RESEARCH ARTICLE JSLS

Pneumoperitoneum Modifies Serum and Tissue CCL2-CCL5 Expression in Mice

Alfonso Papparella, MD, Carmine Noviello, MD, Sara Ranucci, MD, Orlando Paciello, DVM, Serenella Papparella, DVM, Davide De Biase, DVM, Grazia Cirillo, PhD, Giuseppina Rosaria Umano, MD

ABSTRACT

Background and Objectives: Laparoscopy is the preferred method when operating in the abdomen. In this study, we evaluated systemic and morphological peritoneal cytokine modifications (RANTES/CCL5 and MCP-1/CCL2) due to CO_2 pneumoperitoneum in rats.

Methods: Twenty-five prepubertal Sprague-Dawley rats were randomized into three groups. Pneumoperitoneum lasting 30 minutes, was induced with a flow of 0.5 L/min, in two groups (S1 and S2, n = 20), at a P/CO_2 of 6 and 10 mm Hg, respectively. In the control group (C, n = 5), only anesthesia was carried out. All animals were sacrificed after 24 hours. The serum of the rats was collected for ELISA, and the levels of the cytokines RANTES and MCP-1 were investigated. An immunohistochemical analysis of RANTES and MCP-1 was performed on samples of the peritoneum, and the morphological evaluation was conducted with a blinded evaluation by two independent, experienced pathologists by using a grading system (0, 1+, 2+, 3+: no, faint, moderate, and strong reactivity, respectively).

Results: RANTES mean levels were significantly different in the S1, S2, and C groups (70.3 ± 2.26, 58.23 ± 4.32, 29.66 ± 4.03, respectively, $P = .0001$). The levels of MCP-1 were 32.1 ± 1.63 in the S1 group, 27.0 ± 9.26 in the S2 group, and 16.4 ± 9.55 in the C group ($P = .159$). Normal control peritoneum showed little reactivity, whereas a moderate to strong cytoplasmic reaction to anti-CCL5/CCL2 antibodies was observed in mesothelial and inflammatory cells in the S1 and S2 groups.

Conclusion: CO_2 pneumoperitoneum evokes an inflammatory response by modifying plasma RANTES levels and peritoneal CCL5/CCL2 expression.

Key Words: Pneumoperitoneum; Laparoscopy; Inflammation; CCL5; CCL2.

INTRODUCTION

Laparoscopy is now considered a superb diagnostic tool and is the preferred method to explore and operate in the abdominal cavity. The gas used in videoendoscopic laparoscopic surgery to induce pneumoperitoneum (PN) is carbon dioxide (CO_2), which is inert, blood soluble, and not explosive.[1] Many symptoms that are reported after laparoscopy are generally attributed to CO_2 insufflation into the peritoneal cavity. The peritoneum has a large capacity for absorption and reaction; it is one of the rich, vascularized organs with a surface lining that is coated by highly differentiated mesothelial cells. The peritoneum is able to synthesize chemokines, which are cytokines that are significant in driving different elements of inflammation.[2] The creation of CO_2 PN induces morphological alterations in the mesothelium, which are microscopically apparent. We have previously described a peritoneal chronical inflammation in rats 24 hours after PN and consisting mostly of eosinophils, granulocytes, mastocytes, and macrophages.[3] These modifications appear to be related to intraabdominal CO_2 pressure.[4] Volz and Suematsu demonstrated ultrastructural changes after PN, such as swelling up of mesothelium and an enlargement of intercellular junctions.[5,6] The observed uncovering of the mesothelium basement membrane may be dependent from macrophage-evoked changes of peritoneal immunity and could be assessed as the main cause for surface-

Department of Woman, Child, and General and Specialized Surgery, University of Campania "Luigi Vanvitelli," Naples, Italy (Drs Papparella, Ranucci, Cirillo, and Umano).

Department of Pediatric Surgery, Salesi Hospital, Ancona, Italy (Dr. Noviello).

Faculty of Veterinary Medicine, Department of Pathology and Animal Health, University of Naples Federico II, Naples, Italy (Drs Paciello, Papparella, and De Biase).

Disclosures: none.

Funding/Financial Support: none.

Conflicts of Interest: The authors have no conflicts of interest directly relevant to the content of this article.

Informed consent: Dr. Papparella declares that written informed consent was obtained from the patient/s for publication of this study/report and any accompanying images.

Address correspondence to: Alfonso Papparella, MD, Department of Woman, Child, and General and Specialistic Surgery, University of Campania "Luigi Vanvitelli," Largo Madonna delle Grazie n. 1, 8013, Naples, Italy. Phone: 0039 081 566 5352, Fax: 0039 081 5665352, E-mail: alfonso.papparella@unicampania.it.

