Irami Araújo Filho

INFLUÊNCIA DA LAPAROSCOPIA E DA LAPAROTOMIA NA GASOMETRIA, LEUCOMETRIA DIFERENCIAL E CITOCINAS EM MODELO DE SEPSE ABDOMINAL EM RATOS

Dissertação apresentada à Coordenação do Programa de Pós-graduação em Ciências da Saúde, do Centro de Ciências da Saúde da Universidade Federal do Rio Grande do Norte, como requisito para obtenção do título de Mestre em Ciências da Saúde.

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Dedicatória

Dedico esta dissertação aos meus pacientes, que na dor de seus sofrimentos, depositaram nas minhas mãos a sua confiança e esperança de cura, sendo sempre o motivo maior do meu sacrifício, dedicação e estudo.

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Sumário

Resumo

A cirurgia laparoscópica está associada com trauma reduzido e baixa resposta metabólica na fase aguda do trauma, quando comparada com a cirurgia aberta. As citocinas e o balanço ácido-base são fatores importantes da resposta biológica ao trauma cirúrgico-anestésico. O objetivo deste estudo foi determinar se o pneumoperitônio com CO_2 altera a expressão das citocinas intraperitoneais, a gasometria do sangue arterial, dos exsudatos intraperitoneal e subperitoneal, e a contagem diferencial de leucócitos em ratos com sepse abdominal. Método: Ratos Wistar foram aleatoriamente distribuídos em cinco grupos: controle (somente anestesia), laparotomia, pneumoperitônio com CO_2, ligadura e punção do ceco por laparotomia, ligadura e punção do ceco por laparoscopia. Após 30 minutos dos procedimentos, sangue arterial foi colhido para leucometria em hemocitômetro. FNTα, IL-1β e IL-6 foram dosadas no lavado intraperitoneal (por ELISA). Os parâmetros gasosos foram medidos no sangue arterial e nos exsudatos intraperitoneal e subperitoneal. Resultados: Os valores de FNTα, IL-1β e IL-6 foram significantemente menores nos ratos submetidos ao pneumoperitônio do que em todos os outros grupos ($p<0.05$). Expressão de FNTα, IL-1β e IL-6 foi menor no grupo sepse induzida por laparoscopia do que por laparotomia ($p<0.05$). Os ratos submetidos à ligadura e punção do ceco por via laparoscópica desenvolveram acidose hipercárbica no sangue arterial e exsudato subperitoneal, mais intensa do que no grupo sepse laparotômica. Leucopenia e linfopenia foram mais acentuadas no grupo sepse laparoscópica ($p<0.01$). Entretanto, os animais submetidos a

sepse laparotômica desenvolveram significante aumento de neutrófilos e eosinófilos quando comparados com os controles ($p<0.05$). Conclusões: Este estudo demonstrou que o pneumoperitônio com CO_2 contribuiu para reduzir a resposta inflamatória e imunológica em ratos submetidos a modelo de sepse abdominal, no que diz respeito a citocinas intraperitoneais e leucometria diferencial. O pneumoperitônio também contribuiu para instalação de acidose hipercárbica nos ratos sépticos.

Palavras chave: Pneumoperitônio, Gás carbônico, Sepse, Acidose, Leucócitos.

1 INTRODUÇÃO

A cirurgia videoendoscópica teve início em 1987, e tem como base provocar um mínimo trauma aos pacientes, reduzir a permanência hospitalar e a ausência às atividades no trabalho e na família. Na videocirurgia por via laparoscópica é indispensável a insuflação de CO_2 na cavidade peritoneal, para o acesso visual aos órgãos abdominais com segurança[1,2].

De um modo geral, a imunossupressão é uma conseqüência bem estabelecida do estresse cirúrgico e da lesão [4,6,7]. Isto não só tem sido definido no que diz respeito aos níveis séricos de citocinas, mas também em relação às alterações celulares da resposta ao trauma[5,12]. Vários estudos têm examinado como a cirurgia laparoscópica afeta vários componentes da resposta inflamatória e do sistema imunológico[3-6,8,9].

