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Pancreatic Stone Protein and Pancreatitis-associated Protein: Diagnostic Efficacy and Prognostic Value of PSP and PAP as Postoperative Markers of Septic Complications in Patients Undergoing Abdominal Surgery – A Prospective Trial (PSP-Trial)

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Word count: 1941

Abstract: Introduction. Major abdominal surgery leads to a postoperative systemic inflammatory response, making it difficult to discriminate patients with SIRS from those with a beginning postoperative infectious complication. To date, physicians still have to rely on their clinical experience to differ between the two. Pancreatic stone protein (PSP) and pancreatitis-associated protein (PAP), both secretory proteins produced by the pancreas, are dramatically increased during pancreatic disease and have been shown to act as acute-phase proteins. Increased levels of PSP have been detected in polytrauma patients developing sepsis and PSP has shown a high diagnostic accuracy in discriminating the severity of peritonitis and in predicting death in ICU patients. However, the prognostic value of PSP/PAP for infectious complications among patients undergoing major abdominal surgery is unknown.

Methods & Analysis. 160 patients undergoing major abdominal surgery will be recruited preoperatively. On the day before surgery, baseline blood values are attained. Following surgery, daily blood samples for measuring regular inflammation markers (C-reactive protein, procalcitonin, interleukin-6, TNF-alpha, leucocyte counts) and PSP/PAP will be acquired. PSP/PAP will be measured using a validated ELISA developed in our research laboratory. Patient discharge marks the end of trial participation. Complication grade including mortality and occurrence of infectious postoperative complications according to validated diagnostic criteria will be correlated with PSP/PAP values. Total intensive care unit day and total length of stay will be recorded as further outcome parameters.

Objectives. The PSP-Trial is a prospective monocentric cohort study evaluating the prognostic value of pancreatic stone protein (PSP) and pancreatitis associated protein (PAP) for postoperative infectious complications. Additionally, a comparison with established inflammation markers in patients undergoing major abdominal surgery will be performed to help evaluate the role of these proteins in predicting and diagnosing infectious and other postoperative complications.

Ethics. Institution Ethics Board Approval ID: KEKZH-Nr. STV 11-2009

Trial Registration. ClinicalTrials.gov: NCT01258179

STRENGTHS:

- Assessment of the novel sepsis biomarkers PSP/PAP in an abdominal surgical study population
- Comparison of PSP/PAP to other acute-phase reaction proteins, regularly measured in surgical study populations
- Evaluation of ability of PSP/PAP to predict infectious complications in patients undergoing major abdominal surgery
- Evaluation of ability of PSP/PAP to predict postoperative sepsis in patients undergoing major abdominal surgery (independently and in comparison to other inflammatory markers)
- External validation of PSP/PAP as a sepsis marker in an independent, prospective patient cohort

LIMITATIONS:

- Limited ability to generalize to other surgical or medical patient populations

- Limited ability to determine role in guiding treatment decisions
- Non-blinded, non-randomized controlled trial

BACKGROUND & CURRENT KNOWLEDGE

The acute-phase reaction is a systemic, non-specific response of the organism to acute inflammation, infection, and also occurs after surgery and the acute-phase reaction is associated with changes in the plasma protein profile[1]. Endothelial cells, fibroblasts and inflammatory cells such as macrophages secrete endogenous immune mediators in the damaged tissue, such as Interleukin-1 (IL-1), interleukin-6 (IL-6), interferon-γ and tumor necrosis factor alpha (TNF-α). In the presence of cortisol this results in the induction of synthesis of various acute-phase proteins stemming from the liver[2]. These include C-reactive protein (CRP), serum amyloid A (SAA), fibrinogen and the complement C3[3]. These proteins can be used as outcome measures of the acute-phase reaction[3] and have been established in clinical practice. Prolonged increase of acute-phase proteins has been shown to be predictive for postoperative infections and septic complications[4,5]. Additionally, studies have shown, that inflammatory markers such as TNF, IL-10 and IL-6 play an important role in the pathogenesis of postoperative SIRS[6]. But acute-phase proteins aren't only synthesized in the liver. It has been shown that the pancreas increases production of pancreatic secretory proteins such as pancreatitis associated protein (PAP) and the pancreatic stone protein/regenerating protein (PSP/reg), known as secretory stress proteins (SSP)[7,8]. In previous work, our group has shown an increased production and secretion of SSPs in stress induction of a murine pancreatitis-model (WBN / Kob rat)[9,10]. Another group was able to demonstrate a correlation between the course of pancreatitis and the induction of pancreatic secretory stress proteins[11]. However, it has also been demonstrated that both PAP and PSP are secreted in other organs, such as the small intestine[12,13]. PAP was increased under inflammatory conditions of the small intestine, especially in celiac disease[13]. More recently, it was demonstrated that PAP is involved in wound healing processes and it's associated metalloproteinases[14].

In a clinical study in polytrauma patients a significant increase of PSP was observed in those patients who developed infections or sepsis[15]. The same study showed that PSP binds and activates neutrophils, thus acting as an acute-phase protein. The concept that PSP is an early marker of sepsis was further confirmed in subsequent studies on patient populations admitted to the ICU[16-18].

Therefore it seems clear, that PAP and PSP play an important role in various inflammatory events, not only in pancreatitis but also in sepsis subsequent to other inflammatory diseases. However to date, no perioperative study exist, which looks at the value of these proteins and their role as postoperative inflammatory serum markers following major abdominal surgery. Additionally, differentiating between a simple inflammatory response and a true infectious complication following abdominal surgery still remains a challenge in clinical practice. Here, PAP and PSP/reg may provide valuable information and aid in differentiating between both events in the postoperative setting.

METHODS & STUDY DESIGN

The PSP-Trial is a prospective, monocentric cohort study evaluating the role of Pancreatic Stone Protein (PSP) and Pancreatitis Associated Protein (PAP) as new markers for postoperative infectious complications following abdominal surgery. Our study population will consist of patients undergoing liver (n = 30), pancreas (n = 30), upper gastrointestinal tract (n=30) and lower gastrointestinal tract (n=30) surgery as well as patients undergoing emergency abdominal procedures (n=20) and patients undergoing combined renal/pancreas-transplantation (n=10). Hence, a total of 160 patients will be recruited. To ensure adequate data quality, an interim analysis will be performed once 80 patients have been recruited. A power analysis will be performed based on the actual and precise data collected. At interim analysis the potential need to modify the sample size will be investigated. If the external data monitoring committee suggests any changes based on these calculations, the principal investigators will decide on the feasibility of the potential changes and submit a formal addendum to the ethics committee. Unless first approved by the local ethics committee, no changes will be made to the protocol or study design. Any changes to the protocol approved by the ethics committee will be updated at clinicaltrials.gov [NCT01258179].

STUDY OBJECTIVES & ENDPOINTS

Study objectives

1. To determine serum levels of PAP and PSP/reg attained from venous samples of patients undergoing major abdominal surgery.
2. To determine an association between PSP/PAP expression and current, routinely measured inflammatory markers (C-reactive protein, procalcitonin, TNF-alpha, IL-6, white blood cell count, thrombocyte count) and compare these with regards to predicting postoperative complications?
3. To determine, whether PSP/PAP levels correlate with postoperative infectious complications and general postoperative complications as graded by the validated Clavien-Dindo classification for postoperative surgical complications[19]?
4. To determine if PSP/PAP can be used as a predictive marker for postoperative infectious complications and sepsis?

