

Low and standard intra-abdominal pressure during laparoscopic cholecystectomy

An observational study based on colorimetric determination of serum concentration variation of the radical cation of *N,N*-DIETHYL-*para*-PHENYLDIAMINE

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Background: This prospective observational open-label cohort study on patients submitted for elective laparoscopic cholecystectomy is aimed to investigate the association of low (8–10 mm Hg) and high (11–14 mm Hg) intra-abdominal pressure with the variation of serum colorimetric determination of the radical cation of *N,N*-Diethyl-*para*-phenyldiamine (DEPPD), measured immediately before pneumoperitoneum and 10 min after deflation, and intended as a proxy of oxidative stress.

Methods: In 117 patients, subdivided into low-pneumo (n. 49 pts.) and high-pneumo (n. 68 pts.) groups, biochemical analyses were performed and analyzed by *T* tests and a multivariable regression model.

Results: The results of the biochemical analyses showed that the variation of the radical cation of DEPPD values resulted in no significant difference between low and high pneumoperitoneum. Moreover, low intra-abdominal pressure compared with high pressure was not significantly associated with age, body mass index, and previous abdominal surgery. On the contrary, both the female gender and the iatrogenic gallbladder perforation were significantly associated with a positive variation of the radical cation of DEPPD. Notably, when the operation length was longer than 45 minutes, a significant association with a negative variation was observed.

Conclusions: This study on patients submitted for elective laparoscopic cholecystectomy, despite some limitations, suggested that the use of a low-pressure pneumoperitoneum is not associated with a postoperative lower production of oxidants compared with standard pressure.

Keywords: Laparoscopic cholecystectomy; Intra-abdominal pressure; Oxidative stress; d-ROMs test

Introduction

The effects of gas insufflation in the abdominal cavity and the so-called postsurgery oxidative stress still raise great interest because studies in animals and humans correlated intra-abdominal pressure with a portal and splanchnic blood flow, tissue ischemia, and release of reactive oxygen and nitrogen species.^{1,2} Pneumoperitoneum provides a suitable surgical workspace but impacts on peritoneal blood flow, peritoneal acidosis, organ perfusion, capillary microcirculation, possibly

inducing peritoneal adhesions, and an extraordinary number of biochemical changes named oxidative stress (OS).³ Moreover, if the carbon dioxide (CO₂) used to establish the pneumoperitoneum is administered dry and at room temperature, visceral and peritoneal hypoperfusion, rapid evaporative cooling, and damage of the peritoneal surface occur, resulting in disruption of the glycocalyx and mesothelial cell barrier and in a cascade of immunological, proinflammatory, and oxidative events.^{4,5} A 2023 meta-analysis on different laparoscopic procedures also reported that low intra-abdominal pressure (<10 mm Hg)

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The authors declare that they have no conflicts of interest with regard to the content of this report.

Patients were enrolled after obtaining a written informed consent and after the approval of the Ethics Committee (n.19/2020) and of the ASL1 Abruzzo General Director deliberation (n. 116, January 19, 2021).

S.G. is fully accountable for the contents of the submitted manuscript. Conceptualization, Validation and visualization and Writing-review and editing: M.C., F.M., P.P., A.M.S., S.C., and S.G. Investigation and data curation: M.C., F.M., P.P., A.M.S., and S.G. Formal analysis, methodology, resources, and software: A.M.S., P.P., M.C., S.G., and F.M. All authors reviewed and approved the manuscript before submission.

All the datasets are available upon reasonable request to the corresponding author.

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Journal of the Italian Surgical Association (2024) 44:5

Received: 25 June 2024; Accepted 29 July 2024

Published online 6 September 2024

DOI: 10.1097/IA9.0000000000000055

compared with standard intra-abdominal pressure (10 mm Hg or higher) resulted associated with a lower incidence of mild (Clavien–Dindo grade 1–2) postoperative complications, and lower pain scores, not increasing the risk of intraoperative complications.⁶ Regarding cholecystectomy, recent randomized trials on laparoscopic⁷ and robotic approaches⁸ and an Italian meta-analysis,⁹ particularly confirmed the association of low-pressure pneumoperitoneum with lower postoperative pain in patients submitted for minimally invasive cholecystectomy.

