ANTICANCER RESEARCH *42*: 5521-5526 (2022)
doi:10.21873/anticanres.16057

Interleukin-18 (IL-18) Cytokine Serum Concentrations Correlate With Pain Scores and the Number of Analgesic Doses Following Surgery

MAARET ESKELINEN[1], IINA SAIMANEN[1], TUOMAS SELANDER[2], ANU HOLOPAINEN[3], SAMULI ASPINEN[1], ESA HÄMÄLÄINEN[3] and MATTI ESKELINEN[1]

[1]Department of Surgery, and [2]Science Service Center, Kuopio University Hospital (KUH) and School of Medicine, University of Eastern Finland (UEF), Kuopio, Finland; [3]Department of Clinical Chemistry, UEF and Eastern Finland Laboratory Centre (ISLAB), KUH, Kuopio, Finland

Abstract. *Background/Aim: Anti- and proinflammatory cytokines and plasma high-sensitivity C-reactive protein (hs-CRP) are used to assess inflammatory stress response (ISR) following surgery. However, the serum IL-18 (interleukin-18) cytokine values versus numeric rating scale (NRS) pain score and number of analgesic doses (NAD) postoperatively are unknown. Patients and Methods: Blood levels of six interleukins (IL-18, IL-1ra, IL-6, IL-10, IL-1β, and IL-8) and hs-CRP were measured at three time points; before operation (PRE), immediately after operation (POP1), and six hours after operation (POP2) in 114 patients with cholelithiasis. Results: Following surgery, the blood levels of hs-CRP and IL-1ra, IL-6, IL-10, and IL-1β cytokines had a trend for increase ($p<0.001$ and $p=0.014$, respectively). The serum IL-18 concentrations inversely correlated to NRS and NAD during the first 24 h postoperatively. Conclusion: The correlation of IL-18 levels to NRS and NAD values supports the hypothesis that ISR and pain are related.*

Cytokines (CYTs) are cell-signaling molecules that aid cell-to-cell communication and enhance the movement of human cells towards sites of trauma, and inflammation (1). About 200 different CYTs has been reported, with multiple functions in regulating acute phase response (APR), steering cell growth/differentiation, activating antimicrobial defense and regulating homeostasis. CYTs especially mediate innate immunity and assist in the development of adaptive immunity regulating white cell growth and differentiation. Interleukin-18 (IL-18) is a member of the IL-1 family of CYTs (1-3). IL-18 is also known as interferon-gamma inducing factor (IGFN-γ), which in humans is encoded by the IL18-gene (2, 3). IL-18 can modulate both innate and adaptive immunity and its dysregulation can cause autoimmune or inflammatory diseases (2, 3). IL-18 has also been found to increase amyloid-beta production in human neurons in patients with Alzheimer's disease (4). Zhang *et al*. (5) suggested IL-18 as a biomarker of early diabetic nephropathy in patients with type 2 diabetes associated with enhanced urine protein excretion. APR proteins and CYTs are thought to be early measures of inflammatory stress response (ISR) induced by trauma. There is also evidence suggesting that ISR and pain are related (6). Aspinen *et al*. (7) reported in 2016 that the ISR in patients with minilaparotomy cholecystectomy (MC) *versus* laparoscopic cholecystectomy (LC) was equal based on the interleukin (IL)-8, IL-10 and IL-1β values. Interestingly, Purdy *et al*. (6) found a significant correlation in the NRS pain scores *versus* the values of anti-inflammatory CYT IL-10 and pro-inflammatory CYT IL-1β suggesting that ISR and pain are related in midline laparotomy patients. However, the correlation of IL-18 and the ISR in patients with LC *versus* MC is unknown. The aim of this study was to assess the association between the ISR biomarkers and patients' pain experience and number of analgesic doses (NAD) following surgery.

Correspondence to: Matti Eskelinen, MD, School of Medicine, UEF, P.O. Box 100, FI-70029 KYS, Finland. Tel: +358 17173311, Fax: +358 17172611, GSM: +358 400969444, e-mail: matti.eskelinen@kuh.fi

Key Words: Cholelithiasis, surgery, IL-18, NRS pain score, analgesic doses.

