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ORIGINAL ARTICLE

Assessing immunological surgical stress markers in patients undergoing digestive surgery for pancreatic, hepatic and gastric tumors

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Summary

Purpose: *Cytokines like IL-6, IL-10 and other factors like CRP are involved in the postoperative inflammatory-stress response. The association between IL-6, IL-10, CRP, albumin and early postoperative complications and deaths was analyzed on a cohort of cancer patient.*

Methods: *The plasma levels of IL-6, IL-10 and serum levels of c-reactive protein (CRP) and albumin were measured in 85 patients undergoing surgical resection of pancreatic, hepatic and gastric tumors. The measurement of the studied biochemical parameters was made at three time points: before the operation, and on the 1st and 3rd day after the operation.*

Results: *Of the 85 patients, 28 suffered early postoperative complications (14 gastric cancer patients; 11 pancreatic cancer patients; 3 liver cancer patients) and 9 patients died in the early postoperative period (5 gastric cancer patients; 4 pancreatic cancer patients; 0 liver cancer patients). Patients with elevated levels of serum CRP on the 3rd postoperative day had a significant increased risk of death. Also, patients with higher levels of IL-10 on the 3rd postoperative day demonstrated a significantly increased risk of early postoperative complications.*

Conclusion: *This study demonstrates that plasma IL-10 concentration is positively associated with postoperative complications.*

Key words: *albumin, CRP, IL-6, IL-10, tumor immunology*

Introduction

Pancreatic cancer is a deadly disease and one of the foremost causes of cancer-related deaths [1]. So far, the only known treatment is the surgical approach. Unfortunately, only 10-15% of patients suffering from this disease may become candidates for resections [2]. Liver cancer is also a severe malignancy, often discovered at an advanced, non-resectable stage [3]. Surgical resection is seen as the optimal treatment for liver tumors in patients with moderate malignant burdens [4]. Gastric cancer is

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a leading cancer-related cause of deaths the world over and it is also a frequently occuring disease [5]. Such patients are admitted for palliative or curative treatment, the only chance of cure being the complete surgical removal of the malignancy and its adjacent lymphoid structures. While surgical resection seems to be the only hope of cure for patients suffering from such serious diseases, large amounts of data have recently been published [6-8] concerning the wide spectrum of clinical conditions following trauma or infections derived from such operations. Minor or severe, the systemic stress response may lead in some cases to multiple organ failure and death [9].

Understanding the impact of postoperative stress and implementing it on surgical patient's complete care accordingly - before, during and after surgery - becomes a matter of paramount importance. Many published papers use cytokines like interleukin-6, IL-10 and CRP in an attempt to evaluate the systemic inflammatory stress response of organisms subjected to colorectal, pancreatic, hepatic and gastric cancer surgery [10,11]. Such cytokines are released into the bloodstream by immune cells as a response to surgical stress [12]. They are composed of amino acids derived from the host's muscles catabolism, so that during such illnesses, the skeletal muscles are subjected to a decreasing protein content, and the body gradually wastes away, generating a series of complications, ranging from mild to severe. Other studies link cytokine output with postoperative complications [13,14] which prolong hospital stay and raise hospitalization costs.

Our study's objectives were: i) to evaluate the perioperative changes of surgical stress markers (IL-6, IL-10 and CRP) by cancer type; ii) to investigate if the preoperative surgical stress marker levels can predict the postoperative complications and death in gastrointestinal cancer patients; iii) to determine if postoperative surgical stress marker levels can be risk factors for complications and death.

To our knowledge, early postoperative mortality is a topic seldom discussed and debated in other papers with related backgrounds.

Methods

This study included 85 cancer patients treated in the 3rd Surgical Clinic of the Regional Institute of Gastroenterology and Hepatology "Octavian Fodor" in Cluj-Napoca, Romania. The study began in November 2013 and ended in May 2015 and was approved by Ethical Committee of our Institute.

The patients were divided into three categories: pancreatic cancer patients, liver cancer patients and gastric cancer patients. Only patients who had undergone surgical resections were included in the study, while those undergoing paliative procedures were excluded. We analyzed 28 pancreatic cancer patients (mean age±standard deviation 59.10±8.7 years, 15 males and 13 females), 21 liver cancer patients (mean age±standard deviation 57.61 ±12.1 years, 12 males and 9 females) and 36 gastric cancer patients (mean age±standard deviation 68.17±11.6, 27 males and 9 females).

For every patient in each group the following data was recorded: sex, age, hospital stay in days, diagnosis, the types of operations the patients were subjected to, the duration of the procedure, blood loss, complications (fistulas, abscesses, general complications such as cardiac, pulmonary, liver, renal or metabolic, wound dehiscence, wound infections), the type of management of the complications a patient suffered (surgical or non-surgical), the IL-6 and IL-10 plasma level before surgery, on the 1st and 3rd day after the operation and we did the same for serum albumin and CRP. Recorded were also deaths in the aforementioned types of patients that occurred during the postoperative hospital stay. Each and every surgeon was equally experienced and qualified in performing these surgical procedures.

For the plasma IL-6 and IL-10 quantification, we used commercial "sandwich" enzyme-linked immunosorbent assays (ELISA) kits (human IL-6 and human IL-10 ELISA kits from Boster Biological Technology Co., Ltd., Pleasanton, CA, USA). Samples were collected in sterile tubes containing EDTA the evening before surgery, the evening on the 1st postoperative day and the evening on the 3rd postoperative day. After collection, samples were stored at 4°C and then centrifuged for 15 min at 1000g within 30 min after collection. Afterwards, the samples were stored at -80°C. Following dilution, using the provided sample diluent buffer, the working validation range for quantification was 4.69 to 300 pg/mL for IL-6 and 3.4 to 250 pg/mL for IL-10. The quantification of serum CRP was done using an immunoturbidimetric method, with Thermo Fisher Scientific reagents and the quantification of serum albumin was done using spectrophotometry with Thermo Fisher Scientific reagents. The samples were analyzed with a Konelab PRIME 60 Clinical Chemistry Analyzer.

