Pancreatic Stone Protein Predicts Outcome in Patients With Peritonitis in the ICU

Raphael Gukasjan, MD[1]; Dimitri Aristotle Raptis, MD MSc,[2]; Hans-Ulrich Schulz, MD[1]; Walter Halangk, PhD[1]; Rolf Graf, PhD[2]

Objective: To determine the value of pancreatic stone protein in predicting sepsis-related postoperative complications and death in the ICU.

Design: A prospective cohort study of postoperative patients admitted to the intensive care unit. Blood samples were taken within three hours for analysis from admission to the intensive care unit including pancreatic stone protein, white blood cell counts, C-reactive protein, interleukin-6, and procalcitonin. The Mannheim Peritonitis Index and Acute Physiology and Chronic Health Evaluation II clinical scores were also determined. Univariate and multivariate analyses were performed to determine the diagnostic accuracy and independent predictors of death in the ICU [Clinicaltrials.gov, NCT01465711].

Setting: An adult medical–surgical intensive care unit in a teaching hospital in Germany.

Patients: Ninety-one consecutive postoperative patients with proven diagnosis of secondary peritonitis admitted to the ICU were included in the study from August 17, 2007, to February 8, 2010.

Interventions: Peripheral vein blood sampling.

Measurements and Main Results: Univariate analysis demonstrated that pancreatic stone protein has the highest diagnostic accuracy for complications and is the best predictor for death in the ICU. Pancreatic stone protein had the highest overall efficacy in predicting death with an odds ratio of 4.0 vs. procalcitonin (odds ratio 3.2), interleukin-6 (odds ratio 2.8), C-reactive protein (odds ratio 1.3), and white blood cell counts (odds ratio 1.4). By multivariate analysis, pancreatic stone protein was the only independent predictor of death.

Conclusions: In a population of patients with sepsis-related complications, serum-pancreatic stone protein levels demonstrate a high diagnostic accuracy to discriminate the severity of peritonitis and to predict death in the ICU. This test could be of value in the clinical diagnosis and therapeutic decision-making in the ICU. (*Crit Care Med* 2013; 41:0–0)

Key Words: complications; ICU mortality; pancreatic stone protein; peritonitis; sensitivity; specificity; surgery

Peritonitis is a severe complication after abdominal surgery (1, 2). Patients admitted to the ICU following surgery bear the risk of localized infection, generalised peritoneal inflammation, sepsis, or septic shock (3, 4). Due to the loss of the intestinal barrier function or even iatrogenic events, bacterial transfer into the peritoneum is accelerated (5, 6). In the absence of a well-functioning immune system or due to an overwhelming infection, gut bacteria may grow and cause severe infection requiring antibiotic treatment, re-operation, ventilator-assistance, and catecholamine support (7).

Prevention or early detection of such events is important to intervene with an appropriate therapeutic action and avoid risking a potentially life-threatening situation. Several clinically accepted markers indicating the presence of inflammation, infection, and sepsis have been studied. White blood cell counts (WCCs) and C-reactive protein (CRP), interleukin-6 (IL-6), and procalcitonin (PCT) are all established parameters (8–11). However, their clinical impact may be limited in the postoperative period (12). CRP is typically elevated after surgery making it difficult to distinguish surgical stress from a true infection. WCC is even more unspecific and may be suppressed by a weak immune system.

[1] Department of Surgery, Division of Experimental Surgery, Otto-von-Guericke University, Magdeburg, Germany.

[2] Department of Surgery, University Hospital Zurich, Switzerland.

Drs. Gukasjan, Raptis, Halangk, and Graf contributed to this work equally.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (http://journals.lww.com/ccmjournal).

The authors of this manuscript contributed in drafting the article, revising it, and final approval of the version to be published. The authors confirm that there is no one else who fulfills the criteria but has not been included as an author.

This study was supported, in part, by a grant from the Gebert-Rüf-Foundation, Basel, Switzerland.

Rolf Graf is inventor of a patent owned by the University of Zurich for the use of "PSP/reg as a marker of sepsis". Hans-Ulrich Schulz, Walter Halangk, and Rolf Graf have applied for a patent to be owned by the University of Magdeburg and the University of Zurich for the use of "PSP/reg as a marker of peritonitis". The remaining authors have not disclosed any potential conflicts of interest.

For information regarding this article, E-mail: dimitri.raptis@usz.ch

DOI: 10.1097/CCM.0b013e3182771193

Pancreatic stone protein (PSP) was originally identified in calcified concrements from pancreatic ducts. Later, the same protein was independently cloned and sequenced from regenerating pancreatic islets (13). To date, analysis revealed that PSP is expressed in various cell types in the gastrointestinal tract (14). During inflammation, PSP is up-regulated in the pancreas, the small intestine, and the stomach. PSP is known to be constitutively expressed at low levels and is strongly increased under inflammatory situations (15).

Based on these observations, we hypothesize that 1) PSP is significantly up-regulated leading to increased serum levels in patients with secondary peritonitis, and 2) its diagnostic accuracy for severity of sepsis and predictive value for death in the ICU is higher when compared with other markers such as CRP, WCC, IL-6, and PCT that are commonly influenced by surgical stress or other nonsepsis related factors.

MATERIALS AND METHODS

Participants

In this prospective cohort study, 91 consecutive postoperative patients admitted to the ICU with proven diagnosis of secondary peritonitis according to the MPI score were included in the study. For all patients, the data collected were obtained from their first admission to the ICU after their first operation for peritonitis. Age, gender, or pre-existing disease was no reason for exclusion. Patients were not included in the study if blood was not taken within three hours from admission to the ICU or if patients were transferred from other hospitals.

At ICU admission, blood samples were taken within three hours for analysis. The Mannheim Peritonitis Index (MPI) and Acute Physiology and Chronic Health Evaluation II (APACHE II) clinical scores were also determined. Recruitment was from August 17, 2007, to February 8, 2010. Data of all patients were anonymised, prospectively collected, and stored in a password-protected database. **Table 1** shows the patient characteristics. Prior to surgery, patients provided informed consent. The study received approval by the local Ethics committee, adhered to the principles of the Helsinki convention, and was registered to ClinicalTrials.gov (NCT01465711).

Test Method

At admission, blood samples were taken for analysis of WCC, CRP, IL-6, PCT, and PSP.

PSP was determined as previously described (15). Briefly, isoform-specific ELISAs were designed using the sandwich technique. Guinea pig anti-human recombinant PSP/reg antibodies (affinity-purified IgG) were diluted in Tris-buffered saline (TBS) and coated onto Maxisorp plates (FIRMA, Nunc, VWR International, Dietikon, Switzerland) at 4°C overnight. The plates were then blocked with 1% bovine serum albumin (BSA) in TBS for 1 hr at room temperature. Samples were pre-diluted in TBS/BSA and loaded in duplicate wells. The standard curve was generated from serial dilutions of recombinant PSP/reg protein. A second antibody, rabbit anti-PSP/reg, was then incubated and detected by phosphatase-conjugated anti-rabbit IgG.