DOI: 10.4293/JSLS.2020.00017

© 2020 by *JSLS, Journal of the Society of Laparoscopic & Robotic Surgeons.* Published by the Society of Laparoendoscopic & Robotic Surgeons, Inc.

induced adherence. Bertram et al[7] advised that injection and grafting of mesothelial cells into the abdomen was related to reduced development of adhesions. Thus, to decrease postinflammatory and surgical adhesions, it is important to maintain the integrity of the peritoneal surface. Leukocytes have a primary role when the peritoneal serosa is damaged. Activation of these cells and degranulation release active components, such as cytokines and growth factors.[8,9] Among the cytokines, RANTES/CCL5 (Regulated upon Activation of Normal T cell Expressed and Secreted/Chemokine [C-C motif] ligand 5) and MCP-1/CCL2 (Monocyte Chemoattractant Protein-1/Chemokine [C-C motif] ligand 2) exert proinflammatory activities. These chemokines are secreted in infectious and inflammatory diseases and mediate the recruitment of immune cells. In particular, RANTES/CCL5 is produced by different cell types, such as T lymphocytes, macrophages, platelets, and eosinophils playing an active role in the migration of these cells. Nevertheless MCP-1/CCL2 mediates monocyte and macrophage recruitment and migration to the inflammation site.[10]

Furthermore, laparoscopy causes intra-abdominal acidosis, perhaps evoked by the local decrease in partial oxygen pressure. This effect seems to have a beneficial influence on the peritoneal immune system.[11] Following an open surgical procedure, there is a surge in proinflammatory cytokines, such as TNF-α and IL-1, that are attenuated by laparoscopy.[12–14] Therefore, laparoscopic surgery changes not only peritoneal integrity but also its biological environment.

The study was conducted to examine the effect of CO_2 PN on the serum levels of inflammatory cytokines and the corresponding tissue immunohistochemical analysis and expression.

MATERIALS AND METHODS

Animals

The experiment was conducted as previously reported.[3,4] Twenty-five prepubertal Sprague-Dawley rats were in the object of the protocol. Animals were housed following the protocol suggested by Institutional Animal Care and Use Committee of the University of Campania "Luigi Vanvitelli," Faculty of Medicine, in agreement with the recommendations of National Institutes of Health. The rats were hosted in a stable arrangement (12 hours of daylight at a temperature of 22°C) with food and water ad libitum.

Experimental Protocol

On the day of the study, the rats were randomized as follows: PN was evoked in two groups with CO_2 (S1 and S2, n = 20) with a flow rate of 0.5 L/minute. In the last group (C, n = 5), only general anesthesia was executed and acted as control. The designated pressures were chosen according to our previous protocol and other studies.[3,4,15]

Anesthesia was induced by an intramuscular administration in the legs of the animal with a dose of 5 mg/kg tiletamine-zolazepam hydrochloride at a 1:1 ratio and a dose of 0.2 mg/kg and xylazine at a dose of 5 mg/kg into the leg of the animal. Fifteen minutes after, the abdominal wall was shaved and cleaned; a Veress needle was inserted in the abdominal cavity, and PN was created with an inflator (Storz Electronic CO_2 Endoflator, Karl Storz GmbH & Co., Tuttlingen, Germany) that delivered medical CO_2 at 37°C and an outflow of 0.5 L/minute for 30 minutes at different pressures (6 and 10 mm Hg). All rats were sacrificed after 24 hours from surgery with a lethal administration of tiletamine-zolazepam hydrochloride.

Cytokines in rats with PN at 6 mm Hg and 10 mm Hg and in control rats were measured. At 24 hours after PN, the serum was collected and conserved at −20°C until cytokine evaluation, and then, an enzyme-linked immunosorbent assay (ELISA) was performed. In particular, cytokine levels (IL-6, IL-1α, IL-1β, TNF-α, MIP IFNr, MCP-1, and RANTES) were quantified in serum samples, which were optimally diluted.

A multiplex assay kit manufactured by Signosis (Cat. N. EA-1201; Santa Clara, CA, USA) was used for the contemporaneous measurement of the mentioned cytokines.

All optical densities were measured at 450 nm. The levels of cytokines are expressed as ng/mL.