Statistics

The continuous variables were expressed by descriptive statistics as mean±standard deviation (SD) or median and interquartile range [Q1-Q3], while the categorical variables were summarized by absolute and relative frequencies. Evaluation of the perioperative changes in serum albumin and inflammatory cytokines levels (IL-6, IL-10 and CRP) was performed using the Friedman test. *Post hoc* analysis based on Dunn's approach with Bonferroni correction was also done to identify the source of difference.

The influence of preoperative and postoperative levels of serum albumin and plasma inflammatory cytokines levels (IL-6, IL-10 and CRP) on postoperative complications and deaths was tested by logistic regression analysis. The studied markers (IL-6, IL-10, serum albumin and CRP) were transformed into ordinal vari-

ables using tertiles in order to highlight the role of their increased values on postoperative complication/death. The tertiles were created from the values of the studied parameters measured on the 1st and 3rd day. Because the preoperative measurements of IL-6 did not permit the estimation of the first tertile, this marker was not included in the regression model. The effect size of the studied dependencies was then quantified by odds ratio (OR) and 95% confidence interval (CI). The multivariate model performance was described by Hosmer-Lemeshow goodness-of-fit test, R^2 (Nagelkerke) coefficient and C-statistic (equivalent to Area Under the ROC Curve). A ROC curve analysis was performed in order to evaluate discriminant accurracy and to find the cut-off values for the studied markers. For all two-sided statistical tests, significance was achieved if the estimated significance level of p value was ≤ 0.05.

The statistical analysis was performed with the advanced software environment for statistical computing and graphics, R version 3.2.4 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Individual values of IL-6, IL-10, CRP and serum albumin are shown in Figures 1 and 2.

Sample description

The patient age distribution was different between the gastric, liver and pancreas cancer groups (one-way ANOVA, p=0.01). The studied groups were homogeneous regarding gender distribution (x^2 test,=3.61, p=0.164). The baseline patient characteristics are described in Table 1.

Surgical outcomes

The duration of operation by cancer type was different (Kruskal-Wallis test, p<0.001). A *post hoc* analysis, using Dunn's test is shown in Table 2. The analysis of bleeding by cancer type using the one-way ANOVA test showed p<0.001. We also no-

