Systemic Inflammatory Response After Open, Laparoscopic and Robotic Surgery in Endometrial Cancer Patients

RADOVAN PILKA[1], RADIM MAREK[1], TOMAS ADAM[3], MILAN KUDELA[1], DANA ONDROVÁ[1], DAVID NEUBERT[1], JOZEF HAMBÁLEK[1], MARTIN MADĚRKA[1], DAGMAR SOLICHOVÁ[4], LENKA KUJOVSKÁ KRČMOVÁ[4] and BOHUSLAV MELICHAR[2]

[1]*Department of Obstetrics and Gynecology, Medical Faculty Palacký University and Faculty Hospital, Olomouc, Czech Republic;*
[2]*Department of Oncology, Medical Faculty Palacký University and Faculty Hospital, Olomouc, Czech Republic;*
[3]*Department of Laboratory Biochemistry, Medical Faculty Palacký University and Faculty Hospital, Olomouc, Czech Republic;*
[4]*Third Department of Medicine-Gerontology and Metabolic Care, University Hospital, Hradec Králové, Czech Republic*

Abstract. *Aim: To study inflammatory response and nutritional biomarkers in operated endometrial cancer (EC) patients. Materials and Methods: A total 109 consecutive EC patients undergoing open laparotomy (LT), laparoscopic (LS) or robot-assisted surgery (RS) were studied. Twenty four patients served as controls. Pre- and postoperative levels of inflammatory and nutritional biomarkers were analyzed prospectively. Results: The estimated blood loss was significantly lower in RS compared to all other groups. C-reactive protein (CRP) and interleukin-6 (IL-6) correlated with each other and exhibited positive correlation with age, body-mass index (BMI), leukocyte count, platelet count, kynurenine, kynurenine/tryptophan ratio and urinary neopterin and a negative correlation with vitamin D and retinol. Hemoglobin, retinol, alpha-tocopherol, vitamin D and citrulline concentrations decreased and inflammatory biomarkers increased after surgery to a different extent in LT, LS, RS and control groups. Conclusion: The present data demonstrate a differential response to surgical trauma in patients with endometrial carcinoma.*

Endometrial cancer (EC) is the most common cancer of the female genital system (1). Historically, the principal treatment modality of endometrial cancer is surgery that includes abdominal total hysterectomy, salpingo-oophorectomy and pelvic and/or paraortic lymphadenectomy. In 1993, Childers *et al.* were the first to propose the laparoscopic approach in performing complex surgical procedures, such as total hysterectomy (2). The Gynecologic Oncologic group study confirmed the superiority of laparoscopy compared to laparotomy in a randomized study (LAP-2), in terms of complications and hospital stay (3). Subsequently, laparoscopy became the preferred surgical approach in the endometrial cancer staging. Recently, the robotic system Da Vinci (Intuitive Surgical, Sunnyvale, CA, USA) has been introduced. Since the approval of robotic surgery in 2005, the use of robotic surgery has grown exponentially. It has a shorter learning curve compared to conventional laparoscopy and an experience in laparoscopy is not a prerequisite (4, 5).

Surgical intervention elicits an inflammatory response that is accompanied by oxidative stress. There are obvious differences between different therapeutic approaches in terms of tissue trauma and oxidative stress. Different biomarkers of inflammatory response include C-reactive protein (CRP), kynurenine/tryptophan ratio or neopterin. Systemic inflammatory response is also associated with decreased hemoglobin concentrations and higher platelet counts, as well as oxidative stress. Oxidative stress results in decreased concentrations of circulating antioxidants like alpha-tocopherol. The concentrations of alpha-tocopherol, along with other lipid-soluble vitamins like retinol or vitamin D, also reflect the nutritional status of patients. More recently, it was demonstrated that the concentrations of citrulline, a biomarker of bowel mass, also inversely correlates with inflammatory response.

Correspondence to: Professor Radovan Pilka, MD, PhD, Department of Obstetrics and Gynecology, Medical Faculty Palacký University and Faculty Hospital, Olomouc, I.P.Pavlova 6, Olomouc, 779 00, Czech Republic. Tel: +420 585854103, Fax: +420 585414975, e-mail: radovan.pilka@fnol.cz

Key Words: Endometrial cancer, robotic surgery, inflammatory response, nutrition, surgical trauma.

The aim of this study was to compare an association between clinical indicators of surgical trauma with biomarkers of inflammatory response and nutritional balance in endometrial cancer patients treated with three surgical approaches including open laparotomy (LT), laparoscopic (LS) and robotic surgery (RS), as well as in patients operated for benign disorders.

Materials and Methods

Patients. This prospective study was undertaken at the University Hospital Olomouc between October 2012 and June 2015. The study was approved by the hospital's ethical committee and informed, written consent was obtained from all patients. The study group included 109 consecutive patients with histologically confirmed endometrial cancer, aged 65±10 (range=33-88) years who underwent hysterectomy with bilateral salpingo-ophorectomy, pelvic and para-aortic lymphadenectomy. These procedures were performed by LT, RS or LS approaches. Twenty four patients, aged 55±12 (range=36-80) years, who were scheduled for elective total hysterectomy for non-malignant disease, constituted a control group. The patients were randomly allocated to receive either an abdominal hysterectomy or laparoscopic assisted hysterectomy for their benign disease. Venous blood samples were collected from each patient at the following times: a baseline sample the day before surgery (day -1), before skin incision (day 0), 24 h after operation (day 1), second, third, fourth and fifth day after surgery (day 2-5). The samples were separated and the sera were all stored at –80˚C until analysis for CRP, interleukin-6 (IL-6), citrulline, vitamin D, alpha-tocopherol, retinol, kynurenine and tryptophan. Urinary samples, with the same timing, were collected and stored at –20˚C until analysis for urinary neopterin determination. The changes during the postoperative period were assessed by calculation of the area under curve (AUC). Other parameters evaluated were: patient age, body mass index (BMI), blood loss, decline of hemoglobin, increase of white blood cell count, increase of platelet count, number of lymph nodes obtained and visual analogue scale (VAS) scores. BMI was calculated using the formula: body weight/height^2. Preoperative and postoperative hemoglobin, white blood cells and platelets levels were analyzed on the same days (day-1 to day 5) as the venous blood samples were collected. The difference was calculated by subtracting postoperative levels from the preoperative levels. Postoperative pain was evaluated at the time of admission, before surgery and during the postoperative period (day-1 till day-5) using a 10-point VAS score (0, absence of pain; 10, worst possible pain).

Neopterin, kynurenine, tryptophan and creatinine in human serum. The blood samples were centrifuged (1,600 × *g*, 10 min, +4˚C) and serum was separated. Then, 200 µl of serum was diluted with 100 µl phosphate buffer (15 mmol/l, pH 6.5) and deproteinized by 100 µl cooled ethanol (10 min, –25˚C). After centrifugation (14,000 × *g*, 10 min), the supernatant was filtered using 0.2-µm micro-titration plate filters and vacuum manifold. Filtered solution was applied into the HPLC column. The analyses were performed using a high performance liquid chromatography (HPLC) set Prominence LC 20 Shimadzu (Kyoto, Japan) composed by degasser DGU 20A5, pump LC20-AB, special auto sampler SIL/20 AC for micro-titration plates (rack changer), column oven CTO-20 AC, diode array detector SPD-M20A, fluorescence detector RF10 AXL and communication bus module CBM-20A. Sample preparation technique was developed using micro-titration plates with filters AcroPrep 96 filter Plate 0.2 µm/350 µl Pall Corporation (Ann Arbor, MI, USA), vacuum manifold Phenomenex (Aschaffenburg, Germany), vacuum pump VAC Space-50 Chromservis (Prague, Czech Republic) and centrifuge Minispin Eppendorf (Hamburg, Germany). Analysis was performed at HPLC set Prominence LC 20 (Shimadzu) equipped by special auto sampler for micro-titration plates. As stationary phase, two monolithic columns RP- 18e (4.6×50 mm, 3.0×100 mm) were connected together with monolithic security guard (4.6×10 mm). As mobile phase, 15 mmol/l phosphate buffer (KH_2PO_4 +$K_2HPO_4 \cdot 3H_2O$) was used at pH 4.51 and flow rate 1 ml/min (0–3.09) and 2.3 ml/min (3.10–8.20). Injection volume was 1 µl. Kynurenine and creatinine were detected using diode array detection (230 and 235 nm), neopterin and tryptophan were detected using fluorescent detection (excitation and emission wavelengths of 353 nm and 458 nm for neopterin, respectively, and excitation and emission wavelengths of 254 nm and 404 nm for tryptophan, respectively). Separation was held at ambient temperature in 8.2 min.

Retinol and alpha-tocopherol in human serum. The method used in this study for the analysis of retinol and alpha-tocopherol in the serum was modified from the method previously published by Urbanek *et al*. and is briefly described below (6). In the liquid-liquid extraction (LLE) procedure, 500 µl of serum was deproteinized using cold ethanol with 5% of methanol. Then, 2,500 µl n-hexane was added to this mixture and extracted using a vortex apparatus. After centrifugation, an aliquot of the clean extract was separated and evaporated in a concentrator. The residue was dissolved in 400 µl of methanol and analyzed using a Prominence HPLC system (Shimadzu) (7). The separation of retinol and alpha-tocopherol was performed using the Chromolith Performance RP-18e monolithic column (100x4.6 mm; Merck, Darmstadt, Germany). The detection of retinol and alpha-tocopherol was carried out at 325 and 295 nm, respectively, using a diode array detector.