Histological Examination

Immunohistochemical analysis was performed on paraffin-embedded sections following a well-established method.[16] Primary antibodies included CCL5/RANTES AB finity recombinant rabbit oligoclonal antibody (catalog no. 710001) diluted 1:500 in PBS, rabbit polyclonal MCP-1 (ab9669, AbCam, Cambridge, UK) diluted 1:1000 in PBS. Slides were evaluated independently by two experienced pathologists (SP and OP), with good agreement (Cohen's κ, $P < .001$). The κ-value between the two sets of scores was 0.82, indicating good concordance. The expression of CCL5/RANTES and MCP-1 was semi quantitatively evaluated according to the intensity of cytoplasmic tissue stain

from 0 to 3 as follows: (0, 1+, 2+, 3+: no, faint, moderate, and strong reactivity, respectively). To identify the predominant lymphocyte subtype, the labeled immune cells number was determined for each slide and each antibody in all groups and is expressed as the mean of at least 10 fields at 40× magnification.

Statistical Analysis

Variables were tested for normality and were log transformed to achieve normal distribution as appropriate. Differences between different treatments were evaluated by ANOVA to analyze the effect of each cytokine. The results are expressed as the mean ± SD. A value of $P < .05$ was considered statistically significant.

RESULTS

The rats showed no clinically relevant symptoms. To understand the effects of PN treatment on inflammatory response mediators, a probable pattern of cytokines that are involved in immunological mechanisms was examined.

The rats insufflated with 10 mm Hg and 6 mm Hg PN showed higher plasma TNF-α levels (0.32 ± 0.1 and 0.26 ± 0.04, respectively) compared to those of the control group (0.23 ± 0.01), with the 10 mm Hg PN group presenting higher levels compared to those of the 6 mm Hg PN rats with no statistical significance ($P = .38$) (**Figure 1**). Similarly, the three groups had different plasma IL-6 levels, although the observed difference was not significant (10 mm Hg PN, 1.11 ± 0.26; 6 mm Hg PN, 0.93 ± 0.28; controls, 0.73 ± 0.18; $P = .293$) (**Figure 2**).

Interestingly, PN groups showed higher RANTES levels compared to controls, and the differences were statistically significant. In particular, the mean RANTES level in the 6 mm Hg PN samples was 70.3 ± 2.26 vs 29.66 ± 4.03 in the controls ($P = .0000$). Similarly, the 10 mm Hg PN group had significantly higher RANTES levels than controls (58.23 ± 4.32 vs 29.66 ± 4.03, $P = .0001$) (**Figure 3**). In addition, the levels of MCP-1 were more increased in the 6 mm Hg PN group (32.1 ± 1.63) than in the 10 mm Hg PN group (27.0 ± 9.26) compared with those of the control group (16.4 ± 9.55). However, the values between groups were not significantly different ($P = .159$) (**Figure 4**). Interestingly, both RANTES and MCP-1 levels showed a trend toward higher inflammatory cytokine values in low respect to high pressure group, but they were not significant.

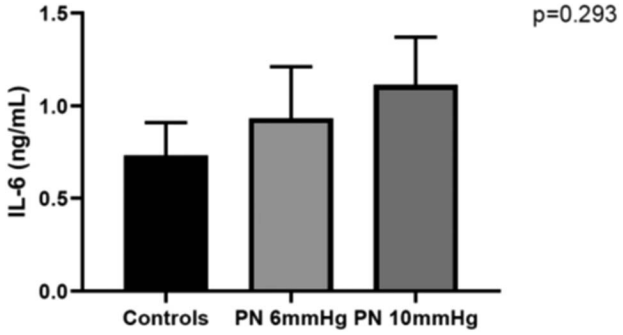

Figure 2. Serum IL-6 levels after PN in the control, PN 6 mm Hg, and PN 10 mm Hg groups. The data are expressed as the mean ± SD.

No differences were reported regarding IL-1α and MIP levels among the three groups. IL-1β value did not exceed the assay limit; however, samples from the 6 mm Hg PN group showed IL-1β concentrations that were 4-fold higher than those of the 10 mm Hg PN group.

Finally, IFN-γ levels were slightly increased in PN rats compared with those of control rats.

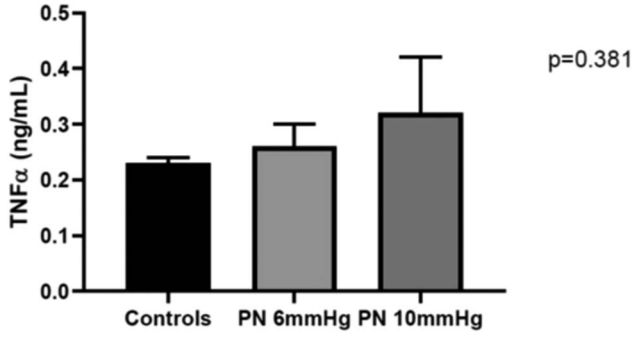

Figure 1. Serum TNF-α levels after PN in the control, PN 6 mm Hg, and PN 10 mm Hg groups. The data are expressed as the mean ± SD.