Endpoints

Primary Endpoint

- Association of PSP/PAP expression values with patients experiencing infectious postoperative complication according to the Systemic Inflammatory Response Syndrome (SIRS) and Sepsis Criteria of the German Interdisciplinary Association of Intensive and Emergency Medicine (DIVI) and the ACCP/SCCM Consensus Conference Criteria[20,21].

Secondary Endpoints

- Correlation/comparison of PSP/PAP expression values to current markers of inflammation in patients undergoing major abdominal surgery.
- Association of PSP/PAP expression values with overall complication grade according to the Clavien-Dindo score for postoperative complications which includes mortality.
- Association of PSP/PAP expression values with overall postoperative infectious complications and the correlation between PSP/PAP expression values and changes/alterations to management of postoperative complications.

- Value of serum PSP/PAP levels at time of admission to ICU to predict infection/sepsis outcome during postoperative course including total ICU days and total length of stay.

Inclusion Criteria

- Age >18 years old
- Patients scheduled for abdominal surgery including liver, pancreas, upper gastrointestinal tract, lower gastrointestinal tract procedures, patients planned for combined kidney-renal transplantation as well as patients requiring emergency abdominal procedures
- Patients able to provide informed consent

Exclusion Criteria

- Age < 18 years old
- Patients unable to provide informed consent

SIRS/Sepsis Criteria

SIRS

- Fever ≥ 38.0°C or hypothermia ≤ 36.0°C measured rectal, intravascular or within the bladder
- Tachycardia ≥ 90/min
- Tachypnea ≥ 20/min or hyperventilation (measured via arterial blood gas analysis showing a PaCO2 ≤ 4.3kPa / 33mmHg)
- Leukocytosis ≥ 12.000/mm^3 or leukopenia ≤ 4.000/mm^3 or ≥10% of immature neutrophils upon differential blood analysis

At least two of the above must be fulfilled to diagnose SIRS [20,21]

Sepsis

- Identified microbiological infection (e.g. positive blood cultures)
- At least two of the above mentioned SIRS criteria

DATA COLLECTION & STATISTICAL METHODOLOGY

Data Collection

Patients will be recruited on the day before surgery or upon admission for emergency abdominal surgery. They will be evaluated for inclusion criteria and informed about the study as well as the possibility of study participation. Once the informed consent form has been read, understood and signed, baseline blood samples will be obtained and sent in for routine as well as separate analysis of PSP/PAP levels. Following surgery, daily blood samples will be obtained and analyzed for the sample values. Blood samples will be marked with patient's names, date of birth and date of sample extraction for identification purposes during transportation to our laboratory. However, following arrival of the blood sample in our laboratory and prior to processing, all patient's information will be modified to allow for anonymous biobanking and study participation.

If postoperative complications occur, they will be documented in the patient's chart by the treating physicians according to the Clavien-Dindo classification of postoperative complications, as is usual clinical practice in our department. In the presence of infectious complications, evaluation for the presence of SIRS/SEPSIS according to the above-mentioned

criteria will occur. If criteria for SIRS are fulfilled, blood cultures will be taken according to the above-mentioned guidelines to document septic complications. Patient demographic data will be collected via chart analysis by the principle investigator and other participating investigators and will be documented in a central, prospective database (see Supplementary File 1). Study participation ends with patient discharge (Fig. 1).
No commercial analysis kit exists for PSP/PAP analysis. Therefore the separate blood samples will be sent to our in-house research, where direct protein detection through a validated ELISA is performed[22]. Excessive material will be stored and catalogued in a central, anonymous bio-bank (-80°C fridge).

Statistical Methods
We will compare continuous variables with the Student t, Mann–Whitney U, one-way ANOVA, and Kruskal-Wallis tests. Differences among proportions derived from categorical data will be assessed using the Fisher's Exact or the Pearson $\chi 2$ tests where appropriate. Paired statistics will be used where appropriate. All p-values will be two-sided and statistical significance will be if $p \leq 0.05$. Sensitivity, specificity, accuracy, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), negative likelihood ratio (NLR), Yuden's index (YI), diagnostic odds ratio (OR), and the receiver operator characteristic (ROC) curve will be calculated. Where appropriate Kaplan-Meier survival curves and survival comparison using the log-rank test will be performed. Where appropriate, data will be presented as mean (SD), median (i.q.r.) and Odds Ratios (95% CI). We will duplicate the blood samples to analyze reproducibility of PSP/PAP measurements and assess the variability by the Pearson's Correlation Coefficient. We will use SPSS Statistics version 20 (SPSS: An IBM company, Chicago IL, 2011) to perform statistical analysis.

STUDY SITE
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Switzerland

ETHICS
This study is conducted in accordance with the principles of the Declaration of Helsinki and "good clinical practice guidelines. The independent medical ethics committee of the canton of Zurich (Kantonale Ethikkommission Zürich, Switzerland) has approved a previous version of this study protocol. Institution Ethics Board Approval ID: KEKZH-Nr. STV 11-2009.

DISCUSSION
To date, no perioperative studies exist looking at the value and role of PAP and PSP/reg as postoperative inflammatory serum markers in patients having major abdominal surgery, although it has been demonstrated that PSP/PAP play an important role in various inflammatory events models [7,9,10,13,14]. Additionally, many serum markers have been studied in the hope of discovering new biomarkers, which will aid in recognizing sepsis [3-5,23]. However, to date no single test exists, which enables physicians to diagnose sepsis and they therefore still have to rely on a combination of physical examination and laboratory findings as well as clinical judgment, hereby potentially delaying adequate treatment. This is crucial, as septic complications following abdominal surgery are treacherous and require early diagnosis and aggressive management due to their high rate of morbidity and mortality [24,25].

Here, PSP/PAP may serve as novel markers for postoperative sepsis, to facilitate early diagnosis of sepsis and hereby hopefully directing resources to those patients at highest risk for death due to septic complications.

CONCLUSION

The PSP-Trial is a prospective, monocentric cohort study evaluating the role of pancreas stone protein (PSP) and pancreatitis associated protein (PAP) as new markers for postoperative infectious complications following abdominal surgery.

ABBREVIATIONS

Interleukin-1 (IL-1); interleukin-6 (IL-6); tumor necrosis factor alpha (TNF-α); C-reactive protein (CRP); procalcitonin (PCT); serum amyloid A (SAA); pancreatic stone peptide (PSP); pancreatic stone protein/regenerating protein (PSP/reg); pancreatitis associated protein (PAP); secretory stress protein (SSP); positive predictive value (PPV); negative predictive value (NPV); positive likelihood ratio (PLR); negative likelihood ratio (NLR); Yuden's index (YI); diagnostic odds ratio (OR); receiver operator characteristic (ROC).

COMPETING INTERESTS

The authors report following conflict of interests: Drs. Fisher, Oberkofler, Raptis, Soll, Béchir and Schiesser have reported that no potential conflicts of interest exist with any companies/organizations whose products or services may be discussed in this article. Prof. Graf is the inventor and the University of Zurich owns the patent for PSP/reg as a marker of sepsis.

AUTHOR CONTRIBUTIONS

OMF and CEO drafted the manuscript and contributed equally to this work. CEO & RG designed the study protocol in its previous version. DAR performed the study design and calculation of the sample size for the study. All other authors participated in the design of the study and are local investigators. All authors were involved in editing the manuscript. All of the listed authors read and approved the final manuscript.