Vitamin D in human serum. A total of 50 µl of 4 % monohydrate zinc sulfate as the precipitation reagent and 400 µl of methanol were added to 200 µl of human serum in an Eppendorf tube. After 20 s of vortexing, the sample was incubated for 10 min at 4˚C. The sample was then centrifuged (14,000 × *g*, 5 min, 21˚C) and 300 µl of supernatant was filtered using 0.2-µm well filter plates and a vacuum manifold. The filtered solution was injected into the UHPLC system. Analysis was performed by using an UHPLC Nexera set (Shimadzu) coupled with an LCMS-8030 triple-quadrupole mass spectrometer operating in positive ESI mode. Chromatographic separation was achieved on a Kinetex C18 analytical column (1.7 µm, 3×100 mm) connected to a security guard ultra cartridge C18, both purchased from Phenomenex (Aschaffenburg, Germany). The column oven, CTO-20 AC, was used to set the temperature of the analytical column at 50˚C. Acetonitrile and water, both with the addition of FA (c=0.01 mol/l) were used as the mobile phase at a flow rate of 0.5 ml/min. The separation of metabolites 25-OHD_2 and 25-OHD_3 was realized in 6 min and under isocratic conditions, 72:28 v/v of acetonitrile/ water. After analyte separation, the switching valve was activated and the pre-column and the analytical column were washed and equilibrated for 3min into the waste by increasing the flow rate to 0.8 ml/min with 90:10 v/v of acetonitrile/water. The sample injection volume was 20 µl. The total analysis time was 9 min. System operation, data acquisition and data processing were controlled using the LabSolutions 5.41 SP1 software. MS conditions were investigated using automated

Table I. *Comparison of baseline parameters between groups*.

Parameter	Mean±SD (range)				*p*-Value	*p*-Value (*post hoc* tests)					
	Robotic surgery (RS)	Laparoscopy (LS)	Laparotomy (LT)	Controls (C)		RS *vs.* C	LS *vs.* C	LT *vs.* C	RS *vs.* LS	RS *vs.* LT	LS *vs.* LT
Age (years)	65±10 (33-81)	59±9 (45-75)	67±10 (52-88)	55±12 (36-80)	<0.0001[a]	0.0004	0.965	0.0001	0.483	1.000	0.141
BMI (kg/m^2)	31.2±6.8 (15-48.9)	29.0±5.6 (19-38)	34.1±6.7 (24-53.4)	26.8±4.2 (21.4-36.5)	0.0003[b]	0.017	1.000	0.0001	1.000	0.597	0.144
Hemoglobin (g/l)	133.7±13.5 (95-163)	139.0±8.8 (124-157)	128±12.2 (105-147)	134.0±11.5 (107-150)	0.113[b]						
Leukocytes (10^9/l)	7.4±1.9 (3.3-12.6)	7.3±1.3 (5.4-9.2)	8.4±2.4 (4.1-14.9)	7.4±1.9 (4.8-12.1)	0.243[b]						
Platelets (10^9/l)	243±69 (52-515)	246±49 (175-369)	274±75 (96-459)	260±61 (144-428)	0.111[b]						
CRP (mg/l)	4.7±5.9 (0.6-29.2)	1.4±0.9 (0.6-3.4)	12.2±18.0 (0.6-90.0)	2.5±2.9 (0.6-12.3)	0.0001[b]	0.367	1.000	0.003	0.052	0.036	0.001
IL-6 (ng/l)	4.8±3.3 (1.5-16.2)	2.8±1.3 (1.5-5.9)	8.5±5.5 (2.0-25.4)	2.7±1.3 (1.5-5.8)	<0.0001[b]	0.003	1.000	<0.0001	0.032	0.002	0.0003
Citrulline (μmol/l)	29.2±8.4 (13-49)	29.3±6.9 (19-43)	31.3±9.9 (12-57)	23.6±7.4 (8-39)	0.011[b]	0.023	0.168	0.014	1.000	1.000	1.000
Vitamin D (nmol/l)	53.0±25.7 (12-140.8)	69.2±27.1 (25.2-134.8)	45.1±16.4 (15.3-86.2)	0.008[b] (27-137.4)	0.358	1.000	0.078	0.178	1.000	0.008	
Alpha-tocopherol (μmol/l)	25.1±5.5 (12.9-40.9)	25.1±5.1 (17.0-33.3)	25.4±5.9 (18.6-43.1)	24.3±5.6 (7.5-33.1)	0.986[b]						
Retinol (μmol/l)	1.1±0.4 (0.3-2.0)	1.5±0.4 (0.7-2.3)	1.2±0.4 (0.5-1.9)	1.0±0.4 (0.3±1.8)	0.005[b]	1.000	0.002	0.588	0.006	1.000	0.408
Kynurenine (μmol/l)	2.4±1.3 (0.4-8.6)	2.1±0.4 (1.2-3.1)	2.5±0.7 (1.0-4.4)	1.6±0.4 (0.7-2.8)	<0.0001[b]	0.003	0.003	<0.0001	1.000	0.746	0.625
Tryptophan (μmol/l)	47.7±11.5 (10.8-72.8)	47.1±8.1 (34.1-59.6)	49.5±15.5 (11.2-83)	43.9±9.9 (20.1-63.4)	0.311[b]						
Kynurenine/ tryptophan ratio (mmol/mol)	53.7±40.1 (15.2-284)	44.6±8.7 (26.4-61.4)	56.4±27.9 (25-163.7)	36.6±9.3 (18.5-54.4)	0.004[b]	0.046	0.072	0.004	1.000	1.000	0.523
Urinary neopterin /creatinine ratio (μmol/mol creatinine)	167±86 (77-502)	151±7š (88-350)	224±179 (85-930)	371±655 (69-2931)	0.325[b]						

[a]ANOVA. [b]Kruskal-Wallis test. NA, Not applicable; SD, standard deviation; BMI, Body mass index.

optimization of software LabSolution as the first step of the analytical method development. Voltages of Q1, Q3, collision cell and five of the most intensive multiple reaction monitoring (MRM) transitions for identification and quantification of 25-OHD$_3$ and 25-OHD$_2$ were found. Other optimized conditions of ion source were as following: interface ESI-positive polarity; interface temperature 350˚C; desolvation line (DL) temperature 250˚C; nebulising gas flow 3ml/min; heat block temperature 400˚C and drying gas flow 15 l/min.

Citrulline in human serum. For citruline analysis, 20 μl of serum samples were mixed with 100 μl of methanol solution containing 0.1% FA and deuterated internal standards (amino acids and acylcarnitines, non-derivatised reagent kit; Chromsystems, Munich, Germany). The final solutions were vortexed and centrifuged (24,400 × *g*, 10 min at 24˚C, 400 × *g* at 4˚C) and 80 μl of supernatant was transferred to a 96-well plate and used for direct injection mass spectrometry analysis. The remaining supernatant was pooled and used for quality control purposes (8). The samples were measured on 4000 (AB Sciex, Framingham, MA, USA) by flow injection mass spectrometry direct analysis infusion with settings as follows: polarity was set to positive mode with IonSpray voltage of 5500 V, capillary temperature of 450˚C, Curtain curtain Gas gas of - 20 arbpsi, Ion ion Source source Gas gas (GS1/GS2) of - 40 arbpsi. Methanol containing 0.1 % FA was chosen as a mobile phase. Flow rate was set at 0.03 ml/min (0.1-0.4 min) and 0.30 ml/min (0.0-0.1 and 0.4-0.5 min). Citrulline was measured in MRM mode under optimized parameters of declustering potential and collision energy for each mass transition. Unit resolution was set for isolation ions in mass analyzer. Data were processed by software Chemoview 2.0 (AB Sciex).

CRP levels in human serum. Cobas 8000 imunoturbidimetry analyzer (Hitachi, Japan) was used.

IL-6 levels in human serum. Detection was performed by electro-chemiluminescence immunoassay (ECLIA) using a Cobas 8000 analyzer.

Table II. *Comparison of surgical parameters between groups of patients.*

Parameter	Mean±SD (range)				p-Value	p-Value (*post-hoc* tests)					
	Robotic surgery (RS)	Laparoscopy (LS)	Laparotomy (LT)	Controls (C)		RS *vs.* C	LS *vs.* C	LT *vs.* C	RS *vs.* LS	RS *vs.* LT	LS *vs.* LT
Sampled lymph nodes (n)	27±12 (11-64)	21±7 (0-30)	17±14 (0-48)	NA	0.001[b]	NA	NA	NA	0.423	0.001	0.506
Blood loss (ml)	127±136 (5-650)	206±106 (30-450)	341±197 (50-1000)	198±112 (5-400)	<0.0001[b]	0.040	1.000	0.007	0.042	<0.0001	0.038
Nadir hemoglobin concentration (g/l)	108±12 (80-136)	107±15 (76-129)	102±10 (79-123)	111±14 (79-123)	0.022[b]	1.000	1.000	0.051	1.000	0.043	0.906
Decrease of hemoglobin concentration (g/l)	25±11 (0-61)	32±14 (14-69)	27±12 (5-48)	23±10 (7-55)	0.072[b]						
Pain (VAS)	2±1.9 (0.8-3.4)	1.9±0.6 (1-3)	2.3±0.7 (1-3.8)	1.9±0.5 (0.8-3.8)	0.110[b]						

[b]Kruskal-Wallis test. NA, Not applicable; VAS, visual analog scale.

Urinary neopterin concentrations. Urine sample were collected and stored at –20˚C until analysis. After centrifugation (5 min, 1,300 × *g*) and diluting 100 μl of urine specimens with 1.0 ml of mobile phase containing 2 g of disodium- EDTA per liter, samples were injected onto a column and neopterin was determined using HPLC, Prominence LC20 (Shimadzu). Neopterin was identified by its native fluorescence (353 nm excitation, 438 nm emission) and quantified by external standard method. Creatinine was determined by the Jaffe reaction after 1:50 dilution of the sample on a Modular analyzer (Roche) using a commercial kit according to the manufacturer's instructions. Neopterin concentrations were expressed as neopterin/creatinine ratio (μmol/mol creatinine).