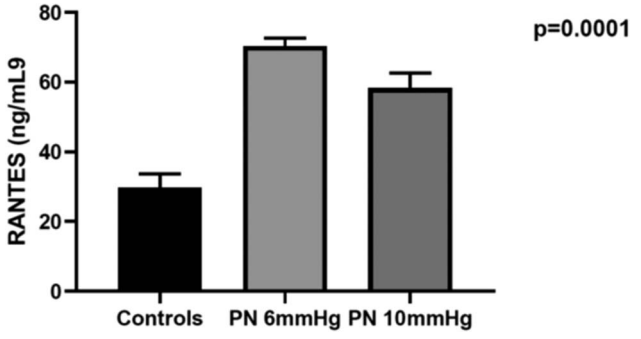

Figure 3. Serum RANTES levels after PN in the control, PN 6 mm Hg, and PN 10 mm Hg groups. The data are expressed as the mean ± SD.

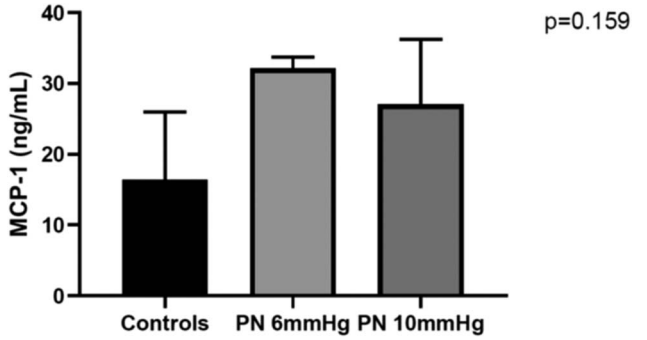

Figure 4. Serum MCP-1 levels after PN in the control, PN 6 mm Hg, and PN 10 mm Hg groups. The data are expressed as the mean ± SD.

Histological Results

Normal control peritoneum shows absent to faint reactivity to the anti-CCL5/RANTES antibody (**Figure 5, A and B**). The visceral peritoneum of the 6 mm Hg PN group showed faint to moderate reactivity of mesothelial cells to the anti-CCL5/RANTES antibody (**Figure 6, A and B**), whereas at 10 mm Hg, PN, there was strong cytoplasmic reactivity of mesothelial cells to the anti-CCL5/RANTES antibody (**Figure 7A** [arrows]), and strong immunoreactivity was observed in omentum adipose tissue (**Figure 7B** [arrows]) and was associated with inflammatory cells **Figure 7B** [arrow heads]). Faint reactivity to the anti-MCP-1 antibody was also observed in the control peritoneum (**Figure 8A**, [arrows]). Adipose tissue and the inflammatory infiltrates showed moderate to strong immunoreactivity to the anti-MCP-1 antibody in 6 mm Hg PN group (**Figure 8B** [arrows]). Strong immunoreactivity to the MCP-1 antibody was observed both in omentum adipose tissue and inflammatory infiltrates (**Figure 8C**).

DISCUSSION

Laparoscopy is widely used and well incorporated in clinical practice as the preferred, minimally invasive technique for operating in the abdominal cavity. The induced PN modifies the peritoneal environment. The gas that is usually employed to lead to PN is CO_2. When the abdomen is distended by CO_2, it exerts a direct pressure on peritoneal cells and reacts with the H_2O found in peritoneal liquid. This phenomenon decreases the pH of abdominal cavity and determines an environment that changes the defense mechanisms of the abdominal cavity,[17,18] which is an element in reducing the inflammatory response.[11]

Vascularized tissue, such as peritoneum, replies to chemical or physical insult through an inflammatory reaction. Soluble molecules such as chemokines have been demonstrated to stimulate diverse inflammatory factors, such as leukocyte influx.[9]

The peritoneum has a great capacity for absorption and action through mesothelial cells, which synthesize cytokines and chemokines that are primary factors in tissue inflammation.

In previous works, we showed that PN causes peritoneal inflammation and the activation of inflammatory cells that release substances responsible for the increase in vascular permeability, complement, and opsin.[3,4] To understand the effects of laparoscopy with CO_2 PN on mediators of inflammatory responses, the cytokines involved in immunological responses were examined. Several papers have confirmed a considerable increase in some cytokines.[19,20] IL-6 represents the most important cytokine determined postoperatively, followed by TNF-α. In our experimental protocol, serum levels of IL-6, TNF-α, IL-1α, IFN-γ, MCP-

