.3–0.39	60–79.9	14,000–15,999	0.2–0.24
4	7–8.9		0.4–0.49	80–99.9	16,000–17,999	0.25–0.29
5	9–		0.5–	100–	18,000–	0.3–

Points	LOH (Days)	Necrosis (%)	Infection (%)	Hospital Readmission (%)	Progression of Severity (%)	Pain Relapse (%)
0	0–4.9	0–0.09	0–0.09	0–0.04	0–0.04	0–0.09
1	5–9.9	0.1–0.19	0.1–0.19	0.05–0.06	0.05–0.06	0.1–0.19
2	10–12.4	0.2–0.29	0.2–	0.07–0.08	0.07–0.08	0.2–0.29
3	12.5–14.9	0.3–	–	0.09–0.10	0.09–0.10	0.3–0.39
4	15–19.9	–	–	0.11–	0.11–	0.4–
5	20–	–	–	–	–	–

Points	VAS-Pain	Nausea/Vomiting (%)	Antibiotics (%)	Opiate-Free Treatment (%)	Start of Oral Intake (%)
0	0–1	0–0.18	0–0.09	0–0.09	0–0.04
1	2–4	0.2–0.39	0.1–0.19	0.1–0.19	0.05–0.09
2	5–7	0.4–0.59	0.2–0.29	0.2–0.29	0.1–0.14
3	8–9	0.6–0.79	0.3–0.39	0.3–0.39	0.15–0.19
4	–	0.8–	0.4–	0.4–0.49	0.2–0.24
5	–	–	–	0.5–	0.25–

Table 14. **Uniform point system.** CRP, C-reactive protein; WCC, white cell count; SIRS, systemic inflammatory response syndrome; LOH, length of hospitalization; VAS, visual analog scale.

calculated with a random-effects model; in the case of intervention, a fixed-effects model was applied as described earlier [132]. Analyses were performed with the Comprehensive Meta-Analysis Software (Biostat, Inc., Englewood, NJ, USA). In the case of binary variables, the differences between EN and NPO were expressed as risk differences or odds ratios with a 95% confidence interval (CI). Heterogeneity was tested between trials with two methods. First, we employed the Q homogeneity test statistic, which exceeds the upper-tail critical value of chi-square on n - 1 degree of freedom (DF), with a *p*-value of less than 0.050 considered suggestive of significant heterogeneity. Second, we used the inconsistency (I^2) index. I^2 is the proportion of total variation contributed by between-study variability. An I^2 value of more than 0.5 suggests a considerable heterogeneity. Heterogeneity was verified using a funnel plot to reduce publication bias. Whenever considerable heterogeneity was observed, random- or fixed-effects models were applied.

In MAP, only two (mortality and multi-organ failure) of the three primary endpoints could be analyzed. With regard to the second endpoints, no forest plot analyses could be calculated due to insufficient data. A uniform point system was developed to make the data analyzable (*Table 14*). Results were also weighted based on the number of patients in the articles. The Mann–Whitney *U* test was used to detect significant differences between the pooled weighted scores. SPSS Statistical Software (version 20, IBM Corporation, Armonk, NY, USA) facilitated this analysis. A *p*-value less than 0.05 was considered as statistically significant, whereas a *p*-value between 0.1 and 0.05 was seen as a trend.

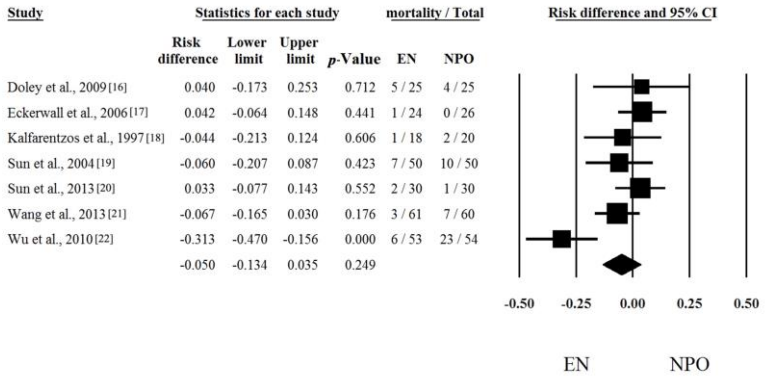


Figure 27. Forest plot of studies evaluating **mortality** data in severe acute pancreatitis (SAP). Risk differences and confidence interval (CI) were calculated to compare the enteral nutrition (EN) with the nil per os diet (NPO). Black squares and lines represent the results for individual studies, the diamond shows the pooled result of the meta-analysis.

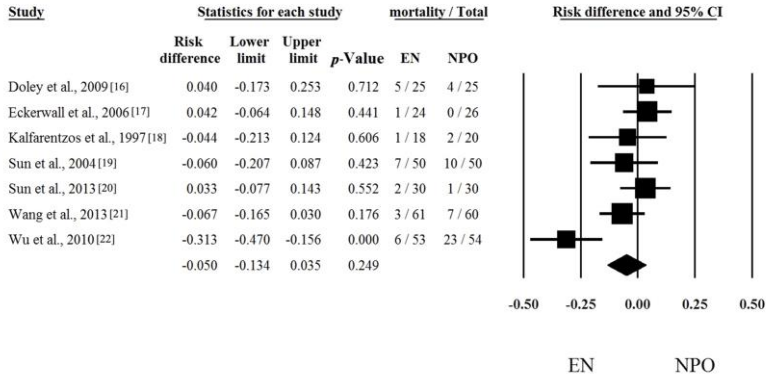


Figure 28. Forest plot of studies evaluating **multi-organ failure (MOF)** in severe acute pancreatitis (SAP). Odds ratio (OR) and confidence interval (CI) were calculated to compare the enteral nutrition (EN) with the nil per os diet (NPO). Black squares and lines represent the results for individual studies, the diamond shows the pooled result of the meta-analysis.

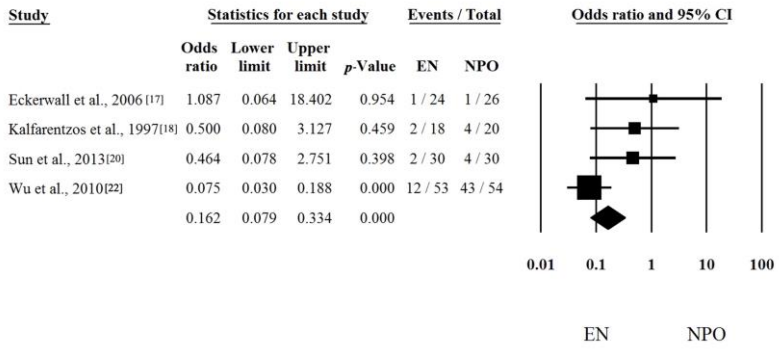


Figure 29 Forest plot of studies evaluating the **intervention** in severe acute pancreatitis (SAP). Odds ratio (OR) and confidence interval (CI) were calculated to compare the enteral nutrition (EN) with the nil per os diet (NPO). Black squares and lines represent the results for individual studies, the diamond shows the pooled result of the meta-analysis.

V.3.5 Developing an RCT

The SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) guideline[133] was used to develop our RCT [5].

V.4 Results

V.4.1 The effects of early enteral feeding in severe AP

Seven out of seven articles contained analyzable data on mortality [100,106,109,117,119,126, 127]. Risk differences and CI were calculated in each article to analyze the effects of EN compared to the NPO nutrition. The calculated average risk difference (RD) was −0.050 (lower limit (LI): −0.134; upper limit (UI): 0.035; p-value: 0.249) (*Figure 27*). Because of the considerable heterogeneity (Q = 16.488; DF: 6; p = 0.011; I^2 = 63.61%) random-effect model was applied. Four out of seven articles contained analyzable data on multi-organ failure (MOF). With regard to MOF, the calculated odds ratio (OR) was 0.258 (LI: 0.072; UI: 0.930; p-value: 0.038; heterogeneity: Q = 13.833; DF: 3; p = 0.003; I^2 = 78.31%) in favor of EN (*Figure 28*). With regard to interventions, a fixed-effect model was used. The calculated average odds ratio (OR) was 0.162 (LI: 0.079; UI: 0.334;

p-value: <0.001; Q = 7.221; DF: 3; p = 0.065; I^2 = 58.45%) also in favor of EN (*Figure 29*). Because of the moderate heterogeneity, the random-effect model was applied as well (OR was 0.274 (LI: 0.073; UI: 1.025; p = 0.054)). These data clearly suggest that EN is beneficial and should be the primary therapy in SAP.

V.4.2 The effects of early enteral feeding in mild and moderate AP

Unfortunately, there is much less research activity in patients suffering from MAP than from SAP. Moreover, the frequency of death and MOF are also much less common in the MAP group vs the SAP group. Not surprisingly, analyses of low amounts of data in which the mortality and MOF are close to zero could not reveal any significant difference between the two groups. With regard to mortality, five out of six articles contained proper data [47,113,101,103,124]. Risk differences and CI were calculated in the articles. The calculated average risk difference (RD) was −0.003 (LI: −0.047; UI: 0.040; *p*-value: 0.879) (*Figure 30*). As predicted, we also saw no significant difference in the frequency of MOF, where we only had four items. Forest plots of OR and CI were calculated. The odds ratio (OR) was 0.849 (LI: 0.369; UI: 1.952; *p*-value: 0.700) (*Figure 31*). Because of the Q and I^2 tests showed negligible heterogeneity (Q = 0.916; DF: 4; *p* = 0.922; I^2 = 0.00% for *Figure 30* and Q = 1.169; DF: 3; *p* = 0.760; I^2 = 0.00% for *Figure 31*), the fixed-effect model was applied.

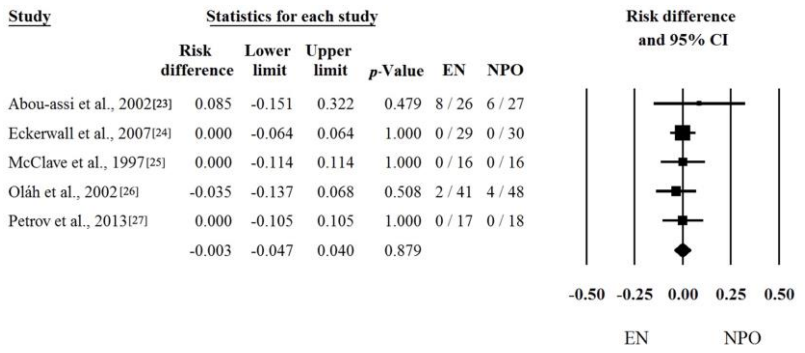


Figure 30 Forest plot of studies evaluating **mortality** data in mild and moderate acute pancreatitis (MAP). Risk differences and confidence interval (CI) were calculated to compare the enteral nutrition (EN) with the nil per os diet (NPO). Black squares and lines represent the results for individual studies, the diamond shows the pooled result of the meta-analysis.

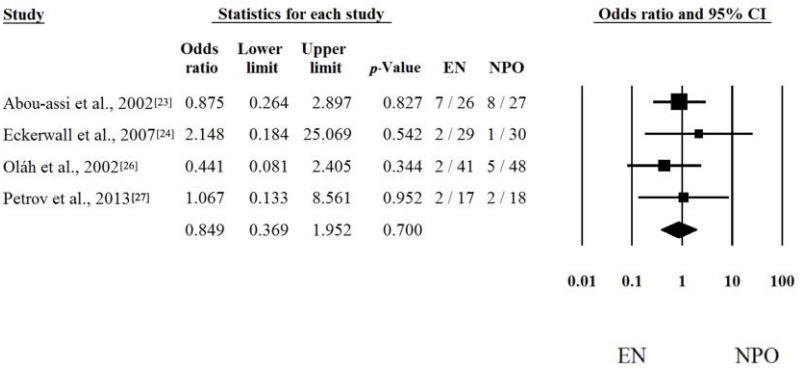


Figure 31 Forest plot of studies evaluating **multi-organ failure (MOF)** in mild and moderate acute pancreatitis (MAP). Odds ratio (OR) and confidence interval (CI) were calculated to compare the enteral nutrition (EN) with the nil per os diet (NPO). Black squares and lines represent the results for individual studies, the diamond shows the pooled result of the meta-analysis.

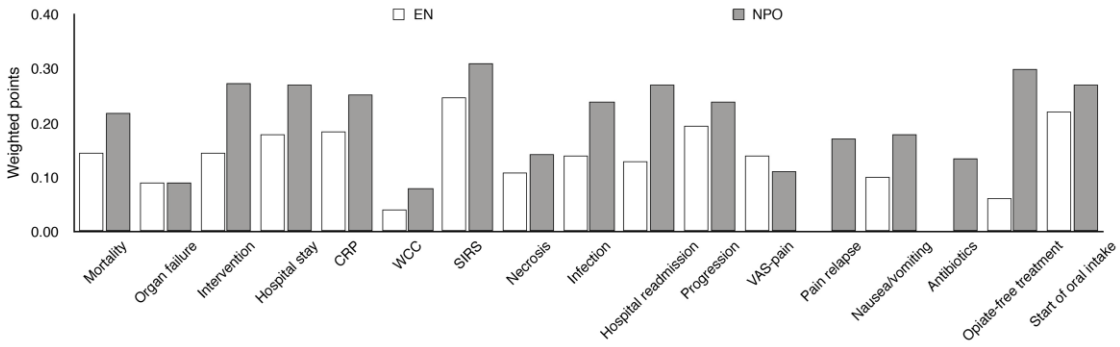


Figure 32. Summary of the uniform data-point system in MAP. EN versus NPO. Due to the low amount of data, 3 primary endpoints and 14 secondary endpoints were collected for MAP. The uniform data point system was then developed (Table 1). Results were weighted based on the number of patients in the articles. CRP, C-reactive protein; WCC, white cell count; SIRS, systemic inflammatory response syndrome; VAS, visual analog scale.

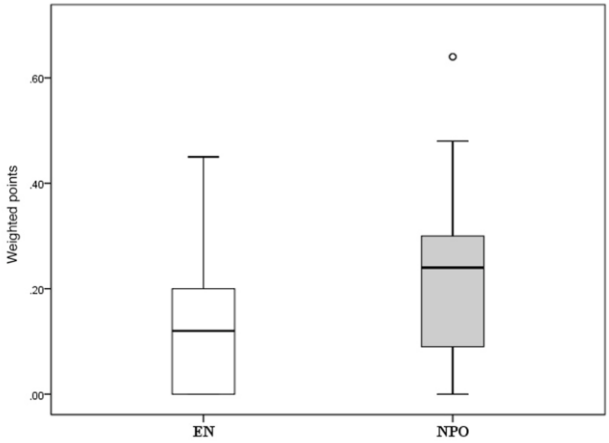


Figure 33. Summary of the uniform data point system in MAP. EN versus NPO. The Mann–Whitney U test was used to detect significant differences between the pooled weighted scores (see Figure 6). o = $p < 0.05$ vs EN

However, the five articles contained several other secondary parameters (see Methods). Unfortunately, each study group concentrated on different parameters, resulting in the fact that almost none of the parameters had a complete data set (*data are shown in supplementary figure 1 in article No.4*[4]). *Figure 32* demonstrates the differences between EN and NPO. Due to the low n number, statistical analyses could not be calculated separately. Importantly, pooling the data from the 17 parameters (3 primary and 14 secondary endpoints) showed a significant difference in favor of EN (*Figure 33*).

These data strongly suggest that early enteral feeding is beneficial in AP. However, due to the several limitations of our meta-analysis we had to develop an RCT (*see V.5*) to answer our question decisively. Until the submission of this thesis 278 patients were already recruited by four centres (Pécs, Székesfehérvár, Gyula, Debrecen). We plan to finish the study in 2022.

V.5 The GOULASH trial - Prestudy protocol of a randomized controlled double-blind clinical trial

V.5.1 Design

This is a randomized controlled two-arms double-blind multicentre trial. Patients suffering from acute pancreatitis will be randomly assigned to groups A (high energy administration starting within 24h of hospital admission) and B (no energy administration after 24h of hospital admission). The study was designed using the SPIRIT guideline (*Figure 34*).

V.5.2 Trial organization, committees and boards

GOULASH is designed and coordinated by the Centre for Translational Medicine at the University of Pécs and the Hungarian Pancreatic Study Group (HPSG). HPSG was established in 2011 in order to stimulate research in pancreatic diseases. Until now HPSG published the relevant guidelines of pancreatic diseases in order to improve patient care in the field of pancreatology and has initiated four prospective clinical trials (EASY, PREPAST, APPLE, and PINEAPPLE).

	STUDY PERIOD							
	Enrolment	Allocation	Post-allocation					Close-out
TIMEPOINT**	*<24h*	0	*day1*	*day2*	*day3*	*dayx*	*discharge*	*1m after discharge*
ENROLMENT:								
Eligibility screen	X							
Informed consent	X							
Laboratory test	X							
CT examination	X							
Allocation		X						
INTERVENTIONS:								
High energy administration			♦	—	—	♦		
Low energy administration			♦	—	—	♦		
ASSESSMENTS:								
Questionnaire A		X						
Questionnaire B			X	X	X	X	X	
Questionnaire C								X

Figure 34 shows the flow chart of participants according to the SPIRIT 2013 statement

The following committees and boards will be involved:

Steering committee (SC): The committee will be led by PH (gastroenterologist, internal medicine specialist). The members will be KM (medical doctor, full time employee on the project), ÁV (gastroenterologist, internal medicine specialist), ZM (intensive care specialist),

TM (clinical research specialist) AS (multidisciplinary unit specialist), MP (gastroenterologist, internal medicine specialist), NF (radiologist), DK (surgeon), IB (interventional radiologist). SC will make decisions concerning all relevant questions including the dropouts during the study.

International translational advisory board (ITAB): The committee will include gastroenterologist (MML), surgeon (JPN) and basic scientists (MST, OHP). ITAB will continuously monitor the progress of the study and will give advice to the SC.

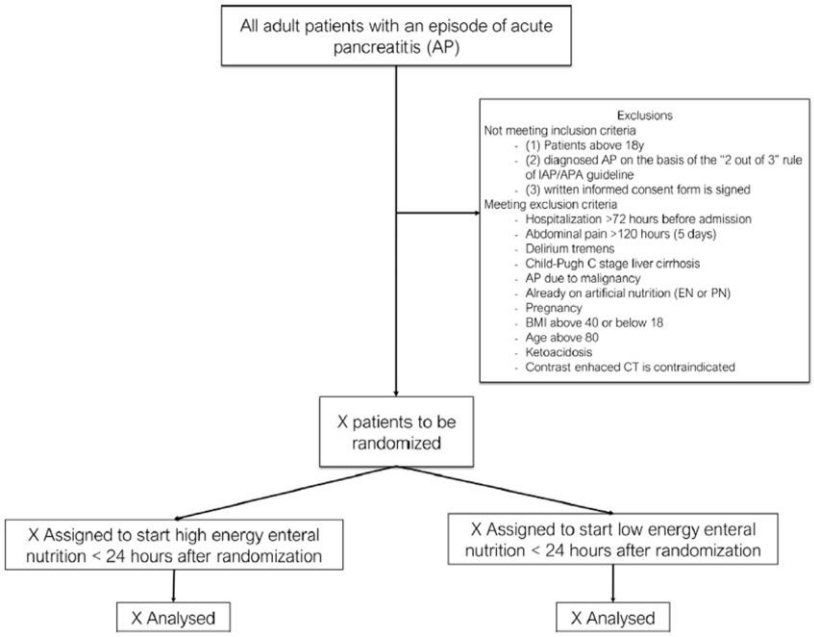


Figure 35 shows the schedule of enrolment, interventions, and assessments according to the SPIRIT 2013 statement

The study was designed by SC and ITAB. The study is financially sponsored by the University of Pécs, the Hungarian Academy of Sciences, and the National Research, Development and Innovation Office. Neither sponsors were involved in the design of the study, and they will have no access to the database management or to the randomization code.

V.5.3 Study population

All patients diagnosed with AP will be informed of the possibility of taking part in the GOULASH study. After the consent form is signed, a computer using a block randomization protocol will randomize the patients (*Figure 35*).

Inclusion criteria: (1) Patients over 18y of age, (2) diagnosed AP on the base of the "2 out of 3" criteria of the IAP/APA guideline: (a) upper abdominal pain; (b) serum amylase or lipase >3x upper limit of normal range; (c) characteristic findings on pancreatic imaging; however those patients without abdominal pain will be excluded because the onset of AP cannot be determined, (3) written informed consent form is signed.

Exclusion criteria: (1) Hospitalization 72 hours before admission, (2) abdominal pain >120 hours (5 days), (3) delirium tremens, (4) Child-Pugh C stage liver cirrhosis, (5) AP due to malignancy, (6) already on artificial nutrition (EN or PN), (7) pregnancy, (8) BMI above 40 or below 18, (9) age above 80, (10) ketoacidosis, (11) whenever CT with contrast is contraindicated.

Sample size: Sample size calculation was based on the National Hungarian Registry operated by the Hungarian Pancreatic Study Group. Our recent analyses indicated that MOF existing more than 48h arises in 9%, whereas mortality is in 2.8% of all patient suffering from AP [34]. Altogether they represent around 10% of all AP patients. In order to detect a treatment effect of at least 50% of the early treatment, a sample size of 957 subjects will be necessary to be recruited using a 10% drop-out rate, 80% power, and 95% significance level. The calculation was performed by the Independent data management and biostatistics provider company (IDMB, Adware Research LTD, Balatonfüred, Hungary).

Randomization: In each centre participants will be divided into 2 groups receiving one of the two study treatments. The allocation of participants to the different groups will be carried out based on predefined randomization lists created separately for each recruiting centre. The randomization lists will be prepared with a block size of 4 and with an allocation ratio of 1:1.

V.5.4 Duration

The planned starting date of the study is; 1 January 2017, and the planned finishing date of the study is; 1 January 2022.

V.5.5 Blinding

The medical staff (e.g., taking the measurements such as blood pressure, examining health records for events such as abdominal pain, reviewing and interpreting examination results such as X-ray or CT) and the patient receiving the intervention will be blinded to the knowledge of treatment assignment. The person providing the intervention cannot be blinded in this study. Sealed envelopes ensure the allocation sequence. Nutritional support equipment will be covered until the fourth day to ensure that only who made the randomization will know which group the patient was enrolled into.

V.5.6 Intervention

Based on the currently available guidelines enteral feeding can be started at any time for the patients suffering from AP. In addition, no calorie restriction/order has been described.

Therefore both groups can be regarded as being treated within accepted practice recommendations.
In this study, early high energy administration will be the intervention. Patients will be randomized to group A or B: see Figure 2.

Groups: In **group A,** high energy will be delivered after admission. Patients will receive a 10 Ch nasogastric (NG) or nasojejunal (NJ) feeding tube on admission. EN will be immediately started as follows: On Day 0 (from admission until the start of EN (can vary from 2-24 h)): calorie intake will be 0 kcal/kg/day. From Day 1 high energy enteral tube feed 30 kcal/kg/day will be provided until the oral feeding starts. In **group B,** low energy administration after hospital admission. Patients will receive a NG or NJ feeding tube at admission as described above. On Day 0 (from admission until the start of EN): calorie intake will be 0 kcal/kg/day. On day 1 0 kcal/kg/day, on day 2 10 kcal/kg/day, on day 3 20 kcal/kg/day and from day 4 30 kcal/kg/day calorie will be delivered until the oral feeding starts. However, between groups A and B, only the amount of calories administered will be different. Patients will receive the same amount of fluid and ions during EN (see below).

Ingredients of enteral tube feed: High Energy Enteral Tube Feed (100ml):
Energy: 150 kcal (630 KJ), **Protein** 6g (16%E), **Carbohydrate**: 18.3g (49%E), **Fat**: 5.8g (35%E) + **Minerals**: 134mg Sodium, 201mg Potassium, 108mg Calcium, 108mg Phosphorus, 34mg Magnesium, 100mg Chloride (0%E). In this study, we will use Nutrison Energy (Numil Ltd, Budapest, Hungary), which is a registered product in Hungary (reg. number: 1217).
Zero Energy Enteral Tube Feed (100ml):
Energy: 0 kcal (0 KJ), **Protein** 0g, **Carbohydrate**: 0g, Fat: 0g + **Minerals**: 134mg Sodium, 201mg Potassium, 108mg Calcium, 108mg Phosphorus, 34mg Magnesium, 5.562g Chloride (0%E) (in this study the local institutional pharmacy will provide it in accordance with the Hungarian regulations). Whenever 10 or 20 kcal/kg/day calories will be delivered, the mixture of the above-mentioned two solutions will be used.

Type of enteral tube: Patients neither vomiting nor having gastric fluid retention >250 ml will receive primarily NG tube. Patients either vomiting or having gastric fluid retention >250 ml will receive NJ tube (placement will be done either endoscopically or radiologically). In the case of GCS 14 or lower in a patient who is not intubated, NG tube will be replaced by NJ tube (risk of aspiration). Abdominal X-ray will be used to check the tube's position.

Start of mixed feeding (around 2620 kcal): Mixed feeding (1000 ml tap water distributed for 24 h and 300 g (around 1900 kcal) biscuits/toasts/low-fat meal (at least 75% CHO containing ones) orally plus enteral tube feed (480ml, 720 kcal/day) will be started on the day when: (1) abdominal pain has been ceased for at least 6 h before the new day started, (2) CRP level has started decreasing and (3) amylase or lipase level has started decreasing

Start of total feeding (around 2000kcal): If the patients have no symptoms during the mixed oral/enteral feeding and the CRP, amylase or lipase levels are not rising again. Total feeding (according to local policy) can be started.

Other issues: The speed of EN will be different for the patients (depends on the body weight), however, the maximum speed of EN cannot exceed 65ml/h. In case of difficulties reaching the 30 kcal/kg/day calories intake (if the patient's body weight is above 75 kg) additional intravenous calorie will be added using Sterofundin G. Maximum of 2000 ml (= 400 kcal) can be delivered in this way. If NG feeding is not tolerated, NG tube will be replaced to NJ tube as described above. If NJ feeding is not tolerated, EN will be reduced by 50% and increased again gradually until tolerated. If the re-increasing process is still not tolerated total parenteral nutrition (TPN) will be started to reach the required energy target. In the case of SAP, TPN has to be delivered via central venous catheter.

V.5.7 Other treatment of subjects

General treatment indicated by the IAP/APA guideline will be utilized[46].