The term “oxidative stress”, introduced approximately 40 years ago, expresses the imbalance between oxidant and antioxidant molecules in favor of oxidants.¹⁰ This qualitatively defined concept implies a quantification problem, requiring the practically impossible measurement of both the rate of free radical production and the rate of their elimination. Consequently, the main problem for the application of OS in clinical medicine is its measurement, considering the complexity of the process and the number of oxidants and antioxidant components that make it up. For OS quantification, recourse was made to different oxidative markers as well as antioxidant markers and indices of the ratio between different tests.^{11,12} Due to the difference among markers, a variety of quantification methods and commercially available tests have been used, having different sensibility and reliability, some of which require very sophisticated techniques. Notably, many different diseases and pathological states (i.e., diabetes, Parkinson’s disease, Alzheimer’s disease, cardiovascular diseases, solid tumors, age-related macular degeneration, chronic obstructive pulmonary disease, atherosclerosis, aging, etc.) were associated with different OS biomarkers¹³ and there is evidence that surgery can result in OS and that the extent of the surgery influences the magnitude of the oxidative response.¹⁴

As concerning cholecystectomy, earlier studies in humans compared the open and laparoscopic approaches, evaluating different oxidative and antioxidative markers, including the enzyme superoxide dismutase (SOD),¹⁵ or the thiobarbituric acid-reactive substances, expressed as nmol of malondialdehyde (MDA) equivalents/ml of plasma,^{16–18} or MDA and the Randox Total Antioxidant test.¹⁹ All these studies reported a lower OS risk for laparoscopy compared with the open procedure when blood samples were taken before and immediately after the procedure. On the contrary, only one study found no difference between laparoscopic and open approaches measuring SOD and performing the Randox Total Antioxidant test when blood samples were taken 1 day before and 1 day after the procedure.²⁰

As concerning different intraabdominal pressures during laparoscopic cholecystectomy, in 2003, Polat et al²¹ measured several substances, including MDA as oxidative markers and thiols as antioxidative markers, and reported no significant difference between low (10 mm Hg) and high (15 mm Hg) pneumoperitoneum in an underpowered randomized clinical trial (24 patients). Ten years later, an animal study in dogs,²² reported that a low-pressure pneumoperitoneum could attenuate OS induced by CO₂ insufflation when plasma “total oxidant status” and “total antioxidant status” levels were determined using a commercially available kit developed by Erel.^{23,24}

For the purposes of this study, as an extraordinary number of exogenous (e.g., drugs, air pollution, smoke, alcohol, toxins in food, radiation, diet, etc.) and endogenous factors (e.g., aging, inflammation, diseases including bacterial, fungal or viral infections, intense and prolonged physical exercise, deficiency of antioxidants, mitochondria age, and dysfunction, etc.)^{25,26} may concur to the production of reactive species in a single subject immediately before a laparoscopic cholecystectomy and as the antioxidant systems that are activated following surgical laparoscopic trauma, including antioxidant enzymes (e.g., SOD, glutathione peroxidase and reductase, catalase, etc.), nutrient-derived antioxidants (e.g., ascorbic acid, tocopherols and tocotrienols, carotenoids, glutathione and lipoic acid, etc.), metal binding proteins (e.g., ferritin, lactoferrin, albumin, and ceruloplasmin, etc.)

and other antioxidant phytonutrients present in plant foods, varies from patient to patient, we thought it appropriate to calculate the variation (in other words the finite difference between two values measured at specific times) of serum colorimetric determination of the radical cation of *N,N*-Diethyl-para-phenyldiamine (DEPPD), immediately before laparoscopic cholecystectomy and 10 minutes after deflation. DEPPD is the substrate chosen to be oxidated by serum radicals in the so-called d-ROMs (determination of reactive oxygen metabolites) test.²⁷

This study on patients submitted for elective laparoscopic cholecystectomy is aimed to investigate the association of low (8–10 mm Hg) and high (11–14 mm Hg) intra-abdominal pressure with the variation of serum colorimetric determination of the radical cation of DEPPD, immediately before pneumoperitoneum and 10 minutes after deflation.