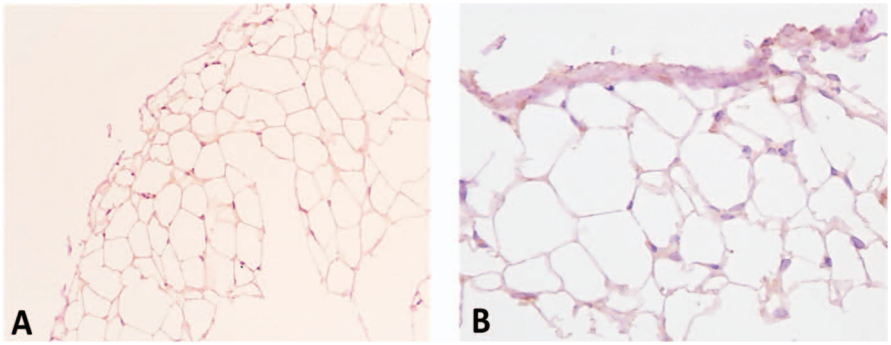

Figure 5. Omentum and visceral peritoneum. **A** and **B**, Normal control peritoneum showing absent to faint reactivity to the anti-CCL5/RANTES antibody. Haematoxylin counterstain. Original magnification, **A**, 20×; and **B**, 40×.

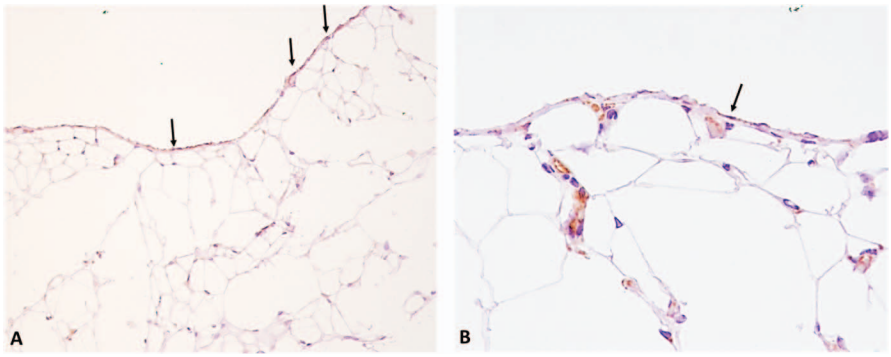

Figure 6. Omentum and visceral peritoneum. **A** and **B**, CO_2 6 mm Hg PN showing faint to moderate reactivity to the anti-CCL5/RANTES antibody of mesothelial cells (arrows). Haematoxylin counterstaining. PN, pneumoperitoneum. Original magnification, **A**, 20×; and **B**, 40×.

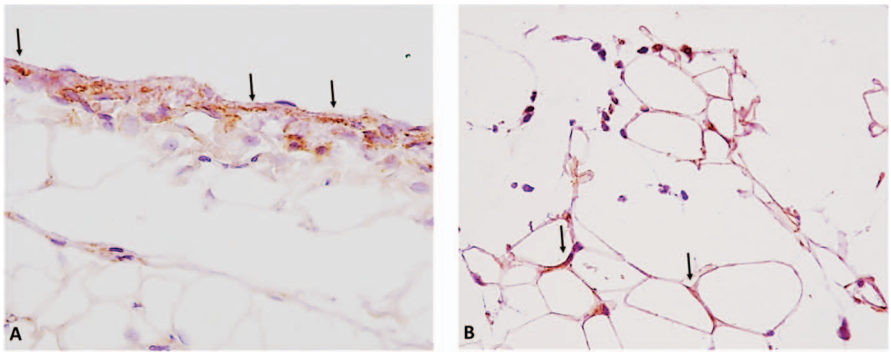

Figure 7. Omentum and visceral peritoneum. **A**, 10 mm Hg PN showing strong cytoplasmic reactivity to the anti-CCL5/RANTES antibody of mesothelial cells (arrows). **B**, A strong immunoreactivity is observed in omentum adipose tissue (arrows), also associated to inflammatory cells (head arrows). Haematoxylin counterstaining. PN, pneumoperitoneum. Original magnification, **A**, 20×; and **B**, 40×.