A cirurgia laparoscópica pode atenuar a imunossupressão celular induzida pelo trauma operatório em termos de contagem de leucócitos totais, populações de leucócitos específicos, e subpopulações de leucócitos[3,4]. Foi demonstrado em alguns estudos um aumento significante nos leucócitos periféricos nas operações pela técnica aberta, quando comparado com pacientes submetidos à colecistectomia laparoscópica [4-7]. Kloosterman et al (1984)[8] demonstraram um aumento passageiro no número de granulócitos após colecistectomia aberta, fato que não ocorreu após colecistectomia laparoscópica.

A fisiopatologia do pneumoperitônio é complexa, com efeitos locais e sistêmicos de um gás injetado na cavidade peritoneal sob pressão[11]. Diferentes gases como hélio, argônio, e óxido nitroso foram avaliados como alternativas ao gás carbônico[12]. Naturalmente surgiu a pergunta se o pneumoperitônio com gás carbônico influencia a resposta metabólica e imune sistêmica após a cirurgia laparoscópica. Dados resultantes de alguns estudos sugerem que possa haver essa influência[4,5,8,9]. West et al(1995)[12] investigaram a produção de citocinas em macrófagos de peritônio incubados em gás carbônico. As respostas de fator de necrose tumoral (FNT) e Interleucina-1 (IL-1) nos macrófagos sob a ação de endotoxinas bacterianas foram mais baixas para os macrófagos incubados em gás carbônico que em ar atmosférico ou hélio. Um mecanismo proposto para esta diferença foi que o gás carbônico afeta o meio

intracelular, criando um ambiente mais ácido. Sabe-se que a função dos macrófagos é prejudicada através da queda no pH intra e extracelular[12,13]. West et al(1995)[12] especulam que a diminuição na produção de citocinas pelos macrófagos peritoneais pode contribuir para uma aparente depressão na resposta sistêmica inflamatória durante a cirurgia laparoscópica. Os autores especulam que um mecanismo molecular potencial, ainda não descrito, possa explicar a imunossupressão dos macrófagos peritoneais.

Uma hipótese alternativa foi sugerida por Watson et al(1995)[14] em um estudo em ratos submetidos à laparoscopia com ar atmosférico e gás carbônico. Os animais do grupo de controle sofreram laparotomia aberta. Neste estudo, macrófagos do tecido peritoneal liberaram superóxido e FNT após laparotomia e laparoscopia com ar atmosférico em quantidades significativamente maiores, quando comparado com o procedimento de controle e laparoscopia com gás carbônico. Porém, a fagocitose dos macrófagos peritoneais foi significativamente menor na laparoscopia com ar e na laparotomia, quando comparados com insuflação de gás carbônico. Os autores especularam que algum fator no ar, talvez uma pequena quantidade de endotoxina contaminante, em lugar de gás carbônico, era responsável pelas alterações na função dos macrófagos. Tudo indica que o pneumoperitônio com gás carbônico, através de mecanismos ainda obscuros, parece atenuar a resposta imune dos macrófagos peritoneais.

Sem dúvida, a eficácia clínica da cirurgia laparoscópica está bem estabelecida. Está cada vez mais aparente que aquelas respostas sistêmicas imunes e metabólicas da cirurgia aberta podem não se aplicar totalmente à cirurgia laparoscópica. A linha de pesquisa nesta área tem procurado explicar esses fenômenos e, como esses esforços se multiplicam, serão em breve melhor entendidas as conseqüências sistêmicas, metabólicas e imunes da cirurgia laparoscópica e os pacientes serão os reais beneficiários. No que diz respeito à cirurgia laparoscópica em casos de peritonite, permanece a dúvida se a atenuação da resposta imune, provavelmente provocada pelo pneumoperitôneo com CO_2, seria prejudicial ou benéfica para os operados.

Tomando por base essas informações, o trabalho principal anexado a essa dissertação, que serviu como um dos requisitos para a obtenção do grau de mestre, teve como objetivo estudar a gasometria arterial, intraperitoneal e

subperitoneal, os níveis de citocinas intraperitoneais e a contagem diferencial de leucócitos periféricos em ratos submetidos ao seguinte modelo experimental: ligadura e punção do ceco através de operação aberta, comparada com a operação laparoscópica, realizada com o pneumoperitôneo com CO_2. Adicionalmente, outros dois trabalhos, um clínico e outro experimental, estão anexados neste volume, publicados em periódicos com indexação internacional, fazendo parte da formação do autor como Mestre em Ciências da Saúde.