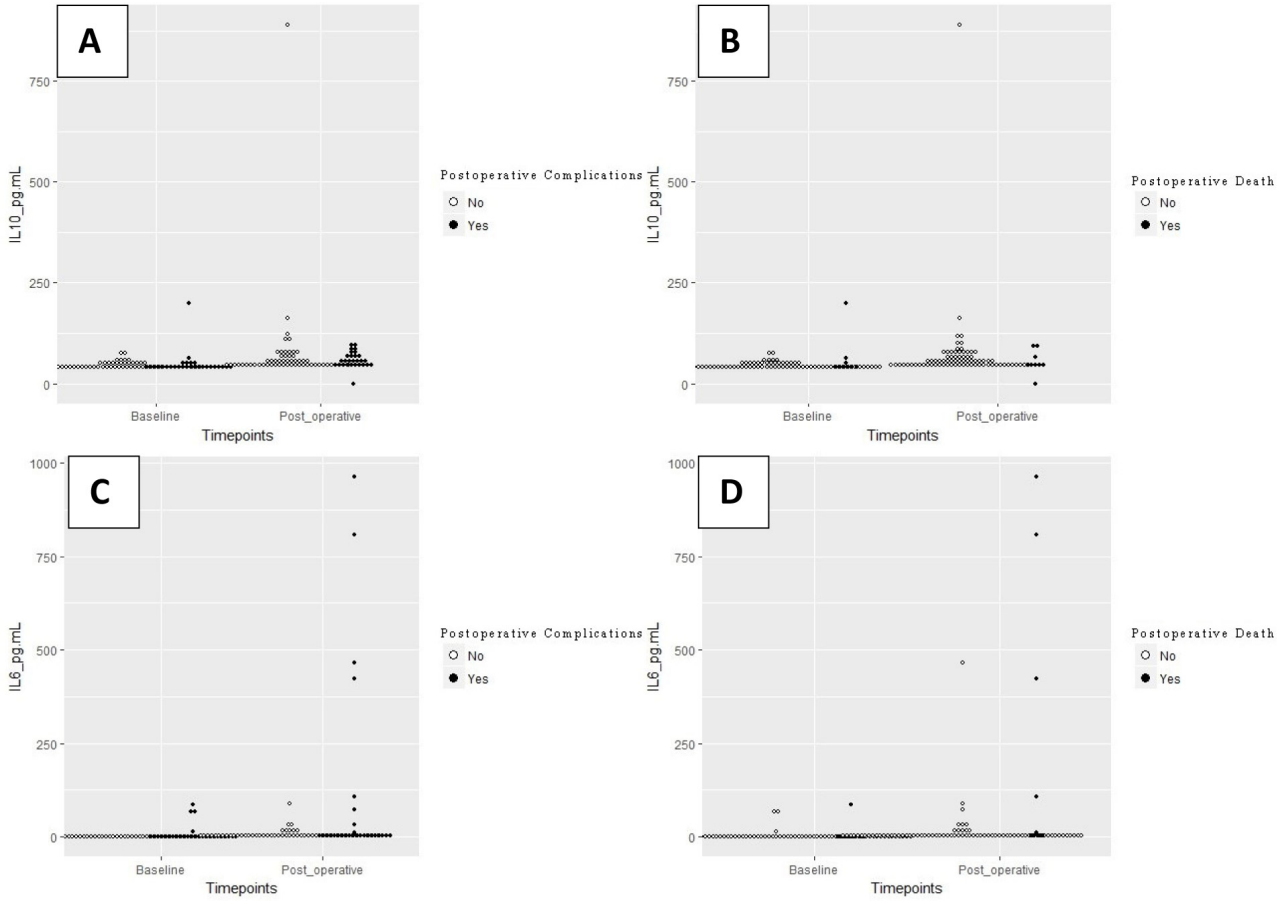

Figure 1. The distribution of preoperative and postoperative values (3 DAO) of IL-6 and IL-10. **(A)** The distribution of postoperative IL10 (pg/ml) regarding postoperative complications at different timepoints (baseline - preoperative values and postoperative values. Black dots= complications present, white dots= no complications). **(B)** The distribution of postoperative IL10 (pg/ml) regarding postoperative deaths at different timepoints (baseline - preoperative values and postoperative values. Black dots= death occured, white dots= survived). **(C)** The distribution of postoperative IL6 (pg/ml) regarding postoperative complications at different timepoints (baseline - preoperative values and postoperative values. Black dots= complications present, white dots= no complications). **(D)** The distribution of postoperative IL6 (pg/ml) regarding postoperative deaths at different timepoints (baseline - preoperative values and postoperative values. Black dots= death occured, white dots= survived).

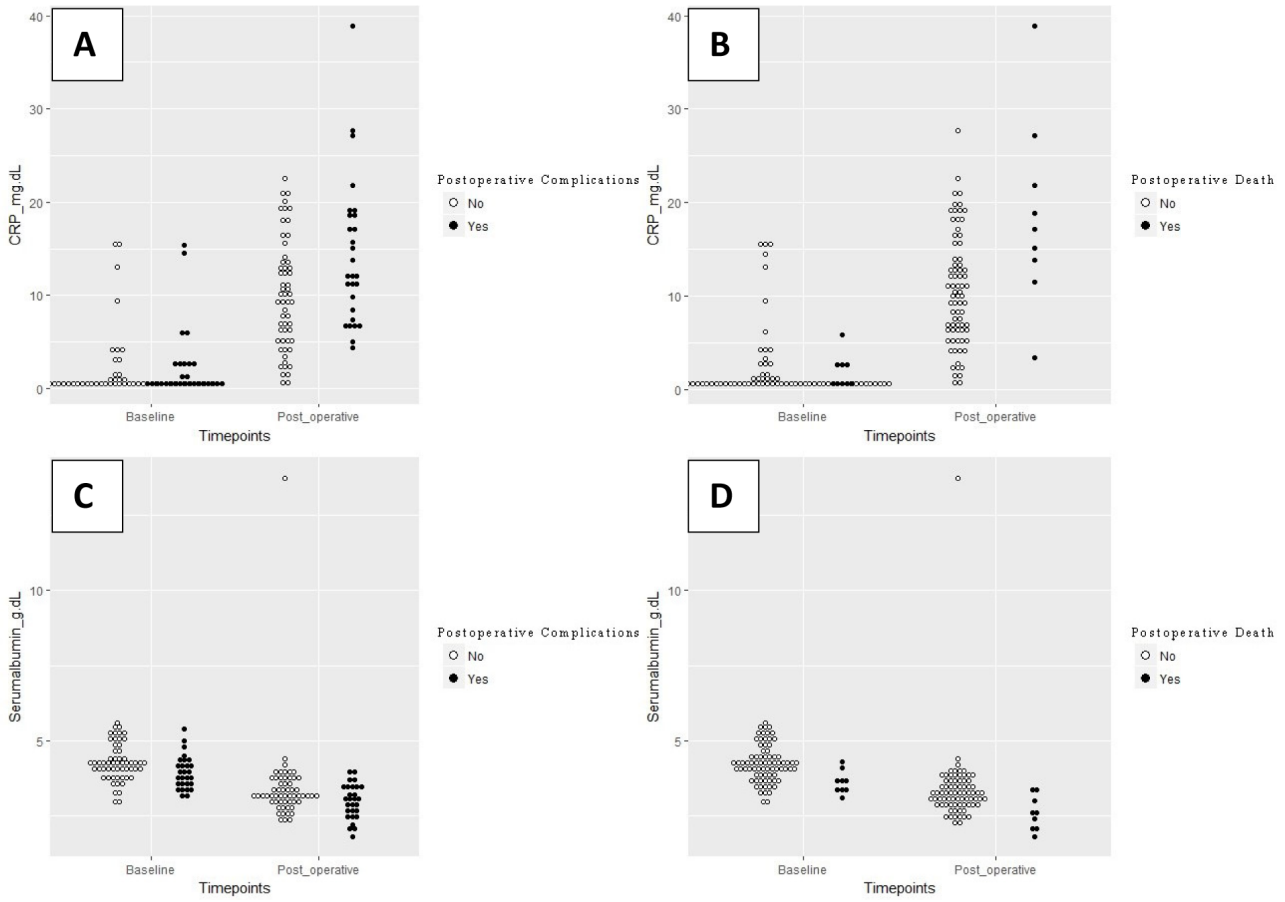

Figure 2. The distribution of preoperative and postoperative values (3 DAO) of CRP and serum albumin. **(A)** The distribution of postoperative CRP (mg/dl) regarding postoperative complications at different timepoints (baseline - preoperative values and postoperative values. Black dots= complications present, white dots= no complications). **(B)** The distribution of postoperative CRP (mg/dl) regarding postoperative deaths at different timepoints (baseline - preoperative values and postoperative values. Black dots= death occured, white dots= survived). **(C)** The distribution of postoperative albumin (g/dl) regarding postoperative complications at different timepoints (baseline - preoperative values and postoperative values. Black dots= complications present, white dots= no complications). **(D)** The distribution of postoperative albumin (g/dl) regarding postoperative deaths at different timepoints (baseline - preoperative values and postoperative values. Black dots= death occured, white dots= survived).

ticed that the mean amount of bleeding was lower for gastric cancer patients than pancreatic cancer patients (Dunnett test, p<0.001) and liver cancer patients (Dunn's test, p=0.008).