FUNDING

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STUDY APPROVAL

This study is approved by the Medical Ethics Commission of the Canton of Zurich, Switzerland via a peer-reviewed process (Kantonale Ethikkommission Zurich; Institution Ethics Board Approval ID: KEKZH-Nr. STV 11-2009).

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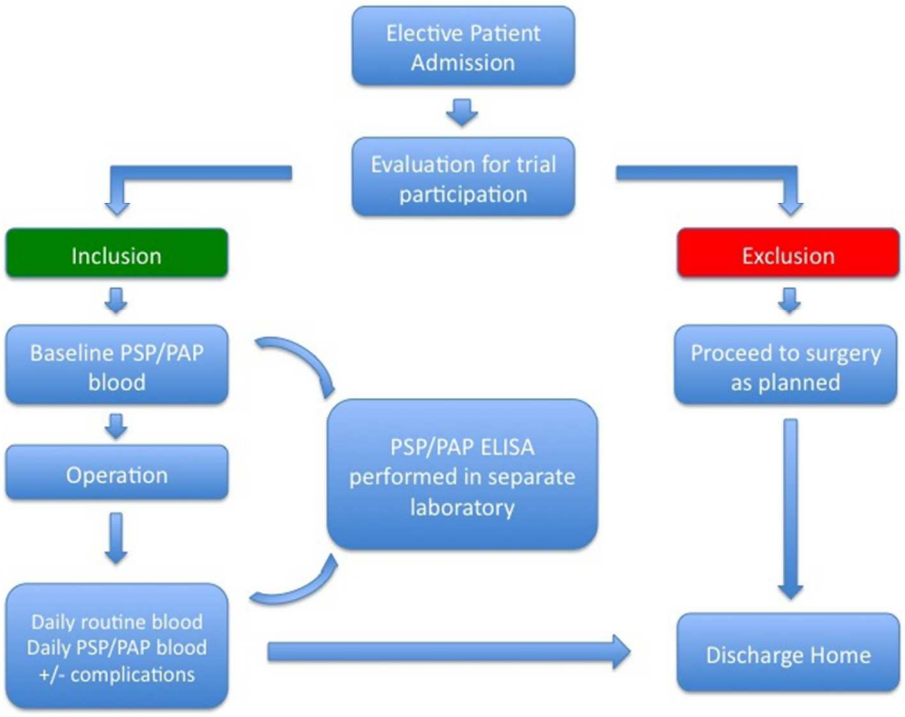

Figure 1. Flow chart of patient recruitment and study participation.
254x190mm (72 x 72 DPI)

Patient Case Report Form - PSP Trial

Patient Baseline Data

Patient Study Number

Birth date (dd/mm/yyyy)

Gender Male ☐ Female ☐

Ethnicity

Caucasian	☐
Asian	☐
African	☐
Other/mix _____	☐

Height (m)
Weight (kg)
BMI (body-mass-index kg/m2)

Underlying Disease / Reason for Admission

Malignant

Yes	☐
No	☐

Admission Date (dd/mm/yyyy)

Patient Case Report Form - PSP Trial

Operation Data		
Operation Date (dd/mm/yyyy)		
Operation Type	General Surgery	☐
	Emergency Abdominal Surgery	☐
	(Major) Endocrine Surgery	☐
	Upper-Gastrointestinal Surgery	☐
	Lower-Gastrointestinal Surgery	☐
	Hepato-Pancerato-Biliary Surgery	☐
	Transplantation	☐
	Undefined	☐
Operation (Text description)		

Patient Case Report Form - PSP Trial

Hospitalisation and Follow-Up Data

Immediate Postoperative Intensive Care Unit	Yes	☐
	No	☐
ICU Length of Stay (immediate postop)	_____	
Complications	Yes	☐
	No	☐
Complication Date (dd/mm/yyyy) (= postoperative Day X)		
Complication Grade (according to Clavien-Dindo Score)	No complications	☐
	Grade I	☐
	Grade II (medications only)	☐
	Grade IIIa (intervention under local anesthetic)	☐
	Grade IIIb (intervention under general anesthesia)	☐
	Grade IVa (organ failure, admission to ICU)	☐
	Grade IVb (multi-organ failure, admission to ICU)	☐
	Grade V (Death)	☐
Type of Complication (text)		

Postoperative SIRS	Yes	☐
	No	☐
Postoperative Sepsis	Yes	☐
	No	☐
Blood Cultures Positive	Yes	☐
	No	☐
Type of Microbiota	Gram positive	☐
	Gram negative	☐
	Fungi	☐
	Viral	☐
	Please specify microbiota type:_____	☐
***Pre*operative Antibiotics (AB)?**	Yes	☐
	No	☐
***Pre*operative AB-Type** (multiple choices possible)	Co-Amoxicillin (or similar)	☐
	Cephalosporin	☐
	Chinolone (Ciproxin and similar)	☐
	Metronidazole	☐
	Piperacillin/Tazobactam	☐
	Carbapenem	☐
	Vancomycin/Daptomycin	☐
	Other, please indicate:_____	☐

***Post*operative AB-Prophylaxis?**	Yes	☐
	No	☐
***Post*operative Prophylaxis AB-Type** (multiple choices possible)	Co-Amoxicillin (or similar)	☐
	Cephalosporin	☐
	Chinolone (Ciproxin and similar)	☐
	Metronidazole	☐
	Piperacillin/Tazobactam	☐
	Carbapenem	☐
	Vancomycin/Daptomycin	☐
	Other, please indicate:_____	☐
***Post*operative AB-Therapy?**	Yes	☐
	No	☐
Indication for Start of *Post*operative AB	Fever	☐
	Increasing inflammatory parameters	☐
	SIRS (empirical)	☐
	Sepsis (empirical)	☐
	Sepsis (directed therapy)	☐
	Other, please indicate:_____	☐
Date Start of *Post*operative AB-Therapy? (= postoperative Day X); (dd/mm/yyyy)		
***Post*operative AB-Therapy Type** (multiple choices possible)	Co-Amoxicillin (or similar)	☐
	Cephalosporin	☐
	Chinolone (Ciproxin and similar)	☐
	Metronidazole	☐

	Piperacillin/Tazobactam	☐
	Carbapenem	☐
	Vancomycin/Daptomycin	☐
	Other, please indicate:_____	☐
AB Change / Escalation	Yes	☐
	No	☐
Date AB Change / Escalation (= postoperative Day X); (dd/mm/yyyy)		
Indication for AB Change/Escalation	Fever	☐
	Increasing inflammatory parameters	☐
	SIRS (empirical)	☐
	Sepsis (empirical)	☐
	Sepsis (directed therapy)	☐
	Microbiology Report with AB-Resistogram	☐
	Other, please indicate:_____	☐
Date AB STOP (regardless of indication) (= postoperative Day X); (dd/mm/yyyy)		
Antifungal Therapy	Yes	☐
	No	☐
***Pre*operative Antifungal Therapy**	Yes	☐
	No	☐