Patients and Methods

The study was approved by the Ethics Committee of Kuopio University Hospital District, Kuopio, Finland (DNRO 27/02/2013), it was registered in the ClinicalTrials.gov database (ClinicalTrials.gov Identifier: NCT01723540, Consort diagram, Figure 1), and was conducted in accordance with the Declaration of Helsinki. The study protocol and inclusion/exclusion criteria of the study patients are detailed in previous reports by Saimanen *et al*. (8, 9).

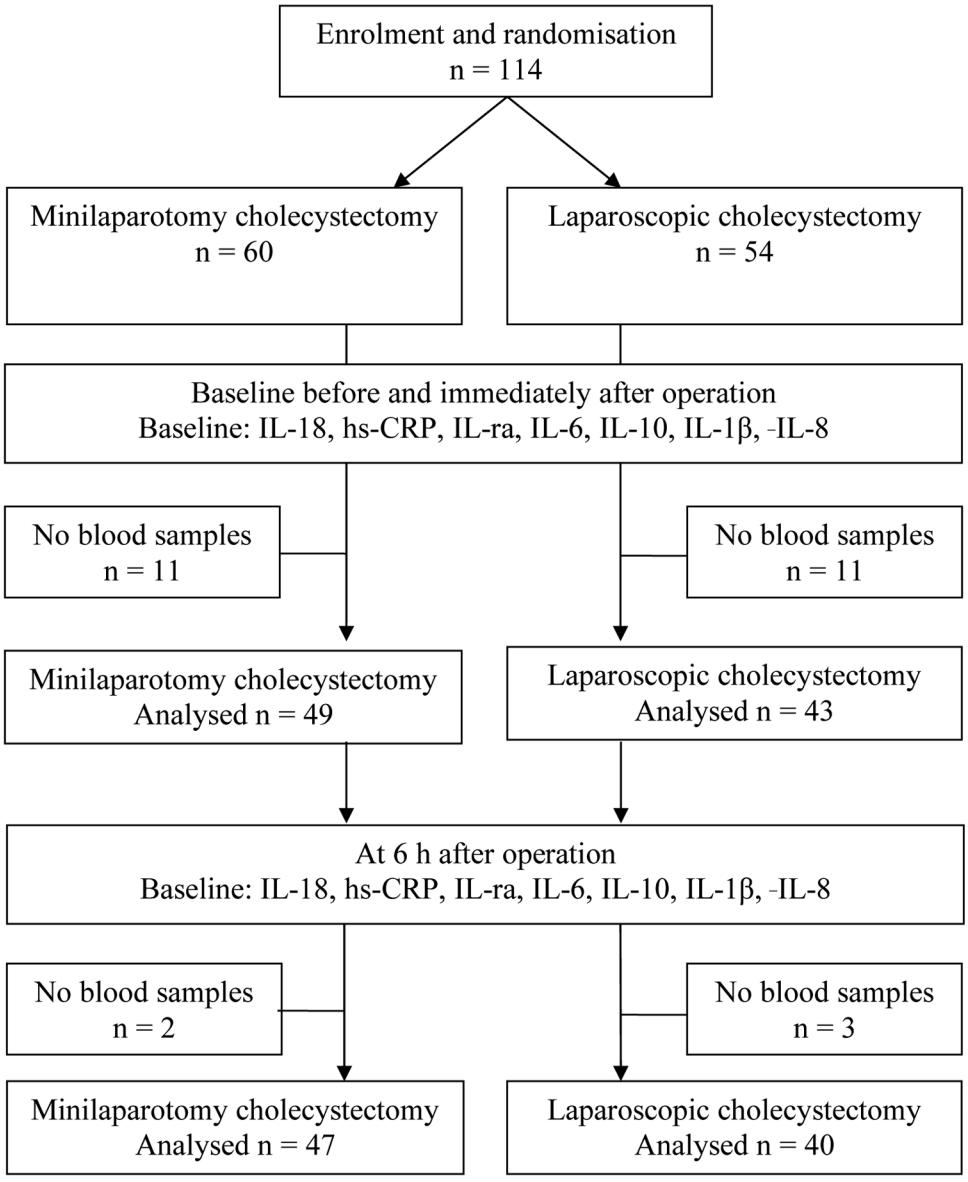

Figure 1. *Study flowchart*.

