

Difusão do Conhecimento Através das Diferentes Áreas da Medicina 3

Lais Daiene Cosmoski
(Organizadora)



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Dados Internacionais de Catalogação na Publicação (CIP) (eDOC BRASIL, Belo Horizonte/MG)	
D569	Difusão do conhecimento através das diferentes áreas da medicina 3 [recurso eletrônico] / Organizadora Lais Daiene Cosmoski. – Ponta Grossa, PR: Atena Editora, 2019. – (Difusão do conhecimento através das diferentes áreas da medicina; v. 3) Formato: PDF Requisitos de sistema: Adobe Acrobat Reader Modo de acesso: World Wide Web Inclui bibliografia ISBN 978-85-7247-882-3 DOI 10.22533/at.ed.823192312 1. Medicina – Pesquisa – Brasil. 2. Saúde - Brasil. 3. Diagnóstico. I. Cosmoski, Lais Daiene. II. Série. CDD 610.9
Elaborado por Maurício Amormino Júnior – CRB6/2422	

Atena Editora
Ponta Grossa – Paraná - Brasil
www.atenaeditora.com.br
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APRESENTAÇÃO

Cada vez mais percebemos, que no mundo da ciência, principalmente da área da saúde, nenhuma profissão trabalha sozinha, é necessário que vários profissionais estão envolvidos e engajados em conjunto, prezando pela, prevenção, diagnóstico e tratamento de diversas patologias, visando sempre a qualidade de vida da população em geral.

A Coletânea Nacional “Difusão do Conhecimento Através das Diferentes Áreas da Medicina” é um *e-book* composto por 4 volumes artigos científicos, que abordam relatos de caso, avaliações e pesquisas sobre doenças já conhecidas da sociedade, trata ainda de casos conforme a região demográfica, onde os locais de realização dos estudos estão localizados em nosso país, trata também do desenvolvimento de novas tecnologias para prevenção, diagnóstico e tratamento de algumas patologias.

Abordamos também o lado pessoal e psicológico dos envolvidos nos cuidados dos indivíduos, mostrando que além dos acometidos pelas doenças, aqueles que os cuidam também merecem atenção.

Os artigos elencados neste *e-book* contribuirão para esclarecer que ambas as profissões desempenham papel fundamental e conjunto para manutenção da saúde da população e caminham em paralelo para que a para que a ciência continue evoluindo para estas áreas de conhecimento.

Desejo a todos uma excelente leitura!

Lais Daiene Cosmoski

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EFFECTS OF INTRA-ABDOMINAL PRESSURE IN RAT LUNG TISSUE AFTER PNEUMOPERITONEUM

Data de aceite: 19/11/2018

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ABSTRACT: Background: Laparoscopic surgeries require pneumoperitoneum achieved by pressure-controlled insufflation with carbon dioxide into the peritoneal cavity. This condition changes the respiratory metabolism and promotes lung damage. Purpose: The aim of the present study was to compare the effect of different levels of intra-abdominal pressure (IAP) in a ventilated rat model with normal lungs. Methods: Forty-eight Wistar rats were selected at random and eight rats were assigned to each of following groups. The Sham group was subjected to a sham operation without pneumoperitoneum, the other groups were subjected to CO₂ pneumoperitoneum with 5, 8, 10, 12 or 14 mmHg intra-abdominal pressure for 60 minutes. All animals were mechanically ventilated. At the end of the experiment, the

animals were euthanatized and had their lungs removed for analysis. Lipid peroxidation, myeloperoxidase activity, measurements of cytokines, and histopathological analysis were performed. Results: In the IAP5 group, all analyses were lower compared with those of other groups. TNF-alpha, IL-1 beta, IL-6, lipid peroxidation and myeloperoxidase activity were higher in groups IAP10, IAP12, and IAP14 compared with those of groups IAP5 and IAP8. These results were supported by histopathological examination. Conclusions: These findings suggest that high pressure increase oxidative stress and inflammatory induced lung damage after pneumoperitoneum. **KEYWORDS:** Laparoscopy; Experimental; Pneumoperitoneum; Cytokines; Oxidative Stress; Lung Injury

INTRODUCTION

Laparoscopy is an important milestone in the era of modern surgery. It is a technique used in the diagnosis and treatment of a large number of specialties. It brings many advantages, but it also requires special care because of the transient physiologic changes promoted by the insufflation of gases in the

abdominal cavity [1-6].

Laparoscopic surgeries require pneumoperitoneum (PNP) usually achieved by pressure-controlled insufflation of carbon dioxide into the peritoneal cavity. In clinical practice, this gas is used with inflation pressures above 10 mmHg in adults. Although PNP is a complex event, it is well tolerated with a pathophysiological condition characterized by increased intra-abdominal pressure (IAP) with low perfusion in abdominal organs. Re-establishment of flow (reperfusion) occurs with deflation of the abdominal cavity with significant hemodynamic and respiratory effects, as well as specific changes in the intra-abdominal organs. This condition of “ischemia-reperfusion” (IR) leads to important effects such as hemodynamic, respiratory and oxidative stress. Thus the intra-abdominal pressure during laparoscopic surgery can cause injury, affecting local and distant tissues [7-10]. Despite the clear advantages of laparoscopic surgery in terms of patient outcome, increased intra-abdominal pressure (IAP) may give rise to significant organ ischemia in the splanchnic organs and even in remote organs such as lung [11]. This IR process leads to production of reactive oxygen species (ROS), and this can cause oxidative cell damage as well as activation of inflammatory mediators initiated immediately after reperfusion, which can last a few hours. [12-18].

