Original Article

Alarmins and Related Molecules in Elective Surgery

(sRAGE / EN-RAGE / calprotectin / HMGB1 / IL-6 / elective surgery)

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Abstract. Surgery is associated with alterations of alarmins' and related molecules' levels. The aim of this study was to investigate which biomarkers are most involved in surgery. The studied group consisted of 58 patients with inguinal or umbilical hernia or cholecystolithiasis and 21 healthy controls for comparison. We also added seven acute patients with appendicitis, cholecystitis and incarcerated hernia. Serum concentrations of soluble receptor of advanced glycation end-products (sRAGE), extracellular newly identified receptor for advanced glycation end-products binding protein (EN-RAGE), calprotectin, high mobility group box 1 (HMGB1) and interleukin 6 (IL-6) were analysed by ELISA before and after surgery. Preoperative concentrations of calprotectin were significantly decreased while concentrations of sRAGE were significantly increased in patients compared to controls; the concentrations of EN-RAGE and HMGB1 did not differ significantly. IL-6 levels were undetectable in elective patients preoperatively and in controls. Postoperatively, there was a significant increase of EN-RAGE, calprotectin, HMGB1, and IL-6 and a significant decrease of sRAGE compared to preoperative levels. In acute patients, all tested molecules except for sRAGE were significantly increased preoperatively, and sRAGE was significantly decreased. In contrast, after surgery, we could observe a further increase in IL-6; the other biomarkers did not differ significantly. We can conclude that the concentrations of all tested biomarkers are significantly influenced by elective surgery. The postoperative levels of all tested molecules increase except for sRAGE, whose level is significantly decreased after surgery. In acute states, these molecules are already increased, and the influence of surgery is, apart from IL-6, insignificant.

Introduction

Our study investigated the levels of alarmins and related molecules in patients with cholecystolithiasis and hernias before and after surgery. Alarmins are damage-associated molecular pattern molecules, which play a key role in inflammation. We focused especially on extracellular newly identified receptor for advanced glycation end-products binding protein (EN-RAGE), calprotectin and high mobility group box 1 (HMGB1). Alarmins bind on pattern recognition receptors; one member of the group is receptor of advanced glycation end-products (RAGE), whose soluble part is designated sRAGE (soluble RAGE). The activation of the signalling pathway leads to production of pro-inflammatory cytokines, and among them interleukin 6 (IL-6). Alarmins can also be described as endogenous proteins that are associated with cell damage or modulation of the immune reaction (Yang et al., 2017).

sRAGE is a soluble receptor of advanced glycation end-products. RAGE is a multiligand membrane receptor that stimulates synthesis of inflammatory factors and

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Abbreviations: ALT – alanine aminotransferase, AST – aspartate aminotransferase, CRP – C-reactive protein, EIA – enzyme immunoassay, ELISA – enzyme-linked immunosorbent assay, EN-RAGE – extracellular newly identified receptor for advanced glycation end-products binding protein, GGT – gamma-glutamyltransferase, GLP-1 – glucagon-like peptide 1, HMGB1 – high mobility group box 1, IBD – inflammatory bowel disease, IL-6 – interleukin 6, IQR – interquartile range, sRAGE – soluble receptor of advanced glycation end-products.

promotes the inflammatory response (Creagh-Brown et al., 2013). sRAGE lacks the transmembrane domain and is a potential marker of disease risk and prognosis (Erusalimsky, 2021). The levels of sRAGE are generally investigated in pneumology, cardiology (Danzig et al., 2010), diabetology (Yamagishi et al., 2007; Skrha et al., 2012), neurology (Moser et al., 2012), nephrology (Kalousová et al., 2006), oncology (Stoetzer et al., 2013; Garrido et al., 2021), and sepsis (Hamasaki et al., 2014) and are promising as new biomarkers of inflammation and tissue damage (Kalousová et al., 2005; Erusalimsky, 2021).

EN-RAGE is the designation for extracellular newly identified receptor for advanced glycation end-products binding protein (also called calgranulin C or S100A12). It is produced by activated granulocytes, macrophages and lymphocytes. It is a pro-inflammatory protein and influences multiple inflammatory and immune diseases (Zakiyanov et al., 2011; Skrha et al., 2012; Wu et al., 2020).