The blood samples were taken before surgery (PRE), immediately after surgery (POP1) and 6 h post-operatively (POP2) and centrifuged at 1,000 × *g* (2,900 rpm) for 15 min. The interleukin assays were performed using ELISA methods from R&D Systems (Minneapolis, MN, USA). The manufacturer's intra-assay and the inter-assay coefficient of variations for IL-18 were 2.5-3.1 % and 7.9-8.7%, respectively. The sensitivity of the other CYT assays were detailed in previous reports by Aspinen *et al*. (7). Plasma high-sensitivity C-reactive protein (hs-CRP) was analyzed with a Cobas 6000-analyzer (Roche Diagnostics, Penzberg, Germany).

Data are presented as means and standard deviations or frequencies and percentages, in Table I. In Table II and Table III, the results of the laboratory measurements are presented as median concentrations with interquartile range as distributions were right-skewed. Differences in baseline characteristics between groups were tested by the Fisher's exact test and in the case of continuous data, the analysis was performed using *t*-test. The blood CYT concentrations and differences in MC and LC groups of patients were tested by the Mann–Whitney *U*-test. The alterations between time points were tested by the Wilcoxon signed rank test. In the linear mixed effect (LME) analysis interleukin and hs-CRP blood concentration were log transformed. The Pearson's method was used to test for correlation of IL-18 concentrations to NRS and NDA values (Figure 2 and Figure 3). Data were analyzed using the IBM SPSS statistical software (IBM SPSS Statistics for Windows, version 26.0, IBM Corporation Armonk, NY, USA).

Table I. *Clinical data. Data are mean (standard deviation) or number of cases.*

Variable	Minilaparotomy n=60	Laparoscopy n=54	*p*-Value
Age, years	50.7 (13.2)	53.2 (13.1)	0.316
Sex male/female	11/49	18/36	0.066
Height, cm	167.4 (7.6)	168.9 (9.9)	0.355
Weight, kg	77.3 (14.4)	83.0 (17.4)	0.057
BMI, kg/m^2	27.6 (4.4)	29.1 (5.6)	0.111
Operative time, min	70.0 (26.9)	70.1 (35.3)	0.976
Time in the operative room, min	120.4 (28.5)	127.1 (35.8)	0.287
Perioperative bleed, ml	41 (59)	31 (39)	0.290
Conversion rate, n	3	3	1.000*
Length of the skin incision(s), mm	49.7 (12.1)	78.3 (22.7)	<0.001

BMI: Body mass index. *t*-test and *Fisher's exact test were used.

Table II. *Changes in blood levels of hsCRP and different cytokines were measured before operation (PRE), immediately after operation (POP1) and 6 h after operation (POP2) in minilaparotomy (MC) and laparoscopic cholecystectomy (LC). Values are median (interquartile range). Mann–Whitney U-test and linear mixed model was used.*

Marker	MC	LC	*p*-Value
IL-18 (pg/ml)			**0.088**
PRE	149 (114-193)	167 (124-244)	0.151
POP1	149 (116-182)	163 (133-219)	0.131
POP2	155 (111-191)	181 (148-242)	0.034
hs-CRP (mg/ml)			**0.033**
PRE	1.87 (0.95-3.35)	2.05 (0.63-3.98)	0.799
POP1	1.95 (0.88-3.10)	1.95 (0.70-3.70)	0.820
POP2	4.10 (2.05-6.35)	2.80 (1.28-4.88)	0.162
IL-1ra (pg/ml)			**0.042**
PRE	258 (221-368)	310 (202-691)	0.369
POP1	366 (251-511)	435 (241-643)	0.482
POP2	460 (330-987)	462 (233-596)	0.184
IL-6 (pg/ml)			**0.080**
PRE	3.11 (3.11-3.15)	3.11 (3.11-3.59)	0.802
POP1	14.1 (5.04-23.5)	7.42 (4.77-10.9)	0.027
POP2	17.5 (8.80-35.7)	8.61 (7.31-19.5)	0.015
IL-10 (pg/ml)			**0.414**
PRE	0.77 (0.77-0.77)	0.77 (0.77-0.77)	0.691
POP1	1.19 (0.77-3.38)	1.76 (0.77-4.42)	0.505
POP2	0.77 (0.77-1.54)	0.77 (0.77-0.77)	0.177
IL-1β (pg/ml)			**0.813**
PRE	0.16 (0.12-0.34)	0.12 (0.12-0.31)	0.698
POP1	0.12 (0.12-0.25)	0.12 (0.12-0.23)	0.729
POP2	0.12 (0.12-0.17)	0.12 (0.12-0.17)	0.608
IL-8 (pg/ml)			**0.065**
PRE	5.06 (3.75-6.02)	6.24 (4.64-8.08)	0.005
POP1	4.74 (3.59-7.94)	5.76 (4.60-8.31)	0.258
POP2	4.97 (3.78-8.12)	5.85 (4.58-8.27)	0.218