In summary the intra-abdominal insufflation with CO₂ elevate the diaphragm and this condition increase the intrathoracic pressure following to decreasing respiratory system compliance associated with hypoxemia, atelectasis, edema and barotrauma [19,20]. The recruitment maneuvers for reduction at atelectasis area increase lung stress and these may be contributed to postoperative pulmonary dysfunction [21-24].

Based on the foregoing issues, we hypothesized that high IAP would promote more pulmonary lesions for increasing inflammatory cytokines and reactive oxygen species production in the lungs. The aim of this study was to evaluate the implications of PNP with different pressures levels of CO₂ and their effects on lung morphological and biochemical parameters.

MATERIALS AND METHODS

All animal care and manipulations were approved by the Institutional Research Committee of the Federal University of São Paulo in accordance with the National Institute of Health (NIH) Guidelines Regarding Animal Experimentation and the guidelines of the 3R's (Council Directive 86/609/EEC and new limits for the use of animals in experiments by the European Parliament in 2010).

The experiment was performed in adult male Wistar albino rats (n= 8/group,

weighing 200-250 g; 3 to 4-months-old). The animals from the Federal University of São Paulo (UNIFESP, SP, Brazil) were housed in the vivarium under controlled temperature ($\pm 22^{\circ}\text{C}$) and photoperiod (12-hour light/dark period) with free access to water and food. A 2-week acclimatization period was allowed before experimental manipulations were initiated. In order to avoid interference factors related to circadian rhythms, all studies were performed between 8 and 10 am.

Anesthetic and Ventilatory Procedures

The rats were anesthetized with intramuscular (IM) injection of ketamine (40 mg/kg; Cetamin™, Syntec, Brazil) and xylazine (10 mg/kg; Anasedan™, Seva, Brazil), put in the supine position on a thermostatically regulated heating pad ($36.7\text{-}37^{\circ}\text{C}$), and had the abdomen shaved and washed with 10% povidone iodine. Muscular relaxation was performed with IM injection of 2 mg/kg of neuromuscular blocking (Pancuron™, Cristália, Brazil) following tracheostomy (16G cannula) for mechanically ventilated (Inspira ASV, Harvard Apparatus, MA, United States) in volume-controlled ventilation mode with tidal volume (VT) of 6 mL/kg, respiratory rate (RR) of 70 incursions/min, PEEP of 5 cmH₂O and inspired oxygen fraction ratio (FiO₂) of 0.21, to maintain end-tidal CO₂ at 30-35 mmHg.

Pneumoperitoneum

Animals were randomized using a specific program (random.org) and divided into 6 groups (n=8/group), using the three R's rules (Reduce, Reuse and Recycle), as follows: Sham: only angiocatheter (18-G cannula) positioned in the peritoneal cavity without insufflation (zero pressure); IAP5: intra-abdominal insufflation with 5 mmHg of CO₂; IAP8: intra-abdominal insufflation with 8 mmHg of CO₂; IAP10: intra-abdominal insufflation with 10 mmHg of CO₂; IAP12: intra-abdominal insufflation with 12 mmHg of CO₂; and IAP14: intra-abdominal insufflation with 14 mmHg of CO₂. After the time of experiment and gradual decompression, the pH concentration in arterial blood gas were analyzed and the animals were euthanized using high anesthetic doses (1 mL/100 g of weight) of T-61 Euthanasia Solution (Hoechst & Roussel, USA).

The capnoperitoneum were performed for 60 minutes using an electronic laparoflator insufflator (Karl Storz GmbH, Germany). Immediately after euthanasia the thoracotomy was performed and the lungs were removed. The right lungs were divided: one part (right cranial lobe) was homogenized in ice-cold potassium chloride solution (1.5%, pH 7.4; Desruptor Ultrasonic, Thorton, Brazil) to yield a 10% (w/v), centrifuged (2.500 rpm for 10 min, at 4°C; VitchLab, DAIKI, Model DTR 16000, SP, Brazil), and supernatants were stored at -20°C until analysis. The biochemistry

analysis was performed using a spectrophotometer (Genesys™, Thermo Scientific, USA), whereas the other part was used for histological analysis.

Enzyme-linked Immunosorbent Assay (ELISA) for Myeloperoxidase Activity (MPO), Malondialdehyde (MDA) and Cytokines

The MPO activity and Malondialdehyde (MDA) were measured to evaluate oxidative stress and determined using an ELISA kit (Zen™ Myeloperoxidase ELISA Kit, Sigma-Aldrich, EUA) and using a OxiSelect™ MDA Adduct ELISA kit (Cell Biolabs, Inc., USA), all according manufacturer instructions.

ELISA kits specific for rats (TNF- α (KAP1751), IL-1 β (KAP1211), and IL-6 (KAP1261), DiaSource, Belgium) were used to determine the concentrations of cytokines in tissue homogenates according to the manufacturer's recommendations.