V.5.8 Discharge of patients

Uniformization of the length of hospital stay is necessary to avoid bias concerning LOH. Re-admission within one week after discharge has to be considered as the same hospital admission. Patients have to be counted as discharged from hospital/from the study when (1) oral feeding was tolerated for 24h, (2) no amylase/lipase level are elevated after total enteral feeding, (3) CRP level is less than 50 mg/L, (4) abdominal pain has completely resolved (5) no other pancreatitis-related complication requiring hospitalization is detected.

V.5.9 Endpoints

The following primary endpoints will be calculated: A combination of MOF more than 48h and Mortality. The following secondary endpoints will be analyzed: (1) pancreatic necrosis, (2) nutrition-related complications: diarrhea, aspiration pneumonia, pneumothorax due to central TPN catheter placement, (3) need for conversion from NG to NJ feeding tube (4) need for conversion from EN to TPN, (5) days until the start of total feeding, (6) use of antibiotics, (7) pain relapse, (8) CRP, (9) WBC, (10) PCT, (11) infection, (12) length of hospital stay, (13) need for ICU admission, (14) length of ICU therapy, (15) organ failure, (16) complications, (17) costs calculation. Notably, only direct costs will be calculated that include all medications, services, salaries of healthcare professionals, equipment and daycare costs.

V.5.10 Monitored parameters during hospitalization

There will be a large variety of parameters monitored during the study (e.g. medical history, physical examination, laboratory tests, diagnostic imaging, therapy, interventions). Form A will contain the parameters collected on admission (Supplementary figure 1). Form B will contain parameters collected every day during hospitalization (Supplementary figure 2). Form C will contain parameters collected 1 month after hospital discharge (Supplementary figure 3). For details see supplementary materials or web page (http://www.pancreas.hu/en/studies/goulash), which will be available from February 2017. Data collection on the case report form (CRF) will be done electronically (see data management).

V.5.11 Data management and statistical analyses

Data handling: Data will be handled by IDMB. Electronic CRF (eCRF) will be used. The Investigator will ensure that the data in the eCRF are accurate, complete and legible. Detailed data flow will be described in the Data Management Plan (DMP). Data from completed eCRFs will be validated under the direction of the Data Manager at IDMB according to Data Cleaning Plan (DCP). Any missing, implausible, or inconsistent recordings in the eCRFs will be referred back to the Investigator using a data query form (DQF), and be documented for each individual subject before clean file status is declared. All changes to eCRF will be recorded. Before Data Base Lock Data Review Meeting will decide and document necessary steps related to any issue in the database and define the analysis sets. Member of the data review meeting are delegated investigator, biostatistician and data manager. Adverse events (AEs) will be coded using MedDRA. AdWare Research Ltd., who will act as IDMB, works according to GCP, GLP, FDA

21CFR PART11, and other relevant regulatory requirements. AdWare Ltd. has GLP and ISO 9001 certificates.

Study populations:

Three analysis populations will be defined:

Safety Analysis Set (SAS):	all patients enrolled in the study.
Per Protocol Set (PPS):	all enrolled patients who finished the study conforming to the requirements of the Study Protocol.
Intention to Treat (ITT)	all randomized participants who start on a treatment, excluding consent withdrawals.

Withdrawal of a subject from PPS: Any participants/investigators and IDMB can submit recommendation for dropouts from the PPS group with reasons given to SC. All recommendations will be filed. SC will discuss all the information and if the alteration in the protocol would be expected to have any bearing on the interventions and outcomes of the study, the patient will not be included in the final per-protocol analysis. Automatic dropout from the per-protocol group shall be ordered if: (1) any of the exclusion criteria diagnosed during the course of AP, (2) at least 50% of the energy requirement is not achieved on any days during the study, (3) parameters required for answering the primary endpoints are missing or (4) serious medical reasons not related to pancreatitis (i.e. accidents, stroke) occur.

Applied softwares: Statistical analysis will be performed using SAS 9.2 or SPSS 19 (or later) statistical packages; Microsoft MSWord will be used for reporting.

Statistical Methods: Baseline patient and disease characteristics will be analyzed by using descriptive analysis. Demographic and baseline characteristics will be summarized for the overall study population. Continuous variables will be described by mean, median, standard deviation, and ranges and categorical variables will be described by absolute and relative frequencies. A graphical presentation of efficacy variables will be prepared, if applicable. Descriptive statistics for both the primary and secondary parameters will be analyzed similarly. Mean changes (and their 95% CI) from baseline to end-of-study visit will be presented as well. Chi-squared tests will be applied to compare proportions between the different groups. Mortality/extended MOF will be investigated using the Kaplan-Meier analysis method; while subgroup comparisons will be performed using the Chi-squared or Fisher's exact test, as

appropriate. For safety data, descriptive statistics and individual listings of adverse events will be also presented.

Subgroups: The following subgroups will be made during statistical analyses: (1) ages (under 40y, 40y-59y, 60y-80y), (2) BMI (below 20, 20-24, 25-29, 30-35, above 35), (3) the start of abdominal pain before admission (≤24h, 24-48h, ≥48h), (4) severity of the disease SAP and MAP. All subgroup analyses, (5) etiologies will be done descriptively. No confirmatory statistical testing will be applied. Hence, statistical tests and the p-values attached to them will be regarded as descriptive and not as tests of hypotheses.
Details of the applied statistical tests will be described in the Statistical Analysis Plan.

V.5.12 Early quality assessment

Early quality assessment check will be performed on the first 100 patients. IDMB (AdWare Ltd.) will perform an independent assessment of the trial related documents and activities, with the aim of ensuring the respect of subject's right, safety, and well-being and to guarantee the plausibility of clinical data. The similarity of groups at baseline will be also checked. IDMB will report to SC. SC will discuss all the information and if the differences would be expected to have any bearing on the interventions and outcomes of the study or the overall dropout rate from PPS is above 20 percent of all participants who were randomized or allocated into each group or the differential dropout rate is above 15 percent between the arms, the study needs to be reassessed and IDMB will make recommendations regarding either reevaluation of power calculation, extension of recruitment period, extension of number of study centers or termination of trial.

V.5.13 Interim analyses and premature termination of the study

IDMB can also recommend to stop the trial early for ethical reasons if one of the groups clearly shows evidence of a significant benefit. An interim analysis will be performed on the primary endpoint when 50% of patients have been randomized and discharged from the hospital. The interim analysis will be performed by the IDMB. IDMB will report to SC.
The Haybittle–Peto boundary approach will be used. If the interim analysis shows a probability of equal to, or less than 0.001 that a difference as extreme between the treatments is found, given that the null hypothesis is true, then the trial will be stopped early.

V.5.14 Centres

The trial will start in two centres (University of Debrecen and University of Pécs), after which the study is open for other centres. In all cases, IDMB will make an audit of the centre and will report to the SC. SC keeps the right to decide whether the centre meets the required quality to join the study. Compulsory requirements for a centres: (1) it needs to treat at least 50 AP patients a year, (2) it needs to have all the equipment required for the study, (3) besides the regular medical team the centre has to appoint at least one doctor and one nurse/administrator fully available for the trial with no additional commitments which can interfere with her/his duty when her/his availability is required, (4) the blinding described above can be fully utilized, (5) all persons need to attend a preliminary meeting where all the details concerning the studies are discussed fully and have qualified as investigators in a GCP course. Centres wish to join need to send a letter of intent to the corresponding author by e-mail.

V.5.15 Publication policy

Centres providing more than 25 patients can provide two authors to the authorship list. Every additional 25 patients will give the opportunity to nominate an additional author.

V.5.16 Feasibility

As a general protocol for the treatment of AP at the Centre for Translational Medicine at the University of Pécs, patients suffering from AP receive early EN (using nasogastric tube). Patients receive 50 ml Nutrison Energy per hour starting immediately when they arrive to the ward from the Emergency Department. Patients data between the period of 1 January – 31 May 2016 were analyzed and the following observations were noted: (1) In 85% of all AP admission early EN could have been started within 24 h. In 15% of the cases, it was not achievable due to the delayed transfer of the patients to the ward or vomiting. In these cases, patients received NG tube later or they received NJ tube whenever X-ray assistance was available. (2) around 80% of NG-fed patients tolerated NG feeding without any complications. The rest of the patients who had gastric retention or vomiting NG feeding was stopped and they received NJ tube whenever X-ray assistance was available. (3) Comparing the outcome (rate of severity, mortality, necrosis, intervention, etc.) of this treatment protocol with the nil per os protocol utilized in most of the Hungarian hospitals revealed that patients enjoy benefits with no risk of early enteral feeding which data confirm the literature data described in the introduction. Concerning the number of patients at the University of Pécs around 250 and at the University

of Debrecen around 150 patients are admitted annually. Therefore, if no other Institution would join the study it can be completed within 3 years.

V.5.17 Safety

Since no unknown drugs/therapy are used in the study no adverse and serious adverse events (SAE) are expected/interpretable that would be attributable to the intervention during the trial. In this trial, IDMB will examine safety variables after every 16 patients have completed. Moreover, investigators will report AE or SAE via a separate form which has to be sent to IDMB and SC. SC will discuss and if the adverse effect is confirmed it will be reported to the relevant institutional and national ethical committee http://www.ett.hu/tukeb.htm.

V.5.18 Additional information and future plan.

Blood samples (serum and plasma) will be stored from all patients in order to study laboratory parameters later if required (e.g. the laboratory could not measure it), and in order to build up a biobank for later clinical studies to which all participants will be given informed consent. The samples will be stored on -80^{o}C. A follow-up study (called GOULASH PLUS) is under preparation in order to follow the patients for up to 5 years after the study. The study protocol will also be published.

V.5.19 Ethics and dissemination.

The trial is registered at the ISRCTN registry (ISRCTN63827758) and got the relevant ethical approval with the reference number of 55961-2/2016/EKU issued by The Scientific and Research Ethics Committee of the Medical Research Council. It is almost needless to say that at the end of the project we will disseminate our results in the medical community. We will publish our results in an open access way.

V.6 Discussion

There are different therapeutic approaches available with regard to nutrition in acute pancreatitis. The recently published IAP/APA (International Association of Pancreatology/American Pancreatic Association) guidelines recommend that enteral tube feeding be the primary therapy in patients with predicted severe and severe acute pancreatitis who require nutritional support (recommendation G. Nutritional support 21-GRADE 1B, strong agreement)[46], whereas point K22 in the Japanese guidelines states that enteral nutrition can reduce the incidence of complications in the early phase of SAP and can contribute to an

increased rate of survival [134]. However, neither of the guidelines provides recommendations on MAP. The reason is understandable. (1) Strong endpoints are missing. The mortality rate is less than 1% in mild AP and 10% in moderate AP, whereas almost no MOF can be detected; (2) since there is a better outcome of the milder disease, researchers have had much less interest in MAP than SAP.

First, we wanted to systematically review the current literature to understand the beneficial effects of early enteral nutrition versus the nil per os diet both in SAP and MAP. Interestingly, there were not many articles in which analyzable data could be found on the two treatments of AP. However, in SAP, the amount of data was sufficient to prove the beneficial effects of enteral feeding. Early enteral feeding was clearly beneficial for MOF and intervention and showed a beneficial tendency for mortality. Nevertheless, as predicted, MAP data analyses revealed no significant difference between enteral nutrition and a nil per os diet. However, analyses of the secondary endpoints in the articles demonstrated that enteral feeding could be beneficial compared to a nil per os diet in mild and moderate AP as well.

The six MAP studies applied different methods for enteral feeding. Eckerwall et al. employed immediate oral feeding[113], Abou-Assi et al. [47], Oláh et al. [103], and McClave et al. administered nasojejunal feeding[101], and Petrov et al. and Ma et al. used nasogastric feeding[124,131]. Immediate oral feeding (EN) significantly cut the length of hospital stay without any adverse events [113]. Nasogastric feeding starting within 24 h of hospital admission was not only well tolerated, but also reduced the intensity and duration of abdominal pain, decreased the necessity of opiates, and almost totally eliminated the risk of oral food intolerance [124]. Moreover, patients in the nasogastric feeding group had significantly improved appetite vs. the NPO group [131]. Nasojejunal feeding lowers the stress response to AP [101] associated with a lower complication rate [103] and cuts the length of hospital stay. Importantly, the fact that all of the studies found merit in early enteral feeding in MAP suggests that it is not the way of feeding that is important, but the feeding itself, i.e., energy. Therefore, finally, we went further and developed the GOULASH trial, which is a randomized controlled two-arm double-blind multicentre trial. It will provide the first evidence concerning the necessity of early energy supply for patients suffering from acute pancreatitis.

In summary, this study provides the first and type A evidence concerning the necessity of energy intake for patients suffering from AP. Please note that this protocol is the first version of the trial completed on 24th May 2017. The latest protocol can be read at https://tm-centre.org/en/trials/goulash/

VI. Limitations

All kind of scientific methodology has its own limitations. The quality of the included articles and the published data in a meta-analysis is questionable. However, in a prospectively collected cohort population the quality of data is much better but on the other hand, the number of recruited patients is significantly less. Concerning the clinical usability of the results of investigations the well designed randomized controlled trials are the most reliable, however, the arrangement of the study requires financial, human resources and valuable time support.

Limitation 1 Concerning the meta-analysis about ageing[1] the included articles presented age of the patients in median, mean or IQR; therefore, the results of this meta-analysis should be interpreted with caution.

Limitation 2 The severity scoring guidelines have changed considerably over the years; therefore, there might be cases in the meta-analysis of ageing where severities have been misclassified in the studies under analysis compared to our current knowledge.

Limitation 3 The large variety of studies caused high heterogeneity which may indicate hidden distorting factors in the meta-analysis about ageing[1].

Limitation 4 We could not explain the reason why the mortality of the 50-59-year age group is lower than that of the 40-49-year age group. Therefore, it can not exclude the possibility that the mortality rate is monophasic and the cutoff A70 is better than the cut off of 59[1].

Limitation 5 Despite the high case number, event numbers concerning some outcomes limited the analysis. To overcome this, we merged similar items of CCI (e.g., malignant tumors) when imputing them in multivariate models, as seen in other works (Murata et al., 2015)[3].

Limitation 6 The non-normal distribution of age and CCI forced us to set up age and comorbidity categories in multivariate analysis[3].

Limitation 7 Despite the four-level data checking system in the Pancreas Registry, imprecision of data recording cannot be excluded[3].

Limitation 8 Distribution of continuous variables proved to be non-normal so that multivariate regression was not performed in terms of LOH. Instead, a dichotomized logistic regression model was used[3].

Limitation 9 The biggest limitation concerning the meta-analysis about enteral feeding is the small number of studies included (especially in MAP) which caused higher

heterogeneity. The low amount of extracted data from the articles caused further difficulties. In MAP, a uniform point system had to be developed to make the data analyzable[4].

Limitation 10 In the Goulash study to detect a treatment effect of at least 50% of the early treatment, a sample size of 957 subjects will be necessary to be recruited which delay the final conclusion of the study[5].

VII. Conclusions - new observations – clinical benefits

1) Pancreatitis-associated mortality is more common with advanced age.
2) The rapid elevation of mortality above the age of 59 suggests the involvement of additional deteriorating factors such as co-morbidity in the elderly. Changing age to comorbidity might be reasonable in predicting scoring systems.
3) Comorbidities determine mortality whereas both comorbidities and ageing predict severity of AP.
4) Enteral feeding is beneficial compared to a nil per os diet not only in severe but also in mild and moderate AP.
5) Development of the GOULASH trial.

The results written in **Chapter 1 change the thinking on severity prediction**. Until now only ageing is included in the scoring systems. However, based on our results it is obvious that **comorbidity** should be included as well. This may lead to the development of more sensitive and specific **risk stratification** in AP.

The results written in **Chapter 2** change our understanding concerning the nutrition in AP. Based on the meta-analysis showing that early enteral feeding is beneficial not only in severe but also in mild AP we started early enteral nutrition in our GI division. Within 1 year **we could decrease the mortality from 30 to 10% in severe AP**, in addition, we could decrease the length of hospitalization with around **400 days/year**.

VIII. My own work

Article No1

I was involved in: i) the study design, ii) article search, iii) data extraction, iv) risk of bias and quality assessment, v) consultation with biostatisticians, vi) developing the data interpretation with biostatisticians and the PI and in vii) developing the publication strategy. I wrote version No1 of the article and took part in developing the final version as well. I also prepared v1 of the ‚answers to the reviewers' and the revision.

Article No2

In this knowledge publication, I was involved in the literature search for relevant publications and helped to develop publication strategy. I wrote the version 1 of the article, and I took part in developing the final version. I prepared the version 1 of the, answers to the reviewers' and the revision.

Article No3

During the three years, I recruited patients suffering from AP to the registry (approximately 50 to 70 patients). I was also actively involved in the monitoring of data quality. I also helped in data interpretation.

Article No4

I was involved in: i) the study design, ii) article search, iii) data extraction, iv) risk of bias and quality assessment, v) consultation with biostatisticians, vi) developing the data interpretation with biostatisticians and the PI, vii) publication strategy plan. I wrote the version 1 of the article, and I took part in developing the final version. I prepared the version 1 of the ‚answers to the reviewers' and the revision.

Article No5

I was involved in: i) the study design, ii) sample size calculation, iii) randomization plan. I wrote the version 1 of the article, and I took part in developing the final version. I prepared the version 1 of the ‚answers to the reviewers' and the revision. I was involved in: iv) the development of the local protocol, v) I coordinated the patient recruitment, vi) I recruited approximately 40 patients in Pécs, vii) I educated and later controlled Székesfehérvár, Debrecen and Gyula centers. I was involved in the safety analysis of the study.

IX. Future carrier plan

During my Ph.D. work, I learned several clinical methodologies such as study designs, retrospective and prospective data analysis, observational and interventional clinical trials, meta-analysis, network meta-analysis, case report, EBM guideline. I also had a chance to be involved in the clinical management of the patients from admission until the discharge of the patients. However, I am also interested in the basic science part of the translational medicine, therefore, I spent 6 months in a high-quality basic science research group focusing on pathomechanism of the pancreatitis at the University of Szeged.

I would like to continue my personal development in basic science, therefore I moved to the USA and joined to one of the best research groups (MITOCARE) led by Professor György Hajnóczky. After my USA training, I want to bring knowledge back to Hungary and wish to be an independent scientist. I wish to continue my clinical development as a trainee gastroenterologist and wish to be a translational gastroenterologist.

X. Acknowledgment

I would like to express my enormous gratefulness to my supervisor **Péter Hegyi,** the head of the Center, Division and Institute for Translational Medicine for his belief in me and in my commitment to science. He managed my studies and always provided support and useful advice throughout my work. I endeavor to have a carrier he might be proud of.
I also would like to thank **Kálmán Tóth** the head of the First Department of Internal Medicine and **Áron Vincze** the head of the GI Division, who allowed me to fulfill the clinical part of my work. I am also grateful to the interdisciplinary research unit led by **Andrea Szentesi**.
I am especially thankful for the **Hungarian Pancreatic Study Group** for data collection including the highly dedicated administrators, local clinical investigators, patient coordinator, and biobank leaders. I wish to express my gratitude to the head of administrators at PTE, **Krisztina Harth**, the biostatisticians especially **Nelly Farkas**, **Péter Mátrai** and **Noémi Gede**.
I thank for the great help of **Tímea Molnár** and **Krisztina Heid** for the ethical management, **Richárd Farkas** for the IT development, **Bálint Erőss, Zsolt Szakács, Dalma Dobszai** and **Dalma Erdősi** for their helpful advice. I am thankful for my mentor in teaching **Márta Balaskó**.
I would like to thank the GI team, nurses, residents for my clinical knowledge improvement.
My basic science knowledge could not have been improved without the help of **Viktória Venglovecz, Emese Tóth, Zsuzsa Miklósné Árva,** and **Edit Magyarné Pálfi.**

I also would like to express my gratitude to **all of my colleagues** at the Institute and Department for Translational Medicine for inspiring my studies, assist my work with useful ideas and the helpfulness in need.

Last but not least, my deepest gratitude goes to **my parents, brother and other members of the family** for their love, encouragement, and support during my studies and

research work; this dissertation would have been impossible to accomplish without their support. I would like to dedicate this thesis to them.

XI. References

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frontiers in Physiology

SYSTEMATIC REVIEW
published: 02 April 2019
doi: 10.3389/fphys.2019.00328

Aging and Comorbidities in Acute Pancreatitis I: A Meta-Analysis and Systematic Review Based on 194,702 Patients

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OPEN ACCESS

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Specialty section:
This article was submitted to Gastrointestinal Sciences, a section of the journal Frontiers in Physiology

Received: *02 October 2018*
Accepted: *11 March 2019*
Published: *02 April 2019*

Citation:
Márta K, Lazarescu A-M, Farkas N, Mátrai P, Cazacu I, Ottóffy M, Habon T, Erőss B, Vincze À, Veres G, Czakó L, Sarlós P, Rakonczay Z and Hegyi P (2019) Aging and Comorbidities in Acute Pancreatitis I: A Meta-Analysis and Systematic Review Based on 194,702 Patients. Front. Physiol. 10:328. doi: 10.3389/fphys.2019.00328

Background: Acute pancreatitis (AP) is one of the most common cause of hospitalization among gastrointestinal diseases worldwide. Although most of the cases are mild, approximately 10–20% of patients develop a severe course of disease with higher mortality rate. Scoring systems consider age as a risk factor of mortality and severity (BISAP; >60 years, JPN>70 years, RANSON; >55 years, APACHE II >45 years). If there is a correlation between aging and the clinical features of AP, how does age influence mortality and severity?

Aim: This study aimed to systematically review the effects of aging on AP.

Methods: A comprehensive systematic literature search was conducted in the Embase, Cochrane, and Pubmed databases. A meta-analysis was performed using the preferred reporting items for systematic review and meta-analysis statement (PRISMA). A total of 1,100 articles were found. After removing duplicates and articles containing insufficient or irrelevant data, 33 publications involving 194,702 AP patients were analyzed. Seven age categories were determined and several mathematical models, including conventional mathematical methods (linear regression), meta-analyses (random effect model and heterogeneity tests), meta-regression, funnel plot and Egger's test for publication bias were performed. Quality assessment was conducted using the modified Newcastle–Ottawa scale. The meta-analysis was registered in the PROSPERO database (CRD42017079253).

Results: Aging greatly influences the outcome of AP. There was a low severe AP incidence in patients under 30 (1.6%); however, the incidence of severe AP showed a continuous, linear increase between 20 and 70 (0.193%/year) of up to 9.6%. The mortality rate was 0.9% in patients under 20 and demonstrated a continuous linear elevation until 59, however from this age the mortality rate started elevating with 9 times

higher rate until the age of 70. The mortality rate between 20 and 59 grew 0.086%/year and 0.765%/year between 59 and 70. Overall, patients above 70 had a 19 times higher mortality rate than patients under 20. The mortality rate rising with age was confirmed by meta-regression (coefficient: 0.037 CI: 0.006–0.068, $p = 0.022$; adjusted r^2: 13.8%), and severity also (coefficient: 0.035 CI: 0.019–0.052, $p < 0.001$; adjusted r^2: 31.6%).

Conclusion: Our analysis shows a likelihood of severe pancreatitis, as well as, pancreatitis-associated mortality is more common with advanced age. Importantly, the rapid elevation of mortality above the age of 59 suggests the involvement of additional deteriorating factors such as co-morbidity in elderly.

Keywords: acute pancreatitis, aging, mortality, severity, co-morbidity

INTRODUCTION

Rationale

Life expectancy has dramatically risen by 16 years (from 55.4 years to 71.4 years) in the last half century, causing a number of changes and challenges to economies and healthcare systems (**Figure 1**). Needless to say, healthcare professionals should focus more intensively on the effects of aging on the course and outcome of diseases.

Acute pancreatitis (AP) is one of the most challenging gastrointestinal disorders: (1) its development is not fully understood (Sahin-Toth and Hegyi, 2017) and it has no specific therapy (Hegyi and Petersen, 2013); (2) its incidence rate is continuously increasing (Peery et al., 2015); and (3) it has an unacceptably high mortality (Parniczky et al., 2016). Unfortunately, gastrointestinal scientists are devoting ever less attention to AP (Szentesi et al., 2016). One of the best examples of this is that mathematical analysis on the effects of aging on many diseases, such as neurophysiological and liver disorders, have been performed (Mizuguchi et al., 2015) but no systematically collected information is available on AP.

Objectives

Age is used as a predictive marker in different scoring systems for AP (**Table 1**). These scoring systems show a great variety in the age group: in the (i) Bedside Index for Severity in Acute Pancreatitis score (BISAP) (Wu et al., 2008), the topmost risk of age is above 60; (ii) in BALI (BUN, Age, LDH, IL-6), it is over 65 (Spitzer et al., 2006); (iii) in the Simplified Acute Physiology Score (SAPS II), it is >40 (Legall et al., 1993); (iv) in Ranson score, it is above 55 (Blamey et al., 1984); (v) in Acute Physiology and Chronic Health Evaluation (APACHE II), it is over 45 (Wagner and Draper, 1984); and (vi) in the Japanese Severity Score (JNP), it is >70 (Hirota et al., 2006). The wide range of age limits suggests that a low number of patients, a selection bias and/or a mathematical inaccuracy could have occurred.

Research Question

In order to minimize these distorting factors, we aimed to (i) comprehensively search and select articles in which all AP cases have been included and (ii) use several mathematical models to understand the effects of aging on the outcome of AP.

METHODS

Study Design, Participants, Interventions, Comparators

The meta-analysis was performed using the preferred reporting items for systematic review and meta-analysis statement (PRISMA) (Moher et al., 2009). We used the classical PICO format to form a question applicable for search in databases: P: acute pancreatitis; I and C: different age categories [under 20 (U20), 20–29, 30–39, 40–49, 50–59, 60–69, and above 70 (A70)]; O: mortality and severity. In order to provide the highest level of quality, the meta-analysis was registered with the PROSPERO registry (CRD42017079253).