***Pre*operative Antifungal Therapy Type**	Fluconazole	☐
	Itraconazle	☐
	Voricanazole	☐
	Caspofungin	☐
	Ampho B	☐
	Other, please indicate:_____	☐
***Post*operative Antifungal Prophylaxis**	Yes	☐
	No	☐
***Post*operative Antifungal Prophylaxis Type**	Fluconazole	☐
	Itraconazle	☐
	Voricanazole	☐
	Caspofungin	☐
	Ampho B	☐
	Other, please indicate:_____	☐
***Post*operative Antifungal Therapy**	Yes	☐
	No	☐
***Post*operative Antifungal Therapy Type**	Fluconazole	☐
	Itraconazle	☐
	Voricanazole	☐
	Caspofungin	☐
	Ampho B	☐
	Other, please indicate:_____	☐
***Post*operative Antifungal Change/Escalation**	Yes	☐
	No	☐

Indication for Antifungal Change/Escalation	Fever	☐
	Increasing inflammatory parameters	☐
	SIRS (empirical)	☐
	Sepsis (empirical)	☐
	Sepsis (directed therapy)	☐
	Microbiology Report with AB-Resistogram	☐
	Other, please indicate:_____	☐
Date Antifungal Therapy Change/Escalation (dd/mm/yyyy)		
Date Antifungals STOP (= postoperative Day X); (dd/mm/yyyy)		
Re-Operation?	Yes	☐
	No	☐
Re-Operation Date (dd/mm/yyyy) (= postoperative Day X)		
Re-Operation Type (text)		
Re-Operation times	1	☐
	2	☐
	3	☐
	4	☐
	5	☐
	>5	☐

Pre-Reoperation Diagnostics?	Yes	☐
	No	☐
Pre-Reoperation Diagnostisc Type	Conventional X-ray	
	CT-Scan	
	Other, please indicate:_____	
Postoperative Intervention (e.g. drain-placement through radiologist etc.)	Yes	☐
	No	☐
Postoperative Intervention Date (= postoperative Day X); (dd/mm/yyyy)		
Postoperative Intervention Type (text)		
Postoperative Intervention Times	1	☐
	2	☐
	3	☐
	4	☐
	5	☐
	>5	☐
ICU-Readmission	Yes	☐
	No	☐
ICU-Readmission Date (dd/mm/yyyy)		

ICU-Readmission LOS	_____	
Hospital Discharge	Yes	☐
	No	☐
	If no, please indicate if	☐
	a) in hospital death	☐
	b) other reason	☐
Hospital Discharge Date (dd/mm/yyyy)		
Hospital Total Length of Stay (LOS)	_____	

Patient Case Report Form - PSP Trial

Laboratory Values		
White Blood Cell Count (WBC)	WBO 0 (Baseline)	☐
	WBC 1 (Day 1)	☐
	WBC 2	☐
	.....	
	WBC XY (Day of discharge)	☐
C-Reactive Protein	CRP 0 (Baseline)	☐
	CRP 1	☐
	CRP 2	☐
	....	
	CRP XY	☐
Procalcitonin	PCT 0	☐
	PCT 1	☐
	PCT 2	☐
	...	
	PCT XY	☐
Thrombocytes	TC 0	☐
	TC 1	☐
	TC 2	☐
	...	
	TC XY	☐

Pancreatic Stone Protein	PSP 0	☐
	PSP 1	☐
	PSP 2	☐
	...	
	PSP XY	☐
Pancreatitis-associated Protein	PAP 0	☐
	PAP 1	☐
	PAP 2	☐
	...	
	PAP XY	☐

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Pancreatic Stone Protein and Pancreatitis-associated Protein: Diagnostic Efficacy and Prognostic Value of PSP and PAP as Postoperative Markers of Septic Complications in Patients Undergoing Abdominal Surgery (PSP-Study)

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Pancreatic Stone Protein and Pancreatitis-associated Protein: Diagnostic Efficacy and Prognostic Value of PSP and PAP as Postoperative Markers of Septic Complications in Patients Undergoing Abdominal Surgery (PSP-Study)

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Keywords: pancreatic stone protein, pancreatitis associated protein, secretory stress protein, abdominal surgery, sepsis
Word count: 2065

STRENGTHS:

- Assessment of the novel sepsis biomarkers PSP/PAP in an abdominal surgical study population
- Comparison of PSP/PAP to other acute-phase reaction proteins, regularly measured in surgical study populations
- Evaluation of ability of PSP/PAP to predict infectious complications in patients undergoing major abdominal surgery
- Evaluation of ability of PSP/PAP to predict postoperative sepsis in patients undergoing major abdominal surgery (independently and in comparison to other inflammatory markers)
- External validation of PSP/PAP as a sepsis marker in an independent, prospective patient cohort

LIMITATIONS:

- Limited ability to generalize to other surgical or medical patient populations
- Limited ability to determine role in guiding treatment decisions
- **Non-blinded, non-randomized single-center observational cohort study**

Introduction. Major abdominal surgery leads to a postoperative systemic inflammatory response, making it difficult to discriminate patients with SIRS from those with a beginning postoperative infectious complication. To date, physicians still have to rely on their clinical experience to differ between the two. Pancreatic stone protein (PSP) and pancreatitis-associated protein (PAP), both secretory proteins produced by the pancreas, are dramatically increased during pancreatic disease and have been shown to act as acute-phase proteins. Increased levels of PSP have been detected in polytrauma patients developing sepsis and PSP has shown a high diagnostic accuracy in discriminating the severity of peritonitis and in predicting death in ICU patients. However, the prognostic value of PSP/PAP for infectious complications among patients undergoing major abdominal surgery is unknown.

Methods & Analysis. 160 patients undergoing major abdominal surgery will be recruited preoperatively. On the day before surgery, baseline blood values are attained. Following surgery, daily blood samples for measuring regular inflammation markers (C-reactive protein, procalcitonin, interleukin-6, TNF-alpha, leucocyte counts) and PSP/PAP will be acquired. PSP/PAP will be measured using a validated ELISA developed in our research laboratory. Patient discharge marks the end of trial participation. Complication grade including mortality and occurrence of infectious postoperative complications according to validated diagnostic criteria will be correlated with PSP/PAP values. Total intensive care unit day and total length of stay will be recorded as further outcome parameters.

Objectives. The PSP-Study is a prospective monocentric cohort study evaluating the prognostic value of pancreatic stone protein (PSP) and pancreatitis associated protein (PAP) for postoperative infectious complications. Additionally, a comparison with established inflammation markers in patients undergoing major abdominal surgery will be performed to help evaluate the role of these proteins in predicting and diagnosing infectious and other postoperative complications.