Results

The distribution of investigated parameters in patients with benign tumors (controls), and cancer patients treated with RS, LS and LT at baseline is shown in Table I. The patients in the control group were significantly younger compared to RS and LT groups. BMI was significantly higher in RS and LT groups compared to controls. Baseline CRP was significantly higher in the LT compared to RS, LS and control patients, while IL-6 was significantly higher in the LT compared to RS, LS and control patients and also significantly higher in the RS compared to LS and control patients. Citrulline was significantly higher in RS and LT groups compared to controls. Vitamin D concentrations were significantly lower in the laparotomy compared to the laparoscopy groups, while retinol concentrations were significantly lower in controls and robotic surgery compared to laparoscopy group. Kynurenine concentrations were significantly higher in all groups of EC patients compared to controls, whereas the tryptophan/kynurenine ratio was higher in laparotomy and robotic surgery groups compared to controls (Table I).

Table II shows the comparison of surgical parameters, including blood loss. As significantly higher number of lymph nodes was sampled during RS compared to LT. The estimated blood loss was significantly lower in RS compared to all other groups, as well as in patients treated with the LS approach and controls compared to LT. The nadir hemoglobin concentration was significantly higher in RS compared to LT patients (Table II).

The concentrations of the biomarkers investigated did not change between day -1 and day 0 indicating that the values were stable. Starting from day 1, however, marked changes were observed. Figure 1A shows the changes of hemoglobin concentrations in the perioperative period in the four groups of patients. Compared to baseline, hemoglobin concentration was decreased on all postoperative days in all four groups of patients (Figure 1A). Figure 1B shows peripheral blood leukocyte counts during the perioperative period. A significant increase in leukocyte counts was evident on day 1 in all four groups and on day 2 in RS and control groups (Figure 1B). Subsequently, the difference of sequential hemoglobin concentrations compared to baseline was not significant. Figure 1C shows platelet counts during the postoperative period. Compared to baseline, CRP and IL-6 concentrations were also significantly increased throughout the postoperative period in all four groups (Figures 1D and 1E). Citrulline concentrations were decreased compared to baseline throughout the postoperative course in the RS and LT groups, as well as on days 1 to 4 in the LS group, with nadir concentrations observed between days 1 and 3. In contrast, in the control group, a significant decrease of citrulline concentrations was evident only on day 1 (Figure 1F). Vitamin D, alpha-tocopherol and retinol concentrations decreased significantly in all four groups with nadir observed between the days 1 and 4 (Figures 1G, H and I). Compared to baseline, kynurenine concentrations decreased in RS group on days 1 and 2 and in the LT group on day 0 (Figure 1J). Tryptophan concentrations were decreased throughout the postoperative