Linear mixed model *p*-values for interaction time between MC and LC groups are in bold.

Table III. *The postoperative alteration in blood levels of hsCRP and different cytokines measured in the groups combined (all patients). Plasma levels were measured before operation (PRE), immediately after operation (POP1) and 6 h after operation (POP2). Median (interquartile range) values are shown. The Wilcoxon signed rank test and linear mixed model was used.*

Marker	All patients	Alteration	*p*-Value
IL-18 (pg/ml)			**0.061**
PRE	155 (119-217)	PRE *vs*. POP1	0.068
POP1	154 (121-204)	POP1 *vs*. POP2	0.034
POP2	166 (129-222)		
hs-CRP (mg/ml)			**<0.001**
PRE	1.95 (0.78-3.63)	PRE *vs*. POP1	0.104
POP1	1.95 (0.80-3.43)	POP1 *vs*. POP2	<0.001
POP2	3.50 (1.70-5.80)		
IL-1ra (pg/ml)			**<0.001**
PRE	289 (210-427)	PRE *vs*. POP1	<0.001
POP1	384 (250-556)	POP1 *vs*. POP2	0.024
POP2	460 (292-815)		
IL-6 (pg/ml)			**<0.001**
PRE	3.11 (3.11-3.51)	PRE *vs*. POP1	<0.001
POP1	9.31 (4.88-17.9)	POP1 *vs*. POP2	<0.001
POP2	11.2 (7.62-27.2)		
IL-10 (pg/ml)			**<0.001**
PRE	0.77 (0.77-0.77)	PRE *vs*. POP1	<0.001
POP1	1.69 (0.77-3.61)	POP1 *vs*. POP2	<0.001
POP2	0.77 (0.77-0.98)		
IL-1β (pg/ml)			**0.014**
PRE	0.13 (0.12-0.33)	PRE *vs*. POP1	0.660
POP1	0.12 (0.12-0.24)	POP1 *vs*. POP2	0.210
POP2	0.12 (0.12-0.17)		
IL-8 (pg/ml)			**0.076**
PRE	5.47 (4.30-6.74)	PRE *vs*. POP1	0.077
POP1	5.67 (4.29-8.20)	POP1 *vs*. POP2	0.863
POP2	5.50 (4.17-8.16)		

Linear mixed model *p*-values for time effect between different time points are in bold.

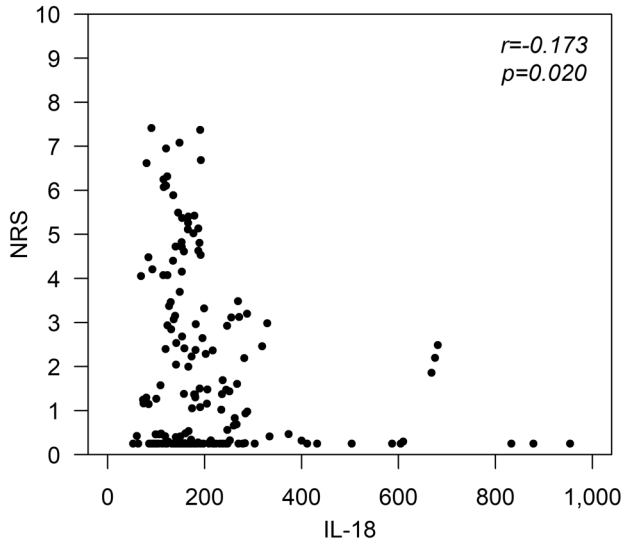

Figure 2. *Scatter plots of the blood IL-18 concentration versus pain assessed using a 11-point numeric rating scale (NRS; 0=no pain; 10=most pain) at 24 h postoperatively (NRS) (r=–0.173, p=0.020).*