2 REVISÃO DA LITERATURA

A partir de 1990 a cirurgia laparoscópica vem progressivamente substituindo com vantagens a cirurgia abdominal aberta, também denominada convencional. Tem resultado em menos dor pós-operatória, retorno mais rápido às atividades normais e melhor efeito cosmético. Outros fatores distinguem a cirurgia laparoscópica da cirurgia aberta: uso do pneumoperitôneo com atmosfera de CO_2, menor dissecção de estruturas intra-abdominais, mínimas mudanças de temperatura e uma melhor preservação da resposta imune tanto sistêmica quanto intraperitoneal. A principal vantagem da cirurgia laparoscópica sobre a cirurgia aberta é que o estado da normalidade é recuperado mais rapidamente. Vários estudos têm mostrado que ocorre menor lesão tecidual e conseqüente menor repercussão na resposta inflamatória[15-19] e preservação da imunidade[10,20-22] . Além disso, a incidência de infecção cirúrgica é reduzida com a cirurgia laparoscópica[23-25]. Várias séries publicadas têm demonstrado que os procedimentos laparoscópicos prolongados, mesmo em casos de infecção intraabdominal, são bem tolerados e não aumentam a translocação bacteriana ou a disseminação intra-abdominal da sepse[26,27].

Desde sua introdução em 1987, a cirurgia laparoscópica e, especialmente a colecistectomia laparoscópica foi rapidamente aceita para uso na clínica cirúrgica. Este procedimento minimamente invasivo requer o pneumoperitôneo para a visualização adequada da cavidade peritoneal e das estruturas a serem operadas[28-31]. O gás carbônico tem sido usado para insuflação por ser de baixo custo e não inflamável. Sua capacidade de difusão é alta, com subseqüente rápida absorção e excreção[29,32]. Alguns efeitos colaterais do pneumopetitôneo com CO_2 têm sido descritos, como hipercapnia, acidose e hipertensão pulmonar[33-36]. Além do CO_2, outros gases como hélio, argônio e óxido nitroso (N_2O), como também a laparoscopia sem gás têm sido investigados[28,30,31,37].

A cirurgia aberta está associada com significante depressão da função immune no pós-operatório e que o grau e duração com que ela ocorre são determinados pela magnitude da lesão inicial[20,38]. Em virtude da cirurgia laparoscópica reduzir a intensidade do trauma cirúrgico, alguns trabalhos têm mostrado que ela está associada com uma menor depressão da resposta

imune[4,39]. Partindo do princípio de que este aspecto é importante, é preciso que seja esclarecido se a laparoscopia está ou não associada com significativas vantagens ou desvantagens imunológicas e se este fato tem implicações nos resultados das ressecções oncológicas e especialmente na presença de sepse abdominal.

2.1. Laparoscopia e função imune

Como a cirurgia laparoscópica causa menos trauma tecidual do que a cirurgia aberta, é de se esperar que esteja associada com uma melhor preservação da função imune sistêmica. A causa exata dos melhores resultados clínicos está sendo investigada, mas a melhor preservação da função imune sistêmica deve estar contribuindo para a melhora na recuperação dos doentes[40-42]. Após a cirurgia aberta convencional, a função imunológica em geral está deprimida, com alterações importantes nos níveis de citocinas e mudanças nas funções dos componentes celulares da resposta sistêmica imune[40,43,44].

A resposta fisiológica ao trauma é um aumento imediato nos níveis de uma série de hormônios e conseqüentemente, diminuição na resposta imune celular. Tal resposta manifesta-se como uma redução na interação entre linfócitos e macrófagos, redução na atividade das células *killer,* diminuição na quimiotaxia de linfócitos e neutrófilos, como também atenuação nas respostas de sensibilidade retardada[45]. Em geral, a resposta imune à cirurgia depende principalmente, de alterações nas funções e níveis das citocinas e do sistema imunológico exercido pelas células.