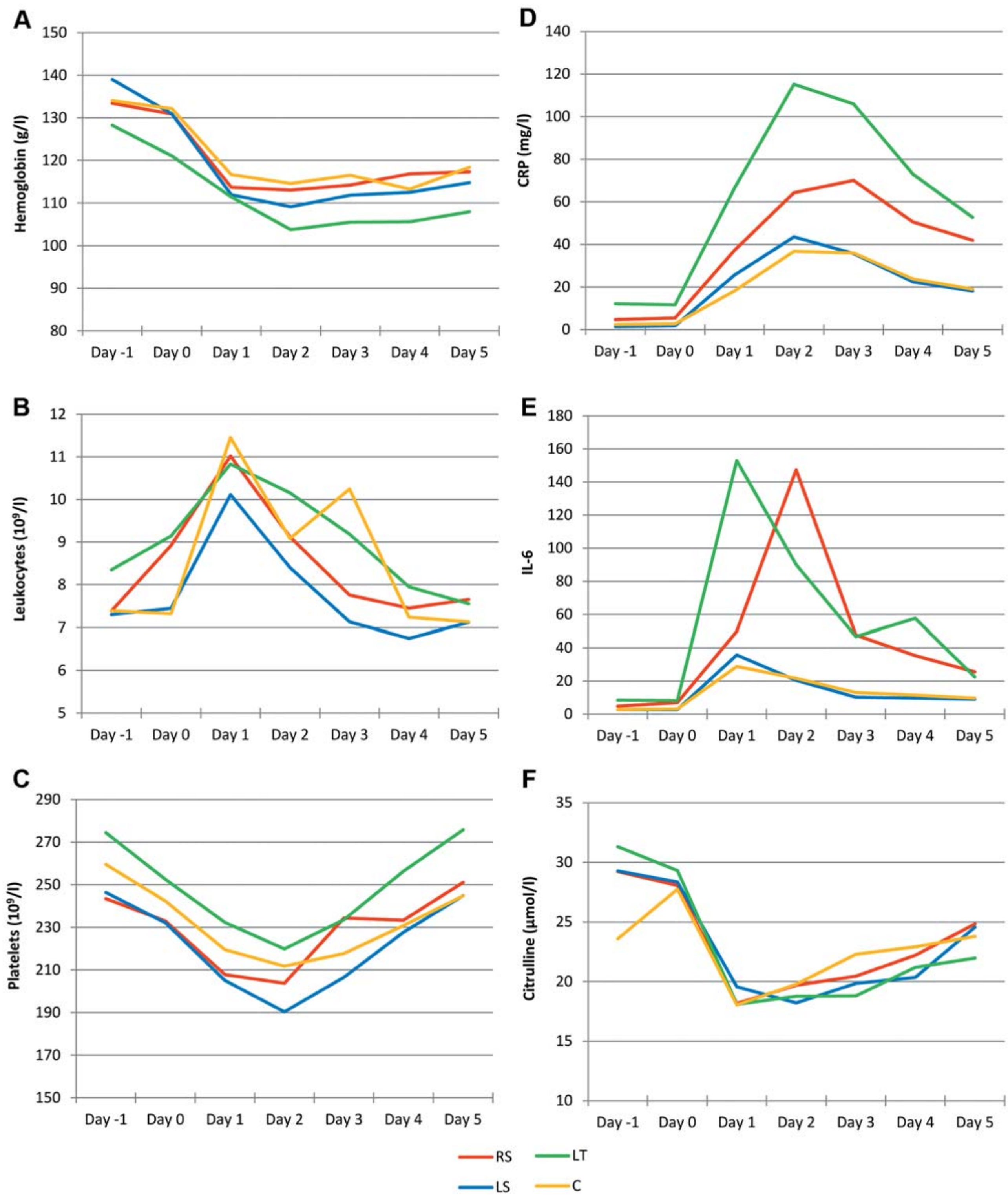

Figure 1. *Continued*

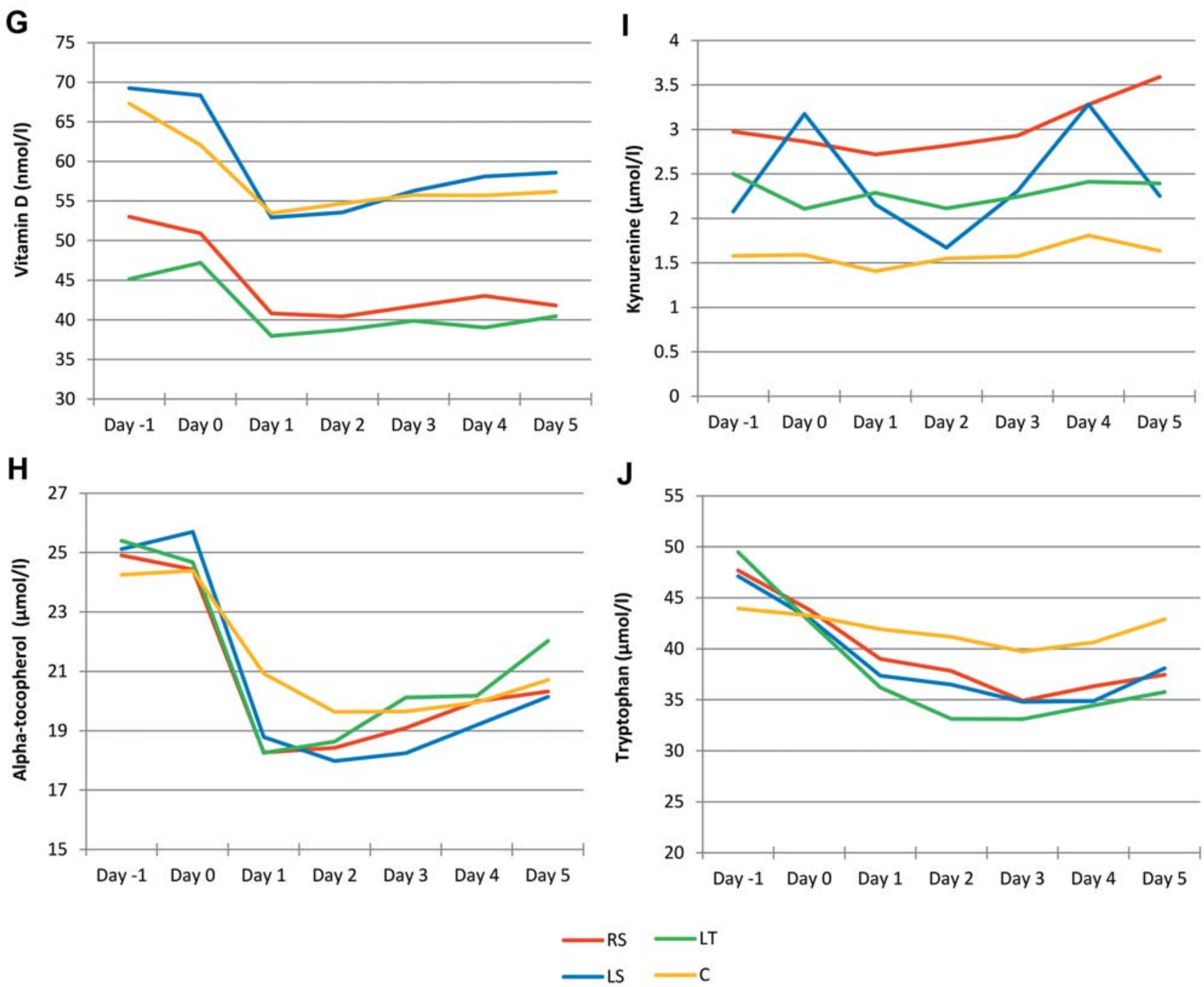

Figure 1. *A: Changes of hemoglobin concentrations in the perioperative period in the robotic (RS), laparoscopic surgery (LS), open laparotomy (LT) and control (C) groups of patients. B: Peripheral blood leukocyte counts during the perioperative period in the robotic surgery (RS), laparoscopic surgery (LS), open laparotomy (LT) and control (C) groups of patients. C: Platelet counts during the perioperative period in the robotic surgery (RS), laparoscopic surgery (LS), open laparotomy (LT) and control (C) groups of patients. D: CRP concentrations throughout the perioperative period in all four groups. E: IL-6 concentrations throughout the perioperative period in all four groups. F: Citrulline concentrations during the perioperative period in the robotic surgery (RS), laparoscopic surgery (LS), open laparotomy (LT) and control (C) groups of patients. G: Vitamin D concentrations during the perioperative period in all four groups. H: Alpha-tocopherol concentrations throughout the perioperative period in the robotic surgery (RS), laparoscopic surgery (LS), open laparotomy (LT) and control (C) groups of patients. I: Retinol concentrations during the perioperative period in the robotic surgery (RS), laparoscopic surgery (LS), open laparotomy (LT) and control (C) groups of patients. J: Kynurenine concentrations during the perioperative period in all four groups. K: Tryptophan concentrations throughout the perioperative period in the robotic surgery (RS), laparoscopic surgery (LS), open laparotomy (LT) and control (C) groups of patients.*

course in the RS, LS and LT groups, but not in controls (Figure 1K). The kynurenine/tryptophan ratio was significantly increased between days 3 and 5 in the RS group and on days 2 to 5 in the LT group and on day 4 in the control group, but not in the LS group (Figure 1L). The urinary neopterin/creatinine ratio did not exhibit significant changes in any of the groups examined (Figure 1M).

AUC of hemoglobin was significantly higher in RS compared to LT group. AUC of CRP and interleukin-6 were significantly higher in RS and LT compared to LS group and controls, as well as in LS compared to RS. AUC of vitamin D was significantly lower in LT compared to the LS patients, while AUC of retinol was significantly lower in RS compared to the LS group. The AUC of urinary

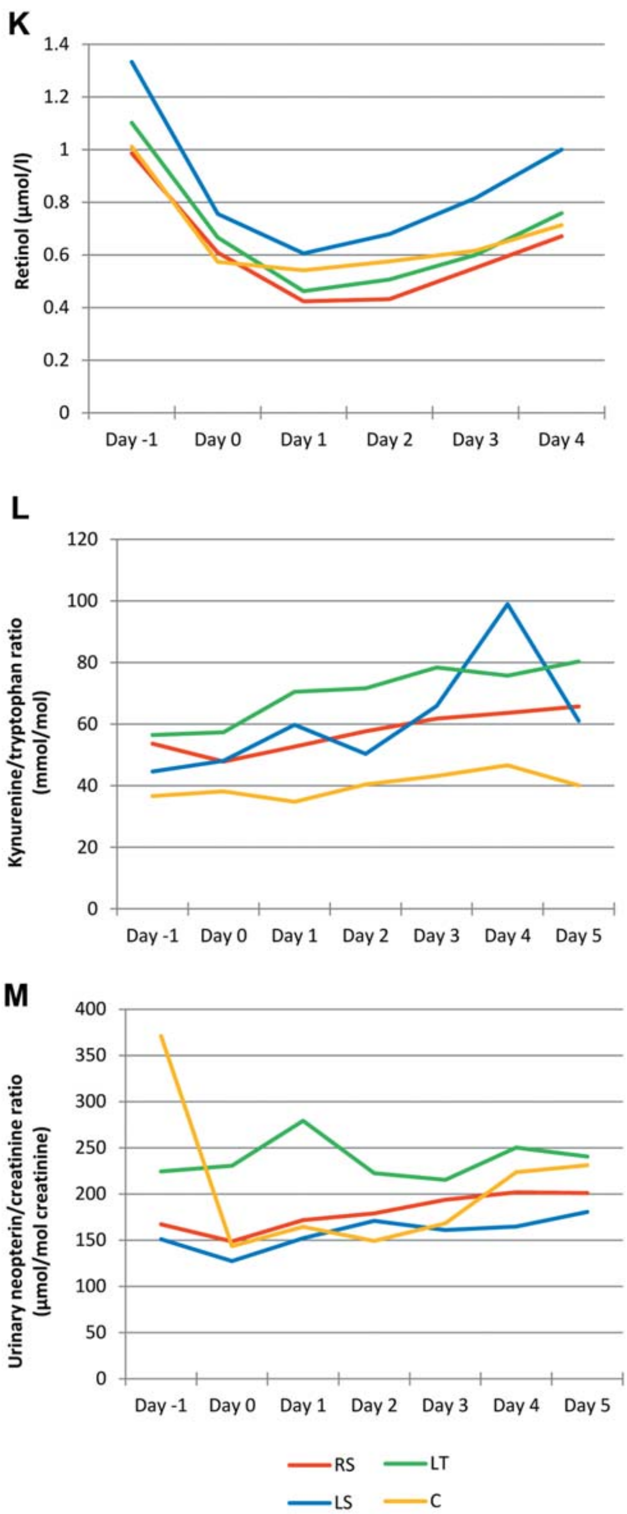

Figure 1. *L: The kynurenine/tryptophan ratio during the perioperative period in the robotic surgery (RS), laparoscopic surgery (LS), open laparotomy (LT) and control (C) groups of patients. M: The urinary neopterin/creatinine ratio during the perioperative period in all four groups.*

neopterin/creatinine ratio was significantly higher in LT compared to LS patients (Table III).

Table IV shows the correlations between investigated biomarkers of nutrition and inflammatory response at baseline in patients with EC. CRP and IL-6 correlated with each other and exhibited positive correlation with age, BMI, leukocyte count, platelet count, kynurenine, kynurenine/tryptophan ratio and urinary neopterin and a negative correlation with vitamin D and retinol. In addition, IL-6 correlated positively with citrulline. Beside the correlation with CRP and IL-6, urinary neopterin correlated positively with age, kynurenine and kynurenine/tryptophan ratio and negatively with vitamin D and retinol. In addition to the correlations mentioned, kynurenine and kynurenine/tryptophan ratio correlated positively with age and citrulline, while kynurenine correlated with BMI.

In controls, CRP correlated positively with IL-6 and negatively with vitamin D concentrations. IL-6 correlated significantly in a positive manner with urinary neopterin and serum kynureninine/tryptophan ratio and negatively with serum tryptophan concentrations. Urinary neopterin/creatinine ratio correlated positively with serum kynurenine concentrations and kynurenine/tryptophan ratios (Table V).

In general, a similar pattern of correlations was observed when AUC values of biomarkers were correlated in EC patients (Table VI) and controls (Table VII) with the exception of a negative correlation being observed between CRP and citrulline in these patients.

Table VIII shows the correlation between the clinical indicators of surgical stress and AUC of investigated biomarkers of nutrition and inflammatory response in patients with EC. Blood loss correlated positively with AUC of kynurenine. Nadir hemoglobin concentrations exhibited significant negative correlation with the AUC of CRP, interleukin-6 and kynurenine/tryptohphan ratio and a positive correlation with AUC of vitamin D, alpha-tocopherol, retinol and tryptophan. Decreased hemoglobin concentrations showed a significant negative correlation with AUC of platelet count and alpha-tocopherol. VAS correlated positively with CRP. The number of removed lymph nodes correlated positively with AUC of tryptophan and negatively with AUC of urinary neopterin/ creatinine ratio. In the control group, only a significant inverse correlation between CRP and nadir hemoglobin concentration (r_s=–0.405; p=0.049) and a positive correlation between citrulline and decrease of hemoglobin concentration (r_s=0.422; p=0.040) were observed.

Discussion

The present data demonstrate significant changes in the investigated biomarkers of nutrition and inflammatory response in the acute postsurgical phase in patients with EC.