Search Strategy

A search was performed in three databases (Embase, PubMed and Cochrane) in January 2017 using the following terms: PubMed: {acute[All Fields] AND ("pancreatitis"[MeSH Terms] OR "pancreatitis"[All Fields])} AND {cohort[All Fields] OR ("clinical trial"[Publication Type] OR "clinical trials as topic"[MeSH

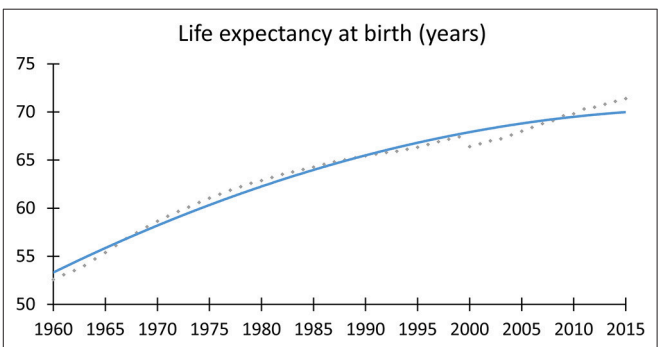

FIGURE 1 | Life expectancy at birth. There is a steadily rising average life expectancy at birth. It has dramatically risen by 16 years (from 55.4 to 71.4 y) in the last half century. Data sources: between 1960 and 1999, World Bank; between 2000–2015, WHO.

Abbreviations: A70, above 70 years; ABP, acute biliary pancreatitis; AP, acute pancreatitis; APACHE II, Acute Physiology and Chronic Health Evaluation; BALI, BUN, Age, LDH, IL-6; BISAP, Bedside Index for Severity in Acute Pancreatitis score; CI, confidence interval; ES, effect sizes; IQR, interquartile range; JNP, Japanese Severity Score; OR, odd's ratio; U20, under 20 years; PRISMA, preferred reporting items for systematic review and meta-analysis statement; SAPS II, Simplified Acute Physiology Score; SD, standard deviation.

TABLE 1 | Characteristics of the scoring systems.

Score system	Publ. (year)	Outcome	Time at measurement	Age cutoff	Patient enrolment	LEB	Age	
							Med.	Mean
Ranson	1974	Severity	48 h	55	1971–1975	60.12		42 50
APACHE II	1982	Severity	24 h	45	1979–1981	62.9	–	
SASP II	1993	Mortality	last 24 h	40	1991	65.6		57.2
JPN	2002	Severity	–	70	1995–1998	66.75	–	
BALI	2006	Mortality	48 h	65	–	–		61 ± 16
BISAP	2008	Morality	24 h	60	2000–2001	66.55	53	

There is a slight elevation in the age of enrolled patients and cut-off values (LEB: Life expectancy at birth). Ranson (Blamey et al., 1984); APACHEII–Acute physiology and chronic health evaluation (Wagner and Draper, 1984); SAPS II–Simplified Acute Physiology Score (Legall et al., 1993); JNP–Japanese Severity Score (Hirota et al., 2006); BALI–BUN, Age, LDH, IL-6 (Spitzer et al., 2006); BISAP–Bedside Index for Severity in Acute Pancreatitis (Wu et al., 2008).

Terms] OR "clinical trial"[All Fields])} AND ("Age"[Journal] OR "age"[All Fields] OR "Age (Omaha)"[Journal] OR "age"[All Fields] OR "Age (Dordr)"[Journal] OR "age"[All Fields] OR "Adv Genet Eng"[Journal] OR "age"[All Fields]) Embase: acute pancreatitis and (cohort or clinical trial) and age; and Cochrane: acute AND pancreatitis AND (cohort OR clinical) AND trial AND age.

Data Sources, Study Selection, and Data Extraction

Two independent authors read the articles for eligibility (age data from cohort and pilot studies) (A-ML, KM). The flow diagram recommended by the PRISMA guidelines shows the article selection procedure (**Figure 2**) (Moher et al., 2009). When conflicts arose, a third participant (PH) made the decision. Two authors collected data in an Excel file (Microsoft Corporation, Redmond, WA98052, USA) according to age (mean, median, range, standard deviation (SD) and interquartile range (IQR), where possible), study type, severity, mortality, and notes (A-ML, KM).

Data Analysis

All meta-analytic calculations were performed with STATA software Version 11 (Stata Corporation, College Station, TX, USA). In our meta-analysis, the pooled effect sizes (ES) were the event rates with a 95% confidence interval (CI) for all outcomes. The random effect model by DerSimonian and Laird was used in all cases (DerSimonian and Laird, 1986). Heterogeneity was tested using Cochrane's Q and the I^2 statistics. I^2 statistics represent the percentage of effect size heterogeneity, which cannot be explained by random chance, but by other factors. I^2-values of 25, 50, and 75% corresponded to low, moderate and high degrees of heterogeneity, based on the Cochrane handbook (Higgins, 2011). If the Q test is significant, it implies that the heterogeneity among effect sizes reported in the observed studies is greater than could be explained only by random error. We considered the Q test significant if $p < 0.1$. The forest plot was evaluated to represent the data. Publication bias was examined by visual inspection as asymmetry in the funnel plot and Egger's test (Sterne et al., 2001). A significant test result ($p < 0.1$) indicates the presence of bias.

A meta-regression was used to consider the effect of aging on mortality and severity. In both cases, we tested the hypothesis that all coefficients are zero. The results are provided as regression coefficients, 95% CIs, *p*-values and the explained variances of the models (R^2 analogs).

A conventional regression analysis was also performed to confirm the results of the meta-regression. In this case, we used the pooled event rates from the subgroup analyses and the middle of the age subgroups as independent variables. We used the IBM SPSS Statistics software for these calculations (IBM Corporation, Armonk, New York, USA, Version 24).

Quality Assessment

The quality of the articles was assessed by 3 main categories recommended by the modified Newcastle-Ottawa scale (**Table 2**, **Supplementary Figure 1**).

RESULTS

Flow Diagram of Studies Retrieved for the Review, Study Selection, and Characteristics

Our search yielded 1,100 articles (704, 379, and 17 in Embase, PubMed, and Cochrane, respectively) (**Figure 2**). Eleven additional articles were found with potential data eligibility for the meta-analysis in the references of the primarily selected articles. After excluding duplicates and irrelevant articles, a total of 33 articles involving 194,702 patients met the inclusion criteria (**Table 2**).

Synthetized Findings

Severity

A total of 23 studies with 22,451 patients were suitable for analyzing severity (**Tables 2**, **3**) (Abou-Assi et al., 2002; Gürleyik et al., 2005; Muller et al., 2006; De Waele et al., 2007; Knoepfli et al., 2007; Uomo et al., 2007; Radenkovic et al., 2009; Gomez Beltran et al., 2013; Gornik et al., 2013; Nijmeijer et al., 2013; Albulushi et al., 2014; de-Madaria et al., 2014; Zuidema et al., 2014; Ho et al., 2015; Ocampo et al., 2015; Wang et al., 2015; Yue et al., 2015; Karpavicius et al., 2016; Mole et al., 2016; Parniczky et al., 2016; Rashidi and Røkke, 2016; Weitz et al., 2016; Zhang et al., 2016).

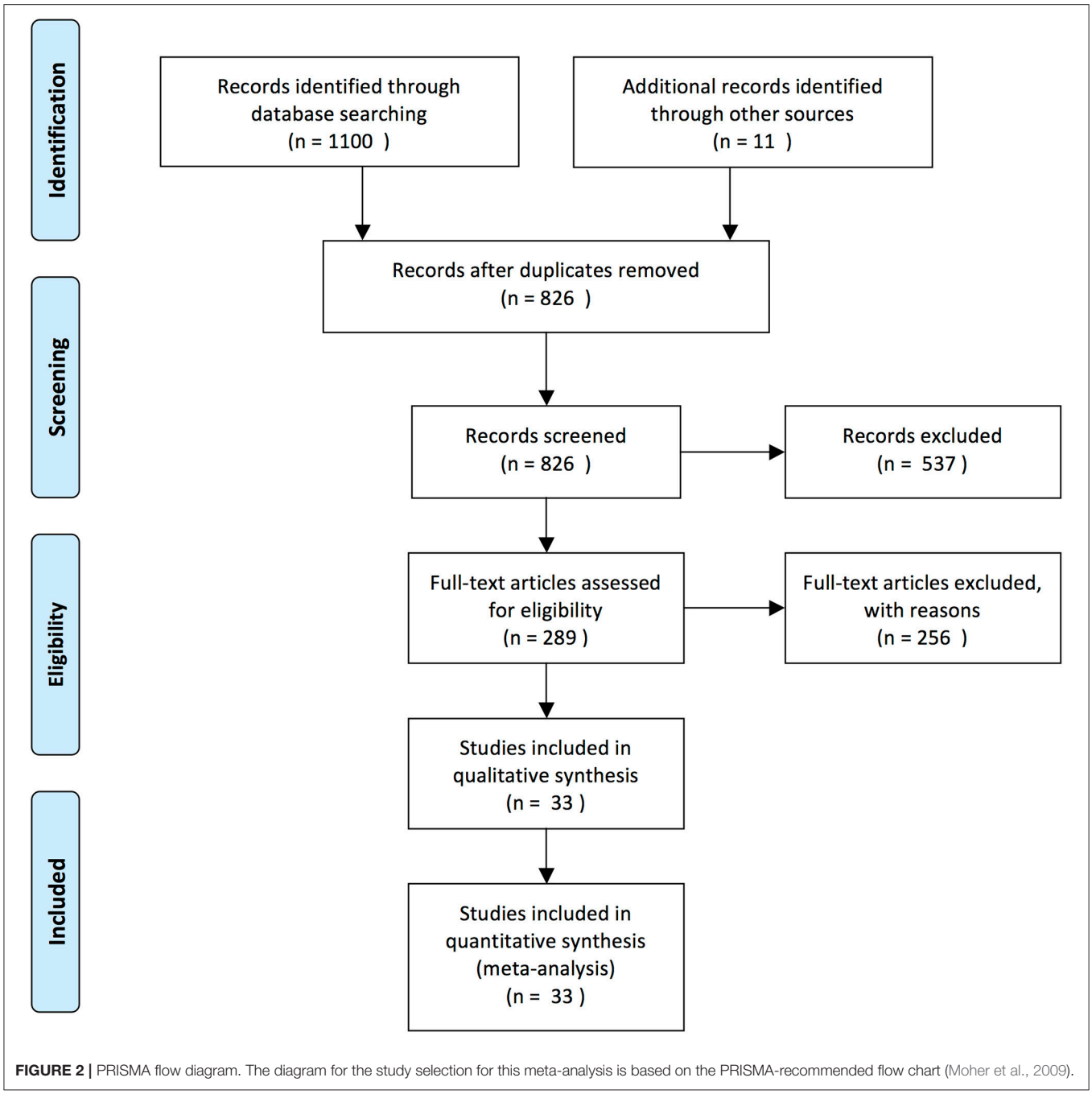

FIGURE 2 | PRISMA flow diagram. The diagram for the study selection for this meta-analysis is based on the PRISMA-recommended flow chart (Moher et al., 2009).

Two thousand Four Hundred Eighty Nine severe cases were found divided into seven age groups with a low severity rate under 30 years. There was a low incidence severe AP rate in patients under 30 and rose continuously between ages 30 and 70 (**Table 3**).

Firstly, a meta-regression was performed to investigate the relationship between age and severity (**Figure 3**). The number of patients in each age group category was extremely diverse (between 24 and 11,933); however, a significant relationship was detected (coefficient: 0.035 CI: 0.019–0.052, $p < 0.001$; adjusted r^2: 31.6%). A conventional regression analysis was also performed showing a linear increase (0.193%/year) from ages U20 to A70 (**Figure 4**).

This continuous elevation was also confirmed by forest plot (**Figure 5**). There was 1 severe AP U20: 4.2% (1/24; pooled event rate: 0.042 CI: −0.077–0.161); 20–29: 0% (0/36; pooled event rate: 0.014 CI: 0.077–0.104); 30–39: 6.7% (5/75; pooled event rate: 0.067 CI: −0.005–0.128); 40–49: 9.2% (726/7882; pooled event rate: 0.109 CI: 0.046–0.172); 50–59: 11.3% (1352/11 933; pooled event rate: 0.201 CI: 0.158–0.245); 60–69: 16.6%

TABLE 2 | The modified Newcastle–Ottawa quality assessment scale.

Study	Sample size	Severe case	Mortality	Study type	Modified Newcastle-Ottawa quality assessment scale								
					Selection				Comparability	Outcome			Sum
					S1	S2	S3	S4	C1	O1.1	O1.2	O2	O3	
Abou-Assi et al., 2002	156	5	14	Prospective	1	1	0	1	1	0	1	1	1	7
Albulushi et al., 2014	174	14	0	Retrospective	1	1	0	0	1	0	1	1	1	6
Gomez Beltran et al., 2013	24	1	0	Retrospective	1	1	1	1	1	0	1	1	1	8
de-Madaria et al., 2014	403	28	17	Prospective	1	1	1	1	0	1	0	1	1	7
Dombernowsky et al., 2016	359	nd	13	Retrospective	1	1	1	1	1	0	1	1	1	8
Gompertz et al., 2012	128	nd	2	Retrospective	1	1	1	1	0	0	0	1	1	6
Gompertz et al., 2013	1367	nd	115	Retrospective	1	1	1	1	0	1	0	1	1	7
Gonzalez-Gonzalez et al., 2012	605	nd	30	Prospective	1	1	1	1	1	1	1	1	1	9
Gornik et al., 2013	1058	210	41	Prospective	1	1	0	1	0	0	1	1	1	6
Gürleyik et al., 2005	55	13	1	Prospective	1	1	1	1	1	1	1	1	1	9
Karpavicius et al., 2016	102	20	5	Prospective	1	1	1	1	0	1	0	1	1	7
Knoepfli et al., 2007	310	63	8	Prospective	1	1	1	1	1	1	0	1	1	8
Lautz et al., 2011	211	nd	0	Retrospective	1	1	1	1	0	1	1	1	1	8
Milheiro et al., 1995	91	nd	10	Retrospective	1	1	0	1	0	0	0	1	1	5
Mole et al., 2016	2053	390	102	Retrospective	1	1	0	1	0	0	1	1	1	6
Muller et al., 2006	109	66	8	Prospective	1	1	1	1	1	1	1	1	1	9
Nijmeijer et al., 2013	622	119	20	Prospective	1	1	0	1	0	0	0	1	1	5
Ocampo et al., 2015	854	140	nd	Prospective	1	1	0	1	1	0	1	1	1	7
Pant et al., 2014	55012	nd	509	Retrospective	1	1	0	0	0	0	0	1	1	4
Parniczky et al., 2016	600	53	17	Prospective	1	1	1	1	1	1	1	1	1	9
Radenkovic et al., 2009	91	24	8	Prospective	1	1	1	1	0	1	0	1	1	7
Rashidi and Røkke, 2016	670	43	37	Prosp and Retrosp	1	1	1	1	1	1	0	1	1	8
Spanier et al., 2013	78257	nd	9515	Retrospective	1	1	0	1	1	0	1	1	1	7
Uomo et al., 2007	1173	167	36	Prospective	1	1	1	1	0	1	1	1	1	8
De Waele et al., 2007	40	14	6	Retrospective	1	1	1	1	0	1	0	1	1	7
Wang et al., 2015	120	31	13	Retrospective	1	1	0	1	0	0	0	1	1	5
Ho et al., 2015	12284	765	nd	Retrospective	0	1	0	1	0	0	0	1	1	4
Weitz et al., 2016	346	21	12	Retrospective	1	1	1	1	0	1	1	1	1	8
Wu et al., 2008	36178	nd	569	Retrospective	1	1	0	0	0	0	0	1	1	4
Yeung et al., 1996	43	nd	1	Retrospective	1	1	0	0	1	0	1	1	1	6
Yue et al., 2015	169	68	nd	Prospective	1	1	0	1	0	0	0	1	1	5
Zhang et al., 2016	974	223	58	Retrospective	1	1	1	1	0	1	1	1	1	8
Zuidema et al., 2014	64	11	3	Prospective	1	1	0	1	0	0	0	1	1	5

Ranks in three categories (green-1: low risk; red-0: high risk, yellow-0: unclear risk) are shown. S1, non-selected etiology AP; S2, all participants have an AP diagnosis; S3, AP diagnosis is confirmed using the latest guidelines; S4, non-selected severity cases. C1: comparability defined by exact age ranges in years. O1.1, severity assigned according to the latest guidelines; O1.2, described mortality (in-hospital and pancreas-related); O2–O3, adequate follow-up for outcome occurrence morality and severity.

(390/2344; pooled event rate: 0.157 CI: 0.110–0.203); A70: 9.6% (15/157; pooled event rate: 0.096 CI: 0.049–0.143). In sum, 11.1% (2489/22 451).

Publication bias was tested by inspection of funnel plot and Egger's test (CI: 1.961–6.728; $p = 0.001$). The visible asymmetry (plots are mostly concentrated to the right side) is most probably due to the fact that authors mostly present data with high volume examinations (**Supplementary Figure 2**).

The cut-off values in sorting articles to U20 and A20, U30 and A30, U40 and A40, U50 and A50, U60 and A60, and U70 and A70 (**Supplementary Figures 3–8**) resulted in significant differences considering three comparison, respectively (U30 vs. A30 $p = 0.036$; U40 vs. A40 $p = 0.009$; U50 vs. A50 $p = 0.021$) (**Figure 6**).

In addition, we performed several sub-group analysis in order to decrease the heterogeneity in our study. Firstly, we used articles only where severity was assessed by the Atlanta or the revised Atlanta classification. This additional analysis could largely decrease the heterogeneity [I^2 = 40–49: 0%, 50–59:96.9%, 60–69:86.6% (**Supplementary Figure 9**)]. Secondly, we excluded the low quality (NOS 4 and 5) studies from the analysis. This analysis also could improve the heterogenity [(I^2 = 40–49: 96.3%, 50–59:96.5%, 60–69:86.6% (**Supplementary Figure 10**)].

And finally, we excluded studies from the analysis where age ranges might overlap between the groups because of given age ranges. We could also successfully decrease the

TABLE 3 | Data of patient's number and severe cases in age groups.

Age	Severe AP	Patient no.	%
U20	1	24	4.2
20–29	0	36	0.0
30–39	5	75	6.7
40–49	726	7882	9.2
50–59	1352	11933	11.3
60–69	390	2344	16.6
A70	15	157	9.6
Sum	2489	22451	11.1

There was only one severe AP in patients under 30; however, the incidence of severe AP rose continuously between ages 30 and 70.

heterogeneity [(I^2 = 40–49: 98%, 50–59:97.1%, 60–69:86.6% (**Supplementary Figure 11**)].

Importantly, none of them modified the outcome of the study which decrease the overall limitations of our results.

Mortality

Thirty studies involving 181,395 subjects contained data on mortality (Milheiro et al., 1995; Yeung et al., 1996; Abou-Assi et al., 2002; Gürleyik et al., 2005; Muller et al., 2006; De Waele et al., 2007; Knoepfli et al., 2007; Uomo et al., 2007; Wu et al., 2008; Radenkovic et al., 2009; Lautz et al., 2011; Gompertz et al., 2012, 2013; Gonzalez-Gonzalez et al., 2012; Gomez Beltran et al., 2013; Gornik et al., 2013; Nijmeijer et al., 2013; Spanier et al., 2013; Albulushi et al., 2014; de-Madaria et al., 2014; Pant et al., 2014; Zuidema et al., 2014; Wang et al., 2015; Dombernowsky et al., 2016; Karpavicius et al., 2016; Mole et al., 2016; Parniczky et al., 2016; Rashidi and Røkke, 2016; Weitz et al., 2016; Zhang et al., 2016) (**Tables 2**, **4**). Eleven thousand one hundred and seventy deceased cases were found in the seven age groups with the highest rates in groups 40–49 and A60 (**Table 4**). Considering that a severe course of AP increases the risk for mortality, we expected a similar regression to severity (**Figure 4**). The mortality rate was 0.9% in patients under 20 and demonstrated a continuous, linear elevation until 59, however from this age the mortality rate started elevating with 9 times higher rate until the age of 70 (**Figure 7**). The mortality rate grew 0.086%/year between ages 20 and 59 and 0.765%/year between 59 and 70 (**Figure 7**). Overall, patients above 70 had a mortality rate 19 times higher than those under 20 (**Table 4**). The mortality rate rising with age was also confirmed by forest plot, showing a clear elevation from pediatric to elderly patients: U20: 0.9% (510/55 290; pooled event rate: 0.009 CI: 0.008–0.010); 20–29: 2.6% (5/1912; pooled event rate: 0.009 CI: −0.011–0.029); 30–39: 1.2% (139/11 527; pooled event rate: 0.012 CI: 0.010–0.014); 40–49: 6.7% (202/3002; pooled event rate: 0.052 CI: 0.025–0.079); 50–59: 2% (838/41 634; pooled event rate: 0.045 CI: 0.032–0.057); 60–69: 8.5% (2153/25 452; pooled event rate: 0.052 CI: 0.015–0.088); and A70: 17.3% (7312/42 322; pooled event rate: 0.112 CI: 0.007–0.217) (**Figure 8**). In summary, 6.2% (11 170/181 395).

A meta-regression analysis on mortality showed a significant difference (coefficient: 0.037 CI: 0.006–0.068, $p = 0.022$; adjusted r^2: 13.8%, **Figure 9**). Publication bias was tested by funnel plot and Egger's test (CI: −0.901–9.234; $p = 0.104$) and showed mild asymmetry, but based on Egger's test publication bias was unlikely (**Supplementary Figure 12**).

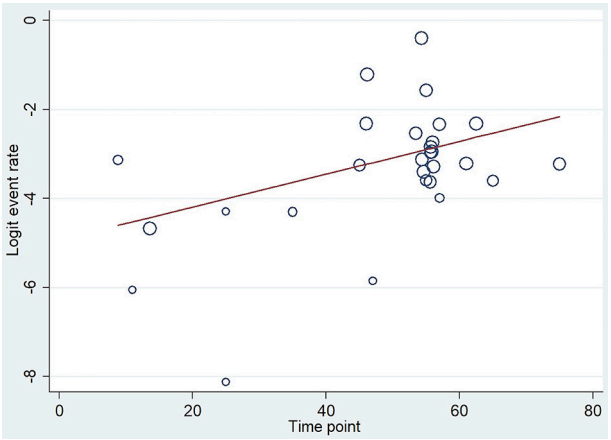

FIGURE 3 | Meta-regression of severity. The figure shows 29 data from 23 reports where x = age (mean), y = logit event rate: ln[p/(1-p)], and circle diameters show the weight of each study based on the random effect model. The meta-regression shows a significant ($p < 0.001$) relationship between age and severity ($r^2 = 31.6$), therefore the risk for developing severe cases is elevated by aging.

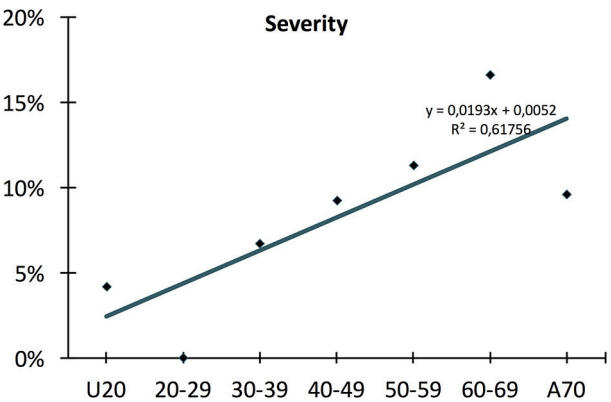

FIGURE 4 | Conventional regression of severity. The conventional regression, which is independent of distortion from diverse numbers of patients, shows a linear rise (0.193%/year) in severity from young to old age.

Forest plot analyses comparing U20 to A20, U30 to A30, U40 to A40 and U50 vs. A50 showed significant differences, respectively (U20 vs. A20 $p < 0.001$; U30 vs. A30 $p = 0.001$; U40 vs. A40 $p < 0.001$; U50 vs. A50 $p = 0.018$; U60 vs. A60 $p = 0.028$, and U70 vs. A70 $p = 0.038$) (**Supplementary Figures 13–18**). Forest plot results are summarized in **Figure 10**.

We excluded the low quality (NOS 4 and 5) studies from the analysis to lower the heterogeneity [I^2 = 40–49: 96.3%, 50–59:96.5%, 60–69:86.6% (**Supplementary Figure 19**)].