Blood samples for later analysis were collected in BD Vacutainers (BD, Plymouth, United Kingdom) and serum was frozen at −80°C. PSP measurements were performed by an experienced technician at the University Hospital of Zurich. The samples were always stored at −80°C and transferred in liquid nitrogen. All other parameters were determined by standard procedures at the Department of Clinical Chemistry in Magdeburg. All blood markers mentioned above were grouped as more vs. less than their individual cut-off point generated by receiver operator characteristic (ROC) curves and the Yuden's Index (16, 17) (the "State/outcome variable" was death in the ICU). Briefly, the YI indicates the specific cut-off point where equal weight is given to both sensitivity and specificity.

MPI (18) was calculated according to Linder et al (19) and APACHE II (20) according to Knaus et al (21). For the intraoperative assessment of the severity of peritonitis several arbitrary factors were taken into account such as the appearance of the exudates, localization of inflammation, i.e., affected quadrant(s), and loss of organ function (22–24).

We used the definition of infection and sepsis as described by Levy et al (25), which was modified according to the original definitions recommended by the American College of Chest Physicians and the Society of Critical Care Medicine (26, 27). All clinicians involved in the study were blinded to the PSP results but were aware of the WCC, CRP, IL-6, and PCT values from the local biochemistry and hematology department, as part of the routine clinical tests performed.

Statistical Methods

Continuous variables were compared with the Student's *t* test, Mann-Whitney *U* test, one-way ANOVA, and Kruskal-Wallis test, where appropriate. Differences among proportions derived from categorical data were compared using the Fischer's exact test or the Pearson chi-square test, where appropriate. All *p* values were two-sided and considered statistically significant if $p \leq 0.05$. The Kruskal-Wallis test (nonparametric ANOVA) was used to identify differences among the median values of WCC, CRP, IL-6, PCT, PSP, APACHE II score, and MPI Score with respect to the grouped severity and localization of peritonitis, as well as for death (28–30). Multiple comparisons were then performed to identify significant differences among the comparisons mentioned above with the use of the Dunnett T3 correction (31–35). Sensitivity, specificity, accuracy, positive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio, Yuden's Index, diagnostic odds ratio (OR), and the ROC curve were also calculated (16, 36–39). Multiple pairwise comparisons of the areas under the curve (AUC) on ROC curve analysis were performed to identify their significant differences among the blood parameters and among the clinical scores, according to the DeLong test. The binary logistic and COX regression models (backward conditional step-wise) were used to identify independent predicting factors (WCC, CRP, IL-6, PCT, PSP and MPI, APACHE, SOFA) of death, adjusted for age and gender. Data are presented as mean (SD), median (interquartile range, IQR), and OR (95% confidence interval) where appropriate. Reproducibility of PSP measurements were performed by duplicating the samples, and the variability was assessed by the Pearson's Correlation Coefficient. Statistical analysis was per-

TABLE 1. Patient Characteristics of the 91 Patients With Peritonitis Included in This Study

Patient Characteristics	*n* = 91
Age, median (IQR)	66 (50–72)
Gender, male/female, number (%)	53/38 (58%/42%)
Diagnosis, number (%)	–
Benign vs. malignant	62/29 (68%/32%)
Colon perforation	20 (22%)
Small bowel perforation	19 (21%)
Gastric perforation	17 (19%)
Pancreatic tumor	14 (15%)
Gall bladder empyema	8 (9%)
Mesenteric ischemia	7 (8%)
Liver abscess rupture	4 (4%)
Appendicular perforation	2 (2%)
Mannheim Peritonitis Index score,[a] median (IQR)	30 (20–33)
Acute Physiology and Chronic Health Evaluation score,[b] median (IQR)	18 (14–26)
Sequential Organ Failure Assessment score, median (IQR)	6 (3–10)
Catecholamines at admission, number (%) yes/no	50/41 (55%/45%)
Dobutamine and noradrenaline, number (%)	25 (27%)
Noradrenaline, number (%)	21 (23%)
Dobutamine, number (%)	4 (5%)
Pa_{O_2}/F_{IO_2} ratio, median (IQR)	226.7 (164.4–322.0)
White cell count, median (IQR)	15 (11–20)
C-reactive protein, median (IQR)	222 (143–291)
Interleukin-6, median (IQR)	88 (34–375)
Procalcitonin, median (IQR)	1.07 (0.27–6.10)
Pancreatic stone protein, median (IQR)	125 (25–419)
Organ failure, number (%) yes/no	61/30 (67%/33%)
Organ failure, median number of organs (IQR)	2 (0–3)
Renal replacement therapy, number (%) yes/no	22/69 (24%/76%)
Intra-abdominal complications[c], number (%) yes/no	4/87 (4%/96%)
Re-laparotomy, number (%) yes/no	48/43 (53%/47%)
Mortality rate, number (%)	23/91 (25%)
Multiorgan failure	19 (20%)
Acute respiratory distress syndrome	2 (2%)
Cardiac arrest	1 (1%)
Hemorrhagic shock	1 (1%)

IQR = interquartile range.

[a]Performed on suspicion of peritonitis. The official cut-off point for a positive Mannheim Peritonitis Index score is ≥ 26.

[b]A severity-of-disease classification system and applied within 24 hrs of admission of a patient to an ICU.

[c]Two patients developed a chronic fistula and two developed intra-abdominal adhesions causing bowel obstruction.

formed using SPSS Statistics version 20 (SPSS: An IBM Company, Chicago IL) with the exception of the multiple pairwise comparisons of the AUC that were performed by MedCalc version 12.3 (MedCalc Software. Mariakerke, Belgium).

RESULTS

Participants

A total of 91 patients were included in this study, with patient characteristics and clinical parameters listed in Table 1.

Association of Blood Parameters With Clinical Conditions and Outcomes

Table 2 demonstrates the association of WCC, CRP, IL-6, PCT, and PSP with different clinical conditions, such as the severity and localization of peritonitis, the presence of organ failure, and mortality in the ICU. PSP was the only blood parameter that significantly differed among all different clinical conditions and nearly all of their subgroups on univariate analysis (Table 2, **Figures 1**–2, **Table S3** and **Fig. S5**, see Supplemental Digital Content 1, http://links.lww.com/CCM/A575). Furthermore, PSP and PCT were the only blood parameters with a predictive value for the need of renal replacement in the ICU (**Fig. S2**, Supplemental Digital Content 1, http://links.lww.com/CCM/A575).