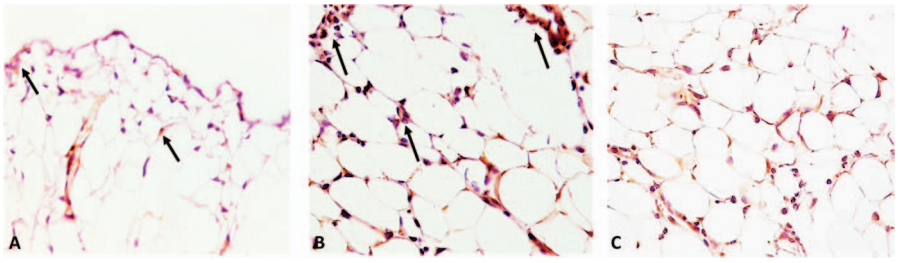

Figure 8. Omentum and visceral peritoneum. **A**, A faint reactivity to the anti-MCP1 antibody is observed in normal control peritoneum (arrows). **B**, CO_2 6 mm Hg PN: adipose tissue and the inflammatory infiltrate showing moderate to strong immunoreactivity to the anti-MCP1 antibody (arrows). **C**, CO_2 10 mm Hg PN: a strong immunoreactivity to MCP1 antibody is observed both in omentum adipose tissue and inflammatory infiltrate. Haematoxylin counterstain. Original magnification, **A**, 20×; and **B** and **C**, 40×.

1/CCL2, IL-1β, RANTES/CCL5, and MIP were assessed after inducing PN at two different pressures and in the control group. We showed that PN modifies serum concentrations and tissue expression of the chemokines CCL5 and CCL2. RANTES/CCL5 showed the most dramatic increase.

RANTES/CCL5 controls the activation and recruitment of inflammatory cells. Moreover, since chemokine receptors are also expressed on other cells, they may also play a role in other regions of the organism, as well as in mechanisms of tissue repair and scarring.[21] There are many studies that establish a role of CCL5 in disease activity and in leading

to leukocyte enrollment, neovascularization, and formation of fibrous tissue.[21] In our study, we showed that there was not only a significant modification of the serum levels but also strong tissue expression, suggesting the influence of this chemokine in the development of inflammation. Kawashima et al[9] showed that during laparotomy, a large amount of CCL5 was produced, and they postulated that this increase may be indicative of mesothelial cell injury. They noted that in the presence of lipopolysaccharide, there was no significant production of CCL5 by exudate cells.[9] Our study showed significant modifications in RANTES/CCL5 serum levels in the groups insufflated with CO_2 compared with those of the control group. This chemokine, together with CCL2, were also strongly expressed in peritoneal tissues, as revealed by histological examination. Therefore, we may speculate that these modifications are directly indicative of mesothelial cellular injury.

Interestingly, we found a trend toward higher inflammatory cytokine levels in the low-pressure group. In our opinion, these results could be related to the experimental model and its variables. However, we might speculate that low pressure is associated with higher CO_2 absorption and mesothelial oxidative stress that evoked a different cytokines' responses.[8,22]

Cytokines have been identified as important mediators in both acute and chronic human inflammatory disorders. The role of inflammatory mediators is not completely understood, especially in morbidity and mortality of intra-abdominal sepsis, and there is no consensus on the clinical use of mediators in diagnosing or managing this problem. However, recently, many researchers have tried to evaluate cytokines and chemokines in order to define a diagnostic and a therapeutic role. In Pediatric appendicitis, through an analysis of inflammatory mediators, the assessment of previously identified mediators such RANTES and MCP-1 demonstrate statistical differences in children with appendicitis when compared with those with abdominal pain not due to appendicitis. Therefore, the cytokines mediate their response in relation to a different inflammatory stimulus.[23] If there is a difference in cytokine response between normal, infected, or previously inflamed peritoneum clearly more studies are necessary to understand how cytokines relate each other and how we could modify this clinical status in order to add a benefit for the patient.

As previously stated, cellular components of mesothelium contribute not only to inflammation but also to tissue repair. PN causes changes in mesothelial cells by extracting the basal membrane and thus leads to the development of postsurgical adherences.[3–5]

Experimental protocols have reported that mesothelium undergoes structural and functional modifications, which are consistent with a mesothelial-mesenchymal transition after an inflammatory stimulation. The capacity of the mesothelium to be a source of fibrogenic cells suggests that it might be an active player in serous fibrosis.[2]

Following serous lesions, mesothelial cells secrete several coagulation factors and inflammatory mediators that may induce the development of adherences.[24] It has been shown that in peritoneal dialysis or in infection of the abdominal cavity, leukocytes migrate from the vasculature into the peritoneal space. Macrophages are able to release cytokines such as TNF-α or IL-1β that stimulate to produce RANTES. This effect further attracts more white blood cells to the place of trauma and allows adhesion and recruitment of leukocyte through the mesothelium.[2] Therefore, different strategies and actions have been proposed to avoid damage to the peritoneal membrane, as well as surgical fluid barriers (4% icodextrin).[24]

Postoperative intra-abdominal adhesions are an important clinical and surgical problem. They are a relevant cause of pain, female sterility, and bowel occlusion that can occur for up to 20 years.[25–27]

When the normal peritoneal healing process is altered, the development of fibrous tissue is thought to occur.[25] The influx of cells such as lymphocytes, fibroblasts, and macrophages have a pivotal role in the serosal layer repair and are influenced by PN.[24] It has been recently discovered, in a murine model, that chemokines are involved in the postoperative adhesions through the mesothelium cellular assembling[9] by the self activation of peritoneal macrophages.