Ethics. Institution Ethics Board Approval ID: KEKZH-Nr. STV 11-2009

Trial Registration. ClinicalTrials.gov: NCT01258179

BACKGROUND & CURRENT KNOWLEDGE

The acute-phase reaction is a systemic, non-specific response of the organism to acute inflammation, infection, and also occurs after surgery and the acute-phase reaction is associated with changes in the plasma protein profile[1]. Endothelial cells, fibroblasts and inflammatory cells such as macrophages secrete endogenous immune mediators in the damaged tissue, such as Interleukin-1 (IL-1), interleukin-6 (IL-6), interferon-γ and tumor necrosis factor alpha (TNF-α). In the presence of cortisol this results in the induction of synthesis of various acute-phase proteins stemming from the liver[2]. These include C-reactive protein (CRP), serum amyloid A (SAA), fibrinogen and the complement C3[3]. These proteins can be used as outcome measures of the acute-phase reaction[3] and have been established in clinical practice. Prolonged increase of acute-phase proteins has been shown to be predictive for postoperative infections and septic complications[4,5]. Additionally, studies have shown, that inflammatory markers such as TNF, IL-10 and IL-6 play an important role in the pathogenesis of postoperative SIRS[6]. But acute-phase proteins aren't only synthesized in the liver. It has been shown that the pancreas increases production of pancreatic secretory proteins such as pancreatitis associated protein (PAP) and the pancreatic stone protein/regenerating protein (PSP/reg), known as secretory stress proteins (SSP)[7,8]. In previous work, our group has shown an increased production and secretion of SSPs in stress induction of a murine pancreatitis-model (WBN / Kob rat)[9,10]. Another group was able to demonstrate a correlation between the course of pancreatitis and the induction of pancreatic secretory stress proteins[11]. However, it has also been demonstrated that both PAP and PSP are secreted in other organs, such as the small intestine[12,13]. PAP was increased under inflammatory conditions of the small intestine, especially in celiac disease[13]. More recently, it was demonstrated that PAP is involved in wound healing processes and it's associated metalloproteinases[14].

In a clinical study in polytrauma patients a significant increase of PSP was observed in those patients who developed infections or sepsis[15]. The same study showed that PSP binds and activates neutrophils, thus acting as an acute-phase protein. The concept that PSP is an early marker of sepsis was further confirmed in subsequent studies on patient populations admitted to the ICU[16-18].

Therefore it seems clear, that PAP and PSP play an important role in various inflammatory events, not only in pancreatitis but also in sepsis subsequent to other inflammatory diseases. However to date, no perioperative study exist, which looks at the value of these proteins and their role as postoperative inflammatory serum markers following major abdominal surgery. Additionally, differentiating between a simple inflammatory response and a true infectious complication following abdominal surgery still remains a challenge in clinical practice. Here, PAP and PSP/reg may provide valuable information and aid in differentiating between both events in the postoperative setting.

METHODS & STUDY DESIGN

The PSP-Study is a prospective, monocentric cohort study evaluating the role of Pancreatic Stone Protein (PSP) and Pancreatitis Associated Protein (PAP) as new markers for postoperative infectious complications following abdominal surgery. Our study population will consist of patients undergoing liver (n = 30), pancreas (n = 30), upper gastrointestinal tract (n=30) and lower gastrointestinal tract (n=30) surgery as well as patients undergoing emergency abdominal procedures (n=20) and patients undergoing combined renal/pancreas-transplantation (n=10). Hence, a total of 160 patients will be recruited. To ensure adequate data quality, an interim analysis will be performed once 80 patients have been recruited. A power analysis will be performed based on the actual and precise data collected. At interim analysis the potential need to modify the sample size will be investigated. If the external data monitoring committee suggests any changes based on these calculations, the principal investigators will decide on the feasibility of the potential changes and submit a formal addendum to the ethics committee. Unless first approved by the local ethics committee, no changes will be made to the protocol or study design. Any changes to the protocol approved by the ethics committee will be updated at clinicaltrials.gov [NCT01258179].

STUDY OBJECTIVES & ENDPOINTS

Study objectives

1. To determine serum levels of PAP and PSP/reg attained from venous samples of patients undergoing major abdominal surgery.
2. To determine an association between PSP/PAP expression and current, routinely measured inflammatory markers (C-reactive protein, procalcitonin, TNF-alpha, IL-6, white blood cell count, thrombocyte count) and compare these with regards to predicting postoperative complications?
3. To determine, whether PSP/PAP levels correlate with postoperative infectious complications and general postoperative complications as graded by the validated Clavien-Dindo classification for postoperative surgical complications[19]?
4. To determine if PSP/PAP can be used as a predictive marker for postoperative infectious complications and sepsis?

Endpoints

Primary Endpoint

- Association of PSP/PAP expression values with patients experiencing infectious postoperative complication according to the Systemic Inflammatory Response Syndrome (SIRS) and Sepsis Criteria of the German Interdisciplinary Association of Intensive and Emergency Medicine (DIVI) and the ACCP/SCCM Consensus Conference Criteria[20,21].

Secondary Endpoints

- Correlation/comparison of PSP/PAP expression values to current markers of inflammation in patients undergoing major abdominal surgery.
- Association of PSP/PAP expression values with overall complication grade according to the Clavien-Dindo score for postoperative complications which includes mortality.
- Association of PSP/PAP expression values with overall postoperative infectious complications and the correlation between PSP/PAP expression values and changes/alterations to management of postoperative complications.

- Value of serum PSP/PAP levels at time of admission to ICU to predict infection/sepsis outcome during postoperative course including total ICU days and total length of stay.

Inclusion Criteria

- Age >18 years old
- Patients scheduled for abdominal surgery including liver, pancreas, upper gastrointestinal tract, lower gastrointestinal tract procedures, patients planned for combined kidney-renal transplantation as well as patients requiring emergency abdominal procedures
- Patients able to provide informed consent

Exclusion Criteria

- Age < 18 years old
- Patients unable to provide informed consent

SIRS/Sepsis Criteria

SIRS

- Fever ≥ 38.0°C or hypothermia ≤ 36.0°C measured rectal, intravascular or within the bladder
- Tachycardia ≥ 90/min
- Tachypnea ≥ 20/min or hyperventilation (measured via arterial blood gas analysis showing a PaCO2 ≤ 4.3kPa / 33mmHg)
- Leukocytosis ≥ 12.000/mm^3 or leukopenia ≤ 4.000/mm^3 or ≥10% of immature neutrophils upon differential blood analysis

At least two of the above must be fulfilled to diagnose SIRS [20,21]

Sepsis

- Identified microbiological infection (e.g. positive blood cultures)
- At least two of the above mentioned SIRS criteria

DATA COLLECTION & STATISTICAL METHODOLOGY

Data Collection

Patients will be recruited on the day before surgery or upon admission for emergency abdominal surgery. They will be evaluated for inclusion criteria and informed about the study as well as the possibility of study participation. Once the informed consent form has been read, understood and signed, baseline blood samples will be obtained and sent in for routine as well as separate analysis of PSP/PAP levels. Following surgery, daily blood samples will be obtained and analyzed for the sample values. Blood samples will be marked with patient's names, date of birth and date of sample extraction for identification purposes during transportation to our laboratory. However, following arrival of the blood sample in our laboratory and prior to processing, all patient's information will be modified to allow for anonymous biobanking and study participation.

If postoperative complications occur, they will be documented in the patient's chart by the treating physicians according to the Clavien-Dindo classification of postoperative complications, as is usual clinical practice in our department. In the presence of infectious complications, evaluation for the presence of SIRS/SEPSIS according to the above-mentioned

criteria will occur. If criteria for SIRS are fulfilled, blood cultures will be taken according to the above-mentioned guidelines to document septic complications. Patient demographic data will be collected via chart analysis by the principle investigator and other participating investigators and will be documented in a central, prospective database (see Supplementary File 1). Study participation ends with patient discharge (Fig. 1).
No commercial analysis kit exists for PSP/PAP analysis. Therefore the separate blood samples will be sent to our in-house research, where direct protein detection through a validated ELISA is performed[22]. Excessive material will be stored and catalogued in a central, anonymous bio-bank (-80°C fridge).