Blood Parameter and Clinical Score Correlation

PSP best correlated with the clinical scores (MPI, APACHE II, and SOFA scores) when compared with WCC, CRP, IL-6, and PCT **Table S1** and **Fig. S2**, see Supplemental Digital Content 1, http://links.lww.com/CCM/A575). Similarly, PSP was the only blood parameter that significantly differed among the clinical scores when grouped according the cut-off points generated by the ROC curves (**Table S2** and **Fig. S3**, Supplemental Digital Content 1, http://links.lww.com/CCM/A575).

TABLE 2. Differences of Blood Parameters of the 91 Patients With Different Clinical Conditions

		WCC		CRP		IL-6		PCT		PSP	
	Number	Median (IQR)	*p*	Median (IQR)	*p*	Median (IQR)	*p*	Median (IQR)	*p*	Median (IQR)	*p*
Localization[a]											
Localized	26	16.5 (8.6–19.6)	0.743	194 (107.1–248.7)	0.015	84.6 (46.4–1086.5)	0.281	1.45 (0.28–7.39)	0.201	30.6 (19.7–262.17)	0.009
Diffuse	65	14.5 (11.6–19.8)	–	237.0 (159.7–334.0)	–	90.8 (26.6–336.3)	–	0.90 (0.18–6.00)	–	140.1 (28.9–518.1)	–
Severity											
Minor	30	14.7 (11.3–19.4)	Reference	212.6 (107.1–255.7)	Reference	99.7 (46.2–1172)	Reference	0.85 (0.17–5.04)	Reference	30.3 (21.7–234.3)	Reference[b]
Moderate	50	14.8 (11.6–21.7)	0.903	213.5 (142.0–337.3)	0.241	75.5 (24.9–312.6)	0.229	1.07 (0.18–6.10)	0.432	122.3 (24.0–521.8)	0.028
Severe	11	15.1 (3.0–19.3)	0.759	243.5 (188.0–316.6)	0.088	147.4 (62.9–496.1)	0.977	1.13 (0.62–7.34)	0.929	201.3 (136.5–514.5)	0.061
Organ failure											
None	30	17.8 (12.9–20.0)	Reference	240.4 (142.7-281-7)	Reference	59.4 (21.6–175.8)	Reference	0.40 (.013–1.42)	Reference	25.4 (19.4–108.0)	Reference
1–3 Organs	55	13.5 (9.3–19.3)	0.264	214.4 (145.8–255.9)	0.885	104.8 (45.0–359.4)	0.983	1.31 (0.47–8.41)	0.140	185.9 (47.8–500.9)	<0.001
>3 Organs	6	18.9 (13.6–27.5)	0.941	248.4 (337.6–1019.6)	0.996	1019.6 (360.0–5534.0)	0.634	14.60 (6.00–40.78)	0.380	721.4 (514.5–830.5)	0.047
Status											
Alive	68	14.7 (11.6–19.7)	0.595	228.6 (143.9–286.5)	0.615	67.9 (24.9–312.6)	0.960	0.83 (0.15–4.84)	0.142	75.0 (20.8–230.5)	0.003
Dead	23	15.1 (8.7–20.0)	–	188.0 (142.7–316.6)	–	159.3 (72.3–511.2)	–	1.19 (0.62–19.52)	–	499.3 (136.5–625.5)	–

IQR = interquartile range; WCC = white cell count, CRP= C-reactive protein, IL-6 = interleukin-6, PCT = procalcitonin, PSP = pancreatic stone protein; – = not applicable.

[a]The Tests for Several Independent Samples procedure compares two or more groups of cases on one variable. The Kruskal-Wallis H test, an extension of the Mann-Whitney U test, is the nonparametric analog of one-way analysis of variance and detects the overall differences in distribution location. Post hoc multiple comparisons determine which of the sub-group medians significantly differ from the first group. As equal variances were not assumed, the Dunnett's pairwise comparison test was chosen. For all subgroups, the first subgroup was used to compare with the remaining groups. For example, for the severity group, the sub-group "None" was used to compare with the "Minor", "Moderate", and "Severe" subgroups.

[b]Reference indicates the subgroup where the two other subgroups were compared with when performing pairwise comparisons.