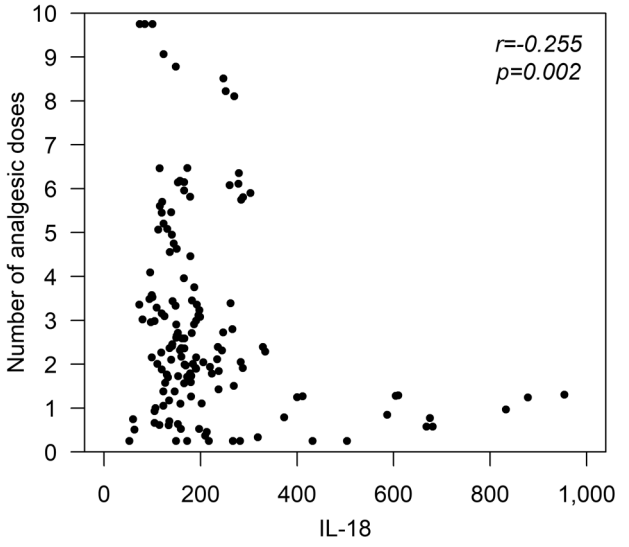

Figure 3. *Scatter plots of blood IL-18 concentration versus number of analgesic doses (NAD) during 24 h postoperatively (r=–0.255, p=0.002).*

Results

The basic patient data of the study. In the MC group there were 60 patients (49 females and 11 males) *versus* 54 patients in the LC group (36 females and 18 males, Figure 1), with the mean (SD) age of 50.7 (13.2) years *versus* 53.2 (13.1) years. There were no significant differences between the MC and LC study groups in mean age, mean height (167.4 and 168.9), mean weight (77.3 and 83.0), mean body mass index (BMI, 27.6 and 29.1), operative time (70 and 70.1 min), time in the operative room (120.4 and 127.1 min), perioperative bleed (41 and 31 ml) or conversion rate (3 and 3). Interestingly, the surgical incision was significantly shorter in the MC than in the LC group (49.7 mm and 78.3 mm, $p<0.001$, Table I).

The APR in MC versus LC patients. The median biomarker concentrations between MC and LC groups were equal. Changes in blood hs-CRP and interleukin concentrations preoperatively and following surgery in different time points between MC and LC patients are shown in Table II. IL-18, hs-CRP, IL-1ra, IL-6, and IL-10 had a trend for increase following surgery in MC and LC patients and the LME interaction time between MC and LC groups was statistically significantly different for hs-CRP and IL-1ra biomarkers ($p=0.033$ and $p=0.042$, respectively, Table II). In all patients, the LME time-effect between different time points was statistically significantly different for hs-CRP, IL-1ra, IL-6, IL-10 and IL-1β biomarkers ($p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$ and $p=0.014$, respectively, Table III).

The IL-18 cytokine versus NRS and NAD. IL-18 concentrations correlated significantly to IL-1ra concentrations (r=0.153, $p=0.03$). However, there were no significant correlations in Pearson's method in between IL-18 concentrations and hs-CRP, IL-ra, IL-6, IL-10, and IL-1β concentrations. There was a significant inverse correlation between IL-18 concentrations and NRS pain scores during the first 24 h following surgery (r=–0.173, $p=0.020$, Figure 2) and a significant inverse correlation between the median IL-18 concentrations and NAD in all patients (r=–0.255, $p=0.002$, Figure 3).