Statistical Methods
We will compare continuous variables with the Student t, Mann–Whitney U, one-way ANOVA, and Kruskal-Wallis tests. Differences among proportions derived from categorical data will be assessed using the Fisher's Exact or the Pearson $\chi 2$ tests where appropriate. Paired statistics will be used where appropriate. All p-values will be two-sided and statistical significance will be if $p \leq 0.05$. Sensitivity, specificity, accuracy, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), negative likelihood ratio (NLR), Yuden's index (YI), diagnostic odds ratio (OR), and the receiver operator characteristic (ROC) curve will be calculated. Where appropriate Kaplan-Meier survival curves and survival comparison using the log-rank test will be performed. Where appropriate, data will be presented as mean (SD), median (i.q.r.) and Odds Ratios (95% CI). We will duplicate the blood samples to analyze reproducibility of PSP/PAP measurements and assess the variability by the Pearson's Correlation Coefficient. We will use SPSS Statistics version 20 (SPSS: An IBM company, Chicago IL, 2011) to perform statistical analysis.

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STUDY STARTING AND ESTIMATED COMPLETION DATE
First patient recruitment for this study occurred following the approval by the local ethics committee in February 2011. Expected study completion date will be December 2014.

ETHICS
This study is conducted in accordance with the principles of the Declaration of Helsinki and "good clinical practice guidelines. The independent medical ethics committee of the canton of Zurich (Kantonale Ethikkommission Zürich, Switzerland) has approved a previous version of this study protocol. Institution Ethics Board Approval ID: KEKZH-Nr. STV 11-2009.

DISCUSSION
To date, no perioperative studies exist looking at the value and role of PAP and PSP/reg as postoperative inflammatory serum markers in patients having major abdominal surgery, although it has been demonstrated that PSP/PAP play an important role in various inflammatory events models [7,9,10,13,14]. Additionally, many serum markers have been studied in the hope of discovering new biomarkers, which will aid in recognizing sepsis [3-5,23].

However, to date no single test exists, which enables physicians to diagnose sepsis and they therefore still have to rely on a combination of physical examination and laboratory findings as well as clinical judgment, hereby potentially delaying adequate treatment. This is crucial, as septic complications following abdominal surgery are treacherous and require early diagnosis and aggressive management due to their high rate of morbidity and mortality [24,25]. Here, PSP/PAP may serve as novel markers for postoperative sepsis, to facilitate early diagnosis of sepsis and hereby hopefully directing resources to those patients at highest risk for death due to septic complications.

CONCLUSION

The **PSP-Study** is a prospective, monocentric cohort study evaluating the role of pancreas stone protein (PSP) and pancreatitis associated protein (PAP) as new markers for postoperative infectious complications following abdominal surgery.

ABBREVIATIONS

Interleukin-1 (IL-1); interleukin-6 (IL-6); tumor necrosis factor alpha (TNF-α); C-reactive protein (CRP); procalcitonin (PCT); serum amyloid A (SAA); pancreatic stone peptide (PSP); pancreatic stone protein/regenerating protein (PSP/reg); pancreatitis associated protein (PAP); secretory stress protein (SSP); positive predictive value (PPV); negative predictive value (NPV); positive likelihood ratio (PLR); negative likelihood ratio (NLR); Yuden's index (YI); diagnostic odds ratio (OR); receiver operator characteristic (ROC).

COMPETING INTERESTS

The authors report following conflict of interests: Drs. Fisher, Oberkofler, Raptis, Soll, Béchir and Schiesser have reported that no potential conflicts of interest exist with any companies/organizations whose products or services may be discussed in this article. Prof. Graf is the inventor and the University of Zurich owns the patent for PSP/reg as a marker of sepsis.

AUTHOR CONTRIBUTIONS

OMF and CEO drafted the manuscript and contributed equally to this work. CEO & RG designed the study protocol in its previous version. DAR performed the study design and calculation of the sample size for the study. All other authors participated in the design of the study and are local investigators. All authors were involved in editing the manuscript. All of the listed authors read and approved the final manuscript.

FUNDING

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STUDY APPROVAL

This study is approved by the Medical Ethics Commission of the Canton of Zurich, Switzerland via a peer-reviewed process (Kantonale Ethikkommission Zurich; Institution Ethics Board Approval ID: KEKZH-Nr. STV 11-2009).

ACKNOWLEDGEMENTS

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(PSP-Study)

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- Comparison of PSP/PAP to other acute-phase reaction proteins, regularly measured in surgical study populations
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- External validation of PSP/PAP as a sepsis marker in an independent, prospective patient cohort

LIMITATIONS:

- Limited ability to generalize to other surgical or medical patient populations
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- **Non-blinded, non-randomized single-center observational cohort study**

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4. To determine if PSP/PAP can be used as a predictive marker for postoperative infectious complications and sepsis?

Endpoints

Primary Endpoint

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Secondary Endpoints

- Correlation/comparison of PSP/PAP expression values to current markers of inflammation in patients undergoing major abdominal surgery.
- Association of PSP/PAP expression values with overall complication grade according to the Clavien-Dindo score for postoperative complications which includes mortality.
- Association of PSP/PAP expression values with overall postoperative infectious complications and the correlation between PSP/PAP expression values and changes/alterations to management of postoperative complications.

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- Fever ≥ 38.0°C or hypothermia ≤ 36.0°C measured rectal, intravascular or within the bladder
- Tachycardia ≥ 90/min
- Tachypnea ≥ 20/min or hyperventilation (measured via arterial blood gas analysis showing a PaCO2 ≤ 4.3kPa / 33mmHg)
- Leukocytosis ≥ 12.000/mm^3 or leukopenia ≤ 4.000/mm^3 or ≥10% of immature neutrophils upon differential blood analysis

At least two of the above must be fulfilled to diagnose SIRS [20,21]

Sepsis

- Identified microbiological infection (e.g. positive blood cultures)
- At least two of the above mentioned SIRS criteria

DATA COLLECTION & STATISTICAL METHODOLOGY

Data Collection

Patients will be recruited on the day before surgery or upon admission for emergency abdominal surgery. They will be evaluated for inclusion criteria and informed about the study as well as the possibility of study participation. Once the informed consent form has been read, understood and signed, baseline blood samples will be obtained and sent in for routine as well as separate analysis of PSP/PAP levels. Following surgery, daily blood samples will be obtained and analyzed for the sample values. Blood samples will be marked with patient's names, date of birth and date of sample extraction for identification purposes during transportation to our laboratory. However, following arrival of the blood sample in our laboratory and prior to processing, all patient's information will be modified to allow for anonymous biobanking and study participation.

If postoperative complications occur, they will be documented in the patient's chart by the treating physicians according to the Clavien-Dindo classification of postoperative complications, as is usual clinical practice in our department. In the presence of infectious complications, evaluation for the presence of SIRS/SEPSIS according to the above-mentioned

criteria will occur. If criteria for SIRS are fulfilled, blood cultures will be taken according to the above-mentioned guidelines to document septic complications. Patient demographic data will be collected via chart analysis by the principle investigator and other participating investigators and will be documented in a central, prospective database (see Supplementary File 1). Study participation ends with patient discharge (Fig. 1).
No commercial analysis kit exists for PSP/PAP analysis. Therefore the separate blood samples will be sent to our in-house research, where direct protein detection through a validated ELISA is performed[22]. Excessive material will be stored and catalogued in a central, anonymous bio-bank (-80°C fridge).