To regulate and mitigate this process, many anti-inflammatory and anticoagulatory substances, such as steroids and heparin, have been tested at the systemic and local levels, but none of these has proven to be effective.[24] Satisfactory results in preventing adhesion development have been obtained experimentally through mesothelial cell transplantation.[7,28] Clearly a better understanding the potential role of RANTES and MCP-1 and the process that cause the development of adhesion formation and inflammation, and how some recognized injuries such as infections or neoplastic condition may have an increased detrimental effect would add a step further in developing new strategies of care. However, since we have conducted our study in a clean peritoneal environment, we

could not evaluate the differences in cytokine response in infection and or neoplastic conditions; nonetheless, when a safe mesothelial environment is present, obtaining less inflammatory cytokine response in the postoperative period as in clean surgery could be considered beneficial to the patient, but to date, targeted therapies remain experimental. We acknowledge that systemic consequences of peritoneal treatments should be investigated.

As reported above, the use of CO_2 for PN, the flow rate and the selected intra-abdominal pressure can cause biochemical and morphological modifications, hypoxia, and dehydration to mesothelium.[3,4,28,29] The development of laparoscopic surgical techniques, the use of modern devices and image magnification support less invasive surgery that can avoid the insult of mesothelial cells and bleeding, which are the origins of adhesions. To avoid these complications, some researchers have tried to change the physical qualities of CO_2, such as humidifying, raising the temperature and the altering composition of the gas used for insufflation.[2,30] Although more studies are needed looking at the potential role of RANTES and MCP-1, we suggest that targeting these chemokines could be beneficial in preventing postoperative peritoneal modifications such inflammation and adhesions. In terms of benefits for the patient, less pain, and fewer postoperative complications could be associated with minimally invasive surgery.

CONCLUSION

In conclusion, we have demonstrated in this study that PN modifies the serum and the corresponding tissue expression of the chemokines CCL5 and CCL2, which are involved in tissue inflammation and response to insults exerted by chemical and physical CO_2 action on the abdominal cavity. Targeting these factors appears to be an important means of providing an additional benefit to laparoscopy by modifying peritoneal inflammation and potential subsequent peritoneal adhesions. More studies are necessary to clarify these mechanisms and their possible influence in humans.

References:

1. Yu T, Cheng Y, Wang X, et al. Gases for establishing pneumoperitoneum during laparoscopic abdominal surgery. *Cochrane Database Syst Rev.* 2017;6:CD009569.

2. Mutsaers SE, Birnie K, Lansley S, Herrick SE, Lim CB, Prêle CM. Mesothelial cells in tissue repair and fibrosis. *Front Pharmacol.* 2015;6:113.

3. Papparella A, Noviello C, Romano M, Parmeggiani P, Paciello O, Papparella S. Local and systemic impact of pneumoperitoneum on prepuberal rats. *Pediatr Surg Int.* 2007;23:453–457.

4. Papparella A, Nino F, Coppola S, Noviello C, Paciello O, Papparella S. Peritoneal morphological changes due to pneumoperitoneum: The effect of intra-abdominal pressure. *Eur J Pediatr Surg.* 2014;24:322–327.

5. Volz J, Köster S, Spacek Z, Paweletz N. Characteristic alterations of the peritoneum after carbon dioxide pneumoperitoneum. *Surg Endosc.* 1999;13:611–614.

6. Suematsu T, Hirabayashi Y, Shiraishi N, Adachi Y, Kitamura H, Kitano S. Morphology of the murine peritoneum after pneumoperitoneum vs laparotomy. *Surg Endosc.* 2001;15:954–958.

7. Bertram P, Tietze L, Hoopmann M, Treutner KH, Mittermayer C, Schumpelick V. Intraperitoneal transplantation of isologous mesothelial cells for prevention of adhesions. *Eur J Surg.* 1999;165:705–709.

8. Mutsaers SE, Whitaker D, Papadimitriou JM. Stimulation of mesothelial cell proliferation by exudate macrophages enhances serosal wound healing in a murine model. *Am J Pathol.* 2002; 160:681–692.

9. Kawashima R, Kawamura YI, Oshio T, et al. Comprehensive analysis of chemokines and cytokines secreted in the peritoneal cavity during laparotomy. *J Immunoassay Immunochem.* 2012; 33:291–301.