Risk of Bias and Quality Assessment

The risk of bias was examined by funnel plot and Egger's test (see above severity and mortality). The quality of the included articles were assessed by using

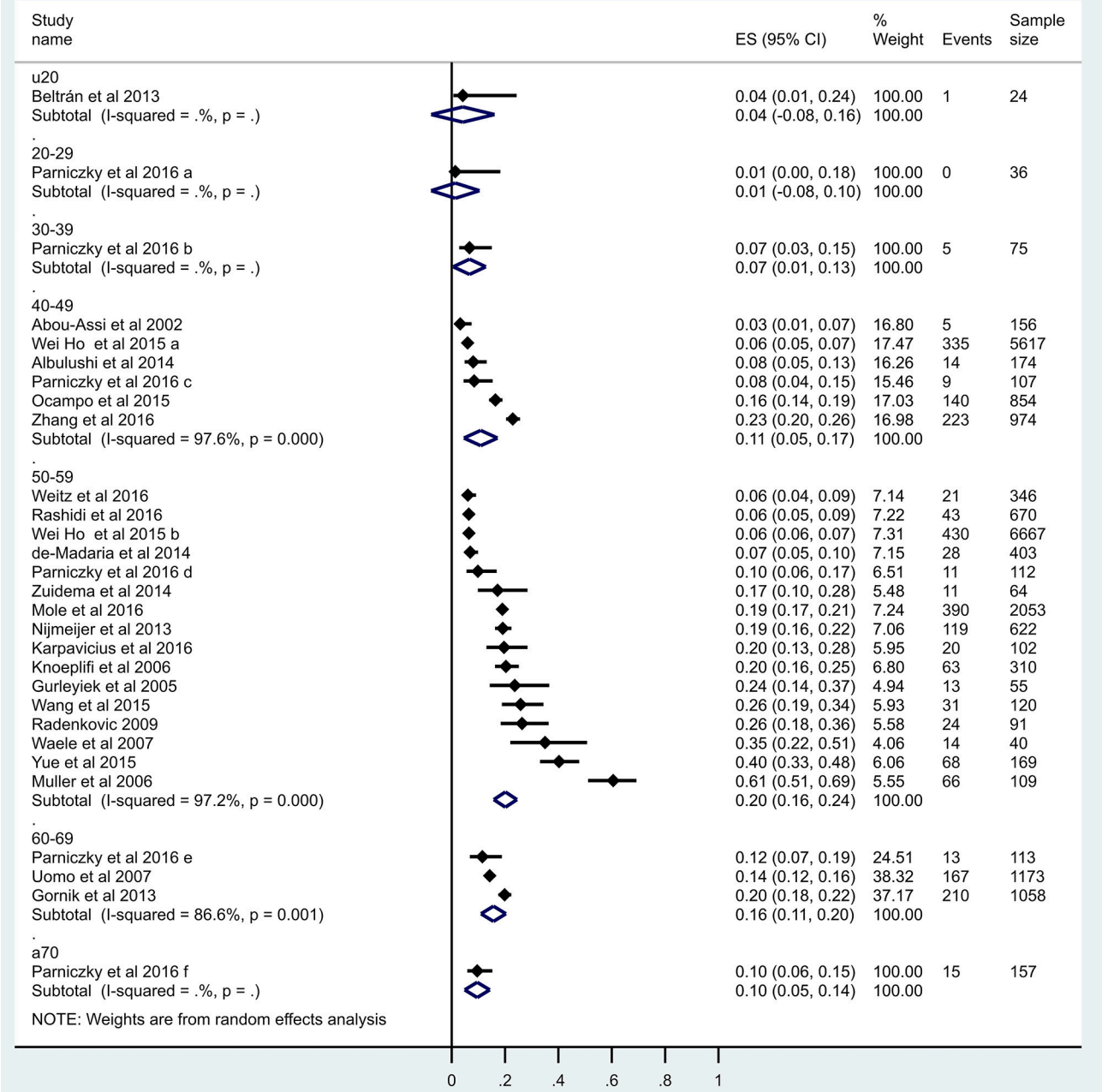

FIGURE 5 | Forest plot of studies evaluating severity in acute pancreatitis in age groups**.** Full diamonds show the weighted event rates for studies, respectively, line represents the 95% confidence interval (CI), and empty diamonds show the pooled results of severe cases with a steadily rising frequency from young to older age. Wideness of the empty diamond represents the confidence limits. Under 40 there is a slight elevation concerning severe cases, from 40 to 60 severity rates differs in the studies, then A60 remains stable.

the modified Newcastle–Ottawa scale as described earlier (Deeks et al., 2003; Mata et al., 2015; Rotenstein et al., 2016).

Two independent investigators have evaluated the articles and classified using a clear guidance described in **Supplementary Figure 1**. The following three main categories were applied: (i) selection of study groups (including four subgroups: S1: non-selected etiology AP; S2: all participants have an AP diagnosis; S3: AP diagnosis is confirmed using the latest guidelines; S4: non-selected severity cases); (ii) comparability of the groups (C1: comparability defined by exact age ranges in years); and (iii) outcome of interest (including four subgroups: O1.1: severity assigned by the latest guidelines; O1.2 described mortality (in-hospital and pancreas-related); and O2–O3: adequate follow-up for outcome occurrence, morality and

Age groups	Severity					Significancy
	ES	95% CI		ES	95% CI	
U20 vs A20	0.04	–0.08-0.16		0.16	0.13-0.18	P=0.188
U30 vs A30	0.02	–0.05-0.10		0.16	0.13-0.19	P=0.036
U40 vs A40	0.05	0.02-0.10	vs	0.16	0.14-0.19	P=0.009
U50 vs A50	0.09	0.04-0.14		0.19	0.15-0.22	P=0.021
U60 vs A60	0.16	0.13-0.18		0.14	0.10-0.19	P=0.994
U70 vs A70	0.16	0.13-0.18		0.10	0.05-0.14	P=0.133

FIGURE 6 | Forest plot results for cut-off values for severity. Summary table of pooled effect with CI and significance levels to detect cut off value. Concerning mortality all comparisons were significant, however examining severity only three. Explanation might be that in young ages there is a low event rate, in middle age groups there is a higher proportion therefore the difference is equalized leading to a non-significant difference. The same occur in the aged vs. middle aged groups.

TABLE 4 | Data of patient's number and deceased cases in age groups.

Age	Fatal event	Patient no.	%
U20	510	55290	0.9
20–29	5	1912	0.26
30–39	139	11527	1.2
40–49	202	3002	6.7
50–59	838	41790	2.0
60–69	2157	25496	8.5
A70	7319	42378	17.3
Sum	11170	181395	6.2

The incidence of severe AP rose continuously between ages 30 and 70.

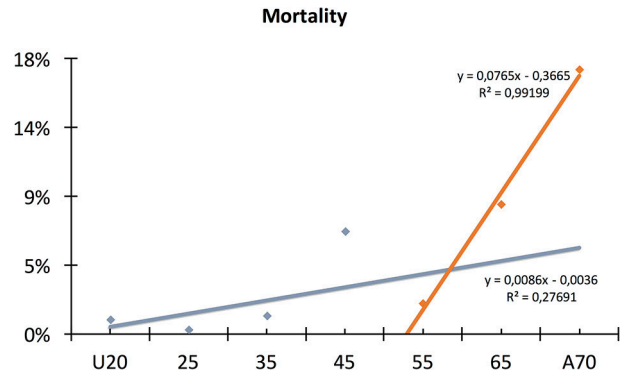

FIGURE 7 | Conventional regression of mortality. The conventional regression shows a linear elevation until 59, however from this age the mortality rate started elevating with 9 times higher rate until the age of 70.

severity). Each item was marked: green-1: low risk; red-0: high risk and yellow-0: unclear risk of bias. A total of 9 points was the maximum that could be assigned (**Table 2**) (Milheiro et al., 1995; Yeung et al., 1996; Abou-Assi et al., 2002; Gürleyik et al., 2005; Muller et al., 2006; De Waele et al., 2007; Knoepfli et al., 2007; Uomo et al., 2007; Wu et al., 2008; Radenkovic et al., 2009; Lautz et al., 2011; Gompertz et al., 2012, 2013; Gonzalez-Gonzalez et al., 2012; Gomez Beltran et al., 2013; Gornik et al., 2013; Nijmeijer et al., 2013; Spanier et al., 2013; Albulushi et al., 2014; de-Madaria et al., 2014; Pant et al., 2014; Zuidema et al., 2014; Ho et al., 2015; Ocampo et al., 2015; Wang et al., 2015; Yue et al., 2015; Dombernowsky et al., 2016; Karpavicius et al., 2016; Mole et al., 2016; Parniczky et al., 2016; Rashidi and Røkke, 2016; Weitz et al., 2016; Zhang et al., 2016).

Whenever different points were given by the investigators a third member of the team made the final decision.

DISCUSSION

Summary of Main Findings

Here we provide the first detailed meta-analysis on the effects of aging on AP. Aging has been demonstrated to play an important role in AP; however, due to the lack of detailed mathematical analysis, there is a great difference between the cut-off values used in predictive scoring systems (Blamey et al., 1984; Wagner and Draper, 1984; Legall et al., 1993; Hirota et al., 2006; Spitzer et al., 2006; Wu et al., 2008).

With regard to severity, unfortunately we only have two articles in which severity was one of the outcome parameters in youth. In one of these studies, Párniczky et al. found no severe cases in the 36 patients under 30 years of age (Parniczky et al., 2016). Similarly, Beltrán et al. found only a single severe case in cohort of 24 patients suggesting a low incidence rate of severe AP in youth (Gomez Beltran et al., 2013). Our situation was far easier regards mortality as data from large nationwide cohorts were available. In a large epidemiology study involving 55,012 patients under 20 years in the USA, Pant et al. showed that mortality is only 0.92% (Pant et al., 2014). Others have also described low mortality in smaller cohorts. Lautz et al. found 0% (0/211 patients) mortality under 20 years, while Yeung et al. reported 2.33% (1/43 patients) (Yeung et al., 1996; Lautz et al., 2011). In contrast, no mortality was found among 1,720 patients between the ages of 20 and 29 in a Hungarian and a Dutch cohort (Spanier et al., 2013; Parniczky et al., 2016). Middle-aged patients (30–59 y) had a mortality rate more than two times higher (Abou-Assi et al., 2002; Gürleyik et al., 2005; Muller et al., 2006; De Waele et al., 2007; Knoepfli et al., 2007; Wu et al., 2008; Radenkovic et al., 2009; Nijmeijer et al., 2013; Spanier et al., 2013; Albulushi et al., 2014; de-Madaria et al., 2014; Zuidema et al., 2014; Wang et al., 2015; Dombernowsky et al., 2016; Karpavicius et al., 2016;

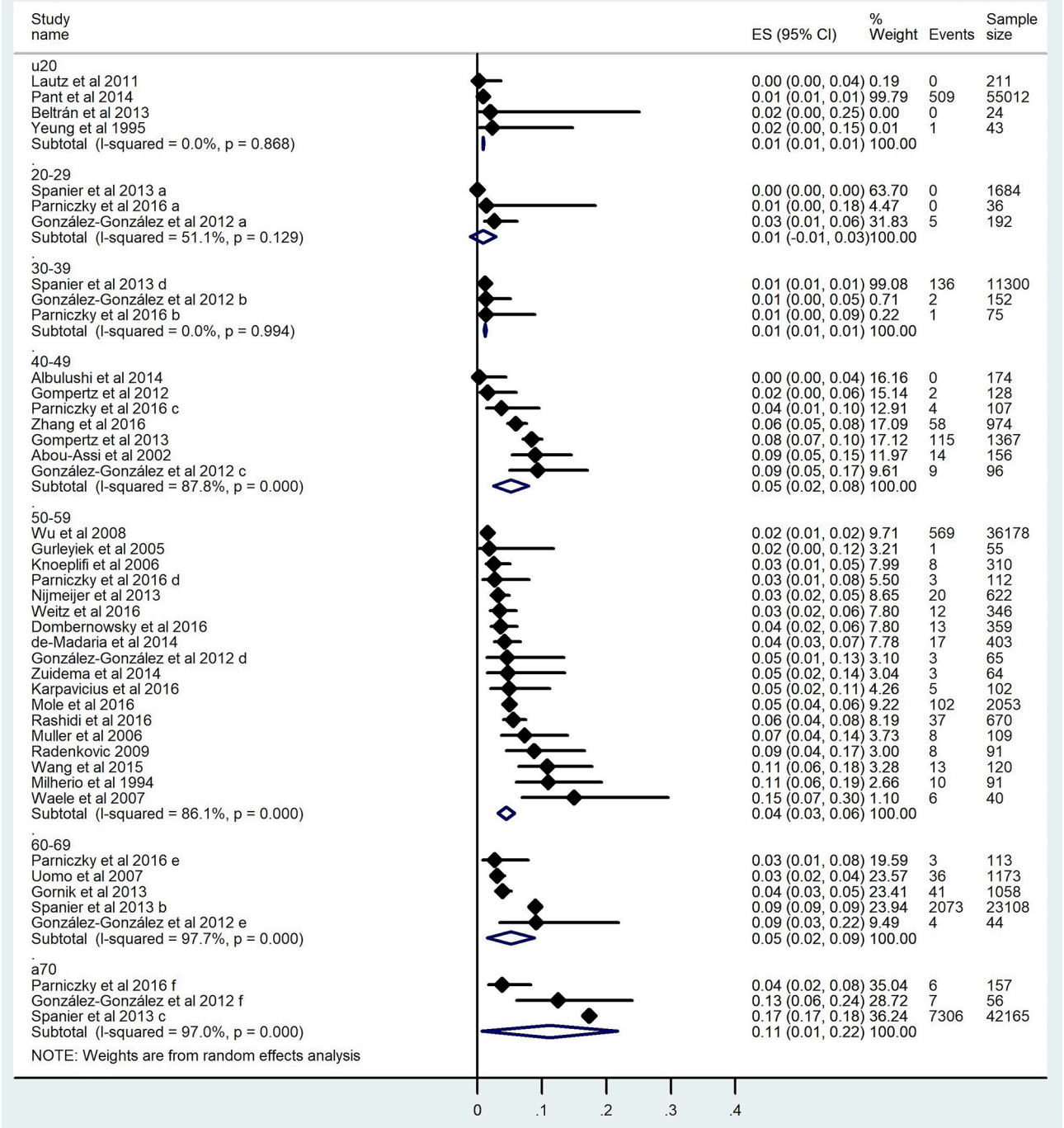

FIGURE 8 | Forest plot of studies evaluating mortality in acute pancreatitis. Full diamonds show the weighted event rates for studies, respectively, line represents the 95% confidence interval (CI), and empty diamonds show the pooled results of mortality with a steadily rising frequency from young to older age. Wideness of the empty diamond represents the confidence limits. The diamonds show a steadily rising frequency in mortality from youth to old age.

Mole et al., 2016; Parniczky et al., 2016; Rashidi and Røkke, 2016; Weitz et al., 2016; Zhang et al., 2016).

Our second main observation was that up until 59 years (this cut-off value was mathematically calculated), both severity and mortality rise linearly (**Figures 4**, **7**). The rate of severity increases 0.193%/year, and mortality grows 0.086%/year. It has been documented that almost all death cases come from the severe AP group; therefore, we can assume that although

the number of severe cases rises every year, the risk for mortality in severe AP remains constant at around 20% (Parniczky et al., 2016).

Thirdly, we found that above 59 years the mortality rate rapidly increases; meanwhile, the rate of severe pancreatitis follows the earlier, slightly elevated pattern (**Figures 4**, **7**). These data clearly suggest that additional factors which are lacking or rare below 59 years also affect mortality in AP. One of the best candidates responsible for the increased elevation of mortality in elderly is definitely co-morbidity. It has been shown that the burden of co-morbidities increases with age (Vasilopoulos et al., 2014; Murata et al., 2015). In addition, it has been also reported that the outcome of AP is worsen by severe co-morbidities (Frey et al., 2007; Murata et al., 2011). Therefore, we can hypothesize that the elevation of severity and mortality with age is attributed to co-morbidity rather than aging.

The incidence of severe AP in patients, however, showed a continuous, linear rise between the ages of 20 and 70 (0.193%/year) of up to 16.6%. The mortality rate was 0.9% in patients under 20 and demonstrated a continuous increase until the age of 70. The mortality rate between 20 and 59 grew 0.086%/year and 0.765%/year between 59 and 70. Overall, patients above 70 had a mortality rate 19 times higher than patients under 20. The rise of mortality rate with age was thus also confirmed.

In adults, the severity of AP clearly increases with age. With regard to mortality, it follows a similar linear rise until 59 years; however, after that a 9-fold change is observed in its steepness. This result completely confirms the observation of Ranson et al. that age is associated with a significantly increased risk of death over 55 years (Ranson and Pasternack, 1977; Blamey et al., 1984). Imrie et al. (1978) modified the scoring system; however, they still considered age above 60 as a valuable parameter. Blamey et al. (1984) evaluated a prospective study with 347 patients in a seven-year period to simplify the system and to improve its accuracy. With regard to age, they also found the cut-off point at 55 years.

The BISAP scoring system was established as the first population-based prognostic scoring system in order to evaluate the risk of in-hospital mortality prior to the onset of organ failure (Wu et al., 2008). The CART analysis identified age above 60 years for prediction of in-hospital mortality based on parameters collected in 2000–2001 in the first 24 h from a patient population of 17,922 suffering from AP (Wu et al., 2008).

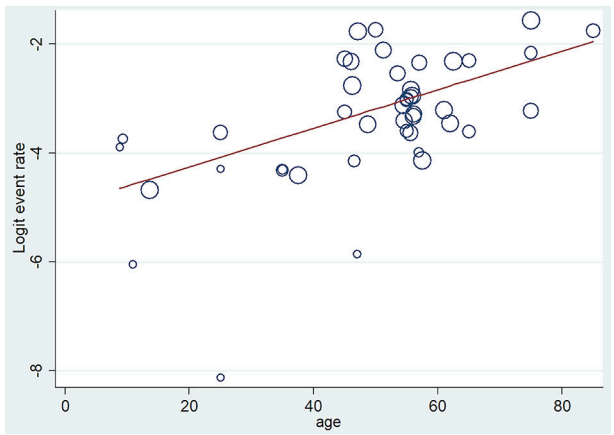

FIGURE 9 | Meta-regression of mortality. The figure shows 43 data from 30 reports where x = age (mean), y = logit event rate: ln[p/(1-p)], and circle diameters show the random size of each study. The meta-regression shows a significant relationship ($p = 0.022$) between age and mortality.

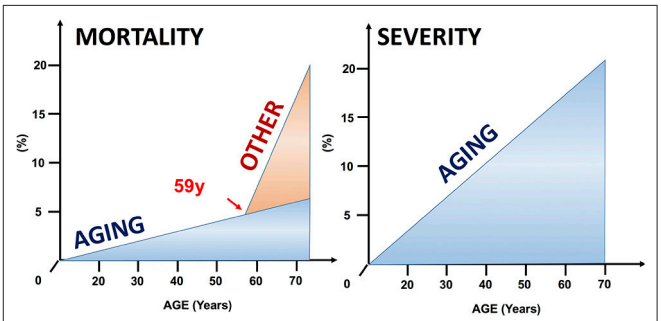

FIGURE 11 | Factors that may prepossess mortality and severity in AP. Our data show that age linearly correlates to higher risk of developing severe AP. Concerning mortality other factors may elevate the risk of decease cases above 59 years of age.

Age groups	Mortality					Significancy
	ES	95% CI		ES	95% CI	
U20 vs A20	0.01	0.01-0.01	vs	0.05	0.03-0.07	P<0.001
U30 vs A30	0.01	–0.00-0.01		0.05	0.03-0.07	P=0.001
U40 vs A40	0.01	0.00-0.01		0.06	0.03-0.09	P<0.001
U50 vs A50	0.02	0.02-0.03		0.06	0.03-0.09	P=0.018
U60 vs A60	0.02	0.01-0.02		0.07	0.02-0.12	P=0.028
U70 vs A70	0.04	0.03-0.05		0.11	0.01-0.22	P=0.038

FIGURE 10 | Forest plot results for cut-off values for mortality. Forest plot results from studies evaluating the cut-off values for mortality in acute pancreatitis with significant results in each of four groups. All comparisons showed a significant difference.

In summary, the predictive scoring systems correspond with our results, which suggests that mortality rises quickly above 59 years of age. Our data suggest that other factors which are associated with older age elevate the mortality in AP (**Figure 11**).

One of the candidates is definitely comorbidity. Fan et al. in 1988 also raised the question and found that concomitant medical and surgical diseases were responsible for the higher in-hospital mortality rate in elderly rather that consequences of AP (Fan et al., 1988). However, they also observed a higher incidence of not local, but systemic complications in older age. They concluded that, if concomitant diseases were ignored, the difference in mortality rate between young and elderly disappeared (Fan et al., 1988). Charlson et al. (1994) validated an Age-Adjusted Charlson comorbidity index (CCI) showing the absent of age from CCI index. Forty years of age have the lowest risk of comorbid death, moreover each decade of age over 40 adds 1 extra point to the risk which is added to the calculated CCI score.

A currently revealed propensity score-matched analysis examined the mortality and severity in the elderly in ABP (Patel et al., 2018). They grouped 184,763 patients in two age groups (<65 years of age vs. ≥65 years) and found that the index admission mortality rate for the elderly was significantly higher (0.32% ($n = 356$) vs. 1.96% ($n = 1473$); $p < 0.001$). The odds of mortality increased progressively in patients aged 75 to 84 years (OR 1.39; 95% CI: 1.06–1.82) and 85 years or older (OR 2.21; 95% CI: 1.70, 2.86). Further, increasing age was also associated with higher odds of severe AP (75 to 84 years: OR 1.20; 95% CI: 1.12, 1.30; 85 y or older: OR 1.28; 95% CI: 1.17, 1.40). However, elderly patients in this analysis had significantly higher ≥3 co-morbidities (based on an Elixhauser score of <3 and ≥ 3) (OR 4.59; 95% CI: 4.33, 4.87; $p < 0.001$), they concluded that age independently contributes to increased mortality in ABP.

However, in order to prove the influence of comorbidity on survivals, we wanted to extend our study with comparing comorbidities at different age categories. Since the articles in this study did not contain sufficient amount of information on comorbidities we have performed a large multinational cohort analysis on a prospective high quality database (Szakács et al., 2018). The analysis of a total of 1,203 patients showed that severe comorbidities (CCI≥3) predict mortality (OR = 4.48; CI: 1.57–12.80) much better than age, suggesting that comorbidity is an important additional predictor for mortality. More details of this investigation can be found in the forthcoming article in Frontiers Physiological Sciences entitled: "Aging and comorbidities in acute pancreatitis II: A cohort-analysis based on 1 203 prospectively collected cases from 12 countries" (DOI: 10.3389/fphys.2018.01776).

Strengths and Limitations

Strength 1 This systematic review and meta-analysis is based on a database which is at least 10 times greater in volume than the database used to develop the largest scoring system

Strength 2 Patients were included independently of etiologies, nationalities, severities and ages, without any limitations in this study.

Strength 3 Aging has serious impact on the healthcare systems worldwide; therefore, scientists' attention must focus on geriatrics.

Limitation 1 In most of the articles, the age of the patients was published in median, mean or IQR; therefore, distortion was alerted.

Limitation 2 The severity scoring guidelines have changed considerably over the years; therefore, there might be cases in which severities have been misclassified in the studies under analysis compared to our current knowledge.

Limitation 3 The co-morbidities of patients involved in the analysis are unknown; therefore, the decisive question as to whether age or age-associated co-morbidity plays an aggravating role remains unanswered in this meta-analysis.

Limitation 4 The large variety of studies caused high heterogeneity which may indicate hidden distorting factors in this analysis.

Limitation 5 We could not explain the reason why the mortality of the 50–59-year age group is lower than that of the 40–49-year age group. Therefore, it cannot exclude the possibility that the mortality rate is monophasic and the cut off A70 is better than the cut off of 59.

CONCLUSIONS

In conclusion, our analysis shows that age has an effect on AP. Both severity and mortality rise linearly, however the rate of elevation in mortality is 9 times higher above 59 than below. Our results rise an important question whether a restorative role is played by aging or other factors like co-morbidity.

CORE TIP

There has been a dramatic increase in life expectancy over the last few centuries. In addition, the incidence rate of one of the most common gastrointestinal disorders, acute pancreatitis (AP), is also growing. Here we provide a detailed mathematical analysis of the effects of aging on AP. Our data clearly shows that (1) younger age has a protective effect in AP, (2) aging raises both the severity and mortality of AP, and, importantly, (3) the mortality rate for patients above 59 years rises with 9 times greater intensity than that in younger patients.

AUTHOR'S NOTE

The results of this article suggested clearly that additional factors play a crucial role in mortality above 59 years of age (**Figures 7**, **11**). There is a Part II of this publication in which a detailed analysis of a 1,203 prospectively collected cases showed that comorbidity is the key factor (**Figure 5** - https://www.frontiersin.org/articles/10.3389/fphys.2018.01776/full; doi: 10.3389/fphys.2018.01776).

AUTHOR CONTRIBUTIONS

KM and A-ML conducted the database search and read the articles for eligibility; when a conflict arose, a third participant, PH, made the decision. KM and A-ML collected the data from the articles in an Excel file. NF and PM analyzed the data. PS, ZR, and LC performed the bias analysis and quality assessment. KM, TH, and BE drafted the manuscript. ÀV, GV, TH, A-ML, MO, LC, PS, ZR, IC, and PH edited the manuscript. KM, MO, and A-ML edited the tables and figures. PS and ZR completed the PRISMA checklist. PH made the critical revision on the finalized manuscript. All authors have read and approved the final manuscript.

FUNDING

This study was funded by a Momentum Grant from the Hungarian Academy of Sciences (LP2014-10/2014) and an Economic Development and Innovation Operative Program Grant and a Highly Cited Publication Grant from the National Research, Development and Innovation Office (GINOP-2.3.2-15-2016-00048 Stay Alive, KH-125678, K116634) (EFOP 3.6.2-16-2017-00006 Live Longer, VEKOP-16-2017-00009) as well as by the Translational Medicine Foundation to PH. We also acknowledge the support by the UNKP-18-3-I New National Excellence program of the Ministry of Human capacities to KM.

ACKNOWLEDGMENTS

This study was presented as a poster presentation at the 50th Meeting of the European Pancreatic Club.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2019.00328/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Digestive and Liver Disease 51 (2019) 559–560

Contents lists available at ScienceDirect

Digestive and Liver Disease

journal homepage: www.elsevier.com/locate/dld

Commentary

Uncommon appearance of concurrent liver cirrhosis and chronic pancreatitis: The alcohol metabolism theory

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ARTICLE INFO

Article history:
Received 19 December 2018
Accepted 28 December 2018

Chand et al. recently reported the frequency of liver diseases (LD) in pancreatitis. In an analysis of 20,931 patients, it was concluded that the incidence rate of LD in chronic pancreatitis (CP) is approximately 5%, whereas that of the end-stage liver disease, liver cirrhosis (LC), is around 2% [1]. Since more than 50% of CP develops due to alcohol consumption [2], we can estimate an incidence rate of LD and LC that is no more than two times higher in alcoholic CP.

Although the rate of LC is variable in each published national cohort of alcoholic CP (2% in a Spanish cohort [3], 16.7% in a Czech one [4], 8.4% in an Indian one [5] and 12.5% in an Italian one [6]), the incidence rate of LC is approximately 10–20%. Further, the incidence of CP is even lower in LC. It was 2.5% in the Czech cohort [4] and 5.3% in the Spanish one [3]. Since the proportion of CP in LC is lower than that of LC in CP and patients with CP are younger than those with LC [6,7], it seems more than likely that alcohol damages the pancreas earlier than it does the liver.

90% of alcohol is metabolized via the oxidative pathway by acetaldehyde dehydrogenase (ADH), whereas 10% is metabolized via the non-oxidative pathway mostly by fatty acid ethyl ester synthase and carboxyl ester lipase. The end product of the oxidative pathway, acetaldehyde, is rather toxic to the liver; however, the end product of the non-oxidative pathway, fatty acid ethyl ester (FAEE), is rather toxic to the pancreas [8–10]. Pharmacological suppression of the oxidative pathway exacerbates ethanol-induced mitochondrial dysfunction and acute pancreatitis, while pharmacological inhibition of the non-oxidative pathway prevents FAEE formation and ameliorates exocrine pancreatic damage and the outcome of acute pancreatitis in experimental models [9]. The same outcomes were observed in genetically altered conditions. In ADH-deficient mice, alcohol administration causes severe pancreatic injury [11]; moreover, mutations of carboxyl ester lipase in humans also increases the risk for alcoholic chronic pancreatitis [12].