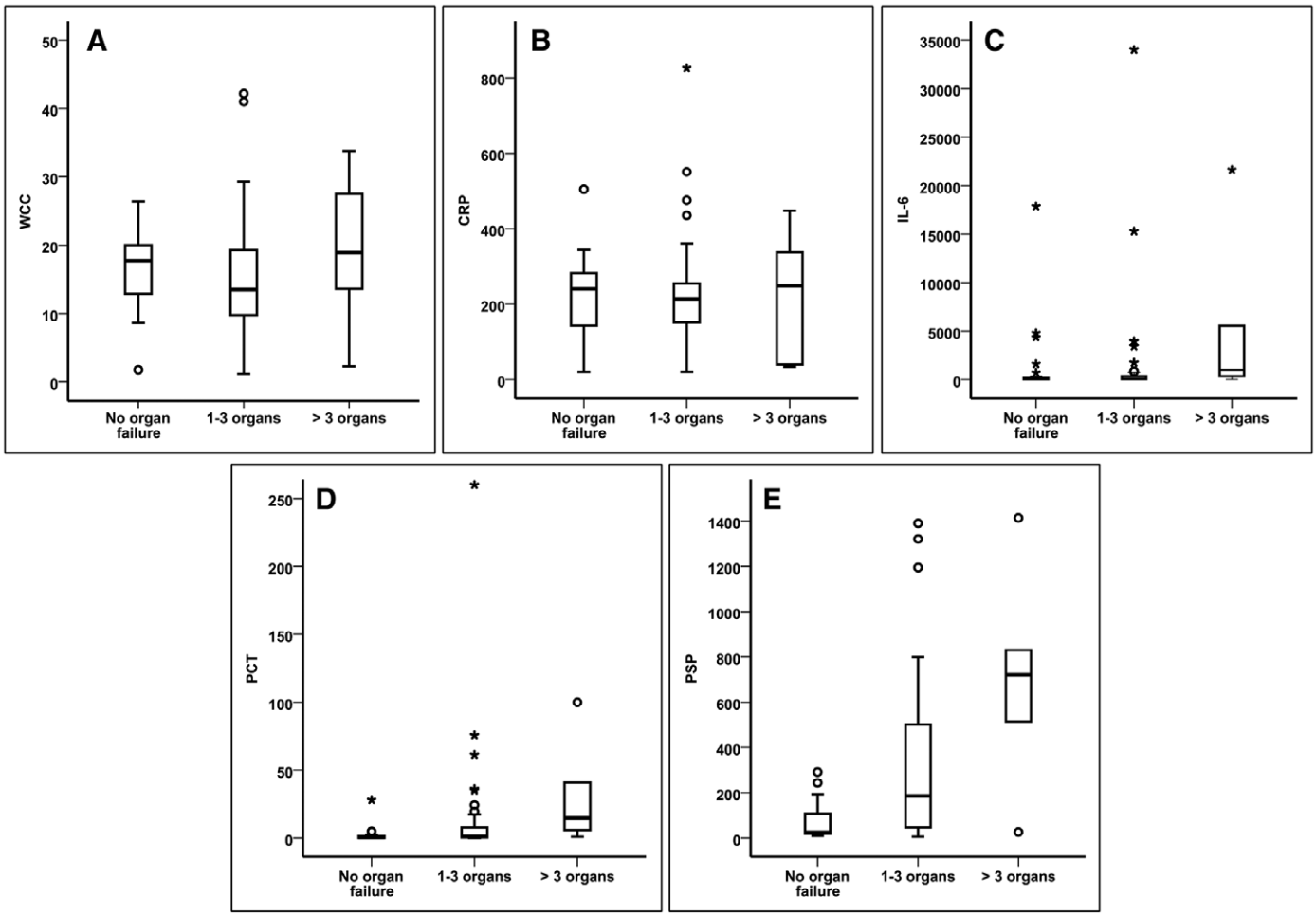

Figure 1. Association of serum markers with organ failure in the intensive care unit. (*A*) White blood cell count (WCC), (*B*) C-reactive protein (CRP), (*C*) interleukin-6 (IL-6), (*D*) procalcitonin (PCT), and (*E*) pancreatic stone protein (PSP) values. For statistical significance, see Table 2. The horizontal line within the boxes represents the median, whereas the lower part of the box represents the 25th and the upper part the 75th percentiles. The whiskers represent the range of the values, whereas the circles and the asterisks, the outliers (extreme values that deviate significantly from the rest of the sample).

Blood Parameters and Clinical Score Prediction of Death in the ICU

To evaluate the predictive value of each blood parameter, we first identified the ideal cut-off points to predict death in the ICU using ROC curve analysis. Based on the Yuden's Index (giving equal weight to both sensitivity and specificity), the cut-off points identified are listed in **Table 3**. The positive and negative predictive value, the positive and negative likelihood ratios, the relative risk, and OR were also calculated. Clearly, PSP was superior to WCC, CRP, IL-6, and PCT in predicting death in the ICU.

Figure 3 illustrates the ROC curves and indicates the area under the curve (AUC for all blood parameters compared among different clinical conditions. On univariate analysis, PSP had the highest diagnostic efficacy of organ failure (presence vs. absence), multiorgan failure (multiple vs. single or none), and predictive value for patient death in the ICU. PSP was superior to CRP, IL-6, and PCT, while WCC had the worst diagnostic efficacy and predictive value. Furthermore, on pairwise comparisons of the AUC, PSP was the only blood parameter that differed significantly among CRP, IL-6, PCT, and WCC.

Similarly, we assessed the clinical scores in predicting death in the ICU using ROC curve analysis. The SOFA score was superior to APACHE II or MPI score in predicting death in the ICU (**Fig. S1**, Supplemental Digital Content 1, http://links.lww.com/CCM/A575). **Figure 4** further illustrates the 90-day survival of patients with a PSP < 130 vs. ≥ 130, being 96% and 74%, respectively [COX regression hazard risk ratio: 6.48 (95% confidence interval 1.45–28.97) $p = 0.015$].

We then compared the AUC generated by ROC curve analysis among the blood parameters and the clinical scores (Table 3). Both SOFA score and PSP were significantly superior to the remaining parameters, while the AUC of the SOFA and PSP did not differ significantly (Table 3).

To further substantiate the potential of PSP as a predictive factor, we performed a multivariate stepwise regression analysis. Among all available blood parameters, PSP was the only indepen-

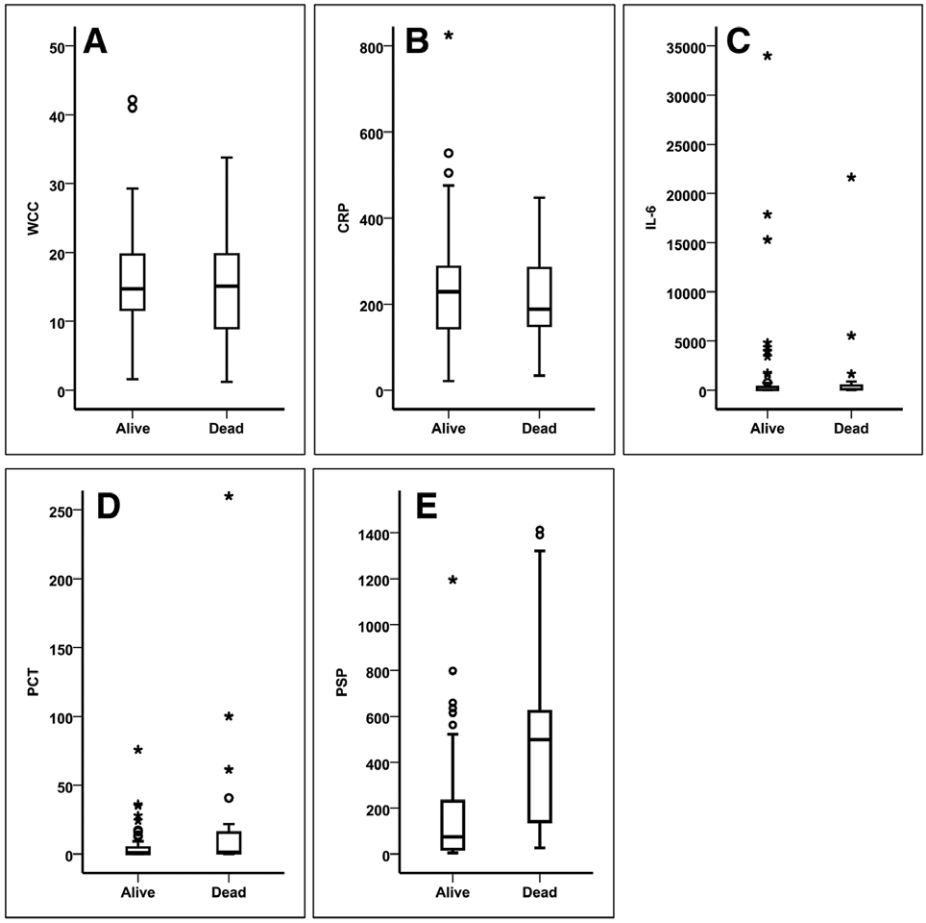

Figure 2. Association of serum markers with mortality in the intensive care unit. (*A*) White blood cell count (WCC), (*B*) C-reactive protein (CRP), (*C*) interleukin-6 (IL-6), (*D*) procalcitonin (PCT), and (*E*) pancreatic stone protein (PSP) values. For statistical significance, see Table 2. For explanation of boxes and whisker, see Figure 1.

dent predicting factor for death in the ICU (**Table 4**). Similarly, among the clinical scores, SOFA and APACHE II were independent predicting factors for death in the ICU (**Table S4**, Supplemental Digital Content 1, http://links.lww.com/CCM/A575).