Discussion

The pain following surgery is the main negative effect associated with surgical procedure. Postoperative pain after cholecystectomy derives from irritation of multiple origins: the peritoneum, abdominal wall, and abdominal viscera. The modern treatment of pain following surgery includes regional pain blocks, paracetamol, nonsteroidal anti-inflammatory drugs (NSAIDs), and opioid analgesics (6). In addition, enhanced recovery after surgery protocols (ERAS) are increasingly used in surgical patients and these programs warrant effective pain treatment that promote early mobilization of the patient (10). It is evident that appropriate pain control is necessary as cholecystectomy decreases pulmonary function by as much as 30% even when effective pain management is administered (11). Future goals include the development of peripherally restricted opioid agonists, selective targeting of opioid-containing immune cells to sites

of trauma, and the augmentation of peripheral ligand and receptor synthesis. The main aim is to avoid harmful side effects of available opioid analgesics such as respiratory depression, cognitive impairment, addiction, gastrointestinal bleeding, and thromboembolic complications (12).

Presently, there is evidence suggesting that ISR and pain are related. Purdy *et al*. (6) found a correlation between NRS pain scores and blood values of anti-inflammatory IL-10 and pro-inflammatory IL-1β, suggesting that ISR and pain are related in midline laparotomy patients. However, the correlation of IL-18 and ISR in patients with LC or MC is unknown. The aim of our study was to assess the correlation between the ISR and patients' pain experience and NAD. The present study showed that the ISR in LC and MC groups was quite similar based on the blood levels of IL-1ra and IL-10, an anti-inflammatory CYT preventing tissue damage caused by ISR and IL-1β, a proinflammatory acute-phase protein. A new finding in the present work is the slightly higher relative elevation in the mean concentrations of IL-18 and IL-8 postoperatively in the LC group. Although, the median concentrations of hs-CRP did increase in the LC group, the plasma levels of hs-CRP were doubled in the MC group at six hours following surgery compared to baseline. In addition, the LME *p*-values for interaction time between the MC and LC study groups in hs-CRP and IL-1ra biomarkers were statistically significant. As the median values of IL-1ra are several folds higher than other CYTs, even quite small relative alterations in median values could be reliably detected. Plasma levels of Il-6, a proinflammatory CYT, were increased in both groups, but the increase was 6-fold in the MC group compared to 3-fold increase in the LC group at six hours after surgery. The blood levels of hs-CRP and six interleukins (IL-18, IL-1ra, IL-6, IL-8, IL-10, IL-1β) measured at three time points; before operation (PRE), immediately after operation (POP1) and six hours after operation (POP2) were log-transformed to avoid showing right skewed values (Table II).

The hypothesis that the ISR and pain could associate is based mostly on suggested interactions between opioid ligands and receptors. Inflammation activates the opioid receptors on peripheral sensory neurons and leads to local production of endogenous opioid substances (1-3, 12). These findings have interesting implications for the development of safer analgesics, where modern treatment can communicate with peripheral sensory neurons to modulate pain. Future strategies take in to account that medication should be particularly active in local pathology rather than producing a suppression of the central nervous system, as with classical opioid analgesics. It is possible that in the future, specific CYTs or antagonists will be developed to act as blockers of the hyperexcitability in the sensory neurons (12, 13). Interestingly, Palat *et al*. found (13), that blockade of IL-18 signaling diminished neuropathic pain and enhanced the efficacy of the opioid analgesics morphine and buprenorphine.

In conclusion, our results suggest that the ISR for cholecystectomy in MC and LC patients was quite equal based on the interleukin and hs-CRP values. A novel finding in the present study is that IL-18, a member of the IL-1 family of CYTs, correlates to NRS pain scores and NAD in cholecystectomy patients. The correlation in IL-18 levels to NRS and NAD values supports the hypothesis that the ISR and pain are related.

Conflicts of Interest

The Authors report no conflicts of interest or financial ties to disclose.

Authors' Contributions

All Authors contributed to the collection and analysis of data, drafting and revising the manuscript, read and approved the final article.

Acknowledgements

The study was funded by the Päivikki and Sakari Sohlberg Foundation, Helsinki, Finland.