Therefore, we hypothesize that in patients in whom alcohol is mostly metabolized via the oxidative pathway, LC develops first and pancreatitis presents in only a minority of patients. This may be due to the fact that (1) non-oxidative metabolism is suppressed and the formation of FAEE is low or (2) since mortality is high in LC, there is no time for CP to develop. Conversely, in patients in whom alcohol is mostly metabolized via the non-oxidative pathway, CP develops first and in some patients LC occurs later. (1) This may be due to the lower activity of oxidative metabolism or (2) since mortality is lower in CP, LC has time to develop (Fig. 1).

All in all, we have at least three independent mechanisms playing a role in the rare incidence of concurrent LC and CP: (1) a patient's genotype does not change during his or her lifetime; therefore, the characteristics of alcohol metabolism remain similar with aging; (2) after one of the diseases develops, the patient's alcohol consumption decreases; and (3) the patient's survival is diminished if comorbidities occur.

Conflict of interest

None declared.

Funding

This study was funded by a Momentum Grant from the Hungarian Academy of Sciences (LP2014-10/2014) and an Economic Development and Innovation Operative Programme Grant and a Highly Cited Publication Grant from the National Research, Development and Innovation Office (GINOP-2.3.2-15-2016-00048 Stay Alive, KH-125678) (EFOP 3.6.2-16-2017-00006 Live Longer) as well as by the Translational Medicine Foundation. The research was

DOI of original article: https://doi.org/10.1016/j.dld.2018.11.001.
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https://doi.org/10.1016/j.dld.2018.12.023

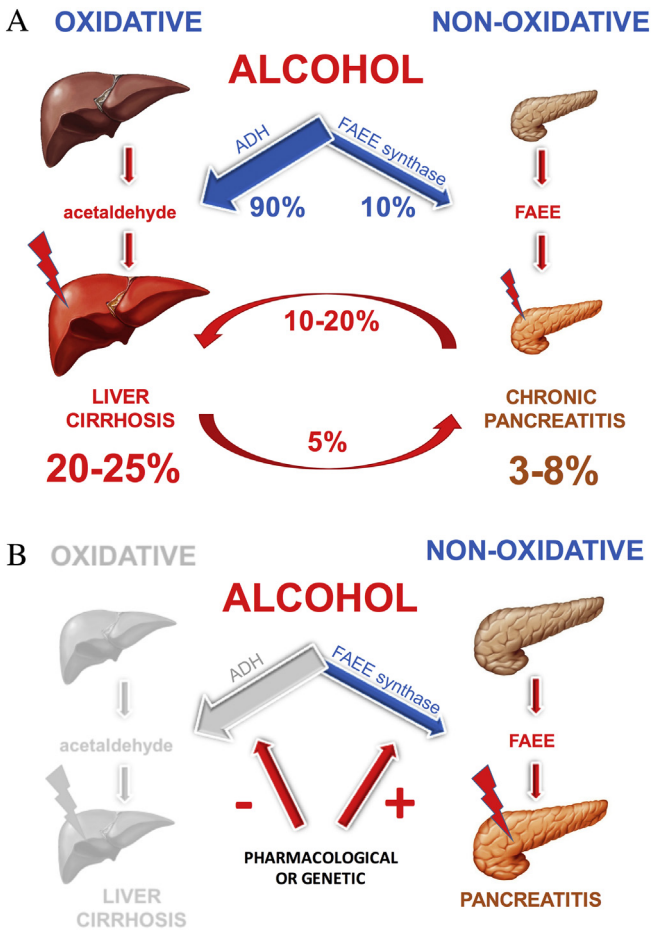

Fig. 1. (A) Alcohol is 90% metabolized via the oxidative pathway; liver cirrhosis is therefore more frequent in alcoholics than chronic pancreatitis (20–25% versus 3–8%, respectively). In patients in which LC develops first, CP is less frequent, whereas the chance for LC is higher in patients where CP develops first. (B) If the non-oxidative pathway is stimulated or the oxidative pathway is inhibited, the pancreas damage is greater, while if inhibited it is less severe.

also supported by the UNKP-18-3-I New National Excellence Programme from the Hungarian Ministry of Human Capacities.

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frontiers in Physiology

ORIGINAL RESEARCH
published: 02 April 2019
doi: 10.3389/fphys.2018.01776

Aging and Comorbidities in Acute Pancreatitis II.: A Cohort-Analysis of 1203 Prospectively Collected Cases

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OPEN ACCESS

Edited by:
Alexei Tepikin, University of Liverpool, United Kingdom

Reviewed by:
Julia Gerasimenko, Cardiff University, United Kingdom
Pawel Ferdek, Jagiellonian University, Poland

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Specialty section:
This article was submitted to Gastrointestinal Sciences, a section of the journal Frontiers in Physiology

Received: *02 October 2018*
Accepted: *23 November 2018*
Published: *02 April 2019*

Citation:
Szakács Z, Gede N, Pécsi D, Izbéki F, Papp M, Kovács G, Fehér E, Dobszai D, Kui B, Márta K, Kónya K, Szabó I, Török I, Gajdán L, Takács T, Sarlós P, Gódi S, Varga M, Hamvas J, Vincze Á, Szentesi A, Párniczky A and Hegyi P (2019) Aging and Comorbidities in Acute Pancreatitis II.: A Cohort-Analysis of 1203 Prospectively Collected Cases. Front. Physiol. 9:1776. doi: 10.3389/fphys.2018.01776

Introduction: Our meta-analysis indicated that aging influences the outcomes of acute pancreatitis (AP), however, a potential role for comorbidities was implicated, as well. Here, we aimed to determine how age and comorbidities modify the outcomes in AP in a cohort-analysis of Hungarian AP cases.

Materials and Methods: Data of patients diagnosed with AP by the revised Atlanta criteria were extracted from the Hungarian Registry for Pancreatic Patients. Outcomes of interest were mortality, severity, length of hospitalization, local, and systemic complications of AP. Comorbidities were measured by means of Charlson Comorbidity Index (CCI) covering pre-existing chronic conditions. Non-parametric univariate and multivariate statistics were used in statistical analysis. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated.

Results: A total of 1203 patients from 18 centers were included. Median age at admission was 58 years (range: 18–95 years), median CCI was 2 (range: 0–10). Only severe comorbidities (CCI $\geq$ 3) predicted mortality (OR = 4.48; CI: 1.57–12.80). Although severe comorbidities predicted AP severity (OR = 2.10, CI: 1.08–4.09), middle (35–64 years) and old age ($\geq$65 years) were strong predictors with borderline significance, as well (OR = 7.40, CI: 0.99–55.31 and OR = 6.92, CI: 0.91–52.70, respectively). Similarly, middle and old age predicted a length of hospitalization $\geq$9 days. Interestingly, the middle-aged patients (35–64 years) were three times more likely to develop pancreatic necrosis than young adults (OR = 3.21, CI: 1.26–8.19), whereas the old-aged ($\geq$65 years) were almost nine times more likely to develop systemic complications

than young adults (OR = 8.93, CI: 1.20–66.80), though having severe comorbidities (CCI $\geq$ 3) was a predisposing factor, as well.

Conclusion: Our results proved that both aging and comorbidities modify the outcomes of AP. Comorbidities determine mortality whereas both comorbidities and aging predict severity of AP. Regarding complications, middle-aged patients are the most likely to develop local complications; in contrast, those having severe comorbidities are prone to develop systemic complications. Studies validating the implementation of CCI-based predictive scores are awaited.

Keywords: acute pancreatitis, comorbidities, mortality, severity, length of hospitalization, complications, prediction, Charlson Comorbidity Index

INTRODUCTION

The annual incidence of AP ranges from 10 to 100 cases per 100,000 persons (Roberts et al., 2013), showing an increasing tendency throughout the past decades (Yadav and Lowenfels, 2013). Multiple theories have been proposed to explain the increment: better diagnostics (e.g., general access to the measurement of pancreatic enzymes) (Yadav et al., 2011), lifestyle factors (e.g., obesity, alcohol consumption, and tobacco use) (Alsamarrai et al., 2014; Samokhvalov et al., 2015) as well as aging of the population (Spanier et al., 2013) have been implicated.

Aging not only increases the risk of AP (Yadav and Lowenfels, 2013) but also may change the clinical course of it, resulting in higher mortality (Fan et al., 1988; Spanier et al., 2013) and longer hospitalization (Murata et al., 2011; McNabb-Baltar et al., 2014), thereby increases the cost for care in the elderly (Fagenholz et al., 2007; Murata et al., 2012). Accordingly, widely accepted predictive scores and severity indices, such as Ranson criteria (age > 55 years) (Ranson et al., 1974), APACHE II (age > 44 years) (Larvin and McMahon, 1989), and BISAP (age > 60 years) (Wu et al., 2008) consider age as a risk factor of worse clinical outcomes, where the potential impact of comorbidities is omitted from these.

Risk of morbidities increases with age (Vasilopoulos et al., 2014). Since the average age of AP onset is around 55–70 years (Yadav and Lowenfels, 2013; Hamada et al., 2014), most AP patients are exposed to the burden of comorbidities (Murata et al., 2015). Sporadic studies reported on how comorbidities affect the outcomes of AP: they increases mortality (Singla et al., 2009; Murata et al., 2011, 2015; Akshintala et al., 2013; McNabb-Baltar et al., 2014) and the length of hospital stay, as well (Murata et al., 2011, 2015; Francisco et al., 2013). However, the predictive role of comorbidities is underutilized regarding AP severity and the development of complications.

Results of the meta-analysis by Marta et al. (under revision) published in the previous issue of Frontiers Science suggested that both mortality and severity of AP are age-dependent, but age alone does not explain the increment of mortality in the elderly. This increment might be attributed to comorbidities, as shown in Figure 11 by Marta et al. (under revision). These findings inspired us to conduct a cohort-analysis of AP cases to provide a comprehensive assessment on how aging and comorbidities alter outcomes of AP including mortality, severity, LOH, and complications; and to decide whether the burden of aging or comorbidities is decisive for determining hard outcomes.

Abbreviations: AP, acute pancreatitis; APACHE, Acute Physiology and Chronic Health Evaluation; BISAP, Bedside Index of Severity in Acute Pancreatitis; CCI, Charlson Comorbidity Index; CI, confidence interval; LOH, length of hospitalization; OR, odds ratio.

MATERIALS AND METHODS

Population

We extracted data from the Hungarian Registry for Pancreatic Patients (AP Registry) established in 2011 by the Hungarian Pancreatic Study Group in order to advance clinical care and research in Pancreatology (Parniczky et al., 2016). AP Registry contains data on consecutive cases of AP attending several Hungarian centers between 2011 and 2017. Accuracy of data recorded is secured by a four-level quality check system involving both medical administrative personnel and gastroenterologist specialists.

Comorbidities

Registry forms of AP cases involve an admission form (A form) and follow-up forms (B-forms) covering the entire hospital stay, as well as the de-identified electronic discharge files. All files were carefully reviewed by an author with a medical degree to aggregate CCI (Charlson et al., 1987) with the International Classification of Diseases 9/10 coding algorithm (Quan et al., 2005). No search engines were used when reviewing charts. CCI items were dedicated to rating common chronic pre-existing diseases along 19 health-related (groups of) conditions. Every CCI item has a weight according to the severity of comorbidities covered (Charlson et al., 1987). CCI of each case was calculated by compiling the weighted items. Earlier studies proved that CCI is an effective predictor of hard outcomes in several acute and chronic conditions (Ng et al., 2013; Frenkel et al., 2014; Marventano et al., 2014).

Eligibility Criteria

To be included in analysis, the following criteria should be met:

(1) Diagnosis of AP ("Two out of three") (Working Group Iap/Apa Acute Pancreatitis Guidelines, 2013):

(i) Abdominal pain
(ii) Serum amylase and/or lipase greater than three times the upper normal limit
(iii) Characteristic findings on abdominal cross-sectional imaging

(2) Age ≥ 18 years
(3) Available history for CCI (Charlson et al., 1987)

Outcomes

Our AP-related outcomes included in-hospital mortality, severity, LOH, local complications (including peripancreatic fluid collections, pseudocysts, and pancreatic necrosis), and organ failure (including respiratory, renal, and cardiac failure).

Ethical Approval

AP Registry has been approved by Scientific and Research Ethics Committee of the Medical Research Council, Hungary (22254-1/2012/EKU). All subjects gave written informed consent in accordance with the Declaration of Helsinki.

Statistical Analysis

An expert biostatistician carried out the analysis with SPSS 19.0.0 (IBM Analytics, United States). Case numbers and percentages were calculated for categorical variables, medians with 25% and 75% quartiles (Q_1 and Q_3, respectively) and ranges were computed for numerical variables in descriptive analysis (due to non-normal distribution of data indicated by the Kolmogorov–Smirnov test). In all analysis, a probability (p) < 0.05 indicated a significant difference, whereas a p-value between 0.05 and 0.10 indicated borderline significance.

Representativeness of study population was tested by binomial, one sample median, and Goodness-of-fit $\chi 2$ tests.

In univariate analysis, Spearmann's rho was calculated to explore correlations between age, CCI, and LOH. ORs with 95% CIs were calculated from 2 × 2 tables. If OR was not calculable, association were investigated with χ^2 - or Fisher's tests.

In multivariate analysis, binary logistic and multinominal regressions were used to investigate the joint effect of age categories and CCI categories or that of age categories and individual comorbidities. We used a three-level age-stratification (young-aged between 18 and 34 years of age, middle-aged between 35 and 64 years of age, and old-aged ≥ 65 years of age) and a four-level comorbidity stratification (none if CCI = 0, mild if CCI = 1, moderate if CCI = 2, and severe if CCI ≥ 3).

RESULTS

Demography

AP Registry contained 1241 cases, of them 1203 (96.9%) from 18 centers were eligible for inclusion. Demography of study population and that of AP Registry are presented in **Table 1** and **Supplementary Appendix 1**, respectively. Distribution of sites of recruitment is presented in **Supplementary Appendix 1**. Study population proved to be representative to that of AP Registry regarding demography and disease outcomes ($p > 0.05$ for all variables analyzed) (**Supplementary Appendices 2, 9**). Data quality for all variables was >99% in study population (**Supplementary Appendix 3**).

TABLE 1 | Demography of study population including a total of 1203 cases of acute pancreatitis (AP).

Age, median (Q_1–Q_3)	58 (44–70)
Sex, n_{male} ($\%_{male}$)	670 (55.7)
Etiology (pure)	
Biliary, n (%)	528 (43.9)
Alcoholic, n (%)	269 (22.4)
Hypertriglyceridemic, n (%)	69 (5.7)
Mortality, n (%)	28 (2.3)
Severity of pancreatitis	
Mild, n (%)	825 (68.6)
Moderate, n (%)	313 (26.0)
Severe, n (%)	65 (5.4)
Length of hospitalization, median (Q_1–Q_3)	9 (7–14)
Local complications, n (%)	358 (29.8)
Fluid collection, n (%)	303 (25.2)
Pseudocyst, n (%)	120 (10.0)
Necrosis, n (%)	111 (9.2)
Systemic complications, n (%)	92 (7.7)
Respiratory failure, n (%)	55 (4.6)
Heart failure, n (%)	19 (1.6)
Renal failure, n (%)	33 (2.7)
Charlson Comorbidity Index, median (Q_1–Q_3)	2 (0–2)
Severity of comorbidities	
No comorbidities, n (%)	444 (36.9)
Mild comorbidities, n (%)	345 (28.7)
Moderate comorbidities, n (%)	190 (15.8)
Severe comorbidities, n (%)	224 (18.6)

Continuous variables are presented in median with quartiles (Q_1–Q_3), categorical variables are presented in frequencies (n) with percentages of total (%).

Association Between Aging and Comorbidities in AP

Aging Strongly Influences the Outcomes of AP in Univariate Models

Median age on admission was 58 years (Q_1–Q_3: 44–70 years, range: 18–95 years) (**Figure 1A**). Deceased were older than survivors [65 years (Q_1–Q_3: 56–78 years) vs. 58 years (Q_1–Q_3: 44–70 years), $p = 0.017$, respectively] (**Figure 1B**). The age difference between severe and non-severe cases was of borderline significance [61 years (Q_1–Q_3: 48–71 years) vs. 58 years (Q_1–Q_3: 43–70 years), $p = 0.076$] (**Figure 1C**), as well as the detected weak positive correlation between age and LOH ($r = 0.055$, $p = 0.058$) (**Supplementary Appendix 4**). Interestingly, patients developing local complications were younger than those not doing so [56 years (Q_1–Q_3: 43–68 years) vs. 59 years (Q_1–Q_3: 44–71 years), respectively, $p = 0.028$]. The association is true for necrosis ($p = 0.049$) and fluid collections ($p = 0.095$), unlike for pseudocysts ($p = 0.839$) (**Supplementary Appendix 5**). On the contrary, patients developing systemic complications were older than those not doing so [62 years (Q_1–Q_3: 50.5–74 years) vs. 58 years (Q_1–Q_3: 43–70 years), respectively, $p = 0.008$].

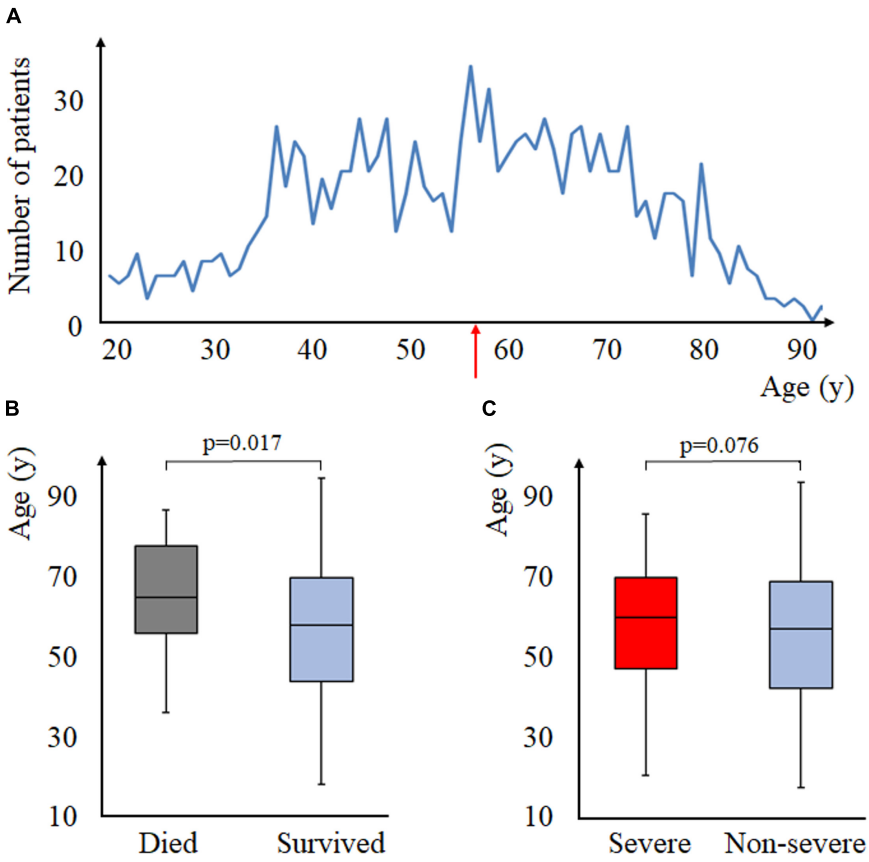

FIGURE 1 | Aging and acute pancreatitis (AP). **(A)** age-distribution of the study population, the red arrow indicates the median age of the population (that is, 58 years of age). **(B)** mortality and age (Mann–Whitney test). **(C)** severity and age (Mann–Whitney test).

Specifically, respiratory ($p = 0.001$) and heart failure ($p = 0.009$) were age-dependent (**Supplementary Appendix 5**).

Comorbidities (CCI) Strongly Influences the Outcomes of AP in Univariate Models

Median CCI was 2 (Q_1–Q_3: 0–2, range: 0–10) (**Figure 2A**). Deceased had higher CCI than survivors [3 (Q_1–Q_3: 1–4) vs. 1 (Q_1–Q_3: 0–2), $p = 0.001$, respectively], as well as those with severe AP [1 (Q_1–Q_3: 0–3) vs. 1 (Q_1–Q_3: 0–2), $p = 0.024$] compared to those with non-severe AP, respectively (**Figures 2B–C**). A weak, significant, positive correlation was detected between age and CCI ($r = 0.073$, $p = 0.012$) (**Supplementary Appendix 4**). Local complications seemed independent of CCI ($p = 0.259$), as were fluid collections ($p = 0.515$), pseudocysts ($p = 0.456$), and necrosis ($p = 0.558$) (**Supplementary Appendix 6**). Systemic complications were associated with higher CCI ($p < 0.001$). This association applies to respiratory failure ($p < 0.001$), as well (**Supplementary Appendix 6**).

Age Correlates With CCI in a Univariate Model

We observed a moderate, positive correlation between age and CCI ($r = 0.334$, $p < 0.001$) (**Figure 3**).

Analyzing the association between the individual comorbidities (i.e., the components of CCI) and age, patients with previous myocardial infarction, co-existing congestive heart failure, peripheral arterial disease, and cerebrovascular disease were significantly older than those without these conditions ($p < 0.001$ for each). These associations applied to chronic pulmonary diseases and dementia ($p < 0.001$ for both), as well as to peptic ulcers/erosions ($p = 0.015$). Both diabetes with and without complications were associated with older age ($p < 0.001$).

Patients with malignant tumors were older ($p < 0.001$) but we failed to detect this association regarding metastatic tumors ($p = 0.112$), probably due to low event rates. The latter may apply to autoimmune diseases ($p = 0.961$).

Interestingly, patients with mild liver disease were younger than their healthy counterparts ($p < 0.001$); however, this difference disappeared regarding moderate and severe liver diseases ($p = 0.555$).

Aging and Comorbidities (CCI) Affect the Outcomes of AP Discrepantly in Multivariate Models

Summaries of multivariate analysis are presented in **Table 2** and **Supplementary Appendix 7**, raw data are presented in **Supplementary Appendix 8**. The exclusive predictor of mortality was a CCI ≥ 3 (ß = 1.50; OR = 4.48; CI: 1.57–12.80); in accordance, the main predictor of severe AP was a CCI ≥ 3

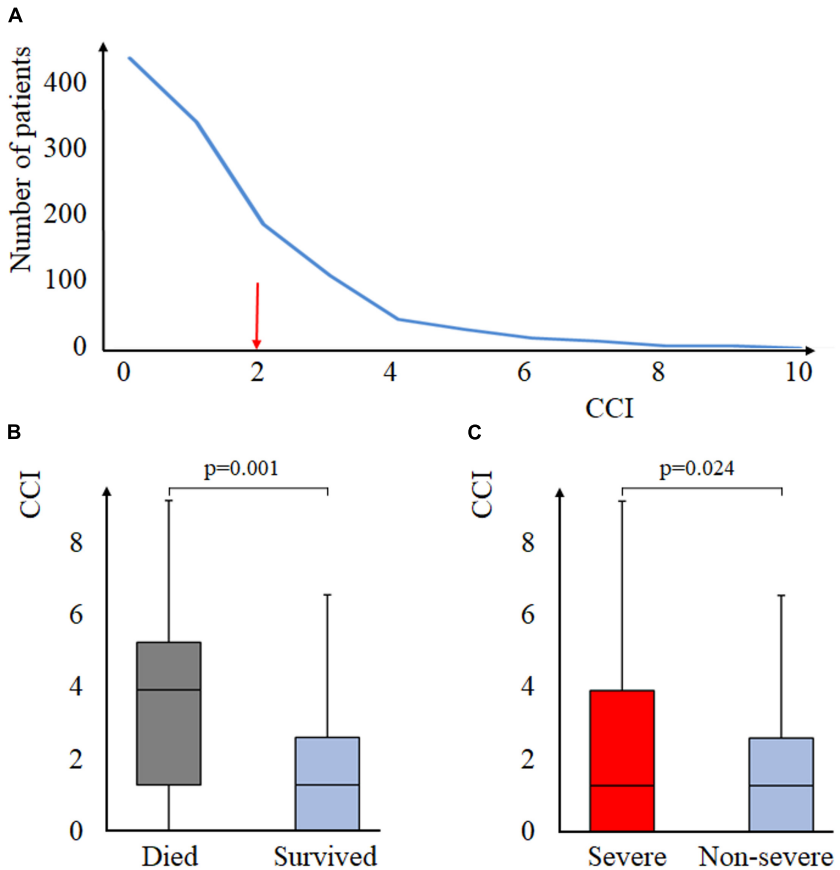

FIGURE 2 | Charlson Comorbidity Score and AP. **(A)** distribution of CCI in the study population, the red arrow indicates the median CCI of the population. **(B)** mortality and CCI (Mann–Whitney test). **(C)** severity and CCI (Mann–Whitney test). CCI, Charlson Comorbidity Index.

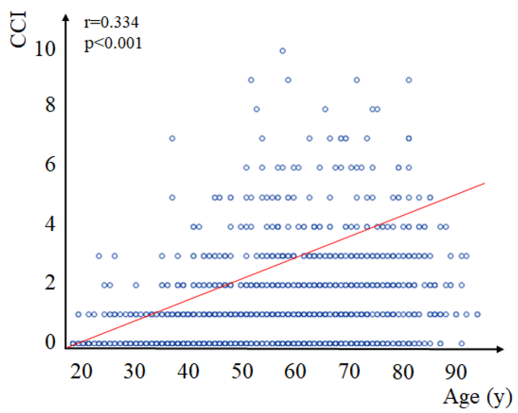

FIGURE 3 | Correlation between age and Charlson Comorbidity Index (CCI). Spearman's correlation established a significant positive correlation of moderate strength ($r = 0.334$, $p < 0.001$) between age on admission and CCI. CCI, Charlson Comorbidity Index.

(ß = 0.74; OR = 2.10, CI: 1.08–4.09), though the middle- and old-aged were exposed to a severe episode with a high OR of borderline significance. Unexpectedly, the middle-aged were more likely to spend ≥9 days in hospital. Along with this, the only predictors of local complications (including pancreatic necrosis) was to be middle-aged (ß = 1.17; OR = 3.21, CI: 1.26–8.19). On the contrary, the middle- and old-aged were about eight times more likely to develop systemic complications than their younger counterparts (β = 2.19, OR = 7.82, CI: 1.06–57.79 and β = 2.06, OR = 8.93, CI: 1.20–66.79, respectively), though comorbidities were important determinants, as well.