Calprotectin is a protein produced by neutrophils (Sejersen et al., 2022). Calprotectin possesses antimicrobial effects; the serum levels of calprotectin increase in bacterial infections. Some studies also describe changes in the serum levels of calprotectin in cancer diagnostics, for example, in laryngeal carcinoma (Topuz et al., 2017), papillary thyroid carcinoma (Tabur et al., 2015) or pancreatic tumours (Holub et al., 2019). In addition, the role of calprotectin was studied in rheumatic diseases (Chen et al., 2009; Ometto et al., 2017) and various respiratory diseases (Kotsiou et al., 2021). Faecal calprotectin is well known in the diagnosis of inflammatory bowel disease (IBD) (Jukic et al., 2021).

HMGB1 is the designation for high mobility group box 1, also known as amphoterin. This protein can be found in all cell types in the nucleus intracellularly or extracellularly after cell death or translocation (Tesarova et al., 2016). Its structure is highly conserved. The main role for HMGB1 is in the influence of innate immunity. The serum levels of HMGB1 increase early in inflammatory states, and a correlation can be observed between HMGB1 levels and the severity of inflammation (Larsen et al., 2019). Various studies have described changes in the HMGB1 levels in sepsis (Andersson and Tracey, 2003), inflammatory diseases, diabetes mellitus (Skrha et al., 2012) and autoimmune diseases (Pilzweger and Holdenrieder, 2015) such as IBD, systemic lupus erythematosus (Liu et al., 2020), vasculitis (de Souza et al., 2013), multiple sclerosis (Bucova et al., 2020), myasthenia gravis (Moser et al., 2012), and in oncology (Stoetzer et al., 2013; Pilzweger and Holdenrieder, 2015; Tesarova et al., 2016; Garrido et al., 2021).

Interleukin 6 (IL-6) is a pro-inflammatory cytokine with a pleiotropic impact on the organism. It participates in immune reactions (Tanaka et al., 2014), acute phase response, bone metabolism (Sims, 2021), trauma (Volpin et al., 2014) and critical illnesses (Jawa et al., 2011). It is commonly used as a very quick and reliable marker of inflammation.

Cholecystolithiasis means the presence of stones in the gallbladder. The cause of the occurrence of gallbladder stones is an imbalance in bile constituents (Ibrahim et al., 2018). It typically manifests as biliary colic or causes variable gastroenterological symptomatology, for example, anorexia, vomiting, diarrhoea after a fatty diet, belching and flatulence (Gutt et al., 2020). Diagnosis of cholecystolithiasis is based on transcutaneous ultrasonography, which is the method of choice with high sensitivity and specificity (Gutt et al., 2020). Cholecystolithiasis is often associated with chronic cholecystitis. Regarding treatment, the preferred treatment for symptomatic cholecystolithiasis is laparoscopic cholecystectomy. In a small number of patients with small cholesterol stones, it is possible to use ursodeoxycholic acid, but in these cases, cholecystectomy is also the method of choice. Extracorporeal lithotripsy is not used (Gutt et al., 2020).

In our study, we focused on umbilical and inguinal hernias. Hernia means a pathological protrusion of tissues from their physiological position (Dabbas et al., 2011; Larsen et al., 2019). The cause of hernia is weakening of the abdominal wall and development of a hernial gap (fascial defect) (Henriksen et al., 2011). Through the hernial gap, tissues and organs can protrude outside the fascia. This state is associated with alterations of the extracellular matrix and alterations of the levels of matrix metalloproteinases, cytokines and alarmins (Antoniou et al., 2009; Henriksen et al., 2011). Risk factors for the development of hernia are all states linked with increased intraabdominal pressure (for example, cough, excessive effort); predisposing factors are obesity, poor nutrition, pregnancy, smoking, diabetes mellitus and various types of collagen diseases (Holzheimer, 2005; Jiang et al., 2015). The treatment is surgical.

The main interest of our study was to demonstrate how circulating levels of sRAGE, EN-RAGE, calprotectin, HMGB1 and IL-6 are influenced by elective surgical treatment.