Histological examination

The other part of the lung (right caudal lobe) was dipped in 10% formalin, embedded in paraffin, and cut in sections of 4 μ m. The slides were stained with hematoxylin and eosin (H&E) and interpreted under optical microscope (Zeiss Axion Image A2™, Germany) for descriptive analysis conducted by two blinded pathologists. The damaged levels in these sections were described according to the extent of interstitial cellular infiltration, alveolar protein exudation, and tissue hemorrhage.

Statistical Analysis

In this study, the mean (M) \pm standard deviation (SD) was used to analyze the data. The biochemistry data were compared using Graph Pad PRISM by one-way analysis of variance with Dunn's least significant difference test. $P < 0.05$ was recognized to state the significant difference statistically.

RESULTS

No animals were dead during of the experimental procedures. Acidosis was most observed in the group IAP14 (6.827 ± 0.1464) and were higher when compared with other groups ($p < 0.05$). No differences were observed between Sham compared with IAP5 (7.29 ± 0.045) and IAP8 (7.281 ± 0.029), IAP5 with IAP8, IAP8 with IAP10 (7.11 ± 0.057), and IAP10 with IAP12 (7.01 ± 0.051).

In the Figures 1 to 5 show the average for the parameters analyzed: TNF-alpha, IL-1beta, IL-6, MDA, and MPO, respectively, as well as the results of histological analyses with illustrative images of the changes.

It is possible to observe that the TNF-alpha (Figure 1) values were significantly higher for all experiments compared with those of the Sham group ($p = 0.001$), except

for the comparisons between the experimental group IAP5 and the Sham group and between the IAP5 and IAP8 experimental groups.

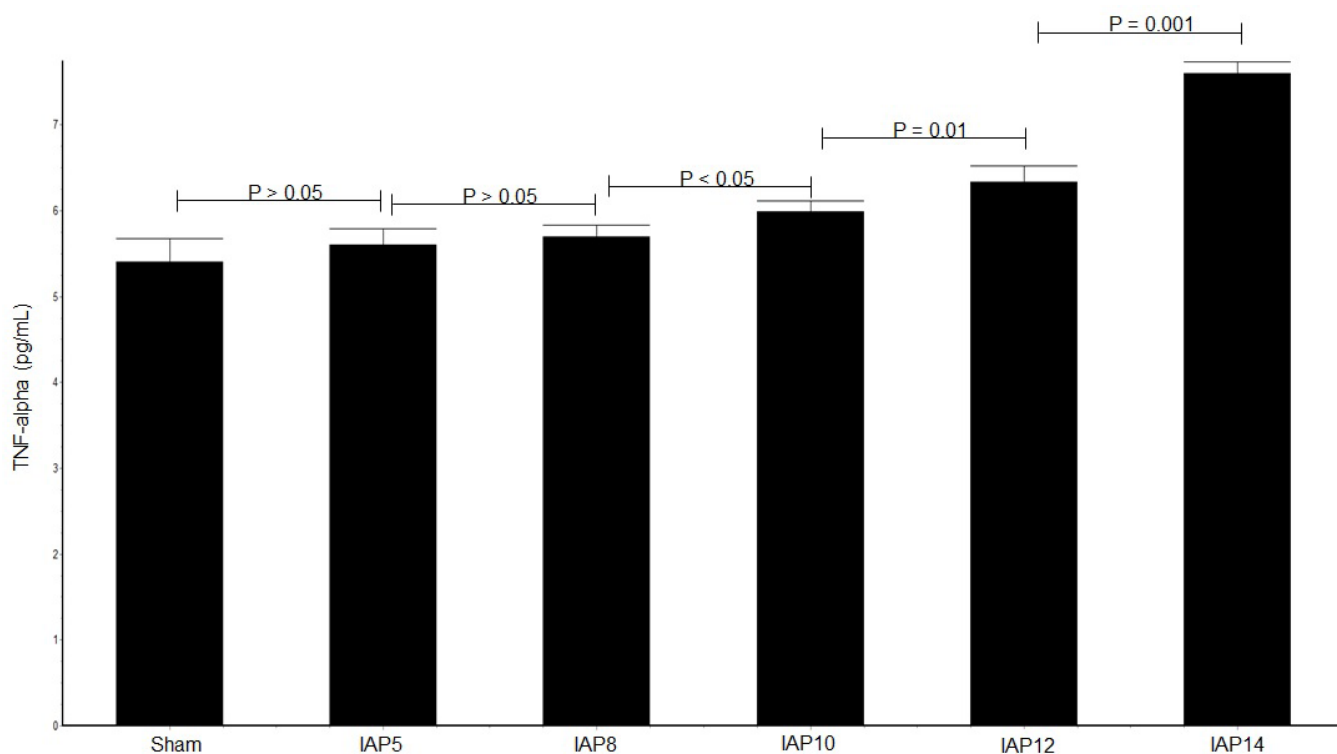


Figure 1: Effect of CO₂ pneumoperitoneum at different IAPs on tissue TNF-alpha concentrations. TNF-alpha levels were not significantly different between groups Sham and IAP-5. Significant differences were observed when comparing the Sham group with all other groups, IAP8 with IAP10, IAP10 with IAP12, and IAP12 with IAP14. TNF-alpha = tumor necrosis factor-alpha; IAP = intra-abdominal pressure.