Individual Comorbidities Are Important Predictors of the Outcomes of AP in Univariate and Multivariate Models

Summaries of univariate and multivariate statistics of individual comorbidities, together with raw data, are presented in **Supplementary Appendices 8–10**. In univariate analysis, out of the six comorbidities associated with higher mortality, moderate/severe liver diseases and metastatic solid tumors proved to be the strongest predictors (OR = 8.04, CI: 2.22–29.13 and OR = 8.47, CI: 1.78–40.23, respectively) (**Figure 4**). Peripheral vascular diseases, cerebrovascular diseases, and diabetes without complications predicted severe AP. Patients with mild liver diseases were two times more likely to develop local complications, including necrotizing pancreatitis

TABLE 2 | Joint effect of aging and comorbidities on the outcomes of AP.

Variables	Deceased vs. survivors			Severe vs. mild AP			LOH ≤9 days vs. LOH >9 days		
	β	OR (95% CI)	*p*-value	β	OR (95% CI)	*p*-value	β	OR (95% CI)	*p*-value
Age categories									
18–34 years (young-aged)	NA[a]	NA[a]	0.961	0	1 (reference)		0	1 (reference)	
35–64 years (middle-aged)	0.76	0.76 (0.35–1.67)	0.493	2.00	7.40 (0.99–55.31)	0.051	0.62	1.86 (1.22–2.83)	0.004
>65 years (old-aged)	0	1 (ref)		1.93	6.92 (0.91–52.70)	0.062	0.40	1.50 (0.96–2.33)	0.073
Comorbidity categories									
CCI = 0 (none)	0	1 (reference)		0	1 (reference)		0	1 (reference)	
CCI = 1 (mild)	0.11	1.12 (0.32–3.90)	0.863	0.04	1.04 (0.52–2.08)	0.911	0.00	1.00 (0.75–1.34)	0.983
CCI = 2 (moderate)	0.09	1.10 (0.26–4.68)	0.900	−0.02	0.98 (0.45–2.24)	0.960	0.30	1.35 (0.95–1.92)	0.092
CCI > 2 (severe)	1.50	4.48 (1.57–12.80)	0.005	0.74	2.10 (1.08–4.09)	0.029	0.15	1.16 (0.83–1.62)	0.387

Red highlights indicate $p < 0.05$, orange highlights indicate $p < 0.10$ but ≥ 0.05. AP, acute pancreatitis; Charlson Comorbidity Index; CI, confidence interval; LOH, length of hospitalization; NA, not applicable; OR, odds ratio. [a]*analysis is impossible due to zero events.*

(OR = 1.86, CI: 1.25–2.75). Congestive heart failure, peripheral vascular diseases, cerebrovascular diseases, chronic pulmonary diseases, and diabetes without complications were associated with a higher rate of systemic complications. Preexisting cardiovascular, renal, and pulmonary diseases predicted the development of respiratory, heart, and renal decompensation, respectively. Interestingly, pre-existing moderate/severe liver diseases and malignant tumors were strongly associated with cardiac decompensation (OR = 7.16, CI: 1.55–33.21 and OR = 4.09, CI: 1.32–12.64, respectively). Multivariate analysis only minimally changed the direction of main associations.

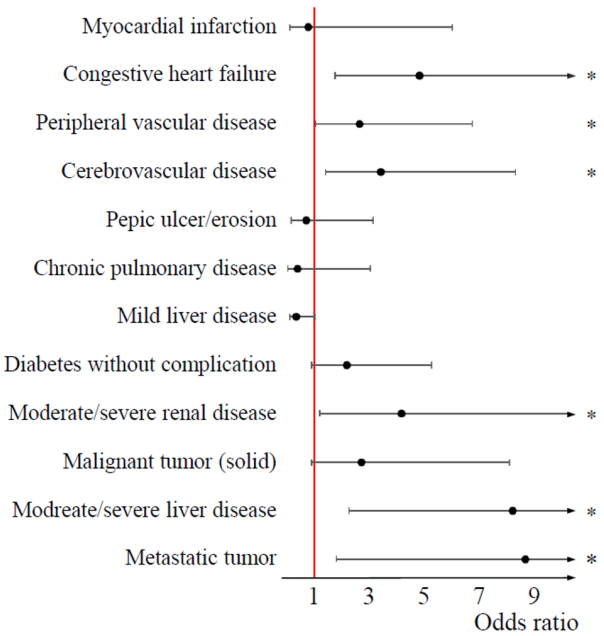

FIGURE 4 | Forest plot on the effect of individual comorbidities on mortality. 95% confidence intervals did not cross the boundary of significance (red, vertical line at an odds ratio of (1) regarding six comorbid conditions: congestive heart failure, peripheral vascular disease, cerebrovascular disease, moderate/severe renal disease, moderate/severe liver disease, and metastatic tumor (asterisks indicate a *p*-value less than 0.05). These comorbidities were associated with higher mortality.

DISCUSSION

Summary of Findings

We aimed to clarify whether aging or comorbidities are decisive for determining the outcomes of AP. All the outcomes, except for local complications, proved to be dependent on both age and CCI in univariate analysis. As opposed to this, multivariate analysis revealed that patients suffering from severe comorbidities were about 4.5 times more likely to have a fatal episode of AP and about two times more likely to develop severe AP than those having no comorbidities, whereas age predicted these outcomes with high OR and borderline significance. In contrast, the middle- and old-aged (but not those with severe comorbidities) were more likely to spend at least 9 days in hospital, as compared to their young counterparts. Moreover, aging and comorbidities influenced the development of local and systemic complications in a completely different manner.

Frequency of comorbidities and distribution of age were similar in our cohort of AP cases to that of the large series in the literature (Frey et al., 2007; Singla et al., 2009; Murata et al., 2011, 2015; Akshintala et al., 2013; McNabb-Baltar et al., 2014).

Although mortality of populations is widely reported, studies on the effect of aging yielded controversial results. Some indicated that each year increase in age may result in an OR = 1.01–1.04 ($p < 0.05$) increase in mortality (Singla et al., 2009; Akshintala et al., 2013; McNabb-Baltar et al., 2014); however, the detection of this statistically significant but probably clinically less prominent increment might have been attained due to large sample sizes. High mortality of older age groups is frequently reported (Frey et al., 2007; Murata et al., 2011; Mendez-Bailon et al., 2015), as are the effects of severe comorbidities: they are strong, independent predictors of mortality in AP (Frey et al., 2007; Singla et al., 2009; Murata et al., 2011, 2015; Akshintala et al., 2013; McNabb-Baltar et al., 2014; Mendez-Bailon et al., 2015; Lee et al., 2016), as confirmed by our study, as well. Our results are in line with

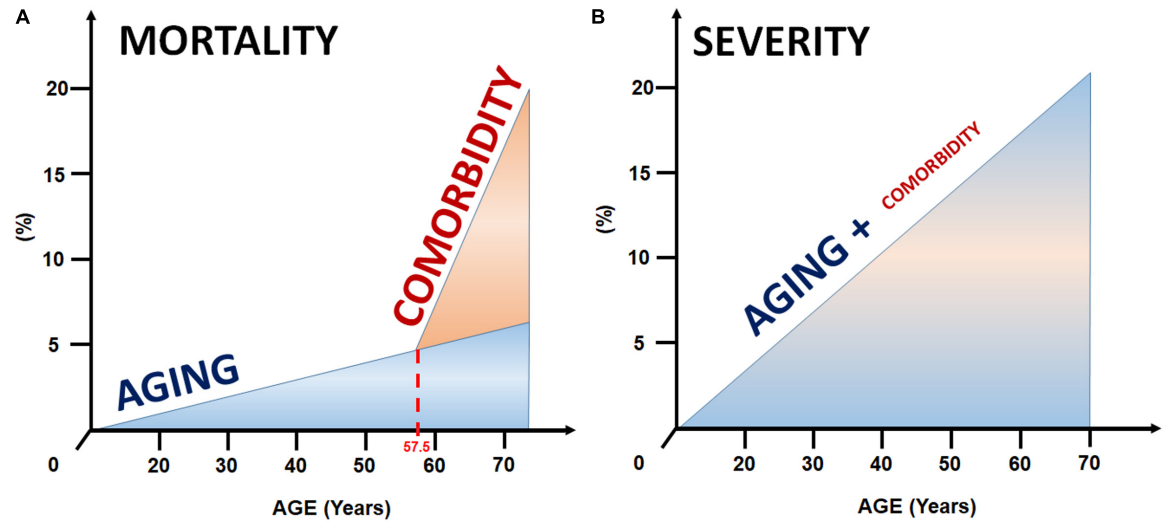

FIGURE 5 | Model for the joint effect of aging and comorbidities on mortality and severity. **(A)** The excess in mortality in the elderly is likely to be explained by the increment in comorbidities with aging. **(B)** In contrast, age seems to be the strongest predictor of the severity of AP, whereas comorbidities have a less prominent effect.

previous findings in a cohort of patients over 70 years stating that pre-existing cardiovascular, malignant, and renal diseases predicted mortality (Murata et al., 2015).

No studies investigated the effects of comorbidities on AP severity graded by the revised Atlanta criteria (Sarr, 2013). In our study, patients with severe AP were older and had higher CCI than those developing moderate AP. Besides that a CCI $\geq$ 3 is an independent predictor of severe AP, middle and old age should be considered a strong risk factor in multivariate analysis (including age and CCI categories).

The middle- and old-aged patients were more likely to stay $\geq$9 day in hospital as compared to younger counterparts. We found no association between LOH and comorbidities, which may oppose previous research (Murata et al., 2011, 2015; Francisco et al., 2013). A possible explanation for this discrepancy may be that we handled LOH as a dichotomous variable in multivariate analysis due to non-normal distribution of data. No studies have analyzed the effect of individual comorbidities on LOH; in our cohort of patients myocardial infarction, mild liver diseases as well as middle and old age predisposed to longer LOH.

Interestingly, patients with local complications and necrosis were younger but do not have higher CCI than those not developing them. Only being middle-aged was an independent predictor of local complications and necrosis. Two small studies reported non-significant associations between comorbidities and local complications (Uomo et al., 1998; Weitz et al., 2016). One study reported on 2-week organ failure, which found that only the number of comorbidities, but not age, was a significant predictor (Frey et al., 2007). On the contrary in our study, the strongest predictor of organ failure was aging: the middle- and old aged were about 8 times more likely to develop organ failure than their younger counterparts, while having severe comorbidities proved to be a weak but significant predictor, as well.

Strengths and Weaknesses

Our study has several strengths. First of all, this is the first report analyzing the joint effect of aging and comorbidities on AP severity and local complications in a non-selected cohort of AP cases with multivariate statistics. Secondly, manual assessment of CCI by a trained investigator provides a sufficient accuracy and might be superior in homogeneity over claims data (Kieszak et al., 1999) upon which most population-based studies rely. Third, precise data collection and consistent data management of the AP Registry with uniform recording of diagnosis, severity, and complications across centers improve the reliability of data and, therefore, strengthen our conclusions (Sarr, 2013).

However, authors must acknowledge that the study is limited by the number of reasons. Data collected are limited to adult (18–95 years). Despite the high case number, event numbers concerning some outcomes limited the analysis. To overcome this, we merged similar items of CCI (e.g., malignant tumors) when imputing them in multivariate models, as seen in other works (Murata et al., 2015). Distribution of continuous variables proved to be non-normal so that multivariate regression was not performed in terms of LOH. Instead, a dichotomized logistic regression model was used. Similarly, the non-normal distribution of age and CCI forced us to set up age and comorbidity categories in multivariate analysis. Despite the four-level data checking system, imprecision of data recording cannot be excluded.

CONCLUSION

Our results confirm that both aging and comorbidities modify the outcomes of AP, however, discrepantly. The increment in mortality associated with an older age in the meta-analysis of Marta et al. might be explained by the additive effects of

comorbidities (**Figure 5**). Taken together, these results support that CCI, together with age, should be incorporated into the predictive scores in AP to increase the accuracy of prediction. Studies validating the implementation of CCI-based predictive scores are awaited.

AUTHOR'S NOTE

There is a Part I of this publication in which a meta-analysis of 194 702 cases showed that additional factors play a crucial role in mortality of acute pancreatitis above 59 years of age (**Figures 7**, **11** – https://www.frontiersin.org/articles/10.3389/fphys.2019.00328/full; doi: 10.3389/fphys.2019.00328). The results of this article proved that mortality of acute pancreatitis is rather determined by the presence of comorbid conditions (**Figure 5** and **Table 2**).

AUTHOR CONTRIBUTIONS

ZS, PH, and ÁV contributed to the design of the research. GK, EF, DD, BK, KM, KK, IS, IT, LG, MP, PS, SG, MV, JH, and TT performed the data collection. AS coordinated data collection and controlled data quality. ZS assessed the comorbidities and calculated comorbidity scores. NG and ZS processed the data, performed the analysis, and drafted the manuscript. ZS and AP designed the figures. DP and FI critically revised the manuscript. PH supervised and coordinated the work. All authors discussed the results and commented on the manuscript.

FUNDING

The study was supported by Project Grants (KH125678 and K116634 to PH, K120335 to TT), the Economic Development and Innovation Operative Programme Grant (GINOP 2.3.2-15-2016-00048 to PH) and Human Resources Development Operational Programme Grant (EFOP-3.6.2-16-2017-00006 and EFOP-3.6.3-VEKOP-16-2017-00009 to PH) from the National Research, Development and Innovation Office, by a Momentum Grant from the Hungarian Academy of Sciences (LP2014-10/2014 to PH), and by the New National Excellence Program of the Ministry of Human Capacities (ÚNKP-17-3-II to DP).

ACKNOWLEDGMENTS

We would like to thank the contribution of centers not meeting the authorship policy. These centers include the Joint Saint Istvan and Saint Laszlo Hospitals (Budapest), the Institute of Surgery, University of Debrecen (Debrecen), the Pándy Kálmán Hospital of County Békés (Gyula), the Bács-Kiskun County Hospital (Kecskemét), the Healthcare Center of County Csongrád (Makó), the Borsod-Abaúj-Zemplén County Hospital and University Teaching Hospital (Miskolc), the Second Department of Medicine, University of Szeged (Szeged), the Emergency Unit, University of Szeged (Szeged), the Department of Surgery, University of Szeged (Szeged), the Department of Gastroenterology, Dr. Bugyi István Hospital (Szentes), and the Markusovszky University Teaching Hospital (Szombathely).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2018.01776/full#supplementary-material

APPENDIX 1 | Tables of demography and representativeness of the study population. Distribution of centers recruiting the study population.

APPENDIX 2 | Data quality.

APPENDIX 3 | Figures of demography and representativeness of the study population.

APPENDIX 4 | Correlation between age and LOH (A panel), and CCI and LOH (B panel).

APPENDIX 5 | Aging and complications in acute pancreatitis.

APPENDIX 6 | Comorbidities and complications in acute pancreatitis.

APPENDIX 7 | Joint effect of aging and comorbidities on local and systemic complications of AP.

APPENDIX 8 | Data used in multivariate analysis.

APPENDIX 9 | Results of univariate analysis on the effects of individual comorbidities on the outcomes of acute pancreatitis.

APPENDIX 10 | Results of multivariate analysis on the effects of individual comorbidities on the outcomes of acute pancreatitis.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

International Journal of
Molecular Sciences

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Article

Meta-Analysis of Early Nutrition: The Benefits of Enteral Feeding Compared to a Nil Per Os Diet Not Only in Severe, but Also in Mild and Moderate Acute Pancreatitis

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Academic Editors: Jaya Padmanabhan and Srikumar Chellappan
Received: 4 July 2016; Accepted: 27 September 2016; Published: 20 October 2016

Abstract: The recently published guidelines for acute pancreatitis (AP) suggest that enteral nutrition (EN) should be the primary therapy in patients suffering from severe acute pancreatitis (SAP); however, none of the guidelines have recommendations on mild and moderate AP (MAP). A meta-analysis was performed using the preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P). The following PICO (problem, intervention, comparison, outcome) was applied: P: nutrition in AP; I: enteral nutrition (EN); C: nil per os diet (NPO); and O: outcome. There were 717 articles found in Embase, 831 in PubMed, and 10 in the Cochrane database. Altogether, seven SAP and six MAP articles were suitable for analyses. In SAP, forest plots were used to illustrate three primary endpoints (mortality, multiorgan failure, and intervention). In MAP, 14 additional secondary endpoints were analyzed (such as CRP (C-reactive protein), WCC (white cell count), complications, etc.). After pooling the data, the Mann–Whitney *U* test was used to detect significant differences. Funnel plots were created for testing heterogeneity. All of the primary endpoints investigated showed that EN is beneficial vs. NPO in SAP. In MAP, all of the six articles found merit in EN. Analyses of the primary endpoints did not show significant differences between the groups; however, analyzing the 17 endpoints together showed a significant difference in favor of EN vs. NPO. EN is beneficial compared to a nil per os diet not only in severe, but also in mild and moderate AP.

Keywords: enteral feeding; acute pancreatitis; early nutrition; energy; meta-analysis

1. Introduction

Acute pancreatitis (AP) is a severe inflammatory disease with high mortality [1]. Despite the extensive research in the field, no specific therapy is available to treat AP [2]. With regard to the pathomechanism of the disease, it is clear that mitochondrial injury and ATP depletion play key roles in the early phase of AP almost irrespectively of the etiology of the disease [3–5]. Bile acids, ethanol, and fatty acids were shown to be responsible for around 80% of the etiological factors initiating AP [6]. All of these factors were shown to induce a toxic calcium signal and severe mitochondrial damage in both acinar and ductal cells [3,7–11]. Importantly, direct administration of ATP (i.e., energy) into the cells restored their functions and prevented cell death [12,13]. Therefore, if we take a translational approach, it is more than likely that patient energy intake would be beneficial. Not surprisingly, enteral nutrition (EN) has almost been the only therapeutic change in recent decades to be highly beneficial and to be widely utilized in severe AP (SAP) [14]. However, in mild and moderate AP (MAP), the primary therapy is still the nil per os diet (NPO) [15]. Since the results in basic science have demonstrated the crucial role of energy breakdown in the early phase of AP, in this study we performed a systemic review of the literature followed by a meta-analysis to understand whether enteral feeding should be the primary therapy not only in severe AP, but in mild and moderate AP as well.

2. Results

2.1. Severe Acute Pancreatitis (SAP) Group

Seven out of seven articles contained analyzable data on mortal [16–22] Risk differences and CI were calculated in each article to analyze the effects of EN compared to the NPO nutrition. The calculated average risk difference (RD) was −0.050 (lower limit (LI): −0.134; upper limit (UI): 0.035; *p*-value: 0.249) (Figure 1). Because of the considerable heterogeneity (Q = 16.488; DF: 6; $p = 0.011$; $I^2 = 63.61\%$) random-effect model was applied. Four out of seven articles contained analyzable data on multiorgan failure (MOF). With regard to MOF, the calculated odds ratio (OR) was 0.258 (LI: 0.072; UI: 0.930; *p*-value: 0.038; heterogeneity: Q = 13.833; DF: 3; $p = 0.003$; $I^2 = 78.31\%$) in favor of EN (Figure 2). With regard to interventions, a fixed-effect model was used. The calculated average odds ratio (OR) was 0.162 (LI: 0.079; UI: 0.334; *p*-value: <0.001; Q = 7.221; DF: 3; $p = 0.065$; $I^2 = 58.45\%$) also in favor of EN (Figure 3). Because of the moderate heterogeneity, the random-effect model was applied as well (OR was 0.274 (LI: 0.073; UI: 1.025; $p = 0.054$)). These data clearly suggest that EN is beneficial and should be the primary therapy in SAP.

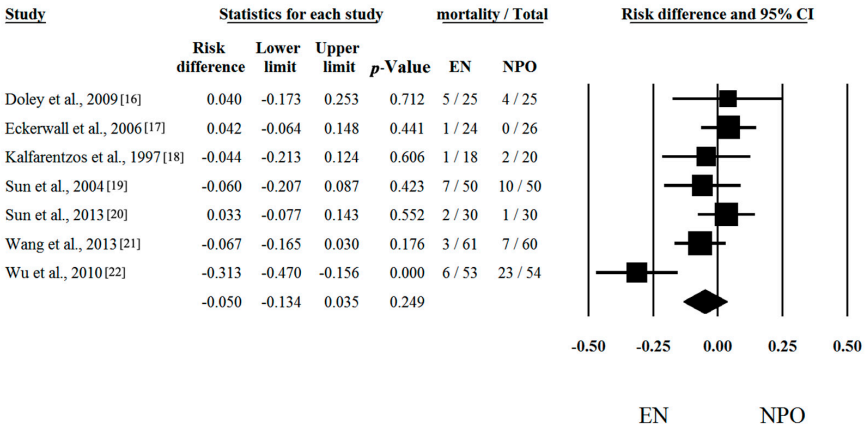

Figure 1. Forest plot of studies evaluating mortality data in severe acute pancreatitis (SAP). Risk differences and confidence interval (CI) were calculated to compare the enteral nutrition (EN) with the nil per os diet (NPO). Black squares and lines represent the results for individual studies, the diamond shows the pooled result of the meta-analysis.

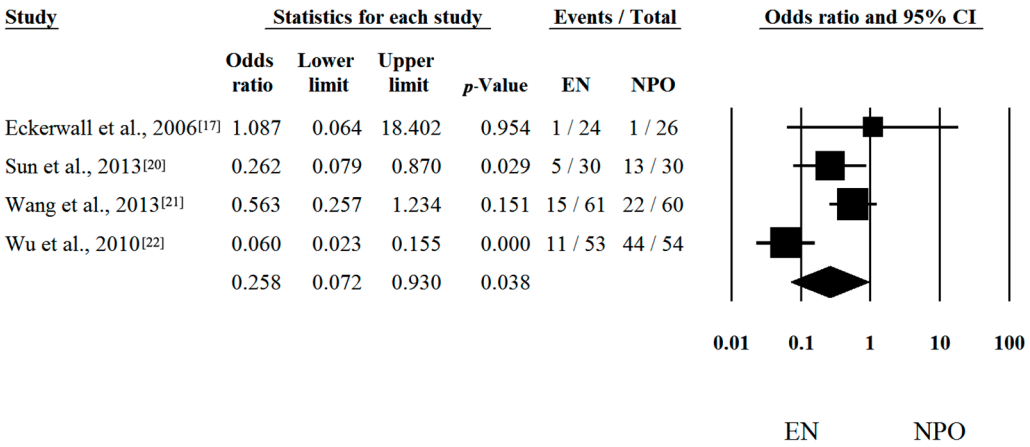

Figure 2. Forest plot of studies evaluating multiorgan failure (MOF) in severe acute pancreatitis (SAP). Odds ratio (OR) and confidence interval (CI) were calculated to compare the enteral nutrition (EN) with the nil per os diet (NPO). Black squares and lines represent the results for individual studies, the diamond shows the pooled result of the meta-analysis.

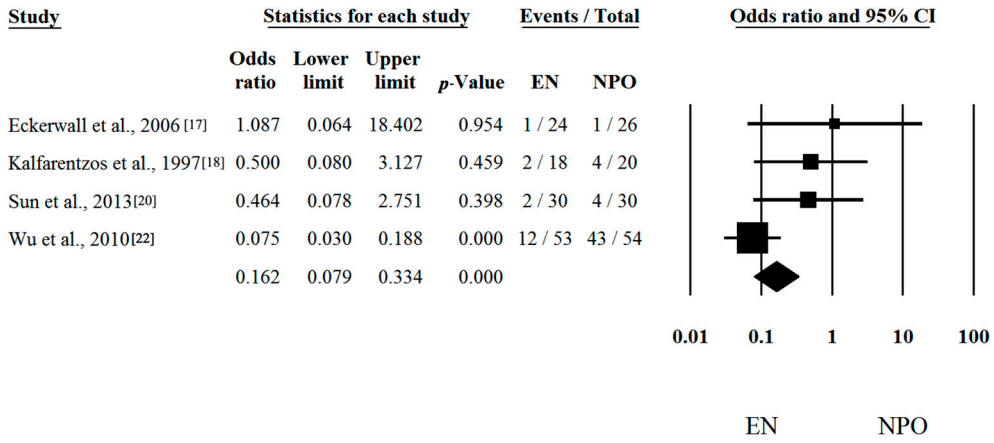

Figure 3. Forest plot of studies evaluating intervention in severe acute pancreatitis (SAP). Odds ratio (OR) and confidence interval (CI) were calculated to compare the enteral nutrition (EN) with the nil per os diet (NPO). Black squares and lines represent the results for individual studies, the diamond shows the pooled result of the meta-analysis.

2.2. Mild and Moderate Acute Pancreatitis (MAP) Group

Unfortunately, there is much less research activity in patients suffering from MAP than from SAP. Moreover, the frequency of death and MOF are also much less common in the MAP group vs. the SAP group. Not surprisingly, analyses of low amounts of data in which the mortality and MOF are close to zero could not reveal any significant difference between the two groups. With regard to mortality, five out of six articles contained proper data [23–27]. Risk differences and CI were calculated in the articles. The calculated average risk difference (RD) was −0.003 (LI: −0.047; UI: 0.040; *p*-value: 0.879) (Figure 4). As predicted, we also saw no significant difference in the frequency of MOF, where we only had four items. Forest plots of OR and CI were calculated. The odds ratio (OR) was 0.849 (LI: 0.369; UI: 1.952; *p*-value: 0.700) (Figure 5). Because of the Q and I^2 tests showed negligible heterogeneity (Q = 0.916; DF: 4; $p = 0.922$; $I^2 = 0.00\%$ for Figure 4 and Q = 1.169; DF: 3; $p = 0.760$; $I^2 = 0.00\%$ for Figure 5), the fixed-effect model was applied.