Table III. *Comparison of AUC of investigated parameters between the groups of patients.*

Parameter	Mean±SD (range)				*p*-Value	*p*-Value (*post hoc* tests)					
	Robotic surgery (RS)	Laparoscopy (LS)	Laparotomy (LT)	Controls (C)		RS *vs.* C	LS *vs.* C	LT *vs.* C	RS *vs.* LS	RS *vs.* LT	LS *vs.* LT
Hemoglobin (g/l)	695±70 (498-844)	679±81 (535-801)	647.2±59 (507-762)	701±85 (568-847)	0.012[b]	1.000	1.000	0.066	1.000	0.012	1.000
Leukocytes (10^9/l)	51.7±16 (29.9-102.8)	47.1±10.8 (27.4-67.7)	55.4±13.8 (34.8-93.8)	50.0±11.5 (29.7-83.5)	0.231[b]						
Platelets (10^9/l)	1359±469 (309-3198)	1307±276 (1060-1998)	1467±386 (643-2269)	1374±284 (674-1997)	0.214[b]						
CRP (mg/l)	249.6±193.2 (39.2-1014.8)	138.9±68.4 (35.3-288)	402.6±262 (69.3-1320)	128.2±70.9 (26.8-301)	<0.0001[b]	0.002	1.000	<0.0001	0.041	0.0004	<0.0001
IL-6 (ng/l)	224.5±484.6 (32.6-3884)	85.0±36.7 (27.7-158.2)	372.4±530.5 (65.4-2859)	84.6±50.6 (30.2-238)	<0.0001[b]	0.0006	1.000	<0.0001	0.011	0.023	0.0002
Citrulline (µmol/l)	135.6±33.6 (68.5-263.0)	133.3±23.9 (94.5-164.5)	134.2±34.3 (71.5-220.0)	134.5±32.2 (67-233.5)	0.986[b]						
Vitamin D (nmol/l)	260.6 ±127.3 (5.9-686.3)	349.3±144.9 (140.4-749.7)	241.3±125.2 (80.2-734.1)	343.3±169.4 (125.6-717.4)	0.010[b]	0.241	1.000	0.069	0.141	1.000	0.034
Alpha-tocopherol (µmol/l)	122.8±27.4 (67.7-205.2)	122.5±27.0 (88.7-183.5)	125.6±28.2 (93.1-195.3)	127.0±22.4 (89.3-162.5)	0.880[b]						
Retinol (µmol/l)	3.9±1.3 (0.2-7.2)	5.4±1.6 (2.0-7.8)	4.3±1.8 (1.3-9.1)	4.2±1.5 (2.1-7.7)	0.017[b]	1.000	0.114	1.000	0.006	1.000	0.259
Kynurenine (µmol/l)	12.8±5.4 (3.3-34.5)	12.8±4.8 (6.4-22.7)	13.7±4.2 (5.6-26.6)	9.5±2.2 (5.2-15.9)	0.0006[b]	0.015	0.129	0.0001	1.000	0.765	1.000
Tryptophan (µmol/l)	234.1±48.8 (67.5-343.9)	226.5±37.4 (173.4-294.9)	218.1±67.1 (44.9-355.8)	250.1±53.5 (97.3-325.3)	0.230[b]						
Kynurenine/ tryptophan ratio (mmol/mol)	333.9±143.6 (98.6-931.8)	311.6±73.7 (184.3-466.5)	409.4±227.3 (145-1301.8)	241.5±69.6 (125.4-438.0)	0.0005[b]	0.007	0.027	0.0007	1.000	0.564	1.000
Urinary neopterin/ creatinine ratio (µmol/mol creatinine)	1077±482 (458-3378)	930±317 (561-1574)	1412.5±773 (519-4364)	1151±541 (422-2723)	0.022[b]	1.000	1.000	0.620	1.000	0.059	0.042

[b]Kruskal-Wallis test.

In addition, the biomarkers of nutrition and inflammatory response correlated significantly with clinical parameters of surgical stress.

There were some remarkable baseline differences among the groups of patients investigated reflecting the presence of tumor and associated risk factors like age or BMI. Baseline parameters of inflammatory response were higher in patients with endometrial cancer scheduled for LT and RS, possibly insinuating higher tumor load. Interestingly, baseline differences were also evident between EC patients selected for different surgical approaches that may imply an association with constitutive factors like BMI or nutritional status. The differences in tumor burden can explain the baseline differences in CRP and IL-6 concentrations in EC patients scheduled for different surgical approaches. As expected, kynurenine concentrations were also significantly higher in all groups of EC patients compared to controls; however, no difference was observed in urinary neopterin concentrations, probably reflecting the early stage of cancer. Urinary neopterin concentrations are known to be increased in EC patients (9) but, similarly to other tumors, neopterin concentrations increase with stage. In patients with early disease, increased concentrations are observed only in a minority of patients. Moreover, neopterin concentrations in the control group were rather high.

Neopterin is a non-specific biomarker of immune activation and, in addition to cancer, increased neopterin concentrations have been observed in a wide range of non-neoplastic conditions, including acute myocardial infarction (10) or increased age (11). In cancer patients, increased neopterin concentrations are associated with poor prognosis (9). Increased neopterin production has been linked to phenotypic changes of the leukocyte population associated with down-regulation of the immune response (12-14). Metabolism of tryptophan to kynurenine may, theoretically, both inhibit and stimulate tumor growth. Tryptophan depletion induces cytostasis in tumor cells (15) and

Table IV. *Correlations between laboratory and clinical parameters at baseline in patients with endometrial carcinoma (n=109).*

EC (n=109)	Age	BMI	Leuko-cytes -1	Platelets -1	CRP -1	IL-6 -1	Citru-lline -1	Vitamin D-1	Alpha-toco-pherol -1	Retinol -1	Kynure-nine -1	Urinary neopterin/ creatinine ratio-1	Trypto-phan -1	Kynurenine/ Trypto-phan ratio-1
Age														
Corr. Coef.	1.000	0.161	0.093	–0.159	0.314	0.418	0.324	–0.098	–0.087	–0.235	0.276	0.217	–0.167	0.332
Sig.		0.095	0.336	0.098	0.001	<0.0001	0.001	0.313	0.369	0.014	0.004	0.025	0.088	0.0005
BMI														
Corr. Coef.	0.161	1.000	0.142	0.053	0.405	0.452	0.117	–0.208	–0.020	0.101	0.275	0.114	0.072	0.168
Sig.	0.095		0.141	0.588	<0.0001	<0.0001	0.225	0.032	0.835	0.297	0.004	0.243	0.464	0.085
Leukocytes-1														
Corr. Coef.	0.093	0.142	1.000	0.464	0.299	0.281	–0.051	–0.169	0.120	0.096	0.008	–0.030	0.053	0.046
Sig.	0.336	0.141		0.000	0.002	0.003	0.599	0.082	0.213	0.322	0.933	0.762	0.587	0.643
Platelets-1														
Corr. Coef.	–0.159	0.053	0.464	1.000	0.282	0.240	0.018	–0.084	0.150	0.016	0.004	–0.131	0.005	0.014
Sig.	0.098	0.588	0.000		0.003	0.012	0.856	0.392	0.121	0.865	0.967	0.178	0.961	0.889
CRP -1														
Corr. Coef.	0.314	0.405	0.299	0.282	1.000	0.751	0.106	–0.206	0.071	–0.214	0.295	0.294	–0.092	0.326
Sig.	0.001	<0.0001	0.002	0.003		<0.0001	0.272	0.033	0.466	0.026	0.002	0.002	0.351	0.001
IL-6 -1														
Corr. Coef.	0.418	0.452	0.281	0.240	0.751	1.000	0.250	–0.324	0.021	–0.237	0.418	0.389	–0.141	0.497
Sig.	<0.0001	<0.0001	0.003	0.012	<0.0001		0.009	0.001	0.830	0.013	<0.0001	<0.0001	0.151	<0.0001
Citrulline-1														
Corr. Coef.	0.324	0.117	–0.051	0.018	0.106	0.250	1.000	–0.073	0.019	–0.080	0.301	0.092	0.003	0.275
Sig.	0.001	0.225	0.599	0.856	0.272	0.009		0.456	0.845	0.408	0.002	0.347	0.979	0.004
Vitamin D-1														
Corr. Coef.	–0.098	–0.208	–0.169	–0.084	–0.206	–0.324	–0.073	1.000	0.112	0.136	–0.117	–0.229	0.117	–0.214
Sig.	0.313	0.032	0.082	0.392	0.033	0.001	0.456		0.250	0.163	0.230	0.018	0.234	0.028
Alpha-tocopherol-1														
Corr. Coef.	–0.087	–0.020	0.120	0.150	0.071	0.021	0.019	0.112	1.000	0.254	–0.096	–0.031	–0.128	–0.009
Sig.	0.369	0.835	0.213	0.121	0.466	0.830	0.845	0.250		0.008	0.321	0.749	0.189	0.926
Retinol-1														
Corr. Coef.	–0.235	0.101	0.096	0.016	–0.214	–0.237	–0.080	0.136	0.254	1.000	–0.130	–0.236	0.110	–0.179
Sig.	0.014	0.297	0.322	0.865	0.026	0.013	0.408	0.163	0.008		0.182	0.014	0.262	0.067
Kynurenine-1														
Corr. Coef.	0.276	0.275	0.008	0.004	0.295	0.418	0.301	–0.117	–0.096	–0.130	1.000	0.205	0.236	0.709
Sig.	0.004	0.004	0.933	0.967	0.002	<0.0001	0.002	0.230	0.321	0.182		0.034	0.015	<0.0001
Urinary neopterin/ creatinine ratio-1														
Corr. Coef.	0.217	0.114	–0.030	–0.131	0.294	0.389	0.092	–0.229	–0.031	–0.236	0.205	1.000	–0.257	0.359
Sig.	0.025	0.243	0.762	0.178	0.002	<0.0001	0.347	0.018	0.749	0.014	0.034		0.008	0.0002
Tryptophan-1														
Corr. Coef.	–0.167	0.072	0.053	0.005	–0.092	–0.141	0.003	0.117	–0.128	0.110	0.236	–0.257	1.000	–0.438
Sig.	0.088	0.464	0.587	0.961	0.351	0.151	0.979	0.234	0.189	0.262	0.015	0.008		<0.0001
Kynurenine/ Tryptophan ratio-1														
Corr. Coef.	0.332	0.168	0.046	0.014	0.326	0.497	0.275	–0.214	–0.009	–0.179	0.709	0.359	–0.438	1.000
Sig.	0.0005	0.085	0.643	0.889	0.001	<0.0001	0.004	0.028	0.926	0.067	<0.0001	0.0002	<0.0001	

Parameter -1 (Leukocytes-1; Platelets-1, *etc*.) shows sample taken the day before surgery (day -1), as explained in the Materials and Methods section. BMI, body mass index; EC, endometrial cancer patients.

kynurenine has been reported to have antitumor activity at high concentration (16); however, it is now thought that tryptophan metabolism to kynurenine promotes tumor growth through the suppression of the antitumor immune response (17).