Regarding IL-1beta (Figure 2), there were significant differences for all comparisons between the control group and the other experimental groups ($p = 0.001$), except for the comparisons between the experimental group IAP5 and the Sham group, and the IAP5 and IAP8 experimental groups.

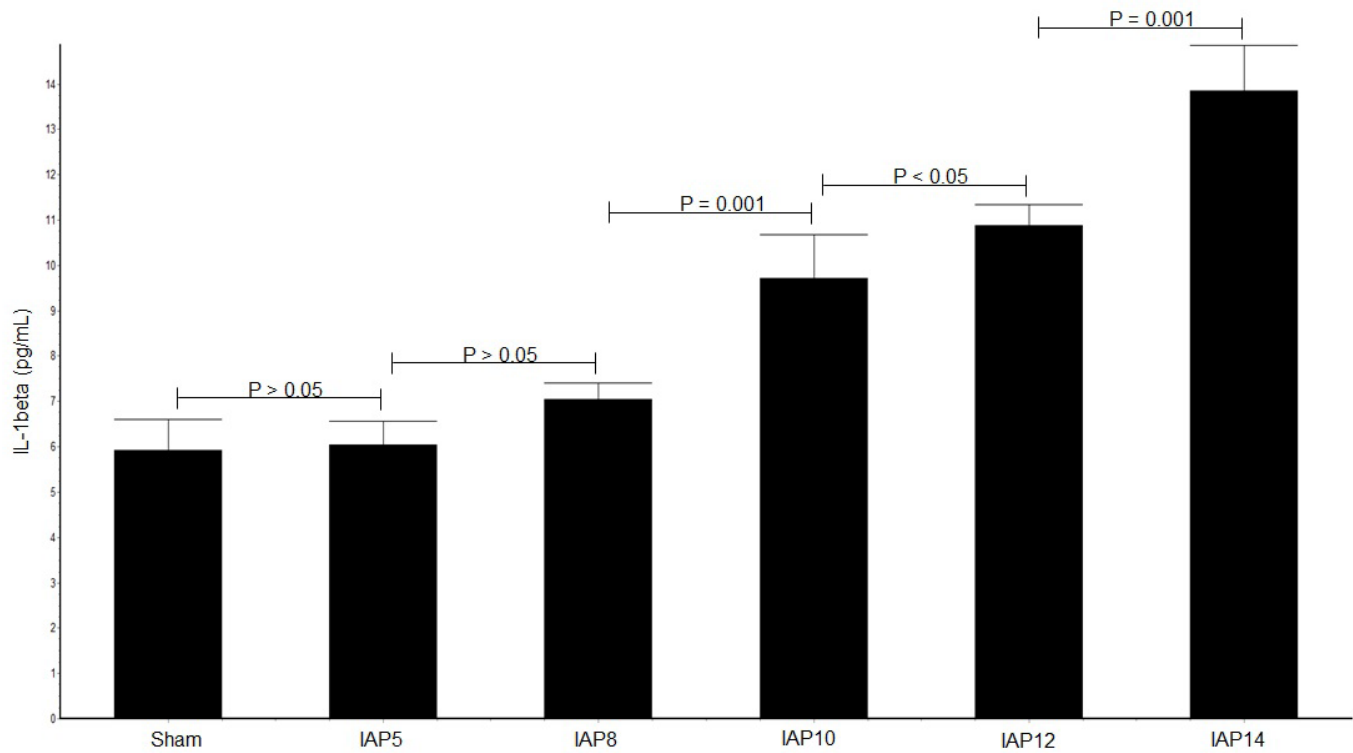


Figure 2: Effect of CO₂ pneumoperitoneum at different IAPs on tissue IL-1b concentrations. IL-1b levels were not significantly different between the Sham, IAP-5 and IAP8 groups. Significant differences were observed when comparing group Sham with all other groups, and comparing IAP8 with IAP10, IAP10 with IAP12, and IAP12 with IAP14. IL1b, interleukin-1beta; IAP, intra-abdominal pressure.

IL-6 levels (Figure 3) were significantly higher for all comparisons between the control group (Sham) and other experimental groups ($p = 0.001$).