Material and Methods

Research participants

The study group consisted of 58 patients (38 men and 20 women, median age 57.5 years) – 23 patients with cholecystolithiasis (10 men and 13 women, median age 50 years), 10 patients with umbilical hernia (6 men and 4 women, median age 53 years), 25 patients with inguinal hernia (22 men and 3 women, median age 72 years) from the 1st Department of Surgery – Department of Abdominal, Thoracic Surgery and Traumatology, First Faculty of Medicine, Charles University and General University Hospital in Prague, and 21 healthy controls (8 men and 13 women, median age 41 years) for comparison. These patients were indicated for elective surgery and had no symptoms of biliary colic, acute cholecystitis, or incarcerated hernia. The group of patients with inguinal hernia and the healthy control group were

the same as in our previous study (Strohalmová et al., 2021). All the patients underwent the first blood collection 1–3 hours before surgery and the second blood collection 24 hours after surgery. All the patients with cholecystolithiasis underwent laparoscopic cholecystectomy, which is a standard type of surgery in our clinic. The patients with umbilical hernia underwent open hernioplasty and patients with inguinal hernia underwent either open Lichtenstein hernioplasty or laparoscopic hernioplasty.

All patients underwent a complete internal preoperative examination and were cardiopulmonary compensated. Twenty-eight patients suffered from arterial hypertension – 23 patients had mild hypertension, five had severe hypertension. Five patients were diagnosed with diabetes mellitus – four patients were on treatment with GLP1 agonist, and one patient was on diet. Three patients had chronic kidney disease, serum level of creatinine below 150 µmol/l, and two patients had known aetiology – hypertensive and diabetic nephropathy. Fourteen patients were treated with heart disease – five patients with ischaemic heart disease, seven patients with atrial fibrillation, one patient with aortic regurgitation, and one patient with mitral regurgitation. Two patients suffered from hypothyroidism and were on therapy with levothyroxine. Two patients were in remission for oncological disease (underwent surgery and chemotherapy in the past) – breast cancer (16 years of remission) and ovarian cancer (8 years of remission). Sixteen patients had no internal comorbidities. The duration of hospitalization after surgery was two to three days; no patients had complications that would require a prolongation of the hospitalization.

Regarding healthy controls, we have chosen volunteers without cholecystolithiasis, without the presence of any type of hernia, without symptoms of acute infection or chronic disease, without elevation of inflammatory markers in the blood (CRP, leukocytes), without hepatic or severe renal failure, and with a blood count in the reference range. The results of routine laboratory examinations of the studied groups are presented in Table 1.

For interest, we also incorporated a small group of acute patients into our study. This group consisted of seven patients (6 men, 1 woman, median age 40 years). These patients suffered from acute abdomen – acute inflammation of the appendix or gallbladder or incarcerated hernia – and were indicated for urgent surgery. Four patients underwent laparoscopic cholecystectomy for acute cholecystitis, two patients underwent laparoscopic appendectomy for acute appendicitis, and one patient underwent hernioplasty for incarcerated umbilical hernia. The preoperative level of CRP was from 1.5 to 160.2 mg/l (median 44.1 mg/l) and leukocytes from 8.9 to 18.1 × 10^9/l (median 11.6 × 10^9/l) according to the

Table 1. Basic laboratory characteristics of the study groups of patients before elective surgery and healthy controls

Parameter	Number of patients = 58			Healthy controls N = 21	P value
	Inguinal hernia N = 25	Umbilical hernia N = 10	Cholecystolithiasis N = 23		
Protein (g/l)	69 (68–71)	73 (72–77)	74 (71–75)	72 (67–73)	0.001
CRP (mg/l)	1.0 (1.0–1.2)	1.4 (1.0–4.2)	1.7 (1.0–2.9)	1.5 (1–3.9)	0.025
Bilirubin (µmol/l)	11.4 (10.1–15.8)	10.4 (8–14.4)	12.2 (9.2–16.0)	5.5 (4.1–8.4)	ns
ALT (µkat/l)	0.34 (0.28–0.47)	0.50 (0.32–0.65)	0.43 (0.32–0.63)	0.40 (0.31–0.47)	ns
AST (µkat/l)	0.42 (0.37–0.48)	0.35 (0.31–0.49)	0.35 (0.33–0.56)	0.37 (0.31–0.44)	ns
GGT (µkat/l)	0.43 (0.31–0.96)	0.68 (0.17–0.91)	0.42 (0.30–1.14)	0.36 (0.30–0.55)	ns
Urea (mmol/l)	6.2 (4.3–6.6)	4.9 (4.0–5.2)	5.0 (4.0–6.3)	4.9 (3.9–6.3)	0.037
Creatinine (µmol/l)	87 (79–98)	73 (66–94)	74 (69–85)	65 (58–76)	0.019
Haemoglobin (g/l)	147 (141–154)	147 (138–162)	145 (135–153)	135 (131–150)	ns
Leukocytes (×10^9/l)	6.45 (4.85–8.12)	6.35 (5.67–9.89)	6.40 (5.31–7.58)	6.5 (5.7–8.4)	ns
Thrombocytes (×10^9/l)	214 (178–252)	253 (237–291)	233 (215–285)	243 (204–301)	0.038