2.2. Resposta ao trauma e citocinas

A resposta metabólica ao trauma e a expressão de citocinas são componentes importantes e necessários da função imunológica. Embora os níveis de citocinas não indiquem diretamente o estado imune, representam uma boa sinalização da ativação do sistema de defesa sistêmico. Entretanto, a superprodução de citocinas, ou sua produção em sítios não

inflamatórios, pode levar a efeitos deletérios nos tecidos. No pós-operatório, a diminuição na produção de citocinas, que pode representar reação inflamatória deduzida, pode ser considerada benéfica.

As citocinas IL-1, FNT e IL-6 desempenham um papel importante na resposta aguda ao trauma[46]. A expressão de IL-6 tem sido considerada diretamente proporcional à extensão do trauma cirúrgico[47]. Seus níveis plasmáticos correlacionam-se fielmente com o porte do trauma cirúrgico e uma significante deferença tem sido encontrada nos níveis plasmáticos pós-operatórios quando comparadas às colecistectomias aberta e laparoscópica[18,48,49]. Leung et al(2000)[50], em estudo randômico de 34 pacientes submetidos a ressecções laparoscopicamente assistidas ou abertas de retosigmoide por câncer, não encontraram aumento nos níveis de FNT em ambos os grupos. No entanto, os níveis séricos de IL-1β e IL-6 tiveram um pico 2 horas após as operações, com menor resposta após a cirurgia laparoscópica. Hill et al(1995)[51] não encontraram qualquer diferença nos níveis de IL-6 após o reparo de hérnias inguinais por vias laparoscópica e convencional. Esta discrepância de resultados pode ter ocorrido porque o nível de trauma na herniorrafia aberta não deve ter sido suficiente para gerar níveis aumentados desses marcadores. Níveis plasmáticos de IL-8, um importante fator de quimiotaxia para neutrófilos, foram encontrados em níveis maiores na cirurgia aberta do que na laparoscópica[52]. Resultados semelhantes têm sido observados por outros investigadores[15].

Em geral, os resultados de estudos experimentais e clínicos têm demonstrado reduzida ativação de citocinas, especialmente IL-1 e IL-6, após a cirurgia laparoscópica, comparada com a equivalente cirurgia aberta. Entretanto, têm sido observadas diferenças na ativação e liberação de outras citocinas, como FNT e IL-8. Como a maioria dos estudos clínicos têm sido realizados usando colecistectomia laparoscópica como modelo, devem ser tomadas precauções ao extrapolar conclusões para outros procedimentos cirúrgicos, como colectomia e herniorrafia laparoscópica. Os resultados com essas operações têm sido inconsistentes em termos de demonstrar vantagens imunológicas para procedimentos laparoscópicos.

2.3. Gasometria

Do ponto de vista fisiopatológico, a insuflação da cavidade peritoneal com gás carbônico parece ser o aspecto mais importante na cirurgia laparoscópica, comparada à cirurgia convencional ou aberta. Várias publicações têm surgido a respeito, relatando que o pneumoperitôneo com CO_2 tem efeitos adversos potenciais no estado ácido-base, função pulmonar e hemodinâmica cardiovascular[53-55].

Em operações em que a insuflação de CO_2 é prolongada, mesmo em pacientes jovens e saudáveis, pode ocorrer importante alteração no balanço ácido-base, devido ao aumento da pressão intra-abdominal e absorção de CO_2 através da serosa peritoneal[35,56].

2.4. Leucócitos periféricos e cirurgia laparoscópica

Os leucócitos polimorfonucleares desempenham um papel chave na defesa do hospedeiro contra a invasão de microorganismos. O estresse cirúrgico afeta a função dos polimorfonucleares durante o período pós-operatório. As atividades fagocítica e quimiotáxica dos neutrófilos, que têm papel importante na defesa, estão reduzidas após a cirurgia e o trauma de um modo geral[4,57]. Esta fagocitose diminuída possivelmente é devida a fatores séricos, em vez de defeitos intrínsecos dos próprios neutrófilos[58]. Em vários estudos os autores têm avaliado a contagem dos leucócitos totais e contagem diferencial após a cirurgia laparoscópica e a cirurgia aberta, e têm demonstrado um significante aumento global dos leucócitos periféricos após a cirurgia aberta, o mesmo não ocorrendo com a cirurgia laparoscópica[59,60]. Um aumento transitório no número de granulócitos tem sido observado nos operados pela cirurgia aberta, mas não na lapacoscópica[61]. Sietses et al(2000)[62] não observaram qualquer diferença na contagem de leucócitos sistêmicos entre pacientes submetidos a fundoplicatura à Nissen por via laparoscópica ou aberta. No entanto, encontraram uma significante redução na atividade fagocítica dos polimorfonucleares após a cirurgia aberta, que não foi notada após a fundoplicatura laparoscópica. Uma maior produção de radicais oxigênio

pelos polimorfonucleares foi observada após técnicas laparoscópicas, quanto comparadas com técnicas abertas, sugerindo um maior estado de ativação dessas células[57,62,63].