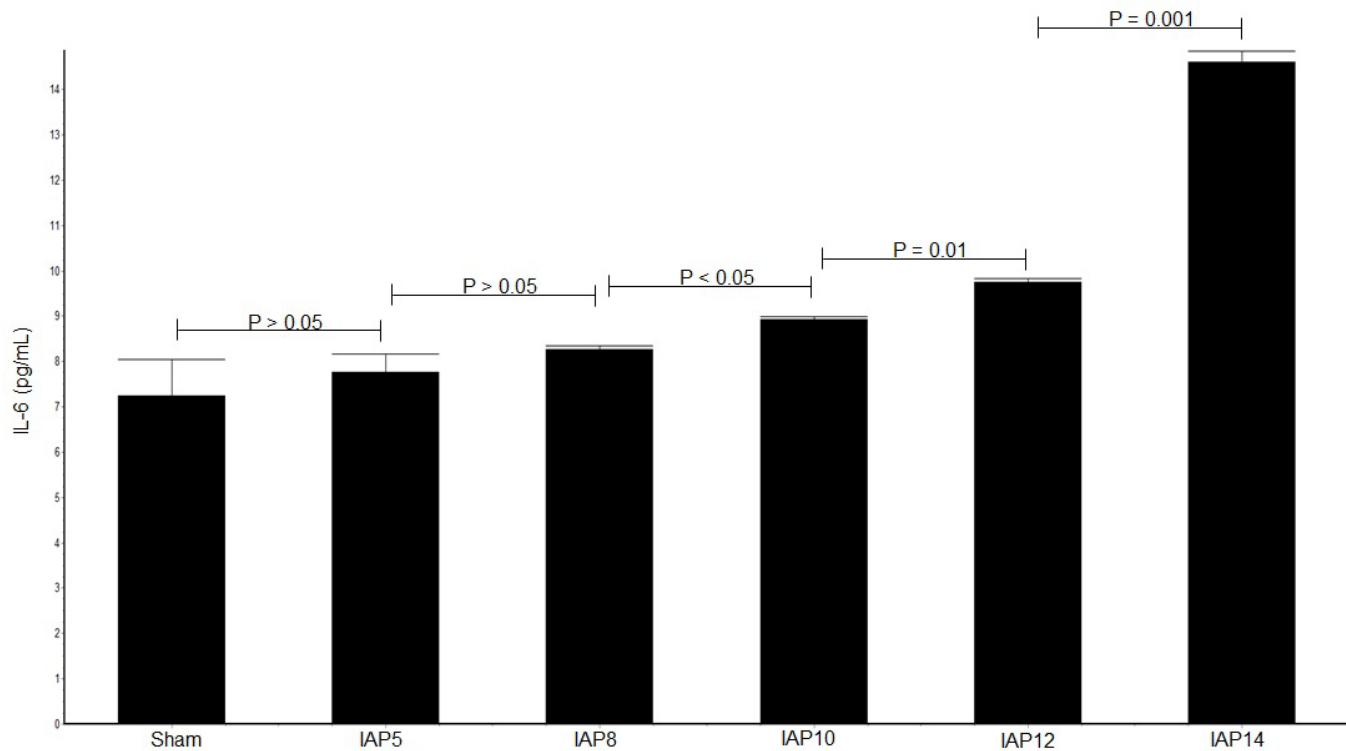


Figure 3: Effect of CO₂ pneumoperitoneum at different IAPs on tissue IL-6 concentrations. IL-6 levels were not significantly different between the Sham and IAP-5 groups and between the IAP5 and IAP8 groups. Significant differences were observed when comparing groups IAP8 with IAP10, IAP10 with IAP12, and IAP12 with IAP14.

Tissue analysis showed increased MDA concentrations (Figure 4) in the lungs for groups IAP8, IAP10, IAP12, and IAP14 when compared with the Sham and IAP5 groups ($p=0.001$). The rats subjected to CO₂ PNP with 8, 10, 12, and 14 mmHg showed significant difference when comparing IAP8 with other groups ($p=0.001$), IAP10 with IAP12 and IAP14 ($p=0.001$), and IAP12 with IAP14 ($p=0.001$).