DISCUSSION

Peritonitis in the postoperative setting is a multifaceted challenge. Patients with peritonitis may require intensive care, antibiotic treatment, percutaneous drainage, or surgical re-intervention. Clinical decision-making is based on physical status, imaging results, and laboratory parameters. To assess the potential peritonitis severity after surgery, we analyzed patients that exhibited various degrees of postoperative sepsis related complications including organ failure and death. This study demonstrates that PSP is an excellent predictor of sepsis related death when compared with other blood parameters such as WCC, CRP, IL-6 and PCT.

PSP was originally identified in calcified concrements from pancreatic ducts (40). Later, the same protein was independently cloned and sequenced from regenerating pancreatic islets (41). Up to date, analyses revealed that PSP is expressed in various cell types in the gastrointestinal tract (42). During inflammation and/or tumour development, PSP is transiently up-regulated, e.g., in the pancreas, the small intestine, and the stomach (43). The active role of PSP is still debated and, depending on the situation, it may act as mitogen, a protective or antiapoptotic molecule, or as a molecule with the potential to aggregate bacteria (44). PSP is part of a multigene family with various isoforms found in the pancreatic acinar and in the intesti-

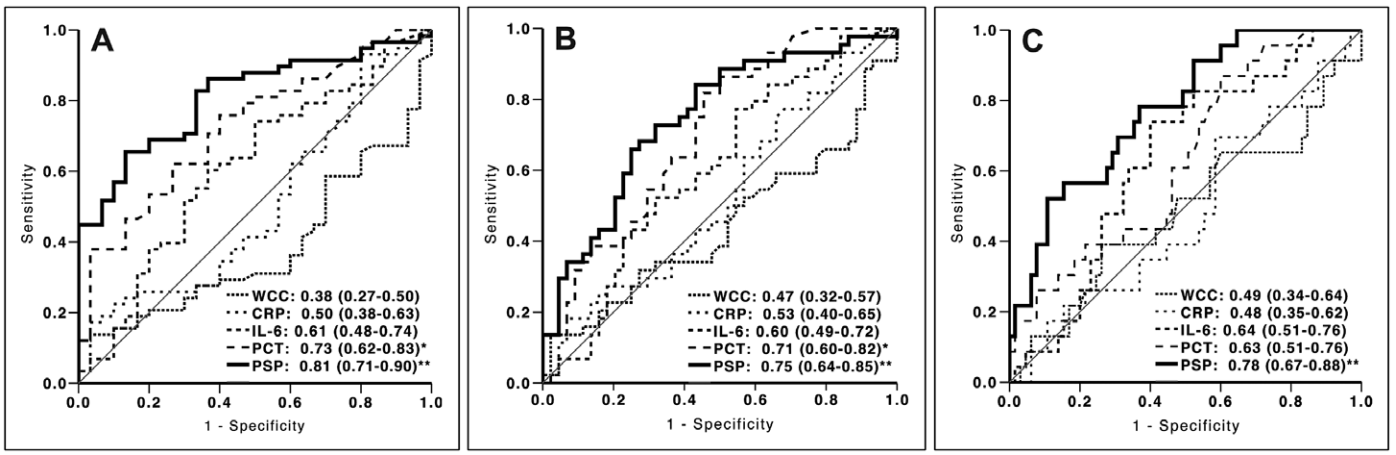

Figure 3. ROC curve analysis of blood parameters with different clinical outcomes. (*A*) Area under the curve (AUC) of serum blood markers for organ failure (yes vs. no), (*B*) multiorgan failure (no or single vs. multiple), and (*C*) death in the ICU. The value of the AUC for each blood parameter and the 95% confidence intervals are listed on the bottom right of each figure. * indicates a p value of < 0.05, whereas ** indicates < 0.001.

TABLE 3. Blood Parameters and Clinical Scores Predicting Death in the Intensive Care Unit

Total: *n* = 91, deaths: *n* = 23, mortality rate: 25%	Yuden's Index[a]	Cut-Off Point[a]	AUC	Accuracy	Sensitivity	Specificity	Positive Predictive Value[b]	Negative Predictive Value	Positive Likelihood Ratio[b]	Negative Likelihood Ratio[c]	Odds Ratio	RR[d]	*p*[e]
WCC	0.130	20	0.488	0.637	0.391	0.738	0.321	0.778	1.400	0.845	1.658	1.446	0.862
CRP	0.111	175	0.484	0.473	0.696	0.415	0.281	0.794	1.154	0.767	1.505	1.363	0.820
IL-6	0.339	90	0.637	0.625	0.739	0.600	0.386	0.864	1.179	0.446	3.988	2.833	0.520
PCT	0.270	0.5	0.634	0.516	0.870	0.400	0.328	0.257	1.442	0.329	4.390	3.279	0.058
PSP	0.413	130	0.775	0.670	0.783	0.615	0.419	0.896	2.129	0.344	6.192	4.019	<0.001
MPI	0.386	30	0.717	0.615	0.783	0.559	0.375	0.884	1.774	0.389	4.560	3.225	0.007
APACHE II	0.649	22	0.818	0.802	0.870	0.779	0.571	0.946	3.942	0.167	23.556	10.667	<0.001
SOFA	0.664	10	0.876	0.835	0.826	0.838	0.633	0.934	5.107	0.207	26.614	9.658	<0.001

Pairwise comparisons of AUC[d]

	WCC	CRP	IL-6	PCT	PSP	MPI	APACHE II
	Δ AUC (*p*)	Δ AUC (*p*)	Δ AUC (*p*)	Δ AUC (*p*)	Δ AUC (*p*)	Δ AUC (*p*)	Δ AUC (*p*)
WCC							
CRP	0.004 (0.965)						
IL-6	0.125 (0.208)	0.121 (0.255)					
PCT	0.122 (0.211)	0.118 (0.292)	0.003 (0.961)				
PSP	0.264 (0.001)	0.259 (0.010)	0.138 (0.016)	0.141 (0.008)			
MPI	0.205 (0.018)	0.201 (0.038)	0.080 (0.387)	0.083 (0.359)	0.058 (0.465)		
APACHE II	0.310 (<0.001)	0.305 (0.003)	0.185 (0.015)	0.188 (0.015)	0.046 (0.440)	0.104 (0.096)	
SOFA	0.363 (<0.001)	0.359 (<0.001)	0.238 (0.003)	0.241 (0.001)	0.099 (0.151)	0.158 (0.007)	0.053 (0.240)

AUC = area under the curve, WCC = white cell count, CRP = C-reactive protein, IL-6 = interleukin-6, PCT = procalcitonin, PSP = pancreatic stone protein MPI = Mannheim Peritonitis Index; APACHE = Acute Physiology and Chronic Health Evaluation; SOFA = Sequential Organ Failure Assessment; RR = relative risk.