Os dados da literatura demonstram que o pneumoperitônio com CO_2 repercute no balanço ácido-base e pode provocar alterações significantes na reação inflamatória e imunológica decorrentes do trauma cirúrgico. Quando são analisados estudos em modelos de sepse, observa-se que os estudos são escassos e controversos. No presente trabalho procurou-se estudar, em modelo animal de sepse abdominal, as conseqüências do pneumoperitônio com CO_2, procurando respostas para os aspectos relacionados com a expressão de citocinas pró-inflamatórias, balanço ácido-base e leucometria.

3 ANEXAÇÃO DOS ARTIGOS

3.1. ARTIGO I - Influence of laparoscopy and laparotomy on gasometry, leukocytes count and cytokines in a rat abdominal sepsis model

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Influence of laparoscopy and laparotomy on gasometry, leukocytes count and cytokines in a rat abdominal sepsis model[1]

Influência da laparoscopia e laparotomia na gasometria, leucometria e citocinas em modelo de sepse abdominal em ratos

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ABSTRACT

Purpose: Laparoscopic surgery is associated with reduced surgical trauma, and less acute phase response, as compared with open surgery. Cytokines are important regulators of the biological response to surgical and anesthetic stress. The aim of this study was to determine if CO_2 pneumoperitoneum would change cytokine expression, gas parameters and leukocyte count in septic rats. **Methods:** Wistar rats were randomly assigned to five groups: control (anesthesia only), laparotomy, CO_2 pneumoperitoneum, cecum ligation and puncture by laparotomy, and laparoscopic cecum ligation and puncture. After 30 min of the procedures, arterial blood samples were obtained to determine leukocytes subpopulations by hemocytometer. TNFα, IL-1β, IL-6 were determined in intraperitoneal fluid (by ELISA). Gas parameters were measured on arterial blood, intraperitoneal and subperitoneal exsudates. **Results:** Peritoneal TNFα, IL-1β and IL-6 concentrations were lower in pneumoperitoneum groups than in all other groups ($p<0.05$). TNFα, IL-1β and IL-6 expression was lower in the laparoscopic than in laparotomic sepsis ($p<0.05$). Rats from laparoscopic cecum ligation and puncture group developed

significant hypercarbic acidosis in blood and subperitoneal fluid when compared to open procedure group. Total white blood cells and lymphocytes were significantly lower in laparoscopic cecum ligation and puncture rats than in the laparotomic ($p<0.01$). Nevertheless, the laparotomic cecum ligation rats had a significant increase in blood neutrophils and eosinophils when compared with controls ($p<0.05$). **Conclusions:** This study demonstrates that the CO_2 pneumoperitoneum reduced the inflammatory and immune response in an animal model of peritonitis with respect to intraperitoneal cytokines, white blood cell count and clinical correlates of sepsis. The pneumoperitoneum produced hypercarbic acidosis in septic animals.

Key words: Pneumoperitoneum. Carbonic gás. Sepsis. Acidosis. Leucocytes.