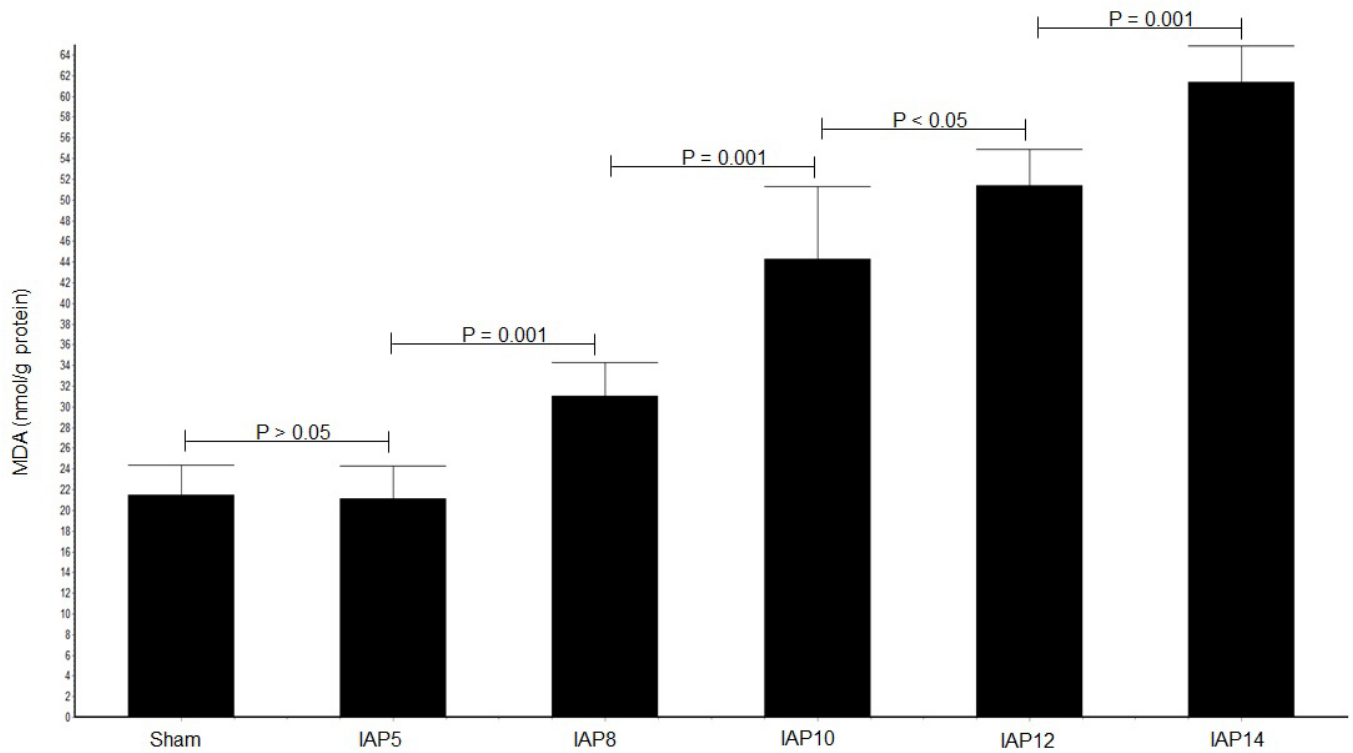


Figure 4: Effect of CO₂ pneumoperitoneum at different IAPs on tissue MDA concentrations. MDA levels were significantly higher in groups IAP8, IAP10, IAP12, and IAP14 than in the Sham and IAP5 group. No difference was observed between groups Sham and IAP5.

MPO levels (Figure 5) were significantly higher in group IAP14 than in others groups ($p = 0.001$). However, there was no difference in MPO levels between groups IAP5 when compared with IAP8.

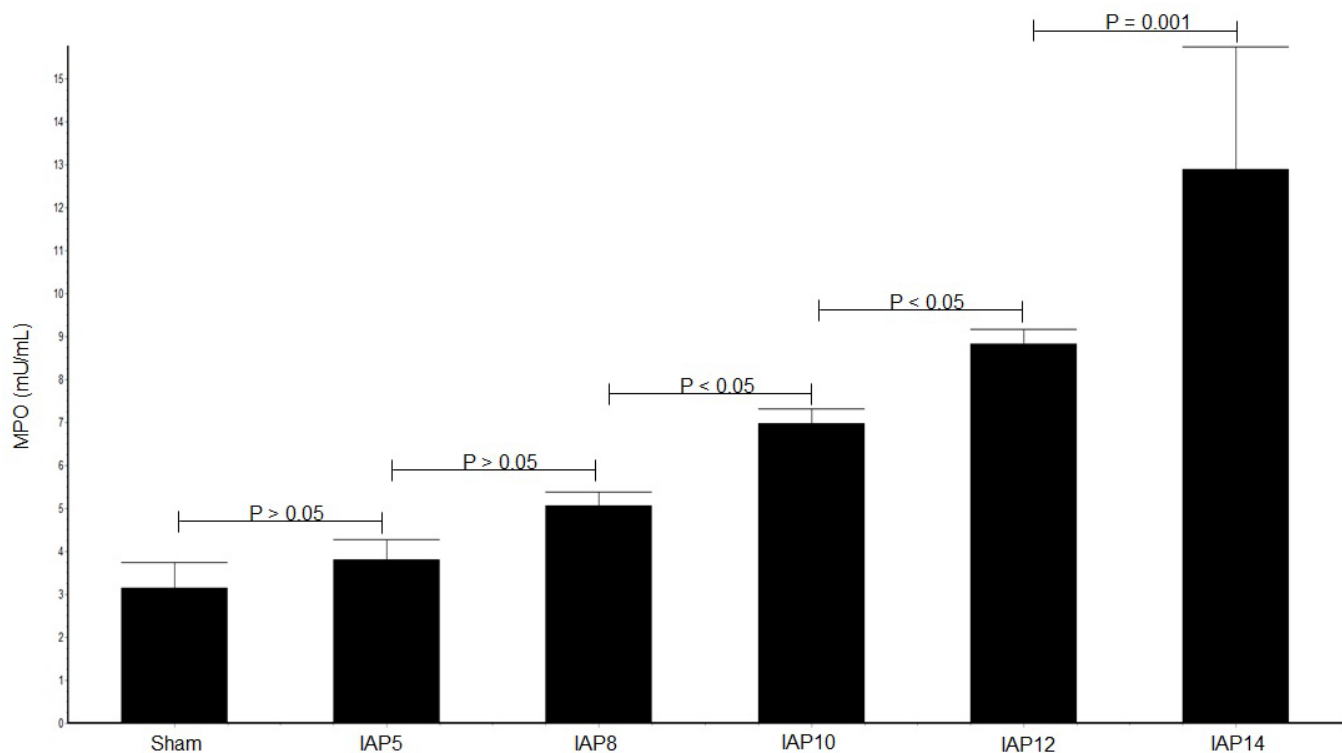


Figure 5: Effect of CO₂ pneumoperitoneum at different IAPs on tissue MPO concentrations. MPO levels were significantly higher in groups IAP8, IAP10, IAP12, and IAP14 when compared with those of the Sham group. There was no statistical difference between groups Sham and IAP5 and IAP5 and IAP8. MPO, myeloperoxidase activity; IAP, intra-abdominal pressure.