10. Griffith JW, Sokol CL, Luster AD. Chemokines and chemokine receptors: Positioning cells for host defense and immunity. *Annu Rev Immunol.* 2014;32:659–702.

11. Ure BM, Niewold TA, Bax NM, Ham M, van der Zee DC, Essen GJ. Peritoneal, systemic, and distant organ inflammatory responses are reduced by a laparoscopic approach and carbon dioxide versus air. *Surg Endosc.* 2002;16:836–842.

12. Menger MD, Vollmar B. Surgical trauma: Hyperinflammation versus immunosuppression? *Langenbecks Arch Surg.* 2004;389: 475–484.

13. Kuntz C, Wunsch A, Bay F, Windeler J, Glaser F, Herfarth C. Prospective randomized study of stress and immune response after laparoscopic vs. conventional colonic resection. *Surg Endosc.* 1998;12:963–967.

14. Redmond HP, Watson RW, Houghton T, Condron C, Watson RG, Bouchier-Hayes D. Immune function in patients undergoing open vs. laparoscopic cholecystectomy. *Arch Surg.* 1994;129: 1240–1246.

15. Avital S, Itah R, Szomstein S, et al. Correlation of CO_2 pneumoperitoneal pressures between rodents and humans. *Surg Endosc.* 2009;23:50–54.

16. De Biase D, Esposito F, De Martino M, et al. Characterization of inflammatory infiltrate of ulcerative dermatitis in C57BL/6NCrl-Tg(HMGA1P6)1Pg mice. *Lab Anim* 2019;53:447–458.

17. Neuhaus SJ, Watson DI, Ellis T, Lafullarde T, Jamieson GG, Russell WJ. Metabolic and immunologic consequences of laparoscopy with helium or carbon dioxide insufflation: A randomized clinical study. *ANZ J Surg.* 2001;71:447–452.

18. Kuntz C, Wunsch A, Bodeker C, et al. Effect of pressure and gas type on intraabdominal, subcutaneous, and blood pH in laparoscopy. *Surg Endosc.* 2000;14:367–371.

19. Bellón JM, Manzano L, Bernardos L, et al. Cytokine levels after open and laparoscopic cholecystectomy. *Eur Surg Res.* 1997;29:27–34.

20. Lingohr P, Dohmen J, Matthaei H, et al. Cytokine expression in the visceral adipose tissue after laparoscopic and conventional surgery in a rodent model. *Eur J Med Res.* 2016;21:4.

21. Russo RC, Garcia CC, Teixeira MM. Anti-inflammatory drug development: Broad or specific chemokine receptor antagonists? *Curr Opin Drug Discov Devel.* 2010;13:414–427.

22. Sahin DA, Haliloglu B, Sahin FK, et al. Stepwise rising CO_2 insufflation as an ischemic preconditioning method. *J Laparoendosc Adv Surg Tech A.* 2007;17:723–729.

23. Shommu NS, Jenne CN, Blackwood J, et al. Metabolomic and inflammatory mediator based biomarker profiling as a potential novel method to aid pediatric appendicitis identification. *PLoS ONE.* 2018;13:e0193563.

24. Brochhausen C, Schmitt VH, Rajab TK, et al. Mesothelial morphology and organization after peritoneal treatment with solid and liquid adhesion barriers: A scanning electron microscopical study. *J Mater Sci Mater Med.* 2012;23:1931–1939.

25. Dizerega GS, Campeau JD. Peritoneal repair and post-surgical adhesion formation. *Hum. Reprod. Update* 2001;7:547–555.

26. Isaksson K, Montgomery A, Moberg AC, Andersson R, Tingstedt B. Long-term follow-up for adhesive small bowel obstruction after open versus laparoscopic surgery for suspected appendicitis. *Ann. Surg.* 2014;259:1173–1177.

27. Attard JA, MacLean AR. Adhesive small bowel obstruction: Epidemiology, biology and prevention. *Can J Surg.* 2007;50:291–300.

28. Kawanishi K, Yamato M, Sakiyama R, et al. Peritoneal cell sheets composed of mesothelial cells and fibroblasts prevent intra-abdominal adhesion formation in a rat model. *J Tissue Eng Regen Med.* 2016;10:855–866.

29. Molinas CR, Koninckx PR. Hypoxaemia induced by CO(2) or helium pneumoperitoneum is a co-factor in adhesion formation in rabbits. *Hum Reprod.* 2000;15:1758–1763.

30. Schlotterbeck H, Greib N, Dow WA, Schaeffer R, Geny B, Diemunsch PA. Changes in core temperature during peritoneal insufflation: Comparison of two CO_2 humidification devices in pigs. *J Surg Res.* 2011;171:427–432.