RESUMO

Objetivo: A cirurgia laparoscópica está associada com trauma reduzido e baixa resposta na fase aguda do trauma, quando comparada com a cirurgia aberta. As citocinas e o balanço ácido-base são fatores importantes da resposta biológica ao trauma cirúrgico-anestésico. O objetivo deste estudo foi determinar se o pneumoperitôneo com CO_2 altera a expressão das citocinas, a gasometria e a contagem diferencial de leucócitos em ratos com sepse abdominal. **Métodos:** Ratos Wistar foram aleatoriamente distribuídos em 5 grupos: controle (somente anestesia), laparotomia, pneumoperitôneo com CO_2, ligadura e punção do ceco por laparotomia, ligadura e punção do ceco por laparoscopia. Após 30 minutos dos procedimentos, sangue arterial foi colhido para leucometria diferencial em hemocitômetro. TNFα, IL-1β e IL-6 foram dosadas no líquido intraperitoneal (por ELISA). Os parâmetros gasosos foram medidos no sangue arterial e nos exsudatos intraperitoneal e subperitoneal. **Resultados:** Os valores de TNFα, IL-1β e IL-6 foram significantemente menores nos ratos submetidos ao pneumoperitôneo do que em todos os outros grupos ($p<0.05$). Expressão de TNFα, IL-1β e IL-6 foi menor no grupo sepse induzida por laparoscopia do que por laparotomia ($p<0.05$). Os ratos submetidos a ligadura e punção do ceco via laparoscópica desenvolveram acidose hipercárbica no sangue arterial e exsudato subperitoneal, mais intensa do que no grupo sepse laparotômica. Leucopenia e linfopenia foram mais acentuadas no grupo sepse laparoscópica ($p<0.01$). Entretanto, os animais submetidos a sepse laparotômica desenvolveram significante aumento de neutrófilos e eosinófilos quando comparados com os controles ($p<0.05$). **Conclusões:** Este estudo demonstrou que o pneumoperitôneo com CO_2 contribuiu para reduzir a resposta inflamatória e imunológica em ratos submetidos a modelo de sepse abdominal, no que diz respeito à expressão de citocinas intraperitoneais e leucometria diferencial. O pneumoperitôneo também contribuiu para instalação de acidose hipercárbica nos ratos sépticos.

Descritores: Pneumoperitôneo. Gás carbônico. Sepse. Acidose. Leucócitos.

Introduction

Operative laparoscopy brought a new dimension to surgical practice, and many experimental and clinical studies have demonstrated feasibility, safety, cost-benefit, and pathophysiologic occurrences. The intraabdominal insufflation of carbon dioxide (CO2) is the most widely used technique for the creation of a pneumoperitoneum. Insufflation under a continuous monitoring of intraabdominal pressure throughout the surgical procedure provides adequate exposure of the operating field. As alternatives, different gases (e.g., helium, argon, nitrous oxide, air) may be used, but they have not been adopted clinically[1,2,3,4,5,6]. The actual knowledge concerning pneumoperitoneum are sometimes inconsistent about the physiologic consequences induced by insufflation of the peritoneal cavity with CO_2. Results from animal models about the effects of a pneumoperitoneum in certain pathologic conditions are often alarming[7]. However, the clinical impact of these changes is unknown, since the majority of patients who undergo laparoscopic procedures do not exhibit any adverse clinical effects either in the short or the long-term course. Laparoscopíc surgery is applied increasingly to abdominal diseases complicated by diffuse or localized peritonitis such as appendicitis, perforated peptic ulcers and diverticulitis[8,9]. A specific paper has reported the use of laparoscopy in diverticulitis complicated by localized peritonitis with intra-abdominal abscess formation[10], and diagnostic laparoscopy is being advocated in diffuse peritonitis after blunt abdominal trauma[11].

However, a theoretical concern with the use of laparoscopic techniques in clinical cases complicated by intra-abdominal infection and peritonitis, is that carbon dioxide pneumoperitoneum may increase the risk of bacteraemia and sepsis by increasing intra-abdominal pressure. Some studies have demonstrated immunossuppressive effects of carbon dioxide on neutrophil and macrophage function. In one study, CO_2 blocked superoxide release from activated polimorphonuclear leukocytes and significantly reduced the secretion of IL-1 from human peritoneal macrophages[12]. Whereas these effects might be considered beneficial from the standpoint of inflammation following elective surgery, experimental evidence suggests that the CO_2 induced immunossuppression might be deleterious in the setting of infection[13]. This may have an adverse effect on clinical outcome when compared with open procedures. Although some evidences, few data exist regarding the effect of pneumoperitoneum and increased intra-abdominal pressure on sepsis and physiological outcome.

The aims of the current study were to investigate the influence of laparoscopic procedures, in particular CO2 insufflation, on the response to sepsis in an animal model—cecal ligation and puncture (CLP) in the rat. Clinical evolution, gasometry, pro-inflammatory cytokines and leukocytes were analyzed.