References

1 Dinarello CA: Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. Blood *117(14)*: 3720-3732, 2011. PMID: 21304099. DOI: 10.1182/blood-2010-07-273417

2 Dinarello CA, Novick D, Kim S and Kaplanski G: Interleukin-18 and IL-18 binding protein. Front Immunol *4*: 289, 2013. PMID: 24115947. DOI: 10.3389/fimmu.2013.00289

3 Kaplanski G: Interleukin-18: Biological properties and role in disease pathogenesis. Immunol Rev *281(1)*: 138-153, 2018. PMID: 29247988. DOI: 10.1111/imr.12616

4 Sutinen EM, Pirttilä T, Anderson G, Salminen A and Ojala JO: Pro-inflammatory interleukin-18 increases Alzheimer's disease-associated amyloid-β production in human neuron-like cells. J Neuroinflammation *9*: 199, 2012. PMID: 22898493. DOI: 10.1186/1742-2094-9-199

5 Zhang D, Ye S and Pan T: The role of serum and urinary biomarkers in the diagnosis of early diabetic nephropathy in patients with type 2 diabetes. PeerJ *7*: e7079, 2019. PMID: 31218128. DOI: 10.7717/peerj.7079

6 Purdy M, Kokki M, Anttila M, Aspinen S, Juvonen P, Korhonen R, Selander T, Kokki H and Eskelinen M: Does the rectus sheath block analgesia reduce the inflammatory response biomarkers' IL-1ra, IL-6, IL-8, IL-10 and IL-1β concentrations following surgery? A randomized clinical trial of patients with cancer and benign disease. Anticancer Res *36(6)*: 3005-3011, 2016. PMID: 27272818.

7 Aspinen S, Kinnunen M, Harju J, Juvonen P, Selander T, Holopainen A, Kokki H, Pulkki K and Eskelinen M: Inflammatory response to surgical trauma in patients with minilaparotomy cholecystectomy *versus* laparoscopic cholecystectomy: a randomised multicentre study. Scand J Gastroenterol *51(6)*: 739-744, 2016. PMID: 26758677. DOI: 10.3109/00365521.2015.1129436

8 Saimanen I, Rahkola D, Kuosmanen V, Kärkkäinen J, Selander T, Holopainen A, Aspinen S and Eskelinen M: Nitrotyrosine (NT), a nitrosative stress biomarker, plasma concentrations in gallstone disease and cancer patients. Anticancer Res *39(2)*: 809-814, 2019. PMID: 30711961. DOI: 10.21873/anticanres.13179

9 Saimanen I, Kuosmanen V, Rahkola D, Selander T, Kärkkäinen J, Harju J, Aspinen S and Eskelinen M: RAND-36-item health survey: a comprehensive test for long-term outcome and health status following surgery. Anticancer Res *39(6)*: 2927-2933, 2019. PMID: 31177131. DOI: 10.21873/anticanres.13422

10 Muallem MZ, Dimitrova D, Pietzner K, Richter R, Feldheiser A, Scharfe I, Schmeil I, Hösl TM, Mustea A, Wimberger P, Burges A, Kimmig R and Sehouli J: Implementation of Enhanced Recovery After Surgery (ERAS) pathways in gynecologic oncology. A NOGGO-AGO* survey of 144 Gynecological Departments in Germany. Anticancer Res *36(8)*: 4227-4232, 2016. PMID: 27466536.

11 Hendolin HI, Pääkönen ME, Alhava EM, Tarvainen R, Kemppinen T and Lahtinen P: Laparoscopic or open cholecystectomy: a prospective randomised trial to compare postoperative pain, pulmonary function, and stress response. Eur J Surg *166(5)*: 394-399, 2000. PMID: 10881952. DOI: 10.1080/110241500750008961

12 Stein C and Machelska H: Modulation of peripheral sensory neurons by the immune system: implications for pain therapy. Pharmacol Rev *63(4)*: 860-881, 2011. PMID: 21969325. DOI: 10.1124/pr.110.003145

13 Pilat D, Piotrowska A, Rojewska E, Jurga A, Ślusarczyk J, Makuch W, Basta-Kaim A, Przewlocka B and Mika J: Blockade of IL-18 signaling diminished neuropathic pain and enhanced the efficacy of morphine and buprenorphine. Mol Cell Neurosci *71*: 114-124, 2016. PMID: 26763728. DOI: 10.1016/j.mcn.2015.12.013

Received August 12, 2022
Revised August 30, 2022
Accepted September 5, 2022