As expected, a significant correlation was observed between different biomarkers of inflammatory phenomena. However, in contrast to increasing concentrations of IL-6, CRP and kynurenine/tryptophan ratio, no significant increase was evident in urinary neopterin concentrations.

Table V. *Correlations between laboratory and clinical parameters at baseline in the control group (n=24).*

C (n=24)	Age	BMI	Leuko-cytes -1	Platelets -1	CRP -1	IL-6 -1	Citru-lline -1	Vitamin D-1	Alpha-toco-pherol -1	Retinol -1	Kynure-nine -1	Urinary neopterin/ creatinine ratio-1	Trypto-phan -1	Kynurenine/ Trypto-phan ratio-1
Age														
Corr. Coef.	1.000	–0.390	0.002	–0.065	–0.009	–0.013	–0.203	0.441	0.012	–0.193	0.129	–0.087	–0.101	0.217
Sig.		0.060	0.992	0.764	0.968	0.952	0.342	0.031	0.956	0.367	0.548	0.687	0.640	0.308
BMI														
Corr. Coef.	–0.390	1.000	0.322	0.255	0.596	0.196	–0.016	–0.376	–0.423	0.153	0.387	–0.152	0.366	0.121
Sig.	0.060		0.125	0.228	0.002	0.358	0.941	0.070	0.040	0.476	0.062	0.478	0.079	0.572
Leukocytes-1														
Corr. Coef.	0.002	0.322	1.000	0.039	0.312	0.464	0.249	–0.080	–0.193	0.082	0.325	0.135	0.255	0.020
Sig.	0.992	0.125		0.857	0.138	0.022	0.240	0.710	0.366	0.704	0.121	0.530	0.230	0.926
Platelets-1														
Corr. Coef.	–0.065	0.255	0.039	1.000	0.281	0.140	–0.136	–0.071	–0.152	–0.335	0.479	–0.203	0.247	0.247
Sig.	0.764	0.228	0.857		0.184	0.514	0.525	0.742	0.478	0.109	0.018	0.342	0.245	0.245
CRP-1														
Corr. Coef.	–0.009	0.596	0.312	0.281	1.000	0.602	0.089	–0.072	–0.330	0.139	0.753	0.142	0.369	0.364
Sig.	0.968	0.002	0.138	0.184		0.002	0.681	0.739	0.115	0.516	<0.0001	0.508	0.076	0.080
IL-6-1														
Corr. Coef.	–0.013	0.196	0.464	0.140	0.602	1.000	0.078	–0.172	–0.016	0.055	0.453	0.487	0.125	0.164
Sig.	0.952	0.358	0.022	0.514	0.002		0.717	0.422	0.940	0.800	0.026	0.016	0.560	0.443
Citrulline-1														
Corr. Coef.	–0.203	–0.016	0.249	–0.136	0.089	0.078	1.000	–0.078	0.072	0.068	0.144	–0.112	0.138	0.061
Sig.	0.342	0.941	0.240	0.525	0.681	0.717		0.718	0.737	0.752	0.502	0.603	0.519	0.777
Vitamin D-1														
Corr. Coef.	0.441	–0.376	–0.080	–0.071	–0.072	–0.172	–0.078	1.000	0.271	–0.157	–0.021	–0.013	0.170	–0.126
Sig.	0.031	0.070	0.710	0.742	0.739	0.422	0.718		0.200	0.465	0.923	0.952	0.426	0.557
Alpha-tocopherol-1														
Corr. Coef.	0.012	–0.423	–0.193	–0.152	–0.330	–0.016	0.072	0.271	1.000	0.151	–0.368	0.040	–0.015	–0.403
Sig.	0.956	0.040	0.366	0.478	0.115	0.940	0.737	0.200		0.483	0.077	0.853	0.945	0.051
Retinol-1														
Corr. Coef.	–0.193	0.153	0.082	–0.335	0.139	0.055	0.068	–0.157	0.151	1.000	–0.301	0.240	–0.087	–0.256
Sig.	0.367	0.476	0.704	0.109	0.516	0.800	0.752	0.465	0.483		0.153	0.259	0.687	0.228
Kynurenine-1														
Corr. Coef.	0.129	0.387	0.325	0.479	0.753	0.453	0.144	–0.021	–0.368	–0.301	1.000	0.008	0.357	0.680
Sig.	0.548	0.062	0.121	0.018	<0.0001	0.026	0.502	0.923	0.077	0.153		0.971	0.087	0.0003
Urinary neopterin/ creatinine ratio-1														
Corr. Coef.	–0.087	–0.152	0.135	–0.203	0.142	0.487	–0.112	–0.013	0.040	0.240	0.008	1.000	–0.328	0.160
Sig.	0.687	0.478	0.530	0.342	0.508	0.016	0.603	0.952	0.853	0.259	0.971		0.118	0.455
Tryptophan-1														
Corr. Coef.	–0.101	0.366	0.255	0.247	0.369	0.125	0.138	0.170	–0.015	–0.087	0.357	–0.328	1.000	–0.312
Sig.	0.640	0.079	0.230	0.245	0.076	0.560	0.519	0.426	0.945	0.687	0.087	0.118		0.138
Kynurenine/ Tryptophan ratio-1														
Corr. Coef.	0.217	0.121	0.020	0.247	0.364	0.164	0.061	–0.126	–0.403	–0.256	0.680	0.160	–0.312	1.000
Sig.	0.308	0.572	0.926	0.245	0.080	0.443	0.777	0.557	0.051	0.228	0.0003	0.455	0.138	

Parameter -1 (Leukocytes-1; Platelets-1, *etc*.) shows sample taken the day before surgery (day -1), as explained in the Materials and Methods section. BMI, body mass index; C, control group.

Increased concentrations of biomarkers of inflammation during the postoperative course were associated with decreasing hemoglobin concentrations and rising leukocyte counts. A concept of AUC of biomarkers during the perioperative course was introduced to assess the longitudinal behavior. As expected, the clinical parameters reflective of surgical trauma, specifically blood loss, differed significantly among the surgical approaches. These differences further accentuated differences in biomarkers of inflammatory biomarkers observed at baseline. A significant correlation was observed between blood loss and biomarkers of inflammatory response.

Table VI. *Correlations between AUC of laboratory parameters in patients with endometrial carcinoma (n=109).*

EC (n=109)	Age	BMI	Leuko-cytes	Platelets	CRP	IL-6	Citru-lline	Vitamin D	Alpha-toco-pherol	Retinol	Kynure-nine	Urinary neopterin/creatinine ratio	Trypto-phan	Kynurenine/Tryptophan ratio
Age														
Corr. Coef.	1.000	0.161	0.178	–0.121	0.214	0.316	0.162	–0.096	–0.057	–0.237	0.286	0.408	–0.037	0.253
Sig.		0.095	0.064	0.208	0.025	0.001	0.092	0.321	0.556	0.013	0.003	<0.0001	0.702	0.008
BMI														
Corr. Coef.	0.161	1.000	0.098	–0.040	0.338	0.229	0.008	–0.220	–0.024	0.088	0.299	0.228	–0.044	0.218
Sig.	0.095		0.311	0.682	0.0003	0.016	0.937	0.021	0.804	0.366	0.002	0.017	0.647	0.023
Leukocytes														
Corr. Coef.	0.178	0.098	1.000	0.337	0.319	0.239	–0.127	–0.227	0.036	–0.043	–0.101	0.164	–0.016	–0.081
Sig.	0.064	0.311		0.0003	0.001	0.012	0.190	0.018	0.710	0.654	0.298	0.089	0.869	0.404
Platelets														
Corr. Coef.	–0.121	–0.040	0.337	1.000	0.157	0.034	–0.026	–0.073	0.272	0.040	–0.138	0.025	–0.182	–0.071
Sig.	0.208	0.682	0.0003		0.103	0.727	0.788	0.449	0.004	0.681	0.153	0.796	0.058	0.463
CRP														
Corr. Coef.	0.214	0.338	0.319	0.157	1.000	0.683	–0.254	–0.248	–0.034	–0.120	0.117	0.502	–0.189	0.244
Sig.	0.025	0.0003	0.001	0.103		<0.0001	0.008	0.009	0.728	0.216	0.225	<0.0001	0.049	0.011
IL-6														
Corr. Coef.	0.316	0.229	0.239	0.034	0.683	1.000	–0.081	–0.326	–0.252	–0.310	0.156	0.449	–0.213	0.300
Sig.	0.001	0.016	0.012	0.727	<0.0001		0.405	0.001	0.008	0.001	0.104	<0.0001	0.026	0.002
Citrulline														
Corr. Coef.	0.162	0.008	–0.127	–0.026	–0.254	–0.081	1.000	–0.151	0.045	0.007	0.138	–0.102	–0.110	0.127
Sig.	0.092	0.937	0.190	0.788	0.008	0.405		0.118	0.641	0.944	0.152	0.289	0.254	0.188
Vitamin D														
Corr. Coef.	–0.096	–0.220	–0.227	–0.073	–0.248	–0.326	–0.151	1.000	0.117	0.217	–0.119	–0.196	0.211	–0.275
Sig.	0.321	0.021	0.018	0.449	0.009	0.001	0.118		0.228	0.024	0.219	0.041	0.028	0.004
Alpha-tocopherol														
Corr. Coef.	–0.057	–0.024	0.036	0.272	–0.034	–0.252	0.045	0.117	1.000	0.307	–0.118	–0.078	0.059	–0.147
Sig.	0.556	0.804	0.710	0.004	0.728	0.008	0.641	0.228		0.001	0.221	0.423	0.542	0.128
Retinol														
Corr. Coef.	–0.237	0.088	–0.043	0.040	–0.120	–0.310	0.007	0.217	0.307	1.000	0.001	–0.180	0.146	–0.177
Sig.	0.013	0.366	0.654	0.681	0.216	0.001	0.944	0.024	0.001		0.995	0.061	0.130	0.065
Kynurenine														
Corr. Coef.	0.286	0.299	–0.101	–0.138	0.117	0.156	0.138	–0.119	–0.118	0.001	1.000	0.089	0.154	0.649
Sig.	0.003	0.002	0.298	0.153	0.225	0.104	0.152	0.219	0.221	0.995		0.360	0.111	<0.0001
Urinary neopterin/creatinine ratio														
Corr. Coef.	0.408	0.228	0.164	0.025	0.502	0.449	–0.102	–0.196	–0.078	–0.180	0.089	1.000	–0.401	0.386
Sig.	<0.0001	0.017	0.089	0.796	<0.0001	<0.0001	0.289	0.041	0.423	0.061	0.360		<0.0001	<0.0001
Tryptophan														
Corr. Coef.	–0.037	–0.044	–0.016	–0.182	–0.189	–0.213	–0.110	0.211	0.059	0.146	0.154	–0.401	1.000	–0.460
Sig.	0.702	0.647	0.869	0.058	0.049	0.026	0.254	0.028	0.542	0.130	0.111	<0.0001		<0.0001
Kynurenine/Tryptophan ratio														
Corr. Coef.	0.253	0.218	–0.081	–0.071	0.244	0.300	0.127	–0.275	–0.147	–0.177	0.649	0.386	–0.460	1.000
Sig.	0.008	0.023	0.404	0.463	0.011	0.002	0.188	0.004	0.128	0.065	<0.0001	<0.0001	<0.0001	