Methods

Study design

This is a prospective observational open-label cohort study on patients submitted for elective laparoscopic cholecystectomy with low (8–10 mm Hg) and high (11–14 mmHg) intra-abdominal pressure.

Patients

At the General Surgery Unit of the University of L’Aquila (Italy), patients were enrolled after obtaining a written informed consent and after the approval of the Ethics Committee (n.19/2020) and of the ASL1 Abruzzo General Director deliberation (n. 116, January 19, 2021), based on the following inclusion criteria: (1) age between 18 and 75 years; (2) ultrasound proven symptomatic gallstones; and (3) gallbladder adenomyomas with a diameter >1 cm, and on the following preoperative exclusion criteria: (1) body mass index (BMI) >30; (2) American Society of Anesthesiologists Class >II; (3) cholecystitis; (4) confirmed or suspected extrahepatic bile duct stones; (5) chronic renal failure; (6) pregnancy; (7) immunological disorders; (8) chronic inflammatory diseases; (9) anticoagulant therapy; (10) continuous intake of immunosuppressants; and (11) cortisone and anti-inflammatory drugs. Moreover, intrapostoperative exclusion criteria were: (1) intraoperative cholangiography; (2) conversion to an open procedure; (3) associate procedure; (3) intraoperative diagnosis of cholecystitis; (4) drainage placement; (5) procedure duration >120 minutes; (6) patients that received one or more blood transfusions (pre-, intra-, postoperative); (7) patients in which a second procedure will be scheduled (re-operation); (8) patients that will develop complications graded >II according to Dindo-Clavien classification of surgical complications²⁸; (9) patients with missing data, including patients with incomplete blood sampling; and (10) inadequate blood sampling. One hundred forty-five patients were enrolled and 117 were evaluated after the intra-postoperative exclusion (Figure 1). For the purposes of this study, the 117 patients were subdivided into two groups: group 1 with low intra-abdominal pressure values (8–10 mmHg) and group 2 or standard group with intra-abdominal pressure values of 11–14 mmHg.

Table 1 shows the descriptive characteristics of the 117 patients treated by laparoscopic cholecystectomy at low and high intra-abdominal pressure.

Operative techniques

All laparoscopic cholecystectomies were performed using a standardized methodology for both anesthesiology and surgery. All 117 patients were orally premedicated with Midazolam (0.1 mg/kg) 40 minutes before operation. The

standardized protocol was based on balanced general anesthesia with remifentanyl and desflurane. Following administration of fentanyl (100 mcg), induction was performed with propofol (2 mg/kg) and rocuronium bromide (1 mg/kg). Maintenance of anesthesia will be guaranteed by the combination of remifentanyl (target-controlled infusion at the effect site—Minto model, range 2–12 ng/ml) and desflurane at a concentration of 1 minimum alveolar concentration, in a mixture of oxygen and air with a fraction of inspired O₂ of 0.40. The adequate curarization of the patient was ensured by the administration of 20 mg of rocuronium bromide every 30 minutes. All patients also received ondansetron (4 mg) and pantoprazole (40 mg). The surgeons were left free to choose the more suitable intra-abdominal pressure. In all patients the surgical technique was performed using the same four small abdominal incisions for trocar placement, the same devices (i.e. the same laparoscopic column for homogeneity in CO₂ temperature and humidity), and the gallbladder was removed from the abdomen in a specimen pouch.

Outcome

The outcome was the variation of serum colorimetric determination of the radical cation of DEPPD.

Biochemical assays

Blood samples were collected before insufflation (preoperative sample), and 10 minutes after deflation (postoperative sample) from each patient. The serum colorimetric determination of the

radical cation of DEPPD was performed according to the published methodology.^{27,29} In particular, monitoring at 505 nm the absorbance of solutions obtained by dissolving the serum samples (10 µl) in 1 ml of pH 4.8 acetate buffer (the serum sample was 100-fold diluted) and adding 10 µl of a 3.7 × 10⁻¹ M solution of the chromogen (DEPPD); the absorbance values being converted to arbitrary Carratelli units (Carr units are obtained multiplying by 10⁴ the optical density values). All biochemical tests were performed double-blind by the analyst.