We also observed 25 cases with postoperative complications for gastric and pancreatic cancer patients vs 3 cases with liver cancer patients. No significant association between cancer type and presence of postoperative complications was found (x^2 test=40, p=0.111). Also, no significant difference was found between the groups in terms of postoperative deaths (Fisher's Exact test, x^2=3.48, p=0.181). The repartition of postoperative complications and death is presented in Table 3.

Evaluation of the perioperative changes in serum albumin and inflammatory cytokines levels

Analysis of median plasma inflammatory marker levels between the studied cancer groups with the Friedman test showed significant differences between groups (p<0.05). Descriptive statistics for all parameter levels for each moment in time was stratified by each studied cancer group and are shown in Table 4. The median values of inflammatory levels and longitudinal comparisons by cancer group estimated significance levels obtained using Friedman's test, applied to compare differences in values between 3 time points.

IL-6

Evaluation of the repeated measurements of plasma IL-6 values revealed a significant difference in time (Table 4) for each studied group while a post-test analysis showed that the median level of IL-6 was different between the 1st day after operation (1 DAO) and baseline for gastric cancer patients (Dunn's test, p=0.0018) and pancreatic cancer (Dunn's test, p=0.0012).

IL-10

Evaluation of repeat measurements of plasma IL-10 values for each studied group is shown in Table 4. A post-test analysis using Wilcoxon signed-rank test identified that the distribution of IL-10 values between the 1 DAO and baseline was different for each group ($p<0.05$). For gastric cancer patients, distribution of Il-10 values in the 1 DAO and 3th after operation (3 DAO) was different (Dunn's test, $p<0.001$), while no difference was found in the distribution of IL-10 values between 3 DAO and baseline (Dunn's test, $p=0.180$). For pancreatic cancer patients, analysis of each time evaluation showed a different distribution of IL-10 values for 1 DAO vs before surgery (Dunn's test $p=0.004$), 3 DAO vs before surgery (Dunn's test, $p=0.001$) and 3 DAO vs 1 DAO (Dunn's test, $p<0.001$). For liver cancer patients, we noticed a different distribution of IL-10 for 1 DAO vs before surgery (Dunn's test, $p=0.001$) and between 1 DAO and 3 DAO (Dunn's test , $p<0.001$), while analysis of IL-10 values between 3 DAO and baseline showed similar distribution ($p=0.054$).

Albumin

Analysis of the post-test evaluation of repeat measurements of albumin values between the 1 DAO and baseline showed a difference in distribution values ($p<0.001$) for each of the studied cancer group. A difference in distribution of albumin values was also seen between the 3 DAO and baseline (Dunn's test, $p<0.001$) for each of the studied cancer groups.

CRP

Analysis of the post-test evaluation of repeat measurements of CRP values between the 1 DAO and baseline showed different distribution values (Dunn's test, $p<0.05$) for each of the studied cancer group. A significant difference in distribution values

Table 1. Distribution of patients' baseline characteristics

Characteristics	*Gastric cancer*	*Pancreatic cancer*	*Liver cancer*
Age,years (mean±SD)	65.56 (11.609)	58.57 (8.668)	57.33 (12.060)
Gender ratio number (M:F)	27:9	15:13	12:9
Tumor type (number, %)	36	28	21
Antropyloric gastric cancer	19 (52.8)		-
Corporeal gastric cancer	12 (33.3)	-	-
Oesophago-gastric cancer	2 (5.6)	-	-
Small curvature gastric cancer	3 (8.3)	-	-
Pancreatic head carcinoma	-	19 (67.9)	-
Vater's ampuloma	-	4 (14.3)	-
Pancreatic body cancer	-	4 (14.3)	-
Malignant duodenal polyp	-	1 (3.6)	-
Cholangiocarcinoma	-	-	3 (14.3)
Hepatocellular carcinoma	-	-	11 (52.6)
Klatskin tumor stage B4	-	-	1 (4.8)
Liver metastases	-	-	5 (23.8)
Gall bladder cancer	-	-	1 (4.8)
IL-6, pg/mL (median, Q1-Q3)	<4.69	<4.69	<4.69
IL-10, pg/mL (median, Q1-Q3)	45.32, 39.56-52.22	41.10, 38.42-43.12	41.26, 38.42-45.70
Albumin, g/dL (median, Q1-Q3)	4.1, 3.5-4.4	4.2, 3.7-4.4	4.1, 4.0-4.4
CRP, mg/dL (median, Q1-Q3)	0.525, 0.44-2.1	0.54, 0.45-0.75	0.52, 0.45-2.44

SD:standard deviation, Q1:25th percentile, Q3:75th percentile, Q1-Q3:interquartile interval

Table 2. Surgical outcomes group distribution

Variables	*Gastric cancer*	*Pancreatic cancer*	*Liver cancer*
Bleeding, ml (mean±SD)	230±161.30	435.00±173.22	586.67±468.38
Operation duration, min (median, Q1-Q3)	150, 120-217.5	270, 240-345	140, 120-180
Hospitalisation days (median, Q1-Q3)	12, 8.00-16.50	18, 13.00-28.50	10, 8.00-15.00

Table 3. Comparisons of postoperative complications and deaths by groups

Variables	*Gastric cancer*	*Pancreatic cancer*	*Liver cancer*
Complications*, number (% from group size)	14 (38.9)	11 (39.3)	3 (14.3)
Type of complications, n (%)			
Wound evisceration	2 (5.6)	1 (3.6)	0 (0)
Haemorrhagic complications	2 (5.6)	1 (3.6)	0 (0)
Abscesses	3 (8.3)	0 (0)	1 (4.8)
General complicaions	6 (16.7)	3 (10.7)	3 (14.3)
Wound infections	1 (2.8)	4 (14.3)	0 (0)
Death	5 (13.9)	4 (14.3)	0 (0)