Methods

Animals and groups - Male Wistar rats (Bioterium from Nucleus of Experimental Surgery, Federal University of Rio Grande do Norte, Brazil), 12 to 13 weeks old, were housed in cages where standard chow and water were available ad libitum. The rats were acclimatized to the laboratory environment for 5 days on arrival and then fasted for 12 hours before any procedures. Anesthesia was obtained using pentobarbital 20 mg/Kg intraperitoneal and ketamine 50 mg/Kg intramuscular. All surgical procedures were performed under aseptic conditions. The animals were allowed to breathe spontaneously for the duration of the experiment. The group C (control) rats were subjeted to anesthesia only (n=6). In the LAP (laparotomy) group (n=7) the following procedures were performed: after anesthesia and antisepsis with povidone, a 5cm laparotomy keeped the peritoneal cavity exposed to the room air during 30 minutes and the abdominal wall was sutured with nylon 4-0. The PNP rats (n=7) were subjected to CO_2 pneumoperitoneum using a Veress needle under 3 mmHg for 30 minutes. On the CLP/LAP (n=6) the CLP was performed after laparotomy. A cecum ligation and puncture (CLP) by laparoscopy under pneumoperitoneum were performed on the CLP/PNP rats (n=6).

Cecal Ligation and Puncture - Pneumoperitoneum was achieved by introducing a Veress needle into the peritoneal cavity and insufflating (Endomed insufflator) the abdomen with 3 mmHg CO_2. Laparoscopic procedures were performed using 3-mm instruments (Henke-Sass, Wolf^{TM}) introduced into the abdomen. Cecal ligation and puncture (CLP) consisted of dissection of the cecum, ligation midway between the ileocecal valve and the terminal cecum using a 3-0 chromic catgut tie, and 8-punctures of the isolated cecum with a hollow 25-gauge needle introduced through the abdominal wall. Laparotomy, for the open CLP group, consisted of a 5-cm midline abdominal incision. The duration of the total procedure, and therefore the duration of anesthesia, pneumoperitoneum, and laparotomy, was standardized to 30 minutes for all groups. Postoperatively, animals were resuscitated with a subcutaneous injection of lactated Ringer's (30 mL/kg) and were again housed in cages where water was available ad libitum. The experimental protocol was approved by the Research Ethics Committee of the Federal University of Rio Grande do Norte, Brazil, and adhered to the Guide for the Care and Use of Laboratory Animals, US National Research Council, 1996.

Gasometry and cytokines dosage - After the surgical procedures, 5mL of buffered saline were injected in peritoneal cavity and the abdomen was softly massaged for 1 minute. Thirty minutes later, whole blood was collected by cardiac puncture and liquid exsudate was collected from peritoneal cavity and from subperitoneal space, using heparinized capilar tube, for determination of pH, pCO_2 and pO_2. An automatic AVL (Roche®) equipment was used. TNFα, IL-1β and IL-6 were determined in the intraperitoneal exsudate, by enzyme-linked immunosorbent assay, using cytokine-kits from PeproTec (Rocky Hill, NJ,USA).

Leukometry - Animals were evaluated 24 hours postoperatively for clinical signs of CLP-induced sepsis (i.e., dark halo around the eyes, piloerection and lethargy). Whole blood was collected by cardiac puncture for

leukocyte cell counts and the rats were then killed via anesthetic overdose. The determination of leukocite cell counts was preformed using a commercially available automated cell counter (Abbott Cell-Dyn 3500R- CD 3500 5L, USA).

Data were expressed as mean±standard deviation. Statistical significance was established using the one way analysis of variance ANOVA followed by Newman-Keuls test, performed by the software BioEstat 2.0. Probabilities less than 0.05 were considered significant.

Results

Cytokines release in peritoneal exsudate - Rats in the control, LAP and PNP groups exhibited normal activity and had no piloerection during the 24 hours after interventions. In contrast, all rats that underwent CLP exhibited decreased activity and significant piloerection. The cytokine levels on LAP rats were higher than PNP ones; however, the difference between these groups was not significant ($p>0.05$).