On the histological evaluation, analyses of different groups showed histological changes more noticeable and characterizable in the groups with major intra-abdominal pressure regimes. The differences were clearer when comparing the extreme groups (Sham and lower pressures vs. higher pressures). In the group undergoing the regime of higher pressures (IAP14) there was markedly severe disruption of the alveolar septa, edema, diffuse bleeding and presence of increased inflammatory infiltration as in the comparative picture below. The IAP10 and IAP12 groups showed changes in the cellular architecture with swelling, rupturing of the septum, and inflammatory infiltration, but at lower levels than the IAP14 group and clearly higher than the IAP8, IAP5 and Sham groups. Samples of lung tissue of IAP8 group showed some changes in the architecture, alveolar damage, increased alveolar septum and inflammatory infiltration, although to a lesser degree than the groups subjected to higher pressure regimes, as shown below on Figure 6.

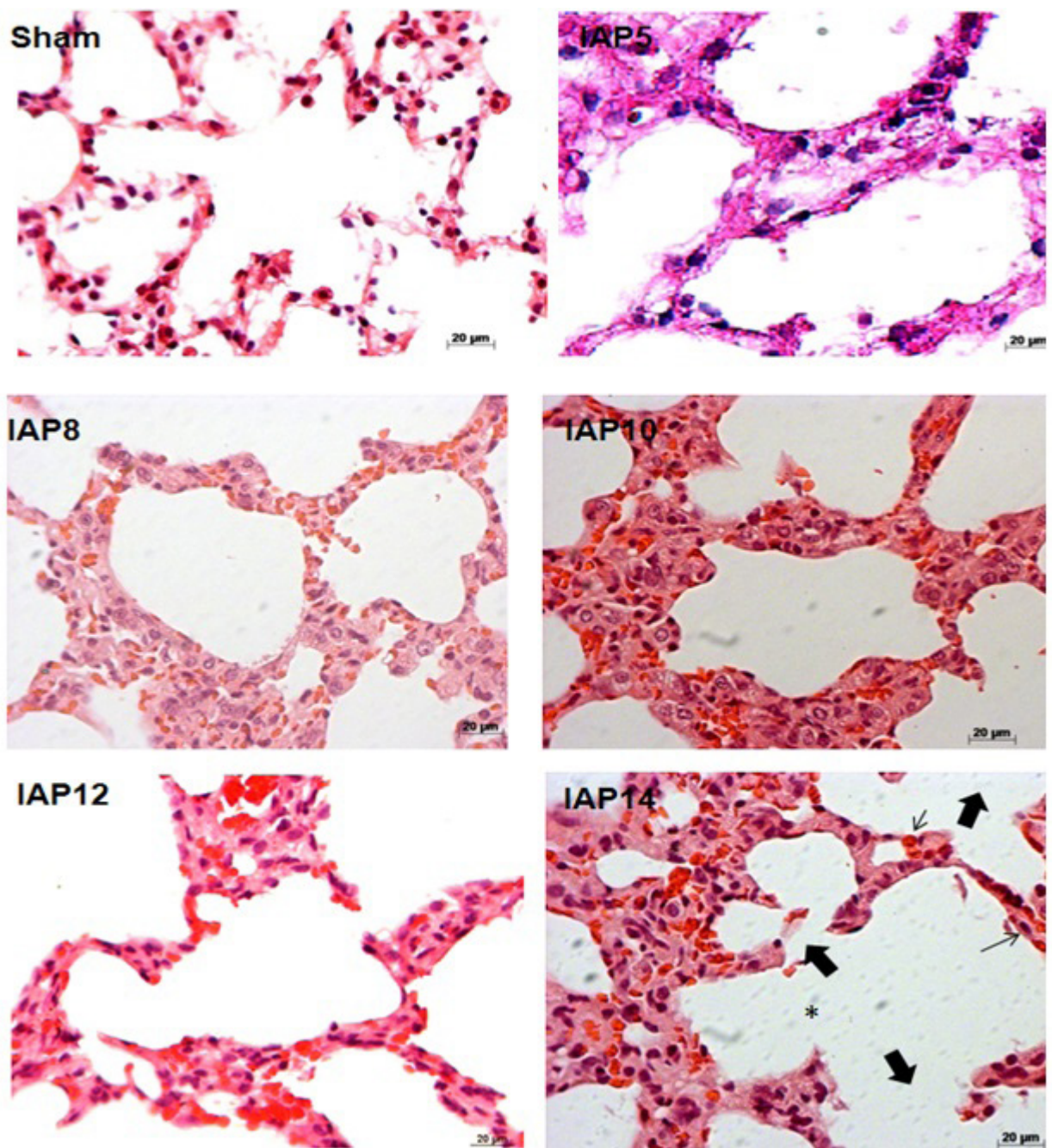


Figure 6: Histological evaluation of the alveoli in group IAP14 showing wall thickening and rupture of alveolar septum (thin full arrow), edema (asterisk), red blood cell (thin small arrow), and PMN influx (thin long arrow). As for the other groups, alterations in the alveolar structure were observed in lesser extension in the lower pressure groups, except for the Sham and IAP5 groups.