EC, Endometrial cancer patients; BMI, body mass index.

A decrease of plasma citrulline concentration, a biomarker of gut mass, was more pronounced in patients undergoing surgery for endometrial carcinoma compared to patients operated for benign disease. The decrease of citrulline in patients undergoing surgery is of significance because citrulline represents a biomarker of gastrointestinal toxicity of chemoradiation in cancer patients (8). Adjuvant therapy in patients with high-risk endometrial carcinoma consists of systemic chemotherapy and radiation; thus, recovery of gut function may be of significance for the planning of postoperative therapy. Surgery was also uniformly associated with a decrease of circulating concentrations of retinol, alpha-tocopherol and vitamin D,

Table VII. *Correlations between AUC of laboratory parameters in controls (n=24).*

C (n=24)	Age	BMI	Leukocytes	Platelets	CRP	IL-6	Citrulline	Vitamin D	Alpha-tocopherol	Retinol	Kynurenine	Urinary neopterin/creatinine ratio	Tryptophan	Kynurenine/Tryptophan ratio
Age														
Corr. Coef.	1.000	–0.390	0.065	–0.017	0.017	0.293	0.141	0.434	0.015	–0.244	0.017	–0.078	–0.225	0.254
Sig.		0.060	0.763	0.936	0.937	0.164	0.512	0.034	0.945	0.251	0.937	0.719	0.290	0.230
BMI														
Corr. Coef.	–0.390	1.000	0.669	0.471	0.117	–0.280	0.137	–0.389	–0.202	0.446	0.070	–0.243	0.321	–0.152
Sig.	0.060		0.0003	0.020	0.586	0.186	0.524	0.060	0.343	0.029	0.745	0.252	0.126	0.478
Leukocytes														
Corr. Coef.	0.065	0.669	1.000	0.423	–0.037	–0.110	0.267	–0.118	–0.130	0.194	0.212	–0.157	0.136	0.040
Sig.	0.763	0.0003		0.039	0.865	0.610	0.208	0.582	0.544	0.364	0.320	0.463	0.527	0.853
Platelets														
Corr. Coef.	–0.017	0.471	0.423	1.000	0.030	–0.201	0.035	–0.158	–0.197	0.066	0.310	–0.245	0.407	–0.091
Sig.	0.936	0.020	0.039		0.891	0.347	0.870	0.460	0.355	0.759	0.140	0.248	0.048	0.671
CRP														
Corr. Coef.	0.017	0.117	–0.037	0.030	1.000	0.655	–0.056	–0.498	–0.236	0.076	0.037	0.141	–0.290	0.216
Sig.	0.937	0.586	0.865	0.891		0.001	0.796	0.013	0.268	0.725	0.865	0.511	0.170	0.312
IL-6														
Corr. Coef.	0.293	–0.280	–0.110	–0.201	0.655	1.000	0.002	–0.366	–0.152	–0.202	0.116	0.405	–0.462	0.415
Sig.	0.164	0.186	0.610	0.347	0.001		0.992	0.079	0.478	0.344	0.590	0.049	0.023	0.044
Citrulline														
Corr. Coef.	0.141	0.137	0.267	0.035	–0.056	0.002	1.000	–0.203	–0.125	0.049	0.395	–0.020	0.020	0.264
Sig.	0.512	0.524	0.208	0.870	0.796	0.992		0.342	0.561	0.821	0.056	0.926	0.926	0.213
Vitamin D														
Corr. Coef.	0.434	–0.389	–0.118	–0.158	–0.498	–0.366	–0.203	1.000	0.319	0.033	–0.053	–0.117	0.026	–0.134
Sig.	0.034	0.060	0.582	0.460	0.013	0.079	0.342		0.129	0.878	0.806	0.585	0.904	0.533
Alpha-tocopherol														
Corr. Coef.	0.015	–0.202	–0.130	–0.197	–0.236	–0.152	–0.125	0.319	1.000	0.317	–0.067	–0.228	0.365	–0.299
Sig.	0.945	0.343	0.544	0.355	0.268	0.478	0.561	0.129		0.131	0.756	0.284	0.079	0.156
Retinol														
Corr. Coef.	–0.244	0.446	0.194	0.066	0.076	–0.202	0.049	0.033	0.317	1.000	0.123	0.035	0.310	–0.028
Sig.	0.251	0.029	0.364	0.759	0.725	0.344	0.821	0.878	0.131		0.565	0.872	0.141	0.897
Kynurenine														
Corr. Coef.	0.017	0.070	0.212	0.310	0.037	0.116	0.395	–0.053	–0.067	0.123	1.000	0.456	0.050	0.555
Sig.	0.937	0.745	0.320	0.140	0.865	0.590	0.056	0.806	0.756	0.565		0.025	0.815	0.005
Urinary neopterin/creatinine ratio														
Corr. Coef.	–0.078	–0.243	–0.157	–0.245	0.141	0.405	–0.020	–0.117	–0.228	0.035	0.456	1.000	–0.337	0.585
Sig.	0.719	0.252	0.463	0.248	0.511	0.049	0.926	0.585	0.284	0.872	0.025		0.108	0.003
Tryptophan														
Corr. Coef.	–0.225	0.321	0.136	0.407	–0.290	–0.462	0.020	0.026	0.365	0.310	0.050	–0.337	1.000	–0.707
Sig.	0.290	0.126	0.527	0.048	0.170	0.023	0.926	0.904	0.079	0.141	0.815	0.108		0.0001
Kynurenine/Tryptophan ratio														
Corr. Coef.	0.254	–0.152	0.040	–0.091	0.216	0.415	0.264	–0.134	–0.299	–0.028	0.555	0.585	–0.707	1.000
Sig.	0.230	0.478	0.853	0.671	0.312	0.044	0.213	0.533	0.156	0.897	0.005	0.003	0.0001	

C, Control group; BMI, body mass index.

possibly reflecting the decreased function of gut mucosa. The decrease of circulating concentrations of antioxidant vitamins is of importance for supportive care.