All data are shown as median (interquartile range).
All results were considered statistically significant at $P < 0.05$. ns – not significant

severity and length of inflammation. Renal and liver function tests were in the physiological range. Three patients had no internal comorbidities, two patients suffered from diabetes mellitus on GLP1 agonist treatment and three patients were treated with mild hypertension. Two patients were diagnosed with hypercholesterolemia.

This study was carried out in accordance with the principles of the Helsinki Declaration and approved on the 21st of July 2016 by the Ethics Committee of the General University Hospital in Prague, reference number 337/16 S-IV. All subjects gave their informed consent to participate in the study.

Sample collection

We took two samples of patients' blood, the first one 1–3 hours before surgery and the second one 24 hours after surgery. All volunteers were subjected to blood collection by cubital vein punction. We used tubes without anticoagulant to obtain the serum and in addition, we took a sample of blood for standard laboratory analysis. After coagulation, the test tubes with blood were centrifuged for 15 min at 900 × *g* and the serum was stored at –80 °C until the measurement of selected alarmins and related molecules – sRAGE, EN-RAGE, calprotectin, HMGB1 and IL-6.

ELISA analysis

Serum samples were analysed using commercial enzyme-linked immunosorbent assay (ELISA) kits. EN-RAGE was measured using Human EN-RAGE DuoSet ELISA (R&D Systems, Minneapolis, MN). To determine IL-6, we used the Human IL-6 Quantikine ELISA Kit. HMGB1 was assessed by HMGB1 Express ELISA (IBL International GmbH, Hamburg, Germany). sRAGE was quantified using the Human RAGE Quantikine ELISA Kit. To measure calprotectin, we used the Human S100A8/S100A9 Heterodimer Quantikine ELISA Kit.

All these sets were processed according to the manufacturer's protocol. The results of sRAGE, EN-RAGE and IL-6 measurements are expressed in pg/ml, and the results for calprotectin and HMGB1 are expressed in ng/ml.

Statistical analysis

Statistical analysis was performed using the Wolfram Mathematica software, version 13.2 (Wolfram Research, Champaign, IL). Due to the strongly non-normal statistical distribution of biochemical variables, we present their descriptive values in the form of median (lower quartile, upper quartile). Moreover, some observations are under the limit of quantification (for one variable also above its upper limit). If the number of such observations impacted some quartile of a variable, we replaced its value with the symbol "< limit" or "> limit", respectively. Because of the non-normality, we also used nonparametric methods for statistical testing, namely, the Mann Whitney test for two-group comparison, the Wilcoxon test for paired comparison, and Spearman's rank correlation coefficient for assessing correlation. All the tests are ordinal, so their results are also valid for non-detect data as a special case of interval data. All the results were considered statistically significant at $P < 0.05$.

Results

The serum concentrations of alarmins in all patients indicated to elective surgery compared to healthy controls are presented in Table 2. More detailed information about the elective patients according to individual diagnoses (cholecystolithiasis, umbilical and inguinal hernia) is presented in Table 3.

Preoperative circulating levels of calprotectin were significantly decreased ($P = 0.013$) and the levels of sRAGE were significantly increased ($P = 0.006$), where-

Table 2. Alarmin and related molecule serum levels of all elective patients before and after surgery and of the control group

Analyte	PATIENTS		Controls	P value before vs after	P value before vs controls	P value after vs controls
	Before	After				
sRAGE (pg/ml)	1256.2 (970.5–1604.2)	864.6 (675.7–1217.6)	1016.1 (822–1161.2)	< 0.0001	0.006	ns
EN-RAGE (pg/ml)	49.4 (38.1–86.7)	108.3 (70.1–155.9)	66.5 (44.6–88.3)	< 0.0001	ns	0.002
HMGB1 (ng/ml)	10.2 (7.5–13.2)	16.3 (11.8–19.4)	11.2 (9.1–14.9)	< 0.0001	ns	0.005
Calprotectin (ng/ml)	2164.6 (1690.4–3488.9)	4700.2 (3261.4–6688.7)	3446.9 (2295.2–4851)	< 0.0001	0.013	0.008
IL-6 (pg/ml)	< 3.13 (< 3.13–< 3.13)	18.5 (8.4–35.7)	< 3.13 (< 3.13–< 3.13)	< 0.0001	ns	< 0.0001