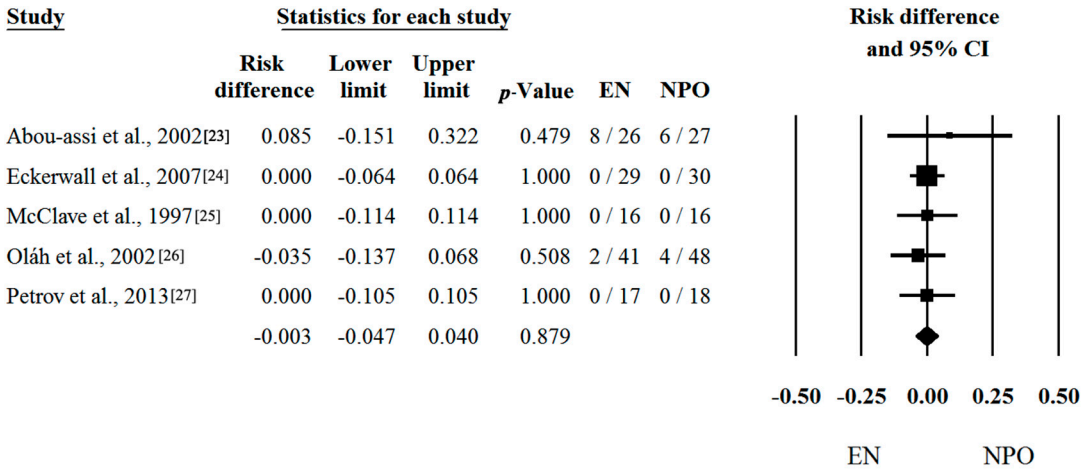

Figure 4. Forest plot of studies evaluating mortality data in mild and moderate acute pancreatitis (MAP). Risk differences and confidence interval (CI) were calculated to compare the enteral nutrition (EN) with the nil per os diet (NPO). Black squares and lines represent the results for individual studies, the diamond shows the pooled result of the meta-analysis.

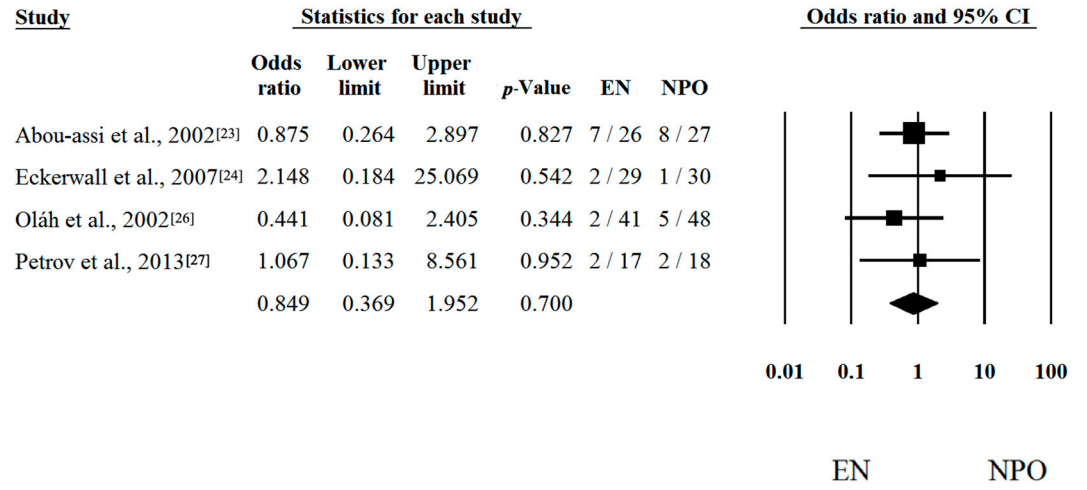

Figure 5. Forest plot of studies evaluating multiorgan failure (MOF) in mild and moderate acute pancreatitis (MAP). Odds ratio (OR) and confidence interval (CI) were calculated to compare the enteral nutrition (EN) with the nil per os diet (NPO). Black squares and lines represent the results for individual studies, the diamond shows the pooled result of the meta-analysis.

However, the five articles contained several other secondary parameters (see Methods). Unfortunately, each study group concentrated on different parameters, resulting in the fact that almost none of the parameters had a complete data set (Figure S1). Figure 6 demonstrates the differences between EN and NPO. Due to the low n number, statistical analyses could not be calculated separately. Importantly, pooling the data from the 17 parameters (3 primary and 14 secondary endpoints) showed a significant difference in favor of EN (Figure 7). The significant difference was also observed when different powers (when primary endpoints were double weighted) of the endpoints were applied. The supplementary data sheet contains all the data used for the statistical analyses.

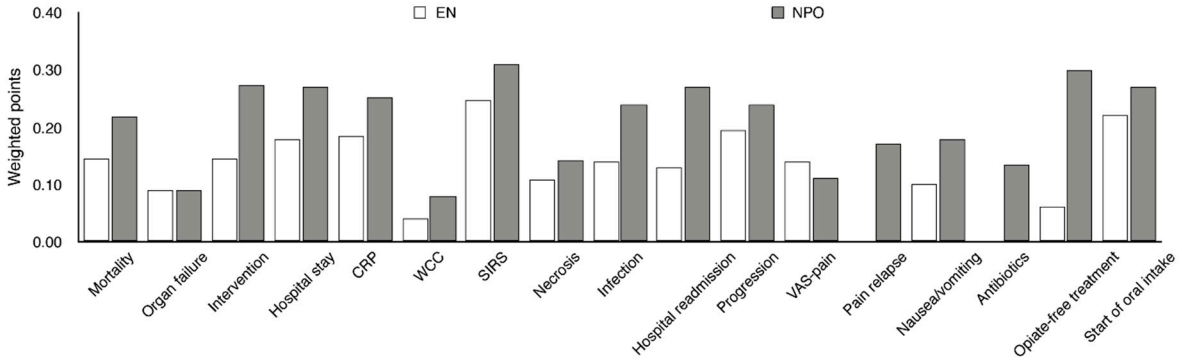

Figure 6. Summary of the uniform data-point system in MAP. EN vs. NPO. Due to the low amount of data, 3 primary endpoints and 14 secondary endpoints were collected for MAP. The uniform data point system was then developed (Table 1). Results were weighted based on the number of patients in the articles. CRP, C-reactive protein; WCC, white cell count; SIRS, systemic inflammatory response syndrome; VAS, visual analogue scale.

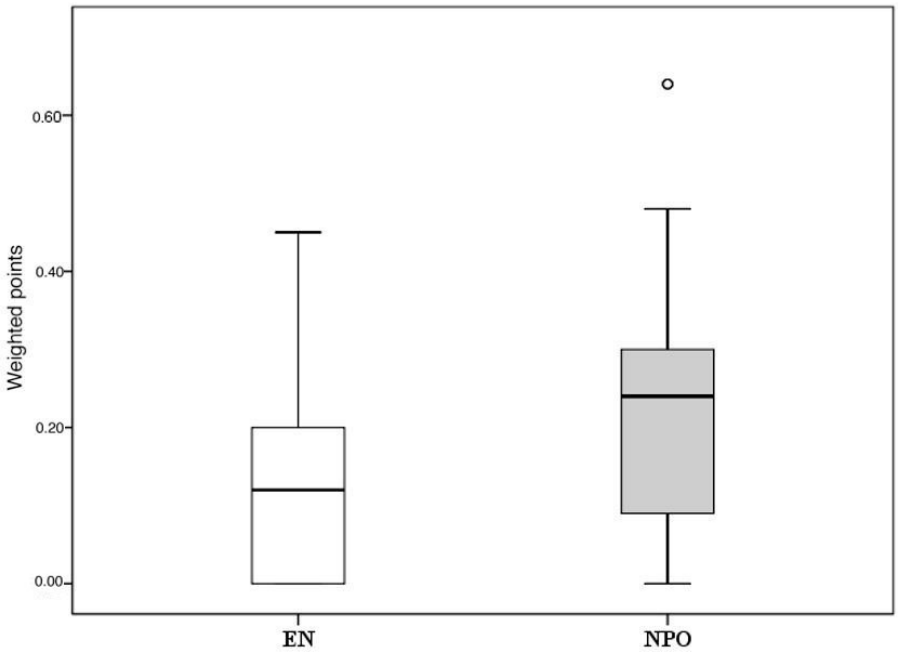

Figure 7. Summary of the uniform data point system in MAP. EN vs. NPO. The Mann–Whitney U test was used to detect significant differences between the pooled weighted scores (see Figure 6). o = $p < 0.05$ vs. EN.

Table 1. Uniform point system. CRP, C-reactive protein; WCC, white cell count; SIRS, systemic inflammatory response syndrome; LOH, length of hospitalization; VAS, visual analogue scale.

Points	Mortality (%)	Organ Failure (%)	Intervention (%)	CRP (mg/L)	WCC (109/L)	SIRS (%)
0	0–0.9	0–0.09	0–0.09	0–19.9	4000–9999.9	0–0.09
1	1–2.9	0.1–0.19	0.1–0.19	20–39.9	10,000–11,999	0.1–0.14
2	3–4.9	0.2–0.29	0.2–0.29	40–59.9	12,000–13,999	0.15–0.19
3	5–6.9	0.3–	0.3–0.39	60–79.9	14,000–15,999	0.2–0.24
4	7–8.9		0.4–0.49	80–99.9	16,000–17,999	0.25–0.29
5	9–		0.5–	100–	18,000–	0.3–

Table 1. *Cont.*

Points	LOH (Days)	Necrosis (%)	Infection (%)	Hospital Readmission (%)	Progression of Severity (%)	Pain Relapse (%)
0	0–4.9	0–0.09	0–0.09	0–0.04	0–0.04	0–0.09
1	5–9.9	0.1–0.19	0.1–0.19	0.05–0.06	0.05–0.06	0.1–0.19
2	10–12.4	0.2–0.29	0.2–	0.07–0.08	0.07–0.08	0.2–0.29
3	12.5–14.9	0.3–	–	0.09–0.10	0.09–0.10	0.3–0.39
4	15–19.9	–	–	0.11–	0.11–	0.4–
5	20–	–	–	–	–	–

Points	VAS-Pain	Nausea/Vomiting (%)	Antibiotics (%)	Opiate-Free Treatment (%)	Start of Oral Intake (%)
0	0–1	0–0.18	0–0.09	0–0.09	0–0.04
1	2–4	0.2–0.39	0.1–0.19	0.1–0.19	0.05–0.09
2	5–7	0.4–0.59	0.2–0.29	0.2–0.29	0.1–0.14
3	8–9	0.6–0.79	0.3–0.39	0.3–0.39	0.15–0.19
4	–	0.8–	0.4–	0.4–0.49	0.2–0.24
5	–	–	–	0.5–	0.25–

3. Discussion

There are different therapeutic approaches available with regard to nutrition in acute pancreatitis. The recently published IAP/APA (International Association of Pancreatology/American Pancreatic Association) guidelines recommend that enteral tube feeding be the primary therapy in patients with predicted severe and severe acute pancreatitis who require nutritional support (recommendation G. Nutritional support 21-GRADE 1B, strong agreement), whereas point K22 in the Japanese guidelines states that enteral nutrition can reduce the incidence of complications in the early phase of SAP and can contribute to an increased rate of survival [2,28]. However, neither of the guidelines provides recommendations on MAP. The reason is understandable. (1) Strong endpoints are missing. The mortality rate is less than 1% in mild AP and 10% in moderate AP, whereas almost no MOF can be detected; (2) since there is a better outcome of the milder disease, researchers have had much less interest in MAP than SAP.

Here, we wanted to systematically review the current literature to understand the beneficial effects of early enteral nutrition vs. the nil per os diet both in SAP and MAP. Interestingly, there were not many articles in which analyzable data could be found on the two treatments of AP. However, in SAP, the amount of data was sufficient to prove the beneficial effects of enteral feeding. Early enteral feeding was clearly beneficial for MOF and intervention and showed beneficial tendency for mortality. Nevertheless, as predicted, MAP data analyses revealed no significant difference between enteral nutrition and a nil per os diet. However, analyses of the secondary endpoints in the articles demonstrated that enteral feeding could be beneficial compared to a nil per os diet in mild and moderate AP as well.

The six MAP studies applied different methods for enteral feeding. Eckerwall et al. [24] employed immediate oral feeding, Abou-Assi et al. [23], Oláh et al. [26], and McClave et al. [25] administered nasojejunal feeding, and Petrov et al. [27] and Ma et al. [29] used nasogastric feeding. Immediate oral feeding (EN) significantly cut the length of hospital stay without any adverse events [24]. Nasogastric feeding starting within 24 h of hospital admission was not only well tolerated, but also reduced the intensity and duration of abdominal pain, decreased the necessity of opiates, and almost totally eliminated the risk of oral food intolerance [27]. Moreover, patients in the nasogastric feeding group had significantly improved appetite vs. the NPO group [29]. Nasojejunal feeding lowers the stress response to AP [25] associated with a lower complication rate [26] and cuts the length of hospital stay. Importantly, the fact that all of the studies found merit in early enteral feeding in MAP suggests that it is not the way of feeding that is important, but the feeding itself, i.e., energy.

4. Materials and Methods

4.1. Article Search

A meta-analysis was performed using the preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) [30]. An article search was performed in the PubMed, EMBASE, and Cochrane databases in February 2016. The PICO process was used to frame and answer our clinical questions.

4.1.1. PICO (Problem, Intervention, Comparison, Outcome)

PICO was broken down as follows: P: nutrition in AP; I: enteral nutrition; C: nil per os diet; and O: outcome. We split our data into two groups: SAP and MAP. In SAP, only three primary endpoints were checked (mortality, multiorgan failure, and intervention), whereas in MAP, due to the low amount of data, 14 secondary endpoints were collected besides the primary endpoints: length of hospital stay (LOH), inflammatory parameters (C-reactive protein (CRP), white cell count (WCC), and presence of SIRS (systemic inflammatory response syndrome)), complications (necrosis, infection, hospital readmission, and progression of severity), intervention, necessity of antibiotic, pain relapse, visual analogue scale (VAS)-pain, opiate-free treatment, start of oral intake, and clinical symptoms (nausea and vomiting).

4.1.2. Search

A search was made using the following terms: in PubMed: (acute (All Fields) and "pancreatitis" (MeSH Terms) or "pancreatitis" (All Fields)) and ("clinical trial" (Publication Type) or "clinical trials as topic" (MeSH Terms) or "clinical trials" (All Fields)) and ("loattrfull text" (sb) and "humans" (MeSH Terms) and English (lang)) in EMBASE: "acute pancreatitis" and (humans)/lim and (English)/lim and (abstracts)/lim and ((controlled clinical trial)/lim or (randomized controlled trial)/lim) and in Cochrane: "acute pancreatitis": ti,ab,kw and "human" and "English" in Trials (the search included various forms of the terms). "Acute pancreatitis" in Title, Abstract and Keywords and "human" and "English" in Trials (the search included various forms of the terms). Altogether, 1634 articles (EMBASE: 717; PubMed: 831; Cochrane: 10) were found (Figure 8).

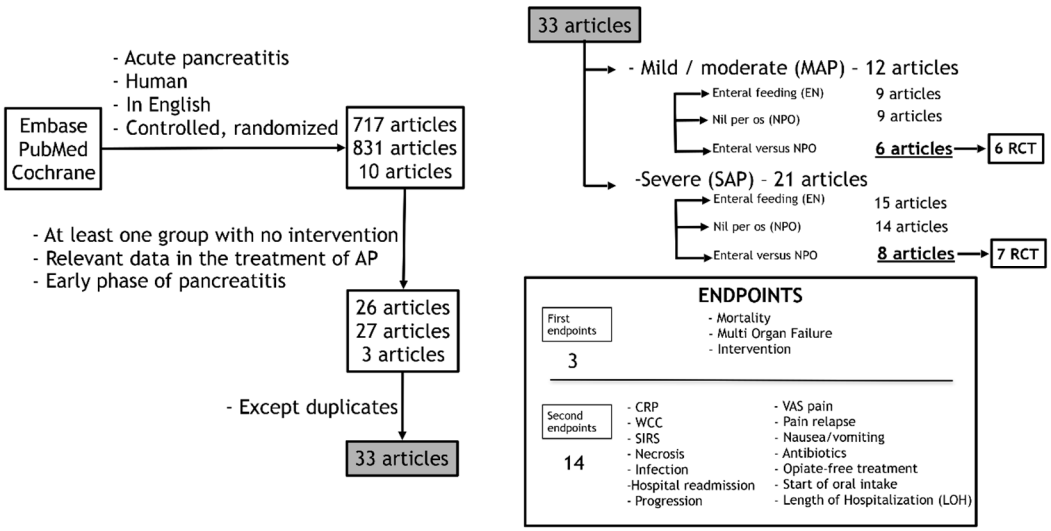

Figure 8. Organogram of article search in PubMed, EMBASE, and Cochrane databases. RCT, randomized and controlled trial; CRP, C-reactive protein; WCC, white cell count; SIRS, systemic inflammatory response syndrome; VAS, visual analogue scale.

4.1.3. Inclusions and Exclusions

A manual search was performed to find the relevant articles. Only articles in English and with relevant data in the early phase treatment of AP were included. Duplications were excluded. Thirty-three articles (21 articles containing patients suffering from SAP as well as 12 articles with MAP patients) were selected. They contained two nonrandomized and 31 randomized controlled clinical trials (Table 2) [16–27,29,31–50]. Finally, statistical analyses were performed on data from articles where both EN and NPO groups were presented, the trial was randomized, and the relevant data were available. Altogether, seven SAP and six MAP articles met these criteria.

Table 2. Articles with data on the early phase of AP. SAP: severe acute pancreatitis; MAP: mild and moderate AP; EN: enteral nutrition; NPO: nil per os diet; RCT: randomized controlled clinical trial.

Article	MAP	SAP	EN	NPO	RCT
Doley et al. 2009 [16]	–	✔	✔	✔	✔
Eckerwall et al. 2006 [17]	–	✔	✔	✔	✔
Kalfarentzos et al. 1997 [18]	–	✔	✔	✔	✔
Sun et al. 2004 [19]	–	✔	✔	✔	✔
Sun et al. 2013 [20]	–	✔	✔	✔	✔
Wang et al. 2013 [21]	–	✔	✔	✔	✔
Wu et al. 2010 [22]	–	✔	✔	✔	✔
Abou-assi et al. 2002 [23]	✔	–	✔	✔	✔
Eckerwall et al. 2007 [24]	✔	–	✔	✔	✔
McClave et al. 1997 [25]	✔	–	✔	✔	✔
Oláh et al. 2002 [26]	✔	–	✔	✔	✔
Petrov et al. 2013 [27]	✔	–	✔	✔	✔
Ma et al. 2016 [29]	✔	–	✔	✔	✔
Li et al. 2013 [39]	✔	–	✔	–	✔
Ockenga et al. 2002 [41]	✔	–	–	✔	✔
Pandey et al. 2004 [42]	✔	–	✔	–	✔
Pongratz et al. 2013 [45]	✔	–	–	✔	✔
Sathiaraj et al. 2008 [46]	✔	–	✔	–	✔
Wu et al. 2011 [49]	✔	–	–	✔	✔
Andersson et al. 2006 [31]	–	✔	–	✔	–
Bakker OJ et al. 2014 [32]	–	✔	✔	–	✔
Besselink et al. 2008 [33]	–	✔	✔	–	✔
Eatock et al. 2005 [34]	–	✔	✔	–	✔
He et al. 2004 [35]	–	✔	–	✔	✔
Karakan et al. 2007 [36]	–	✔	✔	–	✔
Kumar et al. 2006 [37]	–	✔	✔	–	✔
Kyhala et al. 2012 [38]	–	✔	–	✔	✔
Modena et al. 2006 [40]	–	✔	✔	✔	–
Pearce et al. 2006 [43]	–	✔	✔	–	✔
Pettila et al. 2010 [44]	–	✔	–	✔	✔
Singh et al. 2012 [47]	–	✔	✔	–	✔
Vege et al. 2015 [48]	–	✔	–	✔	✔
Zhao et al. 2013 [50]	–	✔	–	✔	✔

4.1.4. Statistical Analyses

In SAP, forest plots were used to illustrate the mortality, multiorgan failure and intervention. In the case of mortality and multiorgan failure, the pooled estimates were calculated with a random-effects model; in the case of intervention, a fixed-effects model was applied as described earlier [51]. Analyses were performed with the Comprehensive Meta-Analysis Software (Biostat, Inc., Englewood, NJ, USA). In the case of binary variables, the differences between EN and NPO were expressed as risk differences or odds ratios with a 95% confidence interval (CI). Heterogeneity was tested between trials with two methods. First, we employed the Q homogeneity test statistic, which exceeds the upper-tail critical value of chi-square on n − 1 degrees of freedom (DF), with a p-value of less than 0.050 considered

suggestive of significant heterogeneity. Second, we used the inconsistency (I^2) index. I^2 is the proportion of total variation contributed by between-study variability. An I^2 value of more than 0.5 suggests a considerable heterogeneity. Heterogeneity was verified using a funnel plot to reduce publication bias. Whenever considerable heterogeneity was observed, random- or fixed-effects models were applied.

In MAP, only two (mortality and multiorgan failure) of the three primary endpoints could be analyzed. With regard to the second endpoints, no forest plot analyses could be calculated due to insufficient data. A uniform point system was developed to make the data analyzable (Table 1). Results were also weighted based on the number of patients in the articles. The Mann–Whitney *U* test was used to detect significant differences between the pooled weighted scores. SPSS Statistical Software (version 20, IBM Corporation, Armonk, NY, USA) facilitated this analysis. A *p*-value less than 0.05 was considered as statistically significant, whereas a *p*-value between 0.1 and 0.05 was seen as a trend.

5. Conclusions

Unfortunately, there are several limitations of this study, therefore, the results of this meta-analysis should be interpreted with caution. The biggest limitation is the small number of studies included (especially in MAP) which caused higher heterogeneity. The low amount of extracted data from the articles caused further difficulties. In MAP, a uniform point system had to be developed to make the data analyzable. Since these limitations attenuate the strength of this meta-analysis, more high-quality randomized controlled clinical trials (RCTs) are still needed to propound more evidence on treatment decisions in MAP.

In conclusion, enteral feeding is beneficial compared to a nil per os diet not only in severe, but also in mild and moderate AP. Additional studies should be performed to understand whether energy supply or enteral passage is more important.

Supplementary Materials: Supplementary materials can be found at www.mdpi.com/1422-0067/17/10/1691/s1.

Acknowledgments: This study was supported by the Hungarian Scientific Research Fund (K116634 to PH) and the Momentum Grant of the Hungarian Academy of Sciences (LP2014-10/2014 to PH).

Author Contributions: Áron Vincze, Imre Szabó, Judit Bajor, Dóra Mosztbacher, Patrícia Sarlós and Dániel Pécsi searched for articles; József Czimmer, Gabriella Pár, Ákos Szűcs, Anita Illés, Andrea Párniczky and Kata Szemes read the papers and collected the data from the relevant manuscripts; Nelli Farkas analyzed the data; and Katalin Márta and Péter Hegyi initiated the study and wrote the article.

Conflicts of Interest: The authors declare no conflict of interest.

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High versus low energy administration in the early phase of acute pancreatitis (GOULASH trial): protocol of a multicentre randomised double-blind clinical trial

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To cite: Márta K, Szabó AN, Pécsi D, *et al*. High versus low energy administration in the early phase of acute pancreatitis (GOULASH trial): protocol of a multicentre randomised double-blind clinical trial. *BMJ Open* 2017;**7**:e015874. doi:10.1136/bmjopen-2017-015874

► Prepublication history and additional material for this paper are available online. To view please visit the journal (http://dx.doi.org/10.1136/bmjopen-2017-015874).

Received 6 January 2017
Revised 21 July 2017
Accepted 25 July 2017

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ABSTRACT

Introduction Acute pancreatitis (AP) is an inflammatory disease with no specific treatment. Mitochondrial injury followed by ATP depletion in both acinar and ductal cells is a recently discovered early event in its pathogenesis. Importantly, preclinical research has shown that intracellular ATP delivery restores the physiological function of the cells and protects from cell injury, suggesting that restoration of energy levels in the pancreas is therapeutically beneficial. Despite several high quality experimental observations in this area, no randomised trials have been conducted to date to address the requirements for energy intake in the early phase of AP.

Methods/design This is a randomised controlled two-arm double-blind multicentre trial. Patients with AP will be randomly assigned to groups A (30 kcal/kg/day energy administration starting within 24 hours of hospital admission) or B (low energy administration during the first 72 hours of hospital admission). Energy will be delivered by nasoenteric tube feeding with additional intravenous glucose supplementation or total parenteral nutrition if necessary. A combination of multiorgan failure for more than 48 hours and mortality is defined as the primary endpoint, whereas several secondary endpoints such as length of hospitalisation or pain will be determined to elucidate more detailed differences between the groups. The general feasibility, safety and quality checks required for high quality evidence will be adhered to.

Ethics and dissemination The study has been approved by the relevant organisation, the Scientific and Research Ethics Committee of the Hungarian Medical Research Council (55961-2/2016/EKU). This study will provide evidence as to whether early high energy nutritional support is beneficial in the clinical management of AP. The results of this trial will be published in an open access way and disseminated among medical doctors.

Trial registration The trial has been registered at the ISRCTN (ISRTCN 63827758).

Strengths and limitations of this study

- Strength 1: This is a randomised controlled two-arm double-blind multicentre trial which provides the first type A evidence concerning the necessity of early energy intake for patients with AP.
- Strength 2: The study enjoys continuous support from an International Translational Advisory Board (ITAB) including several well established experts.
- Strength 3: Data will be separately handled by an Independent Data Management Board (IDMB).
- Strength 4: There are no unknown drugs/therapy used in the study, therefore no adverse and serious adverse events are expected.
- Limitation 1: In order to detect a treatment effect of at least 50% of the early treatment, a sample size of 957 subjects will be necessary to be recruited which will delay the final conclusion of the study.
- Limitation 2: The double-blind arrangement of the study requires many staff members working on the project which may limit the number of joining centres.