*Complications were defined as the presence of at least one type of complications

Table 4. Median (range*) values of inflammatory markers and longitudinal comparisons by cancer group

Variables	*Groups*	*Before surgery n (%)*	*1 DAO n (%)*	*3 DAO n (%)*	*p value*[a]
	Gastric cancer	<4.69 (86.52)	18.24 (908.68)	<4.69 (962.02)	<0.001
IL-6 (pg/mL)	Pancreatic cancer	<4.69 (70.66)	18.69 (290.76)	<4.69 (808.38)	<0.001
	Liver cancer	<4.69 (13.36)	<4.69 (556.88)	<4.69 (72.20)	0.011
IL-10 (pg/mL)	Gastric cancer	45.32 (162.86)	<3.4 (1138.92)	50.30 (122.72)	<0.001
	Pancreatic cancer	41.10 (36.40)	<3.4 (106.62)	56.04 (72.42)	<0.001
	Liver cancer	41.26 (21.46)	<3.4 (ND)	49.54 (848.66)	<0.001
Albumin (g/dL)	Gastric cancer	4.10 (2.30)	3.20 (2.20)	3.20 (2.20)	<0.001
	Pancreatic cancer	4.20 (2.20)	2.80 (2.00)	2.90 (2.00)	<0.001
	Liver cancer	4.10 (2.60)	3.50 (1.90)	3.50 (11.41)	<0.001
CRP (mg/dL)	Gastric cancer	0.53 (15.32)	13.12 (20.21)	11.69 (27.08)	<0.001
	Pancreatic cancer	0.54 (2.95)	12.76 (16.39)	12.07 (35.46)	<0.001
	Liver cancer	0.52 (15.03)	6.02 (25.41)	6.22 (18.50)	<0.001

*Range=maximum-minimum, DAO:days after operation, ND:not determined, [a]estimated significance levels obtained from Friedman Test applied to compare differences in values between three time points

Table 5. The influence of pre-operative surgical stress markers levels on complications

Predictors	*Univariate analysis*		*Multivariate analysis*	
	Crude OR (95% CI)	*p value*	*Adjusted OR (95% CI)*[a]	*p value*
IL-10 (pg/mL)				
Tertiles[b]				
T2 vs. T1	1.32 (0.43, 4.04)	0.632	1.87 (0.47, 7.52)	0.376
T3 vs. T1	1.39 (0.45, 4.29)	0.568	1.53 (0.39, 6.01)	0.539
Albumin (g/dL)				
Tertiles[c]				
T2 vs. T1	0.33 (0.11, 0.99)	**0.048**	0.43 (0.12, 1.54)	0.196
T3 vs. T1	0.32 (0.10, 1.03)	0.055	0.46 (0.11, 1.87)	0.275
CRP (mg/dL)				
Tertiles[d]				
T2 vs. T1	1.82 (0.55, 6.02)	0.329	1.60 (0.43, 5.99)	0.487
T3 vs. T1	3.32 (1.04, 10.66)	**0.044**	3.85 (0.93, 15.91)	0.062

OR:odds ratio, CI:confidence interval, [a]adjusted for age, gender, duration operation and bleeding, [b]T1:<39.85; T2:[39.85, 45.32]; T3:>45.32, [c]T1:<3.2; T2:[3.2, 4.2]; T3:>4.2; [d]T1:<0.7; T2:[0.7, 1.3]; T3:>1.3. Bold numbers denote statistical significance. Multivariate model performance: Nagelkerke R^2=0.34, C-stat=0.82, Hosmer-Lemeshow Test: $x^2(7)$=18.51, p=0.01.

(p<0.05) for each of the studied cancer group was also observed between 3 DAO and baseline.

The impact of preoperative surgical stress markers levels on complications and deaths

Univariate logistic regression analysis demonstrated that the second tertile of albumin was associated with a decreased risk of complications (OR=0.33, 95% CI: 0.11, 0.99), while the third tertile of CRP was associated with an increased risk of complications (OR=3.32, 95% CI: 1.04, 10.66). However, adjusting for age, gender, duration of operation and bleeding, the multivariate model of logistic regression indicated that the third and second tertile of CRP ("high values" of CRP) were not found to be independent risk factors for complications (p=0.062; Table 5).

Adjusting for age, gender, duration of operation and bleeding, the multivariate model of logistic regression showed that the third and second tertile of IL-10 were not found to be independent risk factors for mortality (p=0.10; Table 6).

Table 6. The influence of preoperative surgical stress markers levels on deaths

Predictors	*Univariate analysis*		*Multivariate analysis*	
	Crude OR (95% CI)	*p value*	*Adjusted OR (95% CI)*[a]	*p value*
IL-10 (pg/mL)				
Tertiles[b]				
T2 vs. T1	3.12 (0.30, 31.90)	0.338	9.09 (0.35, 237.82)	0.185
T3 vs. T1	5.87 (0.64, 53.93)	0.118	9.83 (0.64, 151.61)	0.102
Albumin (g/dL)				
Tertiles[c]				
T2 vs. T1	0.20 (0.04, 1.06)	0.059	0.22 (0.02, 2.16)	0.193
T3 vs. T1	ND	0.998	ND	ND
CRP (mg/dL)				
Tertiles[d]				
T2 vs. T1	0.32 (0.03, 3.29)	0.338	0.23 (0.01, 4.04)	0.317
T3 vs. T1	1.88 (0.41, 8.77)	0.419	0.59 (0.04, 8.19)	0.694

OR:odds ratio, CI:confidence interval, [a]adjusted for age, gender, duration operation and bleeding; [b]T1:<39.85; T2:[39.85, 45.32]; T3:>45.32; [c]T1:<3.2; T2:[3.2, 4.2]; T3:>4.2; [d]T1:<0.7; T2:[0.7, 1.3]; T3:>1.3. ND:not determined, Multivariate Model Performance: Nagelkerke R^2=0.34, C-stat=0.94, Hosmer-Lemeshow Test: $x^2(7)$=4.60, p=0.709.