Data are expressed as median (interquartile range).
All results were considered statistically significant at $P < 0.05$. ns – not significant

Table 3. Alarmin and related molecule serum levels before and after surgery in elective patients compared to healthy controls according to diagnoses

Analyte	Before surgery	After surgery	P value before vs after	P value before vs controls	P value after vs controls
Inguinal hernia					
sRAGE (pg/ml)	1474.7 (1188.7–1658.2)	1064.0 (789.8–1270.6)	< 0.0001	0.001	ns
EN-RAGE (pg/ml)	51.4 (40.0–88.9)	112.1 (81.4–157.3)	< 0.0001	ns	0.001
HMGB1 (ng/ml)	11.3 (8.5–19.9)	16.7 (11.6–19.4)	0.001	ns	0.005
Calprotectin (ng/ml)	2227.7 (1761.0–3497.0)	4906.7 (3604.9–6944.8)	< 0.0001	0.040	0.004
IL-6 (pg/ml)	< 3.13 (< 3.13–< 3.13)	35.18 (15.52–57.12)	< 0.0001	ns	< 0.0001
Umbilical hernia					
sRAGE (pg/ml)	1110.2 (941.5–13936.8)	1149.3 (819.9–1602.1)	ns	ns	ns
EN-RAGE (pg/ml)	48.7 (37.5–128.2)	78.6 (64.2–172.5)	ns	ns	ns
HMGB1 (ng/ml)	11.6 (9.8–18.5)	15.3 (11.6–17.5)	ns	ns	ns
Calprotectin (ng/ml)	2164.6 (1932.3–4955.7)	4393.7 (2915.5–6292.4)	0.032	ns	ns
IL-6 (pg/ml)	< 3.13 (< 3.13–< 3.13)	9.99 (5.60–18.90)	0.009	ns	< 0.0001
Cholecystolithiasis					
sRAGE (pg/ml)	1163.0 (915.9–1484.4)	761.3 (603.8–1047.5)	< 0.0001	ns	0.026
EN-RAGE (pg/ml)	49.5 (30.0–66.4)	107.6 (73.6–138.3)	< 0.0001	ns	0.023
HMGB1 (ng/ml)	8.7 (6.8–13.3)	16.8 (13.0–19.8)	< 0.0001	0.032	0.031
Calprotectin (ng/ml)	1937.1 (1473.2–2862.2)	4548.6 (3196.2–6393.7)	< 0.0001	0.008	ns
IL-6 (pg/ml)	< 3.13 (< 3.13–< 3.13)	14.9 (7.3–27.5)	< 0.0001	ns	< 0.0001

Data are expressed as median (interquartile range).
All results were considered statistically significant at $P < 0.05$. ns – not significant

as the levels of EN-RAGE and HMGB1 in patients with all the diagnoses together were without significant changes compared to the levels of healthy controls. Preoperative levels of IL-6 in patients without acute inflammation and in healthy controls were below the limit of quantification (< 3.13 pg/ml).

There was a significant difference in the serum levels of the patients before and after surgery, Table 2. After surgery, there was a significant increase in EN-RAGE, HMGB1, and calprotectin ($P < 0.0001$) and a significant decrease in sRAGE ($P < 0.0001$).

When we compared postoperative patients' serum levels of all tested analytes to the healthy control groups, we found significant differences. After surgery, there was a significant increase in EN-RAGE ($P = 0.002$), HMGB1 ($P = 0.005$) and calprotectin ($P = 0.008$). In contrast, we observed a trend of a slight decrease in sRAGE after surgery compared to healthy controls, but the decrease was not statistically significant ($P = 0.453$).