BACKGROUND

Acute pancreatitis (AP) is an inflammatory disease of the exocrine pancreas which is life threatening in its severe form. Unfortunately, while the overall mortality of AP is around 2–5%, and in its severe form 25–57%, no specific treatment is available. Besides the limited interest of pharmacological companies, the main reasons are (1) the small number of research teams in the field and (2) the lack of collaboration between basic and clinical scientists. Importantly, many new

therapeutic targets were identified in the last decade with clear translational merits.[1–8] One of the main highlights among them is the discovery of energy depletion in the early phase of AP.[1 3–5 7–17]

It has been shown that, almost independently of the aetiological factors, the early phase of AP is almost the same. Bile acids, ethanol, fatty acids and the latter's metabolite fatty acid ethyl esters cause mitochondrial damage and ATP depletion in pancreatic ductal and acinar cells, driving the cells to death and causing pancreatic necrosis.[1 3 4 10–14 18–31] Very importantly, restoration of ATP levels in both cell types prevented cell death and at least partially restored their function.[1 9] In experimental pancreatitis models the same observations have been revealed.[10–21] Although these experimental observations clearly suggest that restoration of the energy level could be a therapeutic tool in AP, this has not been translated into clinical trials.

One of the best and most physiological way of delivering energy to a patient is enteral nutrition (EN). Not surprisingly, besides fluid resuscitation this is almost the only way to significantly reduce mortality in AP.[22–33] Recent analyses of prospectively collected data from 600 patients with AP showed that the mortality is 27% with EN and 57% without EN in the severe form (SAP).[34] Importantly, EN decreases mortality but also reduces the frequency of multiorgan failure and the need for interventions in patients with SAP.[35] No data are available on whether early or on-demand nutrition/energy supply is beneficial in SAP. The recently published Dutch PYTHON study suggests that there is no difference between early and on-demand enteral tube feeding in SAP, but patients may have received an insufficient amount of energy at the early phase of the disease.[36 37] In the early EN group, patients received over 20 kcal/kg/day only from day 3 onwards whereas, in the on-demand group, they received energy supplementation only from day 6.[37] In mild and moderate AP (MAP) much less information is available concerning the usefulness of EN. There is a large variety of protocols on EN in MAP. Immediate oral feeding,[38] nasojejunal feeding[39–41] and nasogastric feeding[42 43] have all been used. Notably, immediate oral feeding significantly decreased the length of hospital stay.[38] Early (within 24 hours) nasogastric EN was well tolerated and reduced the intensity and duration of abdominal pain, decreased the necessity for opiates and almost completely eliminated the risk of oral food intolerance.[42] In order to obtain stronger evidence of the usefulness of early EN in MAP and SAP, we performed a systematic review and meta-analysis which showed that early EN can be beneficial in both MAP and SAP.[35] However, we also realised the lack of multicentre randomised control trials addressing energy intake in the early phase of AP.

The main objective of this trial is to determine whether early energy supplementation is beneficial to patients with AP. Our hypothesis is that early energy supplementation will prevent the cells from death or decrease the size of necrosis if it occurs. This will decrease the systemic immune response that will result in a lower frequency of multiorgan failure and mortality. To prove this concept, a randomised clinical trial involving all patients with AP is needed.

METHODS

Design

This is a randomised controlled two-arm double-blind multicentre trial. Patients with AP will be randomly assigned to groups A (high energy administration starting within 24 hours of hospital admission) and B (no energy administration after 24 hours of hospital admission).

Trial organisation, committees and boards

GOULASH is designed and coordinated by the Centre for Translational Medicine at the University of Pécs and the Hungarian Pancreatic Study Group (HPSG). HPSG was established in 2011 in order to stimulate research in pancreatic diseases. To date, HPSG has published the relevant guidelines of pancreatic diseases in order to improve patient care in the field of pancreatology[44–47] and has initiated four prospective clinical trials (EASY, PREPAST, APPLE and PINEAPPLE).[48–51]

The following committees and boards will be involved:

Steering committee (SC)

The Steering committee (SC) will be led by PH (gastroenterologist, internal medicine specialist). The members will be KM (medical doctor, full time employee on the project), ÁV (gastroenterologist, internal medicine specialist), ZM (intensive care specialist), TM (clinical research specialist), AS (multidisciplinary unit specialist), MP (gastroenterologist, internal medicine specialist), NF (radiologist), DK (surgeon) and IB (interventional radiologist). SC will make decisions concerning all relevant questions including the drop-outs during the study.

International translational advisory board (ITAB)

The committee will include a gastroenterologist (MML), a surgeon (JPN) and basic scientists (MST, OHP). ITAB will continuously monitor the progress of the study and will give advice to the SC.

The study was designed by the SC and ITAB. The study is financially sponsored by the University of Pécs, the Hungarian Academy of Sciences and the National Research, Development and Innovation Office. Neither sponsors were involved in the design of the study, and they will have no access to the database management or to the randomisation code.

Study population

All patients diagnosed with AP will be informed of the possibility of taking part in the GOULASH study. After the consent form is signed, a computer using a block randomisation protocol will randomise the patients (figure 1).

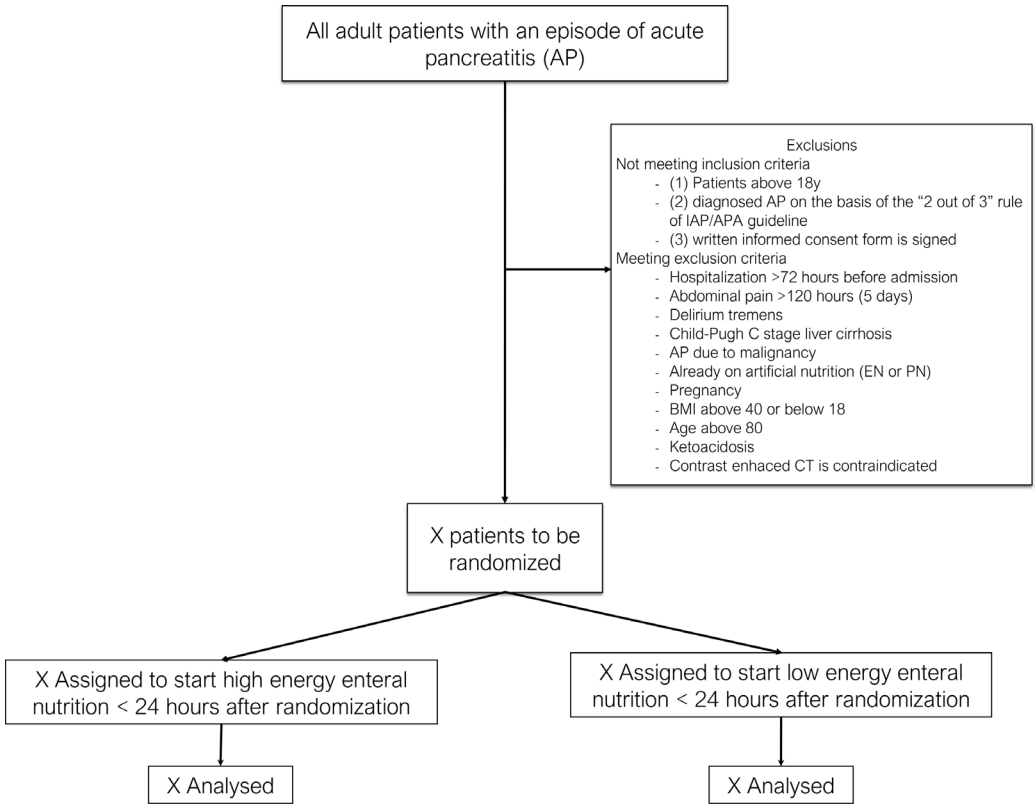

Figure 1 Flow chart of participants according to the SPIRIT 2013 statement.[53]

Inclusion criteria

The inclusion criteria are: (1) patients over 18 years of age; (2) diagnosed AP on the base of the '2 out of 3' criteria of the IAP/APA guideline[52]: (a) upper abdominal pain; (b) serum amylase or lipase >3x upper limit of normal range; (c) characteristic findings on pancreatic imaging; however those patients without abdominal pain will be excluded because the onset of AP cannot be determined; (3) signed written informed consent form.

Exclusion criteria

The exclusion criteria are: (1) hospitalisation 72 hours before admission; (2) abdominal pain >120 hours (5 days); (3) delirium tremens; (4) Child-Pugh C stage liver cirrhosis; (5) AP due to malignancy; (6) already on artificial nutrition (EN or PN); (7) pregnancy; (8) BMI >40 or <18; (9) age >80 years; (10) ketoacidosis; and (11) whenever CT with contrast is contraindicated.

Sample size

Sample size calculation was based on the Hungarian National Registry operated by the HPSG. Our recent analyses indicated that multiorgan failure existing for more than 48 hours arises in 9%, whereas mortality occurs in 2.8% of all patients with AP.[34] Altogether they represent around 10% of all AP patients. In order to detect a treatment effect of at least 50% of the early treatment, a sample size of 957 subjects will be necessary to be recruited using a 10% drop-out rate, 80% power and 95% significance level. The calculation was performed by the independent data management and biostatistics provider company (IDMB, Adware Research Ltd, Balatonfüred, Hungary).

Randomisation

In each centre participants will be divided into two groups receiving one of the two study treatments. The allocation of participants to the different groups will be carried out based on predefined randomisation lists created separately for each recruiting centre. The randomisation lists will be prepared with a block size of 4 and with an allocation ratio of 1:1.

Duration

The planned starting date of the study is 1 January 2017 and the planned finishing date of the study is 1 January 2020.

Blinding

The medical staff (eg, those taking the measurements such as blood pressure, examining health records for events such as abdominal pain, reviewing and interpreting examination results such as X-ray or CT) and the patient receiving the intervention will be blinded to knowledge of treatment assignment. The person providing the intervention cannot be blinded in this study. Sealed envelopes ensure the allocation sequence. Nutritional support equipment will be covered until the fourth day to ensure that only the person who made the randomisation will know into which group the patient was enrolled.

	STUDY PERIOD							
	Enrolment	Allocation	Post-allocation					Close-out
TIMEPOINT**	<24h	0	day1	day2	day3	dayx	discharge	1m after discharge
ENROLMENT:								
Eligibility screen	X							
Informed consent	X							
Laboratory test	X							
CT examination	X							
Allocation		X						
INTERVENTIONS:								
High energy administration			♦	—	—	♦		
Low energy administration			♦	—	—	♦		
ASSESSMENTS:								
Questionnaire A		X						
Questionnaire B			X	X	X	X	X	
Questionnaire C								X

Figure 2 Schedule of enrolment, interventions and assessments according to the SPIRIT 2013 statement.[53] Patients will be randomised to group A (high energy) or B (low energy). Online supplementary figure 1 Form A contains the parameters collected on admission. Online supplementary figure 2 Form B contains parameters collected every day during hospitalisation. Online supplementary figure 3 Form C contains parameters collected 1 month after hospital discharge.

Intervention

Based on the currently available guidelines, enteral feeding can be started at any time for patients with AP. In addition, no calorie restriction/order has been described. Therefore, both groups can be regarded as being treated within accepted practice recommendations.

In this study, early high energy administration will be the intervention. Patients will be randomised to group A or B (see figure 2).

Groups

In group A, high energy will be delivered after admission. Patients will receive a 10 Ch nasogastric (NG) or nasojejunal (NJ) feeding tube on admission. EN will be immediately started as follows: on day 0 (from admission until the start of EN, which can vary from 2 to 24 hours): calorie intake will be 0 kcal/kg/day; from day 1, high energy enteral tube feed 30 kcal/kg/day will be provided until the oral feeding starts. In group B, low energy administration will be delivered after hospital admission. Patients will receive a NG or NJ feeding tube at admission as described above. On day 0 (from admission until the start of EN): calorie intake will be 0 kcal/kg/day; on day 1, 0 kcal/kg/day; on day 2, 10 kcal/kg/day, on day 3, 20 kcal/kg/day and from day 4, 30 kcal/kg/day will be delivered until the oral feeding starts. However, between groups A and B only the amount of calories administered will be different. Patients will receive the same amount of fluid and ions during EN (see below).

Ingredients of enteral tube feed: high energy enteral tube feed (100 mL)

Energy

150 kcal (630 kJ), protein 6 g (16%E), carbohydrate 18.3 g (49%E), fat 5.8 g (35%E) + minerals: 134 mg sodium, 201 mg potassium, 108 mg calcium, 108 mg phosphorus, 34 mg magnesium, 100 mg chloride (0%E). In this study we will use Nutrison Energy (Numil Ltd, Budapest, Hungary), which is a registered product in Hungary (reg. number: 1217).

Zero energy enteral tube feed (100 mL)

Energy

0 kcal (0 kJ), protein 0 g, carbohydrate: 0 g, fat 0 g + minerals: 134 mg sodium, 201 mg potassium, 108 mg calcium, 108 mg phosphorus, 34 mg magnesium, 5.562 g chloride (0%E). In this study the local institutional pharmacy will provide it in accordance with the Hungarian regulations. Whenever 10 or 20 kcal/kg/day calories are to be delivered, a mixture of the abovementioned two solutions will be used.

Type of enteral tube

Patients neither vomiting nor having gastric fluid retention >250 mL will receive a NG tube. Patients either vomiting or having gastric fluid retention >250 mL will receive a NJ tube (placement will be done either endoscopically or radiologically). In case of Glasgow Coma Score (GCS) 14 or lower in a patient who is not intubated, the NG tube will be replaced by a NJ tube (risk of aspiration). Abdominal X-ray will be used to check the position of the tube.

Start of mixed feeding (around 2620 kcal): 1000 mL tap water distributed for 24 hours and 300 g (around 1900 kcal) biscuits/toasts/low fat meal (containing at least 75% carbohydrate) orally plus enteral tube feed (480 mL, 720 kcal/day) will be started on the day when: (1) abdominal pain has ceased for at least 6 hours before the new day started; (2) the C-reactive protein (CRP) level has started decreasing; and (3) the amylase or lipase level has started decreasing.

Start of total feeding (around 2000 kcal): if the patient has no symptoms during the mixed oral/enteral feeding and the CRP, amylase or lipase levels are not rising again, total feeding (according to local policy) can be started.

Other issues

The speed of EN will be different for patients depending on the body weight, however, the maximum speed of EN cannot exceed 65 mL/hour. In case of difficulties reaching an intake of 30 kcal/kg/day calories (if the patient's body weight is >75 kg), additional intravenous calories will be added using Sterofundin G. A maximum of 2000 mL (400 kcal) can be delivered in this way. If NG feeding is not tolerated, the NG tube will be replaced by a NJ tube as described above. If NJ feeding is not tolerated, EN will be reduced by 50% and increased again gradually until tolerated. If the re-increasing process is still not tolerated, total parenteral nutrition (TPN) will be started to reach the required energy target. In patients with SAP, TPN must be delivered via a central venous catheter.

Other treatment of subjects

General treatment indicated by the IAP/APA guideline will be utilised.[52]

Discharge of patients

Uniformisation of the length of hospital stay is necessary to avoid bias concerning length of hospital stay. Readmission within 1 week after discharge has to be considered as the same hospital admission. Patients will be counted as discharged from hospital/from the study when (1) oral feeding is tolerated for 24 hours; (2) amylase/lipase levels are not elevated after total enteral feeding; (3) CRP level is <50 mg/L; (4) abdominal pain has completely resolved; and (5) no other pancreatitis-related complication requiring hospitalisation is detected.

Endpoints

The following primary endpoints will be calculated: a combination of multiorgan failure for more than 48 hours and mortality. The following secondary endpoints will be analysed: (1) pancreatic necrosis; (2) nutrition-related complications (eg, diarrhoea, aspiration pneumonia, pneumothorax due to central TPN catheter placement); (3) need for conversion from NG to NJ feeding tube; (4) need for conversion from EN to TPN; (5) days until the start of total feeding; (6) use of antibiotics; (7) pain relapse; (8) CRP; (9) white blood cells; (10) procalcitonin; (11) infection; (12) length of hospital stay; (13) need for ICU admission; (14) length of ICU therapy; (15) organ failure; (16) complications; (17) costs calculation. Notably, only direct costs will be calculated that include all medications, services, salaries of healthcare professionals, equipment and day care costs.

Monitored parameters during hospitalisation

There will be a large variety of parameters monitored during the study (eg, medical history, physical examination, laboratory tests, diagnostic imaging, therapy, interventions). Form A will contain the parameters collected on admission (online supplementary figure 1). Form B will contain parameters collected every day during hospitalisation (online supplementary figure 2). Form C will contain parameters collected 1 month after hospital discharge (online supplementary figure 3). For details see supplementary materials or web page (http://www.pancreas.hu/en/studies/goulash), which will be available from February 2017. Data collection on the case report form (CRF) will be done electronically (see data management).

Data management and statistical analyses

Data handling

Data will be handled by the IDMB. Electronic CRF (eCRF) will be used. The Investigator will ensure that the data in the eCRF are accurate, complete and legible. Detailed data flow will be described in a Data Management Plan (DMP). Data from completed eCRFs will be validated under the direction of the Data Manager at IDMB according to a Data Cleaning Plan (DCP). Any missing, implausible or inconsistent recordings in the eCRFs will be referred back to the Investigator using a data query form (DQF), and be documented for each individual subject before clean file status is declared. All changes to eCRFs will be recorded. Before Data Base Lock the Data Review Meeting will decide and document necessary steps related to any issue in the database and define the analysis sets. Members of the Data Review Meeting are a delegated investigator, biostatistician and data manager. Adverse events will be coded using MedDRA (AdWare Research Ltd), who will act as IDMB, works according to GCP, GLP, FDA 21CFR PART11 and other relevant regulatory requirements. AdWare Ltd.has GLP and ISO 9001 certificates.

Study populations

Three analysis populations will be defined:

Safety Analysis Set (SAS): all patients enrolled in the study.

Per Protocol Set (PPS): all enrolled patients who finished the study conforming to the requirements of the study protocol.

Intention to Treat (ITT): all randomised participants who start on a treatment, excluding consent withdrawals.

Withdrawal of a subject from PPS

Any participants/investigators and the IDMB can submit recommendations for dropouts from the PPS group with reasons given to the SC. All recommendations will be filed. The SC will discuss all the information and, if the alteration in the protocol would be expected to have any bearing on the interventions and outcomes of the study, the patient will not be included in the final per-protocol analysis. Automatic dropout from the per-protocol group shall be ordered if: (1) any of the exclusion criteria are diagnosed during the course of AP; (2) at least 50% of the energy requirement is not achieved on any days during the study; (3) parameters required for answering the primary endpoints are missing; or (4) serious medical reasons not related to pancreatitis occur (eg, accidents, stroke).

Applied software

Statistical analysis will be performed using SAS 9.2 or SPSS 19 (or later) statistical packages; Microsoft MS Word will be used for reporting.

Statistical methods

Baseline patient and disease characteristics will be analysed using descriptive analysis. Demographic and baseline characteristics will be summarised for the overall study population. Continuous variables will be described by mean, median, SD and ranges and categorical variables will be described by absolute and relative frequencies. A graphical presentation of efficacy variables will be prepared, if applicable. Descriptive statistics for both the primary and secondary parameters will be analysed similarly. Mean changes (with 95% CI) from baseline to end-of-study visit will also be presented. χ^2tests will be applied to compare proportions between the different groups. Mortality/extended multiorgan failure will be investigated using the Kaplan–Meier analysis method, while subgroup comparisons will be performed using the χ^2 or Fisher's exact test, as appropriate. For safety data, descriptive statistics and individual listings of adverse events will also be presented.

Subgroups

The following subgroups will be made during statistical analyses: (1) ages (<40 years, 40–59 years, 60–80 years); (2) BMI (<20, 20–24, 25–29, 30–35, >35); (3) start of abdominal pain before admission (≤24 hours, 24–48 hours, ≥48 hours); (4) severity of the disease SAP and MAP. In all subgroup analyses, aetiologies will be done descriptively. No confirmatory statistical testing will be applied. Hence, statistical tests and p values attached to them will be regarded as descriptive and not as tests of hypotheses.

Details of the applied statistical tests will be described in the Statistical Analysis Plan.

Early quality assessment

Early quality assessment check will be performed on the first 100 patients. The IDMB (AdWare Ltd) will perform an independent assessment of the trial-related documents and activities, with the aim of ensuring the respect of subjects' rights, safety and well-being and to guarantee the plausibility of the clinical data. The similarity of the groups at baseline will also be checked. The IDMB will report to the SC. The SC will discuss all the information and, if the differences would be expected to have any bearing on the interventions and outcomes of the study or the overall dropout rate from PPS is >20% of all participants who were randomised or allocated into each group or the differential dropout rate is >15% between the arms, the study needs to be reassessed and the IDMB will make recommendations regarding either re-evaluation of power calculation, extension of recruitment period, extension of number of study centres or termination of trial.

Interim analyses and premature termination of the study

The IDMB can also recommend to stop the trial early for ethical reasons if one of the groups clearly shows evidence of a significant benefit. An interim analysis will be performed on the primary endpoint when 50% of patients have been randomised and discharged from hospital. The interim analysis will be performed by the IDMB, who will report to the SC.

The Haybittle–Peto boundary approach will be used. If the interim analysis shows a probability of ≤0.001 that a difference as extreme between the treatments is found, given that the null hypothesis is true, then the trial will be stopped early.

Centres

The trial will start in two centres (University of Debrecen and University of Pécs), after which the study is open for other centres. In all cases the IDMB will make an audit of the centre and will report to the SC. The SC has the right to decide whether the centre meets the required quality to join the study. Compulsory requirements for a centre are: (1) it needs to treat at least 50 patients with AP a year; (2) it needs to have all the equipment required for the study; (3) besides the regular medical team, the centre has to appoint at least one doctor and one nurse/administrator fully available for the trial with no additional commitments which can interfere with her/his duty when her/his availability is required; (4) the blinding described above can be fully utilised; (5) all persons need to attend a preliminary meeting where all the details concerning the studies are discussed fully and have qualified as investigators in a GCP course. Centres wishing to join need

to send a letter of intent to the corresponding author by email.

Publication policy

Centres providing more than 25 patients can provide two authors to the authorship list. Every additional 25 patients will give the opportunity to nominate an additional author.

Feasibility

As a general protocol for the treatment of AP at the Centre for Translational Medicine at the University of Pécs, patients with AP receive early EN (using a NG tube). Patients receive 50 mL Nutrison Energy per hour starting immediately when they arrive to the ward from the Emergency Department. Patient data between the period 1 January 2016 to 31 May 2016 were analysed and the following observations were noted. (1) In 85% of all AP admissions early EN could have been started within 24 hours; in 15% of cases it was not achievable due to delayed transfer to the ward or vomiting. In these cases, patients received a NG tube later or they received a NJ tube whenever X-ray assistance was available. (2) Around 80% of NG-fed patients tolerated NG feeding without any complications. For the rest of the patients who had gastric retention or vomiting, NG feeding was stopped and they received a NJ tube whenever X-ray assistance was available. (3) Comparing the outcome (rate of severity, mortality, necrosis, intervention, etc) of this treatment protocol with the nil per os protocol used in most Hungarian hospitals showed that patients enjoyed benefits with no risk of early enteral feeding, which data confirm the literature described in the introduction. About 250 patients at the University of Pécs and about 150 patients at the University of Debrecen are admitted annually. Therefore, if no other institution joins the study, it can be completed within 3 years.

Safety

Since no unknown drugs/therapy are used in the study, no adverse or serious adverse events are expected/interpretable that would be attributable to the intervention during the trial. In this trial the IDMB will examine safety variables after every 16 patients have completed. Moreover, investigators will report adverse or serious adverse events on a separate form which has to be sent to the IDMB and SC. The SC will discuss and, if the adverse effect is confirmed, it will be reported to the relevant institutional and national ethical committee (http://www.ett.hu/tukeb.htm).

Additional information and future plan

Blood samples (serum and plasma) from all patients will be stored in order to study laboratory parameters later if required (eg, the laboratory could not measure it), and in order to build up a biobank for later clinical studies to which all participants will be given informed consent. The samples will be stored at −80°C. A follow-up study (called GOULASH PLUS) is under preparation in order to follow the patients for up to 5 years after the study. The study protocol will also be published.

DISCUSSION

Here we report the protocol of a prospective double-blind randomised controlled trial to study the effects of early energy restoration in AP. The preclinical studies[1 9] and meta-analyses suggest that early energy supplementation should be beneficial. Our main hypothesis is that elevating the energy level of acinar and ductal cells will prevent these cells from injury, therefore decreasing the extent of necrosis during AP. Since both the local and systemic complications (immune response) largely depend on the extent of the necrosis, we propose that this intervention will reduce multiorgan failure and mortality in AP as well. Although nutritional interventions for patients with mild pancreatitis are probably not needed, we must involve all patients with AP in the study. It has to be highlighted that the main aim of the study is not to find new treatments for MAP or SAP but to prevent the development of SAP. This is the reason why severity cannot be a selection criterion but has to be the primary endpoint. Concerning ethical issues, this study has very low risk for patients. The enteral solution (Nutrison Energy) used in this study is widely used in several diseases related to malnutrition in patients and has almost no contraindications, therefore no adverse events are expected during the trial.

ETHICS AND DISSEMINATION

The trial is registered at the ISRCTN registry (ISRCTN63827758) and received relevant ethical approval with the reference number 55961-2/2016/EKU issued by the Scientific and Research Ethics Committee of the Medical Research Council. At the end of the project we will disseminate our results to the medical community and will publish our results via open access.

CONCLUSION

This study provides the first type A evidence concerning the necessity of energy intake for patients with AP. This protocol is the first version of the trial completed on 24 May 2017.

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Contributors All authors were involved in the study design, edited the manuscript, read and approved the final manuscript. During the study KM, ANS, DP and PV are going to randomise the patients and ensure the blinding. AM, KS, JB, SG, MP, PS, ZV and TT are going to manage the treatment of the patients. ÁV will be responsible for the organisation, quality and timing of the endoscopic treatments, ZM and RH for the intensive care, NF and IB for the imaging and interventional radiology, DK and RP for the surgical treatment if needed. PAV and EL will prepare the nutritional solutions. MML, JPN, MST and OHP are members of ITAB. TM and AS will be members of SC. PH and KM drafted the manuscript.

Funding Centre costs (IT, biostatistics, trial organization, etc) are covered by the University of Pécs, Momentum Grant of the Hungarian Academy of Sciences (LP2014-10/2014); and Economic Development and Innovation Operative Programme Grant and Highly Cited Publication Grant of the National Research, Development and Innovation Office (GINOP-2.3.2-15-2016-00015, KH-125678). Since no additional treatment is necessary for the study, the general healthcare costs are covered by the National Healthcare System.

Competing interests None declared.

Patient consent Obtained.

Ethics approval The Scientific and Research Ethics Committee of the Medical Research Council, 55961-2/2016/EKU.

Provenance and peer review Not commissioned; externally peer reviewed.

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