[a]Grouped more vs. less than the cut-off point generated by ROC curves Yuden's Index.

[b]Values > 2 are considered clinically significant.

[c]Values closer to 0 and < 0.20 are considered clinically significant.

[d]Pairwise comparisons of the AUCs was performed according to the DeLong Test on ROC curve analysis. Δ indicates the difference of the AUC between the two blood values.

[e]*p* values were computed by the Fischer's Exact test for categorical variables.

nal paneth cells. PSP (PSP/reg I) is constitutively expressed at low levels and is strongly increased under inflammatory situations. Other family members include pancreatitis associated protein/reg III and reg IV. In the mouse, reg IIIγ an isoform present predominantly in the small intestine, appears responsive to the presence of bacteria (45).

Often times, the postsurgical immune reaction is independent of the severity of disease, basically the human body answers with a cytokine burst, resulting in a rise of white cells and other markers such as CRP. Both WCC and CRP are known markers of septic complications after surgery. In a study of 135 patients that underwent colorectal surgery, CRP at postoperative day 4 was found su-

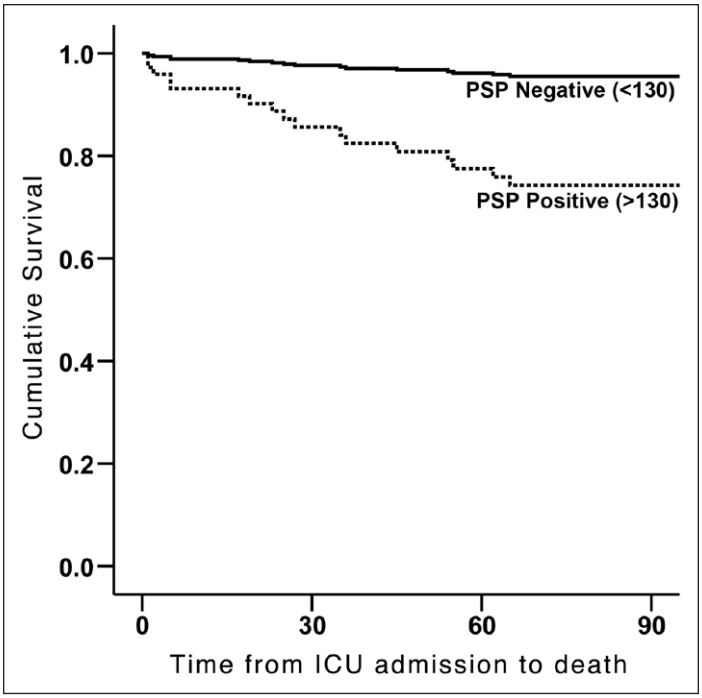

Figure 4. COX regression 90-day survival analysis of the 91 patients admitted to the ICU with peritonitis. The adjusted 90-day survival of patients with pancreatic stone protein (PSP) <130 vs. ≥130 was 96% and 74%, respectively (hazard risk ratio: 6.48 (95% CI 1.45–28.97) $p = 0.015$). PSP values were adjusted to age, gender, white blood cell count, C-reactive protein, interleukin-6, and procalcitonin.

perior to WCC in diagnosing postoperative septic complications (accuracy of 80% and 65%, respectively). On multivariate analysis, CRP was the only independent predictive factor of sepsis (OR: 14 (46)). Similarly, in our study, CRP was superior to WCC in univariate analyses. Recently, we showed that PSP is an excellent biomarker for patients with ventilatory-associated pneumonia (47) and a superior predictor of inflammation and sepsis when compared with CPR, PCT, or IL-6 in patients admitted for severe polytrauma (15). PSP not only binds to neutrophils, but also elicits an activating response in polymorphonuclear cells. This may explain the higher diagnostic efficacy of PSP when compared with CRP or PCT where such as an association has not yet been established (48).

PCT is another promising marker for sepsis and is routinely used in many hospitals (49). PCT and IL-6 appear to be early markers of subsequent postoperative sepsis in patients undergoing major surgery for cancer, when compared with CRP (50). PCT was thought to be helpful in monitoring the severity of infections and subsequent antibiotic treatment discontinuation; however, this has been recently disputed (51). In a study (52) of 69 postoperative patients (compared with 890 controls) diagnosed with severe sepsis within 24 hr preceding their operation, PCT failed to exhibit a discriminative power early (up to day 3) after ICU admission for prediction of mortality. In a meta-analysis of nearly 4,000 patients in the ICU, PCT was found to be a good biological diagnostic marker and superior to CRP in predicting different severities of sepsis. PCT was recommended to be included in the diagnostic guidelines for sepsis and in clinical practice in ICUs (49). However, a recent meta-analysis concluded that PCT cannot reliably differentiate sepsis from other noninfectious causes of systemic inflammatory response syndrome in critically ill patients, discouraging its routine use in the critical care settings (53). PCT is currently met with reservations by many physicians as its use may be restricted to select situations only. A further consideration is the costs associated with the continuous determination of such parameters. It is thought that the cost of testing equals the cost of antibiotics saved without any reduction in mortality (54). PCT and IL-6 appear to be early markers of subsequent postoperative sepsis in patients undergo-

TABLE 4. Independent Blood Parameters as Predictors for Death in the ICU

			ICU Death	
Predictor	**Categories**	**Number**	**Odds Ratio[a] (95% CI)**	***p***
WCC	<20	63	Reference	
	≥20	28	2.850 (0.833–9.758)	0.095
CRP	<175	34	Reference	
	≥175	57	1.133 (0.342–3.761)	0.838
IL-6	<90	44	Reference	
	≥90	47	2.201 (0.611–7.931)	0.228
PCT	<0.5	30	Reference	
	≥0.5	61	2.137 (0.364–12.556)	0.400
PSP	<130	48	Reference	
	≥130	43	4.896 (1.406–17.049)	0.013

WCC = White cell count × 1000, CRP = C-reactive protein, IL-6 = interleukin-6, PCT = procalcitonin, PSP = pancreatic stone protein.
The binary logistic regression analysis method was backward stepwise (conditional).
Reference indicates to which the group was compared.
[a]Adjusted for age and gender.

ing major surgery for cancer. These findings could allow identification of postoperative septic complications (9, 50).