The comparison of serum levels of alarmins and related molecules according to individual diagnoses showed differences. When we compared the alarmin levels before and after the surgery, patients with inguinal hernia and cholecystolithiasis followed the same trend as the entire study group. In umbilical hernia, the comparison of serum levels of all tested molecules before and after the surgery did not differ significantly, with the exception of calprotectin ($P = 0.032$) and IL-6 ($P = 0.009$). When we compared the alarmin levels to the healthy control group, we could see slight differ-

ences between patients with cholecystolithiasis and umbilical hernia. In the case of cholecystolithiasis, preoperative sRAGE levels did not differ significantly compared to healthy controls, while postoperatively, the levels were significantly decreased (P = 0.026). The HMGB1 level was significantly decreased before surgery (P = 0.032) and significantly increased (P = 0.031) after the surgery compared to the healthy controls. The levels of calprotectin did not differ significantly after the surgery. In umbilical hernia, all results were insignificant compared to healthy controls, with the exception of increased postoperative levels of IL-6 (P < 0.0001).

The influence of gender on both preoperative and postoperative levels of all tested molecules was insignificant in the elective patients' group.

We determined the correlations among the tested analytes in elective patients before and after the procedure. In patients before surgery, we found a strong correlation (P < 0.0001) between EN-RAGE and calprotectin (ρ = 0.96) and between EN-RAGE and HMGB1 (ρ = 0.79) levels. This significant correlation (P < 0.0001) was also apparent after surgery (ρ = 0.90, ρ = 0.83, respectively). A significant correlation was also found between the same analytes in the control group (EN-RAGE and calprotectin ρ = 0.59, P = 0.005; EN-RAGE and HMGB1 ρ = 0.69, P = 0.001).

As a matter of interest, we also determined the circulating blood levels of alarmins in a small group of seven patients with acute diagnoses – acute appendicitis and acute cholecystitis – Table 4. The preoperative levels of EN-RAGE (P = 0.020), calprotectin (P = 0.036), HMGB1 (P = 0.030) and IL-6 (P < 0.0001) in the patients were significantly higher and the concentrations of sRAGE were significantly lower (P = 0.020) compared to the healthy controls. The comparison of the patients' levels of alarmins before and after surgery did not show significant changes, with the exception of IL-6. These results illustrate the key role of alarmins as markers of inflammation and acute states; the preoperative levels were increased to such an extent that the influence of surgery was, apart from IL-6, insignificant.

Discussion

In our study, we focused on the changes in circulating levels of alarmins in patients with cholecystolithiasis and abdominal wall hernias before and after elective surgery compared to healthy controls.

The preoperative levels of EN-RAGE and HMGB1 in patients undergoing elective surgery without any acute complication were similar to healthy controls. These patients had no signs of inflammation or complications and were cardiopulmonary stable, which means that the simple presence of hernia or gallstones does not influence the levels of these alarmins. On the other hand, in a small group of acute patients, the preoperative levels were significantly increased. However, we have to admit that the results are limited by the number of patients; our group consisted of seven patients. When we compared the preoperative levels of calprotectin and sRAGE between the patients without the acute group and the healthy controls, we observed differences – the preoperative levels of sRAGE were significantly increased and the levels of calprotectin were significantly decreased in the entire patients' group as well. The decreased levels of calprotectin in our patients were surprising due to the ability of calprotectin to respond to minimal signs of inflammation or tissue alteration (Ometto et al., 2017). Due to its role in IBD, we can just speculate about the possible role of food intake and irritation of the intestine before surgery – patients with cholecystolithiasis should be on diet, and all patients might have had a lighter diet before surgery than healthy controls. It is remarkable that in all groups of patients, the trend of development of sRAGE was opposite to the other biomarkers; sRAGE, unlike other markers, decreased after elective surgery, which might represent a physiological reaction to surgery. In various pathological states, low sRAGE was associated not only with in-

Table 4. Alarmin and related molecule serum levels of acute patients before and after surgery and of the control group

	ACUTE PATIENTS					
Analyte	**Before**	**After**	**Controls**	**P value before vs after**	**P value before vs controls**	**P value after vs controls**
sRAGE (pg/ml)	651.8 (609.1–753.1)	780.7 (539.4–935.6)	1016.1 (822–1161.2)	ns	0.020	ns
EN-RAGE (pg/ml)	174.3 (73.3–272.6)	224.3 (135.6–234.2)	66.5 (44.6–88.3)	ns	0.020	0.002
HMGB1 (ng/ml)	16.89 (13.61–28.61)	19.40 (16.62–22.34)	11.2 (9.1–14.9)	ns	0.030	0.002
Calprotectin (ng/ml)	6968.5 (3589.6–> 8000.0)	7799.2 (5795.7–> 8000.0)	3446.9 (2295.2–4851)	ns	0.036	0.002
IL-6 (pg/ml)	15.57 (5.48–28.22)	23.79 (14.77–74.58)	< 3.13 (< 3.13–< 3.13)	0.047	< 0.0001	< 0.0001