Neoplastic disorders and antitumor therapy are associated with oxidative stress (18-20). The disorders of antioxidant balance may play a role in the toxicity of anticancer treatment. Vitamin E represents a major antioxidant in the serum (21). Although the term vitamin E denotes several naturally occurring tocopherols and tocotrienols, alpha-tocopherol is responsible for most of the activity of vitamin E (21). Disorders of antioxidant balance, involving alpha-tocopherol, may be implicated in the pathogenesis of the

Table VIII. *Correlation between the clinical indicators of surgical stress and AUC of investigated biomarkers of nutrition and inflammatory response in patients with endometrial cancer.*

EC (n=109)	Blood loss	Nadir hemoglobin concentration	Decrease of hemoglobin concentration	VAS	Number of lymph nodes
Leukocytes					
Corr. Coef.	0.046	0.010	0.036	0.009	–0.074
Sig.	0.635	0.921	0.707	0.929	0.447
Platelets					
Corr. Coef.	–0.065	–0.123	–0.215	0.035	0.005
Sig.	0.502	0.202	0.025	0.716	0.956
CRP					
Corr. Coef.	0.195	–0.177	0.070	0.274	–0.051
Sig.	0.042	0.066	0.468	0.004	0.596
IL-6					
Corr. Coef.	0.219	–0.243	0.091	0.154	–0.074
Sig.	0.022	0.011	0.349	0.109	0.446
Citrulline					
Corr. Coef.	–0.004	–0.036	–0.141	–0.116	–0.053
Sig.	0.965	0.713	0.144	0.229	0.581
Vitamin D					
Corr. Coef.	0.004	0.148	–0.042	–0.184	0.075
Sig.	0.965	0.124	0.667	0.056	0.438
Alpha-tocopherol					
Corr. Coef.	–0.062	0.192	–0.225	–0.147	–0.007
Sig.	0.519	0.045	0.019	0.128	0.945
Retinol					
Corr. Coef.	0.123	0.184	0.024	0.014	–0.057
Sig.	0.201	0.055	0.803	0.887	0.553
Kynurenine					
Corr. Coef.	0.223	0.021	–0.119	0.159	–0.021
Sig.	0.020	0.825	0.217	0.099	0.829
Tryptophan					
Corr. Coef.	0.013	0.263	–0.026	0.045	0.245
Sig.	0.893	0.006	0.788	0.641	0.010
Kynurenine/Tryptophan ratio					
Corr. Coef.	0.174	–0.167	–0.002	0.151	–0.143
Sig.	0.071	0.082	0.982	0.117	0.137
Urinary neopterin/creatinine ratio					
Corr. Coef.	0.118	–0.140	–0.002	0.155	–0.231
Sig.	0.221	0.147	0.984	0.107	0.015

EC, Endometrial cancer patients; VAS, visual analog scale.

toxicity associated with external-beam radiation (22, 23) or chemotherapy (24). In prior studies, a decrease in serum alpha-tocopherol has been observed during the administration of systemic chemotherapy (18, 19, 25, 26). Decrease of serum retinol has also been reported in cancer patients and a correlation between the decrease of alpha-tocopherol and retinol, as well as biomarkers of systemic inflammatory response, *e.g*. CRP, has been observed (27, 28).

In conclusion, the present data demonstrate a differential response to surgical trauma in patients with endometrial carcinoma.

Acknowledgements

This project was supported by research grant IGA MH CR NT13566-4.

References

1 Siegel R, Ma J, Zou Z and Jemal A: Cancer statistics, 2014. CA Cancer J Clin *64*: 9-29, 2014.

2 Childers JM, Brzechffa PR, Hatch KD and Surwit EA: Laparoscopically assisted surgical staging (LASS) of endometrial cancer. Gynecol Oncol *51*: 33-38, 1993.

3 Kornblith AB, Huang HQ, Walker JL, Spirtos NM, Rotmensch J and Cella D: Quality of life of patients with endometrial cancer undergoing laparoscopic international federation of gynecology and obstetrics staging compared with laparotomy: a Gynecologic Oncology Group study. J Clin Oncol *27*: 5337-5342, 2009.
4 Marek R, Dzvincuk P, Kudela M, Hambalek P, Maderka M, Zapletalova J and Pilka R: Robot assisted endometrial cancer staging - evaluation the first 100 operations and comparing the first andthe last 30 operations. Ceska Gynekol *80*: 324-332, 2015.
5 Yim GW and Kim YT: Robotic surgery in gynecologic cancer. Curr Opin Obstet Gynecol *24*: 14-23, 2012.
6 Urbanek L, Solichova D, Melichar B, Dvorak J, Svobodova I and Solich P: Optimization and validation of a high performance liquid chromatography method for the simultaneous determination of vitamins A and E in human serum using monolithic column and diode-array detection. Anal Chim Acta *573-574*: 267-272, 2006.
7 Solichova D, Korecka L, Svobodova I, Musil F, Blaha V, Zdansky P and Zadak Z: Development and validation of HPLC method for the determination of alpha-tocopherol in human erythrocytes for clinical applications. Analytical and Bioanalytical Chemistry *376*: 444-447, 2003.
8 Zezulova M, Bartouskova M, Hlidkova E, Adam T, Krcmova LK, Cervinkova B, Solichova D, Zlevorova M, Cwiertka K, Friedecky D, Vrana D and Melichar B: Citrulline as a biomarker of gastrointestinal toxicity in patients with rectal carcinoma treated with chemoradiation. Clin Chem Lab Med *54*: 305-314, 2016.
9 Melichar B, Solichová D and Freedman RS: Neopterin as an indicator of immune activation and prognosis in patients with gynecological malignancies. International Journal of Gynecological Cancer *16*: 240-252, 2006.
10 Melichar B, Gregor J, Solichova D, Lukes J, Tichy M and Pidrman V: Increased urinary neopterin in acute myocardial infarction. Clinical Chemistry *40*: 338-339, 1994.
11 Solichova D, Juraskova B, Blaha V, Bratova M, Kusalova M, Zdansky P and Zadak Z: Bioanalysis of age-related changes of lipid metabolism in nonagenarians. Journal of Pharmaceutical and Biomedical Analysis *24*: 1157-1162, 2001.
12 Melichar B, Solichova D and Jebavy L: Elevation de la neopterine apres traitement par le facteur stimulant des colonies granulo-macrophagiques (GM-CSF). Presse Medicale *23*: 581, 1994.
13 Melichar B, Templin SM, Tomasovic BJ, Kudelka AP, Kavanagh JJ, Edwards CL, Platsoucas CD and Freedman RS: Effects of intraperitoneal injections of interferon-gamma and interleukin-2 on peritoneal monocytes and dendritic cells. Proceedings of the Americal Asssociation for Cancer Research *38*: 631-632, 1997.
14 Melichar B, Touskova M, Dvorak J, Jandik P and Kopecky O: The peripheral blood leukocyte phenotype in patients with breast cancer: Effect of doxorubicin/paclitaxel combination chemotherapy. Immunopharmacology and Immunotoxicology *23*: 163-173, 2001.
15 Burke F, Knowles RG, East N and Balkwill FR: The role of indoleamine 2,3-dioxygenase in the anti-tumour activity of human interferon-gama *in vivo*. International Journal of Cancer *60*: 115-122, 1995.
16 Melichar B, Ferrandina G, Verschraegen CF, Loercher A, Abbruzzese JL and Freedman RS: Growth inhibitory effects of aromatic fatty acids on ovarian tumor cell lines. Clinical Cancer Research *4*: 3069-3076, 1998.
17 Friberg M, Jennings R, Alsarraj M, Dessureault S, Cantor A, Extermann M, Mellor AL, Munn DH and Antonia SJ: Indoleamin 2,3-dioxygenase contributes to tumor cell evasion of T cell-mediated rejection. International Journal of Cancer *101*: 151-155, 2002.
18 Faber M, Coudray C, Hida H, Mousseau M and Favier A: Lipid peroxidation products, and vitamin and trace element status in patients with cancer before and after chemotherapy, including adriamycin. A preliminary study. Biological Trace Element Research *47*: 117-123, 1995.
19 Faure H, Coudray C, Mousseau M, Ducros V, Douki T, Bianchini F, Cadet J and Favier A: 5-Hydroxymethyluracil excretion, plasma TBARS and plasma antioxidant vitamins in adriamycin-treated patients. Free Radical Biology and Medicine *20*: 979-983, 1996.
20 Weijl NI, Hopman GD, Wipkink-Bakker A, Lentjes EGWM, Berger HM, Cleton FJ and Osanto S: Cisplatin combination chemotherapy induces a fall in plasma antioxidants of cancer patients. Annals of Oncology *9*: 1331-1337, 1998.
21 Debier C and Larondelle Y: Vitamins A and E: metabolism, roles and transfer to offspring. British Journal of Nutrition *93*: 153-174, 2005.
22 Delanian S, Porcher R, Balla-Mekias S and Lefaix JL: Randomized, placebo-controlled trial of combined pentoxifylline and tocopherol for regression of superficial radiation-induced fibrosis. Journal of Clinical Oncology *21*: 2545-2550, 2003.
23 Erhola M, Nieminen MM and Ojala A: Human plasma antioxidant capacity during radiotherapy for lung cancer: a clinical study. Journal of Experimental and Clinical Cancer Research *17*: 325-330, 1998.
24 Pace A, Savarese A, Picardo M, Maresca V, Pacetti U, Del Monte G, Biroccio A, Leonetti C, Jandolo B, Cognetti F and Bove L. Neuroprotective effect of vitamin E supplementation in patients treated with cisplatin chemotherapy. Journal of Clinical Oncology *21*: 927-931, 2003.
25 High KP, Legault C, Sinclair JA, Cruz J, Hill K and Hurd DD. Low plasma concentrations of retinol and alpha-tocopherol in hematopoietic stem cell transplant recipients: the effect of mucositis and the risk of infection. American Journal of Clinical Nutrition *76*: 1358-1366, 2002.
26 Jonas RC, Puckett AB, Jones DP, Griffith DP, Szeszycki EE, Bergman GF, Furr CE, Tyre C, Carlson JL, Galloway JR, Blumberg JB and Ziegler TR. Plasma antioxidant status after high-dose chemotherapy: a randomized trial of parenteral nutrition in bone marrow transplantation patients. American Journal of Clinical Nutrition *72*: 181-189, 2000.
27 Mayland C, Allen KR, Degg TJ and Bennet M. Micronutrient concentrations in patients with malignant disease: effect of the inflammatory response. Annals of Clinical Biochemistry *41*: 138-141, 2004.
28 McMillan DC, Talwar D, Sattar N, Underwood M, O Reilly DSJ and McArdle C. The relationship between reduced vitamin antioxidant concentrations and the systemic inflammatory response in patients with common solid tumours. Clinical Nutrition *21*: 161-164, 2002.

Received March 10, 2016
Revised April 15, 2016
Accepted April 19, 2016