Clinical scores have a long tradition to assess complex clinical pictures. The limitations of scores are dictated by either being fast but of low predictive value or time consuming with a higher accuracy. Both SOFA (55) and APACHE II (21) scores definitely show a higher association with other clinical parameters. It was shown that during the first few days of ICU admission, the SOFA score is a good indicator of prognosis and that, independent of the initial score, an increase in SOFA score during the first 48 hr in the ICU predicts a mortality rate of at least 50% (55). Similarly in our study, the SOFA score had the highest predictive value of death in the ICU followed by the APACHE II score that was also an independent predictor of death. However, both scores require time-consuming data acquisition resulting in a lag time of approximately 24 hrs. For this reason, the MPI score (19) was also used which can be completed easier and faster. In comparison, PSP levels can be determined within a few hours after admission and hence provide a simple and fast parameter.

As the SOFA and APACHE II scores seems to be a more reliable predictor of sepsis, we focused on this score to compare PSP and other parameters. Indeed, the correlation was much higher than with the MPI. Despite its highly predictive value, the APACHE II score was not designed exclusively to patients with sepsis or peritonitis. It does not take into account parameters such as the type of operation (emergency vs. elective) or exudates' properties. Malik et al (56) demonstrated an association between increasing MPI or APACHE II and mortality, respectively, in 101 patients with peritonitis. With our data we would come to a similar conclusion, although the mortality was lower in our hospital. Furthermore, when patients were grouped according to a range of MPI points or APACHE II score (56), the mortality was 82% for an MPI score above 25 points and over 91% in patients with an APACHE II score over 20. Patients in our hospital with a similar disease severity had a somewhat lower mortality.

We also compared the AUC generated by ROC curve analysis among the blood parameters and the clinical scores. The SOFA score followed by the APACHE II and PSP were the best predictors of death in the ICU. However, PSP did not differ significantly when compared with the SOFA and APACHE II scores.

This study has some limitations. There was no sample size calculation performed during the study design; however, the post hoc power calculation showed that the endpoint comparisons in this study were all adequately powered (data not shown). The values of WCC, CRP, IL-6, and PCT were readily available to the clinicians, while PSP blood values were not. Possible false positive and false negative blood results may have altered the patient management confounding the outcomes. However, WCC and CPP, for example, are routine blood tests and the clinicians could not have been blinded to them for ethical purposes. This was a single-point study where blood parameters were assessed at the very beginning of the admission to the ICU. Multiple blood tests throughout the whole course after admission might have added more to this study. However, blood tests taken at admission to the ICU were considered clinically most relevant. Thus, those patients, for whom blood was not taken within three hours from admission to the ICU, were not included in this study. However, this was a random and not systematic situation and thus is unlikely to affect the results. Another limitation is that this was a single-center study, and the generalization of the results has to be confirmed in ICUs from other centers.

CONCLUSIONS

In conclusion, we show that the postoperative serum PSP measured at admission to the surgical ICU is a reliable marker to discriminate the severity of peritonitis and the prediction of death in the ICU, when compared with CRP, WCC, IL-6, and PCT. PSP might therefore provide a complementary parameter in the ICU or emergency department to assess the health status in patients suspected or at risk for septic events that may lead to death.

ACKNOWLEDGMENTS

We thank Christiane Jechorek for managing the entire blood sample bio-bank and Martha Bain for her excellent technical assistance.

REFERENCES

1. Roehrborn A, Thomas L, Potreck O, et al: The microbiology of postoperative peritonitis. *Clin Infect Dis* 2001; 33:1513–1519
2. Sotto A, Lefrant JY, Fabbro-Peray P, et al: Evaluation of antimicrobial therapy management of 120 consecutive patients with secondary peritonitis. *J Antimicrob Chemother* 2002; 50:569–576
3. Hotchkiss RS, Karl IE: The pathophysiology and treatment of sepsis. *N Engl J Med* 2003; 348:138–150
4. Wittmann DH: Intraabdominal infections–Introduction. *World J Surg* 1990; 14:145–147
5. Guillou PJ: Biological variation in the development of sepsis after surgery or trauma. *Lancet* 1993; 342:217–220
6. Wakefield CH, Carey PD, Foulds S, et al: Changes in major histocompatibility complex class II expression in monocytes and T cells of patients developing infection after surgery. *Br J Surg* 1993; 80:205–209
7. Hall JC, Heel KA, Papadimitriou JM, et al: The pathobiology of peritonitis. *Gastroenterology* 1998; 114:185–196
8. Castelli GP, Pognani C, Meisner M, et al: Procalcitonin and C-reactive protein during systemic inflammatory response syndrome, sepsis and organ dysfunction. *Crit Care* 2004; 8:R234–R242
9. Dahaba AA, Metzler H: Procalcitonin's role in the sepsis cascade. Is procalcitonin a sepsis marker or mediator? *Minerva Anestesiol* 2009; 75:447–452
10. Rau BM, Kemppainen EA, Gumbs AA, et al: Early assessment of pancreatic infections and overall prognosis in severe acute pancreatitis by procalcitonin (PCT): A prospective international multicenter study. *Ann Surg* 2007; 245:745–754
11. Reith HB, Mittelkötter U, Debus ES, et al: Procalcitonin in early detection of postoperative complications. *Dig Surg* 1998; 15:260–265
12. Llewelyn M, Cohen J; International Sepsis Forum: Diagnosis of infection in sepsis. *Intensive Care Med* 2001; 27 (Suppl 1):S10–S32
13. Graf R, Schiesser M, Reding T, et al: Exocrine meets endocrine: Pancreatic stone protein and regenerating protein–Two sides of the same coin. *J Surg Res* 2006; 133:113–120
14. Jing D, Kehoe DE, Tzanakakis ES: Expression of Reg family proteins in embryonic stem cells and its modulation by Wnt/beta-catenin signaling. *Stem Cells Dev* 2010; 19:1307–1319
15. Keel M, Härter L, Reding T, et al: Pancreatic stone protein is highly increased during posttraumatic sepsis and activates neutrophil granulocytes. *Crit Care Med* 2009; 37:1642–1648
16. Youden WJ: Index for rating diagnostic tests. *Cancer* 1950; 3:32–35
17. Fluss R, Faraggi D, Reiser B: Estimation of the Youden Index and its associated cutoff point. *Biom J* 2005; 47:458–472
18. Billing A, Fröhlich D, Schildberg FW: Prediction of outcome using the Mannheim peritonitis index in 2003 patients. Peritonitis Study Group. *Br J Surg* 1994; 81:209–213