Statistical Methods
We will compare continuous variables with the Student t, Mann–Whitney U, one-way ANOVA, and Kruskal-Wallis tests. Differences among proportions derived from categorical data will be assessed using the Fisher's Exact or the Pearson $\chi 2$ tests where appropriate. Paired statistics will be used where appropriate. All p-values will be two-sided and statistical significance will be if $p \leq 0.05$. Sensitivity, specificity, accuracy, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), negative likelihood ratio (NLR), Yuden's index (YI), diagnostic odds ratio (OR), and the receiver operator characteristic (ROC) curve will be calculated. Where appropriate Kaplan-Meier survival curves and survival comparison using the log-rank test will be performed. Where appropriate, data will be presented as mean (SD), median (i.q.r.) and Odds Ratios (95% CI). We will duplicate the blood samples to analyze reproducibility of PSP/PAP measurements and assess the variability by the Pearson's Correlation Coefficient. We will use SPSS Statistics version 20 (SPSS: An IBM company, Chicago IL, 2011) to perform statistical analysis.

STUDY SITE
Department of Surgery
University Hospital Zurich
Raemistrasse 100
CH-8091 Zurich
Switzerland

STUDY STARTING AND ESTIMATED COMPLETION DATE
First patient recruitment for this study occurred following the approval by the local ethics committee in February 2011. Expected study completion date will be December 2014.

ETHICS
This study is conducted in accordance with the principles of the Declaration of Helsinki and "good clinical practice guidelines. The independent medical ethics committee of the canton of Zurich (Kantonale Ethikkommission Zürich, Switzerland) has approved a previous version of this study protocol. Institution Ethics Board Approval ID: KEKZH-Nr. STV 11-2009.

DISCUSSION
To date, no perioperative studies exist looking at the value and role of PAP and PSP/reg as postoperative inflammatory serum markers in patients having major abdominal surgery, although it has been demonstrated that PSP/PAP play an important role in various inflammatory events models [7,9,10,13,14]. Additionally, many serum markers have been studied in the hope of discovering new biomarkers, which will aid in recognizing sepsis [3-5,23].

However, to date no single test exists, which enables physicians to diagnose sepsis and they therefore still have to rely on a combination of physical examination and laboratory findings as well as clinical judgment, hereby potentially delaying adequate treatment. This is crucial, as septic complications following abdominal surgery are treacherous and require early diagnosis and aggressive management due to their high rate of morbidity and mortality [24,25]. Here, PSP/PAP may serve as novel markers for postoperative sepsis, to facilitate early diagnosis of sepsis and hereby hopefully directing resources to those patients at highest risk for death due to septic complications.

CONCLUSION

The **PSP-Study** is a prospective, monocentric cohort study evaluating the role of pancreas stone protein (PSP) and pancreatitis associated protein (PAP) as new markers for postoperative infectious complications following abdominal surgery.

ABBREVIATIONS

Interleukin-1 (IL-1); interleukin-6 (IL-6); tumor necrosis factor alpha (TNF-α); C-reactive protein (CRP); procalcitonin (PCT); serum amyloid A (SAA); pancreatic stone peptide (PSP); pancreatic stone protein/regenerating protein (PSP/reg); pancreatitis associated protein (PAP); secretory stress protein (SSP); positive predictive value (PPV); negative predictive value (NPV); positive likelihood ratio (PLR); negative likelihood ratio (NLR); Yuden's index (YI); diagnostic odds ratio (OR); receiver operator characteristic (ROC).

COMPETING INTERESTS

The authors report following conflict of interests: Drs. Fisher, Oberkofler, Raptis, Soll, Béchir and Schiesser have reported that no potential conflicts of interest exist with any companies/organizations whose products or services may be discussed in this article. Prof. Graf is the inventor and the University of Zurich owns the patent for PSP/reg as a marker of sepsis.

AUTHOR CONTRIBUTIONS

OMF and CEO drafted the manuscript and contributed equally to this work. CEO & RG designed the study protocol in its previous version. DAR performed the study design and calculation of the sample size for the study. All other authors participated in the design of the study and are local investigators. All authors were involved in editing the manuscript. All of the listed authors read and approved the final manuscript.

FUNDING

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STUDY APPROVAL

This study is approved by the Medical Ethics Commission of the Canton of Zurich, Switzerland via a peer-reviewed process (Kantonale Ethikkommission Zurich; Institution Ethics Board Approval ID: KEKZH-Nr. STV 11-2009).

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Patient Case Report Form - PSP-Study

Patient Baseline Data

Patient Study Number

Birth date (dd/mm/yyyy)

Gender Male ☐ Female ☐

Ethnicity

Caucasian	☐
Asian	☐
African	☐
Other/mix _____	☐

Height (m)
Weight (kg)
BMI (body-mass-index kg/m2)

Underlying Disease / Reason for Admission

Malignant

Yes	☐
No	☐

Admission Date (dd/mm/yyyy)

Patient Case Report Form - PSP-Study

Operation Data		
Operation Date (dd/mm/yyyy)		
Operation Type	General Surgery	☐
	Emergency Abdominal Surgery	☐
	(Major) Endocrine Surgery	☐
	Upper-Gastrointestinal Surgery	☐
	Lower-Gastrointestinal Surgery	☐
	Hepato-Pancerato-Biliary Surgery	☐
	Transplantation	☐
	Undefined	☐
Operation (Text description)		

Patient Case Report Form - PSP-Study

Hospitalisation and Follow-Up Data		
Immediate Postoperative Intensive Care Unit	Yes	☐
	No	☐
ICU Length of Stay (immediate postop)	_____	
Complications	Yes	☐
	No	☐
Complication Date (dd/mm/yyyy) (= postoperative Day X)		
Complication Grade (according to Clavien-Dindo Score)	No complications	☐
	Grade I	☐
	Grade II (medications only)	☐
	Grade IIIa (intervention under local anesthetic)	☐
	Grade IIIb (intervention under general anesthesia)	☐
	Grade IVa (organ failure, admission to ICU)	☐
	Grade IVb (multi-organ failure, admission to ICU)	☐
	Grade V (Death)	☐
Type of Complication (text)		

Postoperative SIRS	Yes	☐
	No	☐
Postoperative Sepsis	Yes	☐
	No	☐
Blood Cultures Positive	Yes	☐
	No	☐
Type of Microbiota	Gram positive	☐
	Gram negative	☐
	Fungi	☐
	Viral	☐
	Please specify microbiota type:_____	☐
***Pre*operative Antibiotics (AB)?**	Yes	☐
	No	☐
***Pre*operative AB-Type** (multiple choices possible)	Co-Amoxicillin (or similar)	☐
	Cephalosporin	☐
	Chinolone (Ciproxin and similar)	☐
	Metronidazole	☐
	Piperacillin/Tazobactam	☐
	Carbapenem	☐
	Vancomycin/Daptomycin	☐
	Other, please indicate:_____	☐