DISCUSSION

This study was designed to determine the impact of different IAP in the lungs. The findings were quite consistent and maintained a standard throughout the different groups, showing the direct relationship between the inflation pressure of the PNP and the pulmonary changes, evaluating oxidative stress (MDA and MPO), inflammatory cytokines (TNF-alpha, IL-1beta and IL-6), and histological findings. The study analyzed six different groups using five different levels of IAP to measure

the consistence and magnitude of difference of pulmonary changes with respect to oxidative stress and inflammatory response. Most studies in the literature use only two to three different IAP regimes [25-28] and the used other gases (e.g. nitrous oxide, helium, or room air), but the most used is CO₂ [29].

Reduction of the antioxidant defenses make the cells more susceptible to oxidative attack [30] 30. After 30 min of abdominal deflation, reperfusion in the abdominal organ resulted in increased oxidative stress and lipid peroxidation [27]. Cevrioglu et al., reported increased plasma oxidative stress (MDA) and cytokine response (TNF-alpha and IL-6) in a group receiving 15 mmHg IAP [18]. The present study corroborates and expanded their results by analyzing MDA and cytokines in the lung tissue, finding higher values for the IAP14 group compared with those of the other groups ($p < 0.05$).

In another study, Runck et al. observed decreased compliance of the respiratory system after increase of the IAP in mechanically ventilated mice [21]. The progressive increase of IAP promotes inversely proportional reduction in the respiratory compliance. Decrease in the compliance of the respiratory system implies average increase in the inspiratory pressure values for compensation, leading to increased driving pressure, exposure of the respiratory system under physiological systems with higher pressures, and consequently more inflammation and ROS production. To prevent atelectasis, hypoxemia, and pulmonary lesions, PEEP should not be neglected to counteract the IAP. Therefore, the higher the IAP, the greater the resistance in the respiratory system, which is directly associated with the increase of inflammatory markers [31,32] in accordance with the results obtained in this study.

Pneumoperitoneum increases the inflammation of cytokines causing damage in cell structures, capillary endothelium, and pulmonary tissue, resulting in lung injury [31]. During laparoscopy surgery the production of TNF-alpha was associate with the activity of peritoneal macrophages and after pneumoperitoneum associate with increased levels of IL-6 and TNF-alpha [18,33-36]. Our results show the increase inflammatory mediators in the lung tissue as the IAP increased.

Strang et al. [37] in a porcine model, demonstrated the direct correlation between increased IAP pressures and atelectasis incidence. Different IAP CO₂ levels were applied to mechanically ventilated pigs in the supine position and the proportion of atelectasis was assessed after the application of PNP. The following results were found: the control group (without PNP inflation) presented 4% of atelectasis on average, group IAP8 showed 9%, group IAP12, had 12%, and group IAP16, presented 16 % of atelectasis, showing the direct relationship between the increase in the PNP inflation pressure and the presence of atelectasis.

In our study, the histopathological examination of the lung tissue was consistent with biochemical data analyses. The histological indicators show significant tissue

damage in the IAP12 and IAP14 groups, considering that there is a clash of pressures due to mechanical ventilation associated with intra-abdominal insufflations (PNP), likely leading to some pulmonary hypoperfusion, which is in accordance with the literature. In contrast, low intra-abdominal pressure levels reduce pulmonary damage, confirming the beneficial effects of the use of lower intra-abdominal pressure levels for the PNP inflation corroborated with the other studies [38-42].

PNP induces oxidative stress systemically. The same occurs in the lung submitted to a regime of non-physiological pressure levels, which further accentuates the change in homeostasis. Inflammatory factors caused by the surgery trauma, PNP pressure, and mechanical ventilation promote the formation of these ROS, time-exposure dependent [39-43].

This study has several limitations. First, while the results this study clearly show a no different ventilatory parameters when we used different PNP. Secondly, the position of the rats was not studied, this condition may be affecting the concentration of oxidative stress and inflammatory mediators in the lung. We were also not able to differentiate the changes in activity and expression of other analyses of anti-oxidative and anti-inflammatory mediators. Thirdly, this study was conducted in healthy rats. Lastly, we not evaluated the activity of lung and peritoneal macrophage.

CONCLUSION

In conclusion, our study shows that while lung inflammatory mediators and oxidative stress when we increased intra-abdominal pressure, confirmed in the histological analysis. Although these findings are not transferrable to clinical practice, they highlight the future potential in protocols with the use of low intra-abdominal pressures during CO₂ pneumoperitoneum in laparoscopic surgery.

ACKNOWLEDGEMENTS

The authors are grateful to the Sao Paulo Research Foundation (FAPESP; 14/12730-6) and to the Saul Goldemberg vivarium at the Federal University of São Paulo (UNIFESP) for the maintenance of the animals during the preoperatively phase. Pilot study result was presented at the ATS 2016 International Conference, May 13-18, 2016, San Francisco, California.

COMPLIANCE WITH ETHICAL STANDARDS

Disclosures C.A. Dato, K. Vasconcelos, L.C. Teixeira, and Drs. J.C.M. Brandão, L.F.R. Falcão, D. Ferez, M. Munechika and I.S. Oliveira-Júnior have no conflict

of interest or financial ties to disclose. Dr. I.S. Oliveira-Júnior reports a restricted educational Grant the Sao Paulo Research Foundation during the conduct of this trial.

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