19. Linder MM, Wacha H, Feldmann U, et al: [The Mannheim peritonitis index. An instrument for the intraoperative prognosis of peritonitis]. *Chirurg* 1987; 58:84–92
20. Bosscha K, Reijnders K, Hulstaert PF, et al: Prognostic scoring systems to predict outcome in peritonitis and intra-abdominal sepsis. *Br J Surg* 1997; 84:1532–1534
21. Knaus WA, Draper EA, Wagner DP, et al: APACHE II: A severity of disease classification system. *Crit Care Med* 1985; 13:818–829
22. Lippert H. Peritoneum und Omentum majus. In: Lippert H, editor. *Praxis der Chirurgie Allgemein- und Viszeralchirurgie.* Stuttgart: Georg Thieme Verlag, S; 1998. p. 613–621.
23. Pichlmayr R, Weimann A, Klempnauer J: [Continuous irrigation or staged lavage in peritonitis?]. *Chirurg* 1992; 63:162–168
24. Lefering R, Goris RJ, van Nieuwenhoven EJ, et al: Revision of the multiple organ failure score. *Langenbecks Arch Surg* 2002; 387:14–20
25. Levy MM, Fink MP, Marshall JC, et al: International Sepsis Definitions Conference: 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Intensive Care Med* 2003; 29:530–538
26. Bone RC, Balk RA, Cerra FB, et al: Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 1992; 101:1644–1655
27. Bone RC, Grodzin CJ, Balk RA: Sepsis: A new hypothesis for pathogenesis of the disease process. *Chest* 1997; 112:235–243
28. Siegel S, Castellan NJ: Nonparametric Statistics for the Behavioral Sciences. New York, Mcgraw-Hill, 1988
29. Conover WJ: Practical Nonparametric Statistics. Second Edition. New York, John Wiley and Sons, 1980
30. Daniel WW: Biostatistics. Sixth Edition. New York, John Wiley and Sons, 1995
31. Brown MB, Forsythe AB: Robust tests for the equality of variances. *J Am Stat Assoc* 1974; 69:364–367.
32. Levene H: Robust tests for the equality of variance. *In*: Contributions to Probability and Statistics. Olkin I (Ed). Palo Alto, CA, Stanford University Press, 1960, pp 278–292
33. Milliken G, Johnson D: Analysis of Messy Data: Volume 1. Designed Experiments. New York, Chapman & Hall, 1992
34. Neter J, Wassermann W, Kutner MH: Applied Linear Statistical Models. Third Edition. Homewood, Irwin, 1990
35. Welch BL: On the Comparison of Several Mean Values: An Alternative Approach. 38th edition. *Biometrika* 1951; 38:330–336
36. Kirshwood BR, Sterne JAC: Essential Medical Statistics. Second Edition. Malden, MA, Blackwell Science, 2003
37. Kraemer HC: Reconsidering the odds ratio as a measure of 2x2 association in a population. *Stat Med* 2004; 23:257–270
38. Glas AS, Lijmer JG, Prins MH, et al: The diagnostic odds ratio: A single indicator of test performance. *J Clin Epidemiol* 2003; 56:1129–1135
39. Obuchowski NA: Receiver operating characteristic curves and their use in radiology. *Radiology* 2003; 229:3–8
40. De Caro A, Lohse J, Sarles H: Characterization of a protein isolated from pancreatic calculi of men suffering from chronic calcifying pancreatitis. *Biochem Biophys Res Commun* 1979; 87:1176–1182
41. Terazono K, Yamamoto H, Takasawa S, et al: A novel gene activated in regenerating islets. *J Biol Chem* 1988; 263:2111–2114
42. Carrère J, Figarella-Branger D, Senegas-Balas F, et al: Immunohistochemical study of secretory proteins in the developing human exocrine pancreas. *Differentiation* 1992; 51:55–60
43. Asahara M, Mushiake S, Shimada S, et al: Reg gene expression is increased in rat gastric enterochromaffin-like cells following water immersion stress. *Gastroenterology* 1996; 111:45–55
44. Iovanna J, Frigerio JM, Dusetti N, et al: Lithostathine, an inhibitor of CaCO3 crystal growth in pancreatic juice, induces bacterial aggregation. *Pancreas* 1993; 8:597–601
45. Cash HL, Whitham CV, Behrendt CL, et al: Symbiotic bacteria direct expression of an intestinal bactericidal lectin. *Science* 2006; 313:1126–1130
46. Ortega-Deballon P, Radais F, Facy O, et al: C-reactive protein is an early predictor of septic complications after elective colorectal surgery. *World J Surg* 2010; 34:808–814
47. Boeck L, Graf R, Eggimann P, et al: Pancreatic stone protein: A marker of organ failure and outcome in ventilator-associated pneumonia. *Chest* 2011; 140:925–932
48. Simon L, Gauvin F, Amre DK, et al: Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: A systematic review and meta-analysis. *Clin Infect Dis* 2004; 39:206–217
49. Uzzan B, Cohen R, Nicolas P, et al: Procalcitonin as a diagnostic test for sepsis in critically ill adults and after surgery or trauma: A systematic review and meta-analysis. *Crit Care Med* 2006; 34:1996–2003
50. Mokart D, Merlin M, Sannini A, et al: Procalcitonin, interleukin 6 and systemic inflammatory response syndrome (SIRS): Early markers of postoperative sepsis after major surgery. *Br J Anaesth* 2005; 94:767–773
51. Jensen JU, Hein L, Lundgren B, et al; Procalcitonin and survival study (PASS) Group: Procalcitonin-guided interventions against infections to increase early appropriate antibiotics and improve survival in the intensive care unit: A randomized trial. *Crit Care Med* 2011; 39:2048–2058
52. Dahaba AA, Hagara B, Fall A, et al: Procalcitonin for early prediction of survival outcome in postoperative critically ill patients with severe sepsis. *Br J Anaesth* 2006; 97:503–508
53. Tang BM, Eslick GD, Craig JC, et al: Accuracy of procalcitonin for sepsis diagnosis in critically ill patients: Systematic review and meta-analysis. *Lancet Infect Dis* 2007; 7:210–217
54. Heyland DK, Johnson AP, Reynolds SC, et al: Procalcitonin for reduced antibiotic exposure in the critical care setting: A systematic review and an economic evaluation. *Crit Care Med* 2011; 39:1792–1799
55. Ferreira FL, Bota DP, Bross A, et al: Serial evaluation of the SOFA score to predict outcome in critically ill patients. *JAMA* 2001; 286:1754–1758
56. Malik AA, Wani KA, Dar LA, et al: Mannheim Peritonitis Index and APACHE II–prediction of outcome in patients with peritonitis. *Ulus Travma Acil Cerrahi Derg* 2010; 16:27–32