***Post*operative AB-Prophylaxis?**	Yes	☐
	No	☐
***Post*operative Prophylaxis AB-Type** (multiple choices possible)	Co-Amoxicillin (or similar)	☐
	Cephalosporin	☐
	Chinolone (Ciproxin and similar)	☐
	Metronidazole	☐
	Piperacillin/Tazobactam	☐
	Carbapenem	☐
	Vancomycin/Daptomycin	☐
	Other, please indicate:_____	☐
***Post*operative AB-Therapy?**	Yes	☐
	No	☐
Indication for Start of *Post*operative AB	Fever	☐
	Increasing inflammatory parameters	☐
	SIRS (empirical)	☐
	Sepsis (empirical)	☐
	Sepsis (directed therapy)	☐
	Other, please indicate:_____	☐
Date Start of *Post*operative AB-Therapy? (= postoperative Day X); (dd/mm/yyyy)		
***Post*operative AB-Therapy Type** (multiple choices possible)	Co-Amoxicillin (or similar)	☐
	Cephalosporin	☐
	Chinolone (Ciproxin and similar)	☐
	Metronidazole	☐

	Piperacillin/Tazobactam	☐
	Carbapenem	☐
	Vancomycin/Daptomycin	☐
	Other, please indicate:_____	☐
AB Change / Escalation	Yes	☐
	No	☐
Date AB Change / Escalation (= postoperative Day X); (dd/mm/yyyy)		
Indication for AB Change/Escalation	Fever	☐
	Increasing inflammatory parameters	☐
	SIRS (empirical)	☐
	Sepsis (empirical)	☐
	Sepsis (directed therapy)	☐
	Microbiology Report with AB-Resistogram	☐
	Other, please indicate:_____	☐
Date AB STOP (regardless of indication) (= postoperative Day X); (dd/mm/yyyy)		
Antifungal Therapy	Yes	☐
	No	☐
***Pre*operative Antifungal Therapy**	Yes	☐
	No	☐

***Pre*operative Antifungal Therapy Type**	Fluconazole	☐
	Itraconazle	☐
	Voricanazole	☐
	Caspofungin	☐
	Ampho B	☐
	Other, please indicate:_____	☐
***Post*operative Antifungal Prophylaxis**	Yes	☐
	No	☐
***Post*operative Antifungal Prophylaxis Type**	Fluconazole	☐
	Itraconazle	☐
	Voricanazole	☐
	Caspofungin	☐
	Ampho B	☐
	Other, please indicate:_____	☐
***Post*operative Antifungal Therapy**	Yes	☐
	No	☐
***Post*operative Antifungal Therapy Type**	Fluconazole	☐
	Itraconazle	☐
	Voricanazole	☐
	Caspofungin	☐
	Ampho B	☐
	Other, please indicate:_____	☐
***Post*operative Antifungal Change/Escalation**	Yes	☐
	No	☐

Indication for Antifungal Change/Escalation	Fever	☐
	Increasing inflammatory parameters	☐
	SIRS (empirical)	☐
	Sepsis (empirical)	☐
	Sepsis (directed therapy)	☐
	Microbiology Report with AB-Resistogram	☐
	Other, please indicate:_____	☐
Date Antifungal Therapy Change/Escalation (dd/mm/yyyy)		
Date Antifungals STOP (= postoperative Day X); (dd/mm/yyyy)		
Re-Operation?	Yes	☐
	No	☐
Re-Operation Date (dd/mm/yyyy) (= postoperative Day X)		
Re-Operation Type (text)		
Re-Operation times	1	☐
	2	☐
	3	☐
	4	☐
	5	☐
	>5	☐

Pre-Reoperation Diagnostics?	Yes	☐
	No	☐
Pre-Reoperation Diagnostisc Type	Conventional X-ray	
	CT-Scan	
	Other, please indicate:_____	
Postoperative Intervention (e.g. drain-placement through radiologist etc.)	Yes	☐
	No	☐
Postoperative Intervention Date (= postoperative Day X); (dd/mm/yyyy)		
Postoperative Intervention Type (text)		
Postoperative Intervention Times	1	☐
	2	☐
	3	☐
	4	☐
	5	☐
	>5	☐
ICU-Readmission	Yes	☐
	No	☐
ICU-Readmission Date (dd/mm/yyyy)		

ICU-Readmission LOS	_____	
Hospital Discharge	Yes	☐
	No	☐
	If no, please indicate if	☐
	a) in hospital death	☐
	b) other reason	☐
Hospital Discharge Date (dd/mm/yyyy)		
Hospital Total Length of Stay (LOS)	_____	

Patient Case Report Form - PSP-Study

Laboratory Values		
White Blood Cell Count (WBC)	WBO 0 (Baseline)	☐
	WBC 1 (Day 1)	☐
	WBC 2	☐
	.....	
	WBC XY (Day of discharge)	☐
C-Reactive Protein	CRP 0 (Baseline)	☐
	CRP 1	☐
	CRP 2	☐
	....	
	CRP XY	☐
Procalcitonin	PCT 0	☐
	PCT 1	☐
	PCT 2	☐
	...	
	PCT XY	☐
Thrombocytes	TC 0	☐
	TC 1	☐
	TC 2	☐
	...	
	TC XY	☐

Pancreatic Stone Protein	PSP 0	☐
	PSP 1	☐
	PSP 2	☐
	...	
	PSP XY	☐
Pancreatitis-associated Protein	PAP 0	☐
	PAP 1	☐
	PAP 2	☐
	...	
	PAP XY	☐

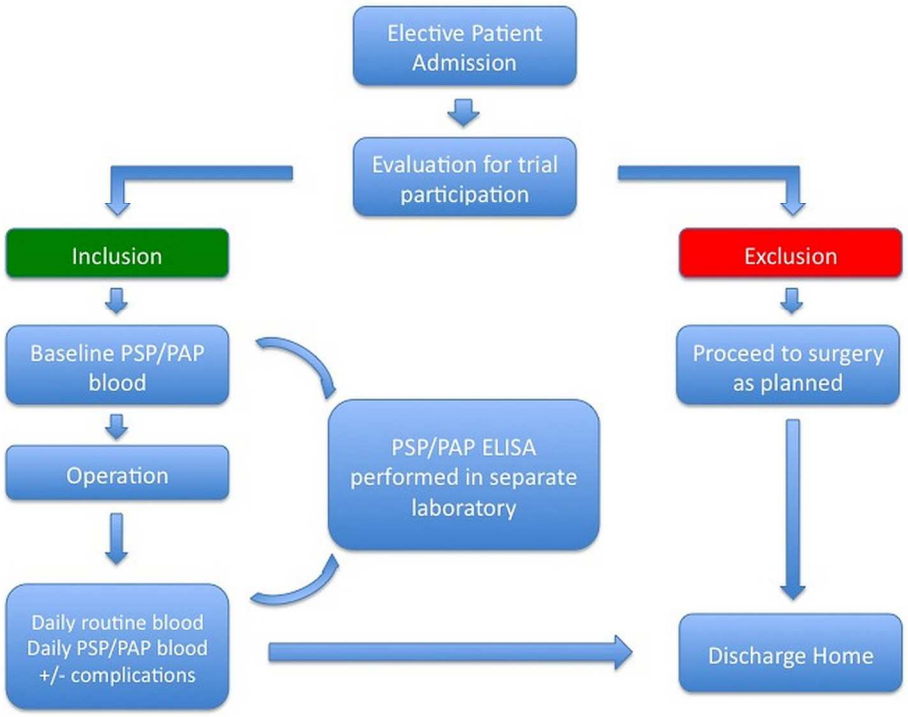

Figure 1. Flow chart of patient recruitment and study participation.
119x90mm (300 x 300 DPI)