Sample size

As the methods of estimating the sample size are subject to the study design and to the planned multivariate model for the statistics analysis and considering the need to adjust the estimate of the outcome according to 7 predictors (including the treatment variable represented by low or high pneumoperitoneum), a medium f² effect size of 0.15, according to Cohen, was used.³⁰ The “pwr.f².test” command, running under the library “pwr” of the software R (Version 4.2.2, The R Foundation for Statistical Computing, Vienna, Austria, 2022), assuming a type I error of 5% and an 80% power, adjusting for a 20% dropout, provided a sample size estimate of 120 patients.

Statistical analysis

The statistical analysis provided descriptive summary statistics for continuous and categorical variables, stratifying by low and high pneumoperitoneum pressure. The variation of the radical cation of DEPPD, above described, measured immediately before

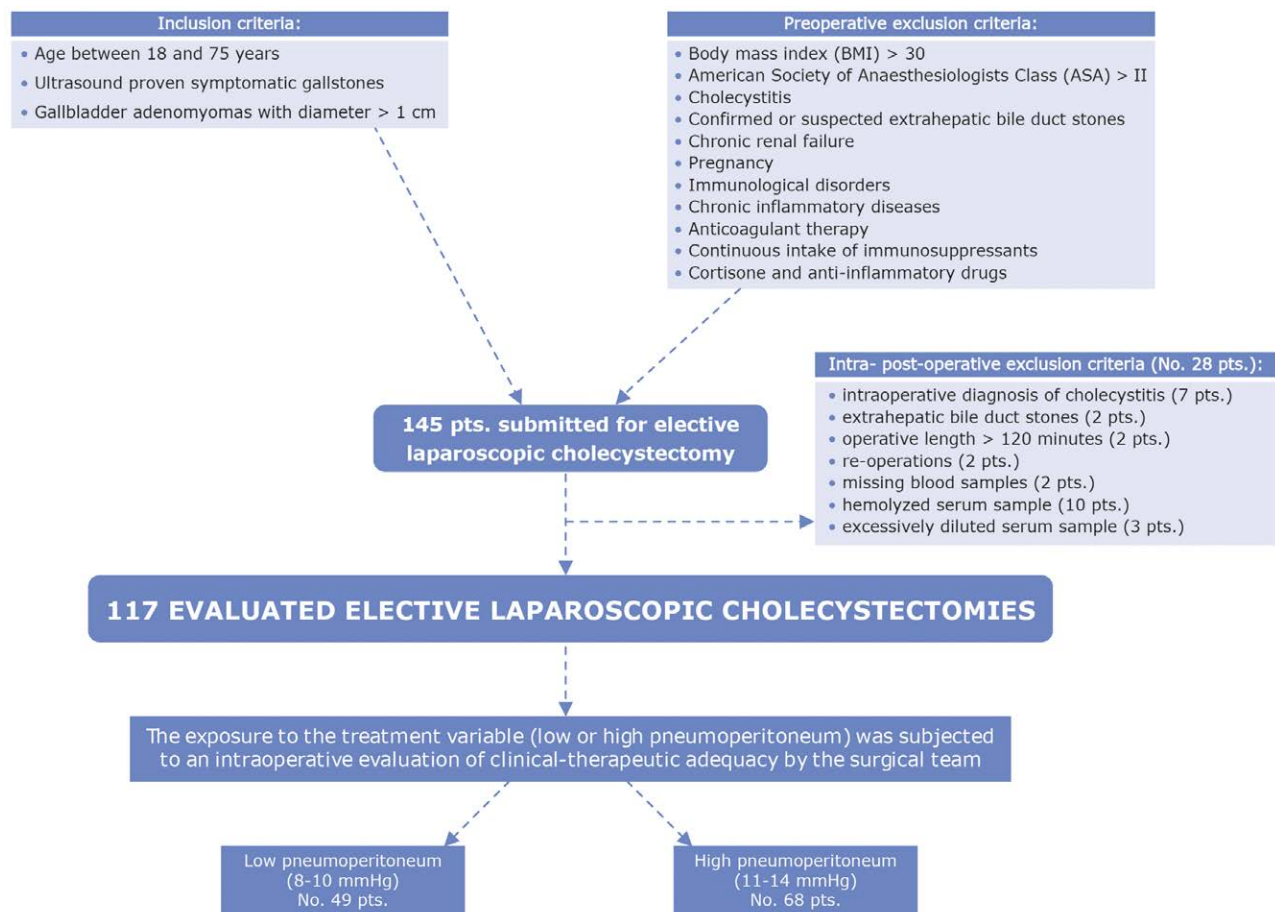


Figure 1. Study flowchart.

Table 1.
Descriptive analysis of the variables considered in the 117 patients evaluated

		Low pneumoperitoneum (8–10 mmHg) No. 49 patients	High pneumoperitoneum (11–14 mmHg) No. 68 patients	P
Continuous variables				<i>T</i> Student's test ^a
Age (years) mean (±S.D.)		49.1 (± 15.8)	50.4 (±15.8)	0.761
BMI (Kg/m ²) mean (±S.D.)		26.5 (± 3.0)	27.0 (± 2.2)	0.036
Operation length (minutes) mean (±S.D.) ^b		49.3 (± 15.6)	52.4 (± 18.4)	0.005
Categorical variables				<i>T</i> Student's test between proportions
Gender	Pattern	No. (%)	No. (%)	
	Female	77 (65.8)	35 (71.4)	0.277
Comorbidity	Male	40 (34.2)	14 (28.6)	
	Yes	65 (55.5)	31 (63.3)	0.154
Previous therapy	No	52 (44.5)	18 (36.7)	
	Yes	67 (57.3)	32 (65.3)	0.135
Previous abdominal surgery	No	50 (42.7)	17 (34.7)	
	Yes	66 (56.4)	22 (44.9)	0.033
Adherences	No	51 (43.6)	27 (55.1)	
	Yes	47 (40.2)	19 (38.8)	0.793
Iatrogenic gallbladder perforation	No	70 (59.8)	30 (61.2)	
	Yes	22 (18.8)	8 (16.3)	0.560
Operation length (minutes)	No	95 (81.2)	41 (83.7)	
	>45	44 (37.6)	12 (24.5)	0.013
	≤45	73 (62.4)	36 (52.9)	

Bold values express significant statistical significance.

^aAdjusted for unequal variances.