Table 7. The influence of postoperative surgical stress markers on complications

Predictors	*Univariate analysis*		*Multivariate analysis*	
	Crude OR (95% CI)	*p value*[a]	*Adjusted OR (95% CI)*[a]	*p valuea*
IL-10 (pg/mL)				
Tertiles[b]				
T2 vs. T1	2.24 (0.69, 7.25)	0.178	3.60 (0.87, 14.98)	0.078
T3 vs. T1	2.37 (0.73, 7,71)	0.151	4.64 (1.05, 2052)	**0.043**
Albumin (g/dL)				
Tertiles[c]				
T2 vs. T1	0.33 (0.10, 1.07)	0.064	053 (0.12, 2.28)	0.395
T3 vs. T1	0.50 (0.17, 1.45)	0.200	1.45 (0.30, 6.95)	0.643
CRP (mg/dL)				
Tertiles[d]				
T2 vs. T1	1.65 (0.50, 5.46)	0.412	1.14 (0.29, 4.51)	0.848
T3 vs. T1	3.18 (0.99, 10.23)	0.053	2.23 (0.55, 8.99)	0.261

OR:odds ratio, CI:confidence interval; [a]adjusted for age, gender, duration operation and bleeding; [b]T1:<46.08; T2:[46.08, 59.45]; T3:>59.45; [c]T1:<3.3; T2:[3.3, 3.9]; T3:>3.9; [d]T1:<6.6; T2:[6.6, 12.58]; T3:>12.58. Bold numbers denote statistical significance. Multivariate model performance: Nagelkerke R^2=0.49, C-stat=0.81, Hosmer-Lemeshow Test: $x^2(7)$=1.12, p=0.993.

Table 8. The influence of postoperative surgical stress markers levels on deaths

Predictors	*Univariate analysis*		*Multivariate analysis*	
	Crude OR (95% CI)	*p value*	*Adjusted OR (95% CI)*[a]	*p value*
IL-10 (pg/mL)				
Tertiles[b]				
T2 vs. T1	096 (0.18, 5.22)	0.964	2.07 (0.17, 25.23)	0.570
T3 vs. T1	1.00 (0.18, 5.45)	1.00	2.30 (0.16, 33.21)	0.540
Albumin (g/dL)				
Tertiles[c]				
T2 vs. T1	0.29(0.05, 1.61)	0.157	1.66 (0.12, 22.69)	0.705
T3 vs. T1	0.13(0.01, 1.13)	0.064	0.52 (0.02,11.16)	0.675
CRP (mg/dL)				
Tertiles[d]				
T2 vs. T1	0.96(0.06, 16.21)	0.980	0.65 (0.03, 15.32)	0.789
T3 vs. T1	9.00(1.03, 78.94)	**0.047**	9.97 (0.60, 165.99)	0.109

OR: odds ratio, CI: confidence interval, T1, T2, T3: tertiles. a adjusted for age, gender, duration operation and bleeding T1:<46.08; T2:[46.08, 59.45]; T3:>59.45; b T1:<3.3; T2:[3.3, 3.9]; T3:>3.9; c T1:<6.6; T2:[6.6, 12.58]; T3:>12.58. Bold numbers denote statistical significance. Multivariate model performance: Nagelkerke Test R2=0.49, C-stat=0.93, Hosmer-Lemeshow Test: x2(7)=1.12, p=0.993.

The impact of postoperative surgical stress markers levels on complications and deaths

Univariate logistic regression analysis didn't show significant correlation concerning the associations between the second tertile of albumin (p=0.064), third tertile of CRP (p=0.053) and risk of complications. However, the multivariate model of logistic regression analysis indicated that only the third tertile of IL-10 was independent risk factor for postoperative complications (Table 7). Adjusting for age, gender, operation duration and bleeding, none of the postoperative studied markers levels remained as independent risk factor for mortality (Table 8).

Additional analysis: ROC analysis for distinguishing between patients with and without complications

The median of postoperative plasma IL-10 values of patients with complications and those without complications were 53.8 pg/mL (interquartile range: 46.7-68.9 pg/mL) vs 49.5 pg/mL (interquartile range: 44.2-63.7), respectively. The median albumin values of patients with complications and those without complications were 3.0 g/dL (interquartile range: 2.6-3.4 g/dL) vs 3.2 g/dL (interquartile range: 2.9-3.7), respectively, while the median CRP values of patients with complications and those without complications were 12.1 mg/dL (interquartile range: 7.9-18.6 mg/dL) vs 9.4 mg/dL (interquartile range: 5.2-13.4 mg/dL).

Postoperative determination of plasma IL-10 at a cut-off value of 47.05 pg/mL had an estimated sensitivity of 75%, specificity of 43.86%, and AUC=0.55 (95% CI: 0.42-0.68) for distinguishing between patients with complications and those without complications. The ROC results obtained for serum albumin were: cut-off value=3.05 g/dL, AUC= 0.64 (95% CI: 0.51-0.77), sensitivity=53.57%, specificity=68.42% and CRP: cut-off value=10.97 mg/dL, AUC= 0.66 (95% CI: 0.54-0.78), sensitivity=67.86% and specificity=59.65%.

Discussion

The present study highlights the surgical stress level measured by IL-6, IL-10 and CRP in pancreatic, gastric and liver cancer patients. In our study, evaluation of perioperative changes in plasma IL-6, IL-10 and in serum albumin and CRP yielded statistically significant results. Measuring plasma IL-6 values at different times revealed a significant difference for each studied group, the median level of IL-6 being different from the baseline on the 1 DAO for gastric cancer patients (p=0.0018) and pancreatic cancer patients (p=0.0012). Concerning the perioperative changes in plasma IL-10, we found significant changes in the median level of IL-10 at different times for each group, being different from the baseline on the 1 DAO (p<0.05) and the same was found comparing values on the 1 and 3 DAO (p<0.001). According to the data in Table 4, the immune response of IL-10 manifested on the 3 DAO. Also, Table 4 shows that CRP values measured on 3 DAO did not drop below the values measured on the 1 DAO. Thus we conclude that the immune response of IL-10 starts after the release of CRP, which is an acute phase protein.