Data are expressed as median (interquartile range).
All results were considered statistically significant at P < 0.05. ns – not significant

flammation but also disease progression and worse prognosis (Tesarova et al., 2007; Erusalimsky, 2021), while decreased renal function increased sRAGE (Kalousová et al., 2005), and an increase in sRAGE might also be of prognostic significance (Erusalimsky, 2021).

When we compared the patients' serum levels of alarmins and related molecules according to individual diagnoses, we found that the levels of alarmins in the inguinal hernia and cholecystolithiasis mostly followed a similar trend, while the results in the umbilical hernia differed. In umbilical hernia, the comparison of results before and after surgery and to the healthy control group was not significant. The differences were probably caused by the presence of chronic inflammation and tissue damage and remodelling, which is present both in cholecystolithiasis (Liu et al., 2018) and in inguinal hernia (Antoniou et al., 2009; Henriksen et al., 2011). Umbilical hernia is smaller and often contains only fat, and the extent of surgical procedure is also smaller (Liang et al., 2017; Sigley et al., 2020).

Several studies were also interested in the changes of serum levels of alarmins associated with surgery, but none of them has covered such a number of alarmins in similar diagnoses as our study.

Creagh-Brown et al. (2013) have reported that the serum levels of sRAGE are significantly higher in patients after cardiac surgery and the higher levels of sRAGE also correlate with critical illness and the length of hospitalization. In our study, on the contrary, the postoperative patients' levels of sRAGE were lower. The patients in the study by Creagh-Brown et al. (2013) underwent extensive surgery and subsequent intensive care. They required pulmonary ventilation and often suffered from various organ failures. Another possible difference between the two studies may also be due to the different times of blood collection after surgery. We collected the blood sample 24 hours after surgery, whereas Creagh-Brown et al. (2013) took the blood samples two hours after surgery. Creagh-Brown et al. (2013) also found a correlation between the preoperative level of sRAGE and critical illness and the duration of the hospitalization. In our patients, we did not observe complications that would prolong the length of hospitalization or require intensive care unit treatment.

Several studies were also interested in the serum levels of calprotectin. Sejersen et al. (2022) described an increase in the serum levels of calprotectin and IL-6 in patients after hernioplasty over time and showed that calprotectin is a very quick and promising marker of possible postoperative complication. Their results are consistent with our study; the levels of IL-6 were significantly higher after surgery and also differed in acute diagnoses both preoperatively and postoperatively. The preoperative levels of IL-6 in our patients with hernias and cholecystolithiasis without any signs of acute infection indicated for elective surgery were below the limit of detection, which demonstrates the importance of IL-6 as a marker of inflammation.

Holub et al. (2019) studied the changes in calprotectin and EN-RAGE serum levels in patients with pancreatic cancer. They described a correlation between the increased levels of calprotectin and EN-RAGE in patients diagnosed with pancreatic cancer both preoperatively and postoperatively in the event of development of postoperative pancreatic fistulas.

IL-6 was the subject of interest of Jawa et al. (2019). They demonstrated changes in the serum levels of IL-6 after surgery, trauma and critical illness. Their primary focus was on monitoring the adverse complications in intensive care. The postoperative levels of IL-6 found by them were significantly increased, which corresponds with our research.

Regarding HMGB1, to our knowledge, there are no studies focused on changes in the serum levels of HMGB1 in patients undergoing elective surgery, and our study is the first to demonstrate the increase of HMGB1 after surgery.

Unfortunately, we have to admit that our study has some limitations. The group of patients was slightly older than the group of healthy controls, and the female ratio in the group of healthy controls was notably higher than in the group of patients.

Conclusions

Our results illustrate the considerable impact of elective surgery on the levels of alarmins and related molecules. In acute patients, where alarmins are already changed due to inflammation, only a further increase of IL-6 was observed, while the effect of surgery on other alarmins seems to be limited.

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Data availability

Research data are available on reasonable request to the corresponding author.

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