^bOperation length (minutes) in 117 patients: mean 49.3 (S.D. ± 15.6), median 45, range 30–120.

pneumoperitoneum and 10 min after deflation, was assumed to be a proxy variable that characterizes OS. This variation in time response was modeled using a multivariable linear regression. This choice of design addresses three main features: first, it adjusts for baseline oxidative and antioxidative conditions of the patients; second, it avoids the cost of estimating the covariance matrix with the consequent loss of power of the analysis; third, it makes the results easier to interpret. Explorative univariate analysis was carried out testing the variation of the radical cation of DEPPD and the model covariates variations. Continuous variables were tested by performing *T* tests for independent samples, adjusting for unequal variances with the Satterthwaite correction, and differences in the case of dichotomic categorical variables were tested using *T* tests for proportions. The regression model of the outcome included seven covariates, five dichotomic variables, and two continuous. The overall model fitting was assessed using the *F* statistics. Collinearity was checked using the variables individual variance inflation factor (VIF), and the mean variance inflation factor score. Model consistency with the linear regression hypothesis was assessed by carrying out a residual analysis and performing a Skewness and Kurtosis test for the normality of residuals (*P* = 0.09).

The calculations have been worked out using the statistical software STATA version 18 (Copyright 1985-2021 StataCorp LLC, 4905 Lakeway Drive, College Station, Texas 77845 USA).

Results

The results of the biochemical analyses showed that, although the mean values of the serum colorimetric determinations of the radical cation of DEPPD (expressed in Carr units, measured before but also after the laparoscopic cholecystectomy using low intra-abdominal pressure) were lower than those measured when high intra-abdominal pressure was used (Table 2), the variation of the mean Carr units values resulted not significantly different in the two groups of patients (Table 2).