Evaluation of the repeat measurements of serum albumin revealed a significant difference between the 1 DAO and baseline ($p<0.001$) and between the 3 DAO and baseline ($p<0.001$) for each studied group of patients. The evaluation of CRP values revealed a significant difference between the 1 DAO and baseline as well as between the 3 DAO and baseline for each group of patients ($p<0.05$). We also observed a greater number of postoperative complications for gastric and pancreatic cancer patients as opposed to liver cancer patients (25 vs 3 cases). Due to the restricted number of studied patients, the number of postoperative deaths was small (9 cases).

Some biochemical parameters have been reported in the literature as predictors for postoperative complications in cancer patients. The IL-6/IL-10 ratio could possibly reflect the balance of pro- and anti-inflammatory cytokines in liver surgery [15]. IL-6 was associated with postoperative site infection [16]. There is evidence that IL-6 plasma concentration is associated with postoperative morbidity in gastric cancer patients [17]. Plasma IL-6, through p-STAT3 rather than p-STAT1 signal pathway, affected hepatic function, tumor progression, and determined patient survival with hepatocellular carcinoma [18]. The release or pro-inflammatory cytokines, i.e. TNF-alpha, IL-1, IL-6 and IL-8 was synchronized with the release of antagonistic mediators (i.e. IL-1ra, IL-10, IL-2 and IL-6 soluble receptors) [19]. Hypoalbuminemia was associated with early mortality after liver resection [20]. CRP alone is a strong prognostic factor in colorectal cancer patients with liver metastases [21] and the CRP/Alb ratio could be a predictor of poor long-term outcomes in patients with colorectal liver metastases after hepatic resection [22]. The prognostic use of CRP and other inflammatory markers was proven in certain types of cancer, such as prostate or colorectal, but the overall evidence for the diagnostic or etiological role of serum CRP in cancer patients has so far been inconsistent [23]. The results of the present study showed that preoperative elevated levels of serum CRP were associated with an increased risk of postoperative complications in univariate logistic regression analysis. Adjusting for age, operation duration and intraoperative blood loss, the multivariable logistic regression analysis showed that there was an association between increased level of CRP and risk of postoperative complications with a tendency toward statistical significance ($p=0.06$). Concerning early postoperative deaths linked to the preoperative values of stress markers, the multivariate model of logistic regression showed that only the elevated level of IL-10 was an independent risk factor for mortality with tendency toward statistical significance. Other authors also studied the correlation between preoperative plasma IL-6 and CRP and gastric tumors. While they found that increased values of IL-6 and CRP before surgery predict a more severe malignant disease, only IL-6 was found to influence the overall survival [24]. Since this article focused on preoperative measurements and overall survival for cancer patients undergoing surgery, our study focused on both pre- and postoperative measurements and ultimately on the early postoperative period and the complications and deaths occuring therein.

Regarding the impact of the postoperative values of the studied stress markers on postoperative complications, the univariate analysis found that an increased level of serum albumin was associated with a lower risk of postoperative complications ($p=0.064$), while a high level of serum CRP tended to be associated with an increased risk for complications ($p=0.053$). The multivariate model showed that only the elevated level of plasma IL-10 was an independent risk factor for the said complications. While in many papers [12,25-28] raised levels of serum (plasma) IL-6 were positively linked to postoperative complications, no papers were found with a similar patient sample. We included almost equal groups of patients, each suffering from a different cancer type (gastric, pancreatic and liver cancer), while other studies only included one type of cancer.

The influence of postoperative surgical stress markers studied by univariate logistic regression analysis revealed that the elevated levels of CRP were significantly associated with an increased risk of early postoperative death. However, adjusting for age, gender, operation time and bleeding, none of these markers studied remained as independent risk factors for mortality.

Abdominal surgery will generate a certain amount of surgical stress by producing a systemic inflammatory response, as any injury of a given magnitude. This complex phenomenon is initialised by tissue damage and bleeding and involves the nervous system as well as the endocrine and both immune and hematopoietic systems [25]. As this process is naturally occuring, it is often self-limited. Despite this, there are many cases, especially after prolonged laborious procedures, in elderly or clinically impaired patients, where this process runs out of control, leading to cachexia, depletion of the systems' biological reserve and, clinically, to postoperative complications and even death [26,27].

In conclusion the results of the present study are in favor of the following hypothesis: First, cy-

tokines and CRP levels change over time regardless of the type of cancer, the change being due to a significant difference in serum concentrations between 1 DAO and baseline. Second, high preoperative values of serum CRP and albumin were associated with increased risk of postoperative complications while IL-10 and CRP were independent risk factors for early postoperative complications. It can also be concluded that postoperative increased values of certain biormarkers, such as CRP, were associated with risk of early postoperative death but none of the studied parameters can be regarded as independent prognostic factor for death. Regarding the impact of the aforementioned postoperative markers on the early onset of postoperative complications, the elevated serum albumin concentrations can be positively linked to a lower rate of complications, while high values of CRP can be linked to an increased risk. From the set of the studied biomarkers, only IL-10 can be used as an independent risk factor for early postoperative complications.

Conflict of interests

The authors declare no conflict of interests.