To understand how the predictive variables, combined together, were significantly related to the dependent variable (variation of serum colorimetric determination of the radical cation of DEPPD), a multivariable model (with 7 predictive

variables) was used. Table 3 shows the results of the multivariable linear regression analysis.

A low intra-abdominal pressure compared with high pressure was not significantly associated with the radical cation of DEPPD variation. The same is true for age, BMI, and previous abdominal surgery. On the contrary, both the female gender and the iatrogenic gallbladder perforation were significantly associated with a positive variation of the radical cation of DEPPD (in other words resulted in a serum imbalance in favor of oxidants). Notably, when the operation length was longer than 45 minutes, a significant association with a negative variation was observed (in other words resulted in a serum imbalance in favor of antioxidants).

A subgroup analysis considering the interaction between female gender and BMI, failed to find a significant association with the variation of the radical cation of DEPPD (*P* = 0.396), although patients with BMI >30 were excluded a priori.

Discussion

The aim of this article was to investigate in patients submitted for elective laparoscopic cholecystectomy, using standard intra-abdominal pressure values (11–14 mmHg) or low values (8–10 mmHg), how the immediately preoperative serum levels of some oxidative molecules varied at the end of operation by the colorimetric determination of the radical cation of DEPPD. The choice of extended exclusion criteria adopted in the study enabled us to focus on the estimation of the role of intra-abdominal pressure on the production of oxidative substances, as all the conditions/comorbidities/complications selected for the exclusion criteria can increase the concentration of oxidants during laparoscopic cholecystectomy, as reported in recent literature.^{13,14} This study showed that the serum variation of the radical cation of DEPPD was not significantly associated with the use of low intra-abdominal pressure. Moreover, independently from the pneumoperitoneum values, a positive variation of the radical cation resulted significantly associated with iatrogenic gallbladder perforation and to female gender. On the contrary, a negative variation resulted significantly associated with operation length >45 minutes. These observations, as well as the statistical significance, are corroborated by both

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Table 2.

Serum colorimetric determinations of the radical cation of *N,N*-DIETHYL-*para*-PHENYLDIAMINE in patients submitted for laparoscopic cholecystectomy using low or high pneumoperitoneum values

	Low pneumoperitoneum (8–10 mm Hg) No. 49 patients (mean ± standard error)	High pneumoperitoneum (11–14 mm Hg) No. 68 patients (mean ± standard error)	Two-sample <i>T</i> Student's test with unequal variances (Satterthwaite's correction)
Preoperative radical cation of DEPPD (Carr units)	283.9 ± 11.5	334.1 ± 12.1	<i>p</i> = 0.003
Postoperative radical cation of DEPPD (Carr units)	327.0 ± 11.2	366.3 ± 9.7	<i>p</i> = 0.009
Variations between postoperative versus preoperative radical cation of DEPPD (Carr units)	43.1 ± 12.9	32.2 ± 9.5	<i>p</i> = 0.499

Carr units are obtained multiplying by 10⁴ the Optical Density values.

Table 3.

Multivariable linear regression analysis of the dependent variable (variation between postoperative versus preoperative serum colorimetric determination of the radical cation of *N,N*-DIETHYL-*para*-PHENYLDIAMINE) and 7 predictive variables, combined together

Variations between postoperative versus preoperative radical cation of DEPPD (Carr units)	Coefficient	S.E.	<i>p</i>	(95% C.I.)	VIF
Low intra-abdominal pressure	−0.7	15.6	0.963	−31.7; +30.2	1.18
Operation length >45 minutes	−38.6	16.3	0.019	−70.8; −6.3	1.23
Age	0.3	0.5	0.533	−0.6; +1.2	1.11
BMI	1.9	2.5	0.427	−2.9; +6.9	1.11
Female gender	39.6	17.2	0.023	+5.5; +73.7	1.32
Iatrogenic gallbladder perforation	50.4	21.3	0.020	+8.2; +92.7	1.38
Previous abdominal surgery	−12.9	17.3	0.458	−47.2; +21.5	1.46

Bold values express significant statistical significance.

Carr units are obtained multiplying by 10⁴ the optical density values.

Low intra-abdominal pressure = 8–10 mmHg.

the predictive capacity (fitting Fisher-Snedecor's test) and the adequacy of the multivariable regression model in providing unbiased estimates (Gaussian distribution of residuals).

There is evidence that low pneumoperitoneum compared with the standard one is associated with lower postoperative pain scores and mild complications,^{6–8} but few data are available about the serum balance of oxidative and antioxidative molecules in laparoscopic cholecystectomy.²¹

It is appropriate, above all, to clarify that the choice of an observational study design in comparison to a randomized one, was mainly linked to a feasibility concept, as it has been preferred to leave the surgeons free to adopt the more suitable intra-abdominal pressure.

As an extraordinary number of intra- and extra-cellular systems concur to the redox steady state, it is practically impossible

to quantify all the involved oxidant and antioxidant molecules and their ratios. The published studies selected and used different indicative markers and different quantitative methods, also suggesting indices of OS and often using the arbitrary term “total.” For the present study, the indicative marker of measured oxidants, mainly represented by alkoxy and peroxy radicals,³¹ was the serum colorimetric determination of the radical cation of DEPPD. This marker characterizes the so-called commercial d-ROMs test, which received criticism and favorable reviews but is diffusely worldwide used.^{29,32–34}

As the redox environment in serum is extremely complex and an extraordinary number of exogenous and endogenous factors may concur to the level of reactive species before the laparoscopic cholecystectomy and the antioxidant systems activated following surgical laparoscopic trauma varies from patient to patient, this study, for the first time in literature, evaluated the variation of the marker from the time immediately preceding the operation to 10 min after deflation.

Although using a different marker, this study confirmed the findings of Polat et al,²¹ suggesting that the use of low intra-abdominal pressure during elective laparoscopic cholecystectomy, compared with standard pressure, does not associate with a significant difference of the variation between preoperative and postoperative values of DEPPD radical cation level, which indicates the serum concentration of some oxidative molecules. In other words, for this type of operation, the use of a low-pressure pneumoperitoneum is not associated with a lower OS.

Concerning the variables significantly associated with a variation of the selected oxidative marker, this study demonstrated that the iatrogenic gallbladder perforation, in addition to resulting in a longer operative time and length of hospital stay, as reported in a recent meta-analysis,³⁵ is associated with an increased presence of serum oxidants, speculatively related to the peritoneum damage promoted by the bile salts.

The significative association between a negative variation of the oxidative marker and an operation length >45 minutes, may be related to the activity of the antioxidant systems and, particularly, to those antioxidants which slower act against free radicals (i.e., uric acid, cysteine, and polyphenols, etc.) in comparison to those which are fast-acting, such as Vitamin C or Vitamin E.³⁶ In other words, mainly when the operation length is >45 minutes, become relevant the effect of the slow-acting antioxidant systems (having lower rate constants for the reaction) that, concurring with the fast-acting antioxidant systems effect, determines a reduction of the oxidant serum concentration below the preoperative level.

Concerning the female gender, although female hormones, sex gene dosage, and maternal inheritance of the mitochondria, constitute the basis of superior antioxidant response that has been observed in females, compared with males, as reported in a recent meta-analysis,³⁷ the significant positive variation in the female gender observed in this study, was not related to an interaction with obesity/BMI, as the multivariable regression model measured (*P* = 0.396) and may be speculatively related to the high percentage of females (35%) assuming a chronic preoperative therapy with thyroid hormones, which elicit free radical generation and OS.³⁸

Shortly, this study has several limitations that require caution in emphasizing the results: (1) it is not a randomized study; (2) the biochemical methodology does not measure all oxidative and antioxidative molecules in the patient serum; (3) the study design does not measure all known and unknown impacting variables on redox steady state.

Conclusions

This prospective observational open-label cohort study on patients submitted for elective laparoscopic cholecystectomy, despite the limitations mentioned above, suggested that the use

of a low-pressure pneumoperitoneum is not associated with a postoperative lower production of oxidants compared with standard pressure.

Acknowledgments

This article has been written in memory of Simona Bacchi.

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