References

1. Kim HJ, Lee WJ, Kang CM et al. Risk Factors Associated with Loco-Regional Failure after Surgical Resection in Patients with Resectable Pancreatic Cancer. PLoS One 2016;11(6):e0157196.
2. Brennan MF, Moccia RD, Klimstra D. Management of adenocarcinoma of the body and tail of the pancreas. Ann Surg 1996;223:506-11.
3. Park JW, Chen M, Colombo M et al. Global patterns of hepatocellular carcinoma management from diagnosis to death: the BRIDGE Study. Liver Int 2015;35: 2155-66.
4. Benson AB 3rd, Abrams TA, Ben-Josef E et al. NCCN clinical practice guidelines in oncology: hepatobiliary cancers. J Natl Compr Canc Netw 2009;7:350e-91.
5. Mrena J, Mattila A, Böhm J, Jantunen I, Kellokumpu I. Surgical care quality and oncologic outcome after D2 gastrectomy for gastric cancer. World J Gastroenterol 2015;21:13294-301.
6. Jhanji S, Thomas B, Ely A, Watson D, Hinds CJ, Pearse RM. Mortality and utilisation of critical care resources amongst high-risk surgical patients in a large NHS trust. Anaesthesia 2008;63:695-700.
7. Head J, Ferrie JE, Alexanderson K, Westerlund H, Vahtera J, Kivimaki M. Diagnosis-specific sickness absence as a predictor of mortality: the Whitehall II prospective cohort study. Br Med J 2008;337:a1469.
8. Pearse RM, Harrison DA, James P et al. Identification and characterisation of the high-risk surgical population in the United Kingdom. Crit Care 2006;10:R81.
9. Fisher J, Jones D, Pomposelli F, Upchurch G (Eds): Fisher's Mastery of Surgery (6th Edn). Philadelphia (PA): Lippincott Williams & Wilkins; 2012, pp 1058-67.
10. Wang Y, Liu R, Zhang Z et al. A safety study of transumbilical single incision versus conventional laparoscopic surgery for colorectal cancer: study protocol for a randomized controlled trial. BioMed Central 2015; 16:539.
11. Hildebrandt U, Kessler K, Plusczyk L, Pistorius G, Vollmar B, Menger MD. Comparison of surgical stress between laparoscopic and open colonic resections. Surg Endosc 2003;17:242-6.
12. Aoyama T, Yoshikawa T, Hayashi T et al. Randomized Comparison of Surgical Stress and the Nutritional Status Between Laparoscopy-Assisted and Open Distal Gastrectomy for Gastric Cancer. Ann Surg Oncol 2014;21:1983-90.
13. Deyle S, Banz VM, Wagner M et al. Estimation of Physiologic Ability and Surgical Stress Score Does Not Predict Immediate Outcome After Pancreatic Surgery. Pancreas 2011;40:723-9.
14. Hashimoto D, Takamori H, Sakamoto Y, Tanaka H, Hirota M, Baba H. Can the Physiologic Ability and Surgical Stress (E-PASS) Scoring System Predict Operative Morbidity After Distal Pancreatectomy? Surg Today 2010;40:632-7.
15. Jerin A, Požar-Lukanovič N, Sojar V, Stanisavljevič D, Paver-Eržen V, Osredkar J. Balance of Pro- and Anti-Inflammatory Cytokines in Liver Surgery. Clin Chem Lab Med 2003; 41:899-903.
16. Kimura F, Shimizu H, Yoshidome H et al. Increased Plasma Levels of IL-6 and IL-8 are Associated With Surgical Site Infection After Pancreaticoduodenectomy. Pancreas 2006;32:178-85.
17. Szczepanik AM, Scislo L, Scully T et al. IL-6 serum levels predict postoperative morbidity in gastric cancer patients. Gastric Cancer 2011;14:266-73.
18. Kao JT, Feng CL, Yu CJ et al. IL-6, through p-STAT3 rather than p-STAT1, activates hepatocarcinogenesis and affects survival of hepatocellular carcinoma patients: a cohort study. BMC Gastroenterol 2015; 15:50.
19. Maruna P, Gürlich R, Frasko R et al. Cytokines and soluble cytokine receptors in the perioperative period. Sb Lek 2002;103:273-82.
20. Lee CW, Tsai HI, Sung CM et al. Risk factors for early mortality after hepatectomy for hepatocellular carcinoma. Medicine (Baltimore) 2016;95:e5028.
21. Køstner AH, Kersten C, Löwenmark T et al. The prog-

nostic role of systemic inflammation in patients undergoing resection of colorectal liver metastases: C-reactive protein (CRP) is a strong negative prognostic biomarker. J Surg Oncol 2016;114:895-9.

22. Haruki K, Shiba H, Horiuchi T et al. Impact of the C-reactive protein to albumin ratio on long-term outcomes after hepatic resection for colorectal liver metastases. Am J Surg 2017; pii: S0002-9610(17)30272-6. doi: 10.1016/j.amjsurg.2017.02.001.
23. Petekkaya I, Unlu O, Roach E et al. Prognostic role of inflammatory biomarkers in metastatic breast cancer. JBUON 2017;22:614-22.
24. Kim DK, Oh SY, Kwon HC et al. Clinical significances of preoperative serum interleukin-6 and C-reactive protein level in operable gastric cancer. BMC Cancer 2009;9:155.
25. Hu X, Xie S, Wen W, Tian D, Bao Q. Comparative evaluation of surgical stress of different lymph node dissection for gastric cancer. Chinese-German J Clin Oncol 2007;6:305-10.
26. Orci LA, Toso C, Mentha G, Morel P, Majno PE. Systematic review and meta-analysis of the effect of perioperative steroids on ischaemia-reperfusion injury and surgical stress response in patients undergoing liver resection. Br J Surg 2013;100:600-9.
27. Bulut O, Aslak K, Levic K et al. A randomized pilot study on single-port versus conventional laparoscopic rectal surgery: effects on postoperative pain and the stress response to surgery. Tech Coloproctol 2015;19: 11-22.
28. Soda K, Kano Y, Kawakami M, Konisi F. Excessive increase of serum interleukin 6 jeopardizes host defense against multi-bacterial infection. Cytokines 2003;21:295-302.