Both surgical procedures, PNP/CLP and LAP/CLP, induced higher TNFα, IL-1β and IL-6 in the peritoneal fluid than were found in the control group. In contrast, the peritoneal fluid TNFα, IL-1β and IL-6 levels in the pneumoperitoneum (PNP) group were significantly lower than in the other groups ($p<0.05$). The PNP/CLP rats had a significantly lower elevation of TNFα, IL-1β and IL-6 expression in the peritoneal fluid than LAP/CLP rats ($p<0.05$) (Figures 1,2,3).

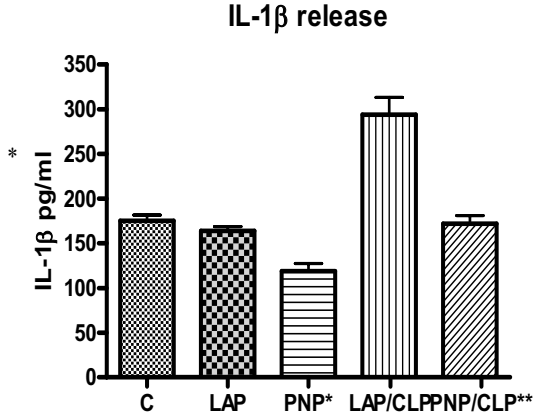

Figure 1 - No statistical difference was observed between groups C and LAP. When comparing IL-1β expression in groups LAP/CLP and PNP/CLP**, the difference was significant. ($p<0.05$).

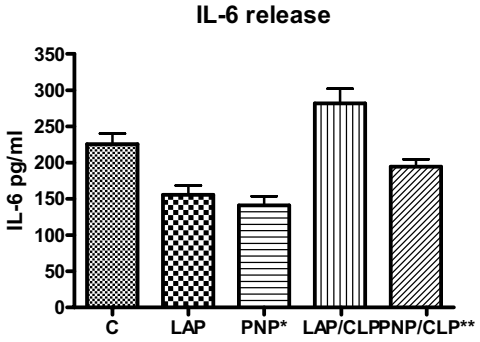

Figure 2 - The laparoscopic sepsis group (PNP/CLP) expressed IL-6 significantly lower than laparotomic group ($p<0.05$). *p<0,05 vs C, PNP/CLP, LAP/CLP

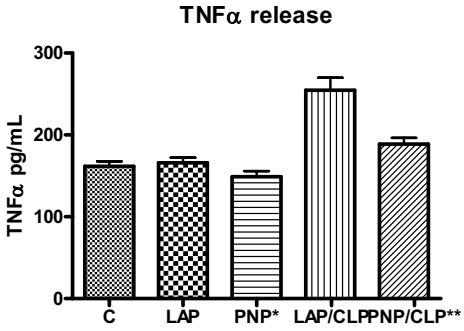

Figure 3 - TNF-α levels were significantly lower in laparoscopic sepsis rats (PNP/CLP) than when sepsis was induced by laparotomy (LAP/CLP) ($p<0.05$).

Gasometry - Arterial blood gas parameters (pH, pO2) in rats from the control group (C) remained significantly higher than in rats from LAP, PNP and PNP/CLP groups ($p<0.01$). Rats from PNP/CLP group developed significant hypercarbic acidosis with mean pH of 7.18±0.05 and pCO_2 60.7±10.2 when compared to LAP/CLP group. The LAP/CLP rats acidosis was not hypercarbic (Table 1). The pCO_2 was significantly higher on PNP/CLP rats than controls ($p<0.01$). Significantly reduced pCO_2 was observed following LAP/CLP, compared to LAP, PNP and PNP/CLP ($p<0.01$).

Table 1 – Arterial blood gas parameters

GROUP	pH	pCO_2	pO_2
C	7.36±0.08*	43.4±4.7§	88.5±9*
LAP	7.23±0.03	50.1±9.2	62.4±19
PNP	7.22±0.07	48.6±9.6	61.2±15
PNP/CLP	7.18±0.05**	60.7±10.2	63.7±18
LAP/CLP	7.26±0.04	35.45±7.9†	49.4±19

*P<0,01 vs LAP, PNP, PNP/CLP; **p<0.01 vs LAP/CLP; § p<0,05 vs PNP/CLP;
† p<0.05 vs LAP, PNP, PNP/CLP

Table 2 – Intraperitoneal exsudate gas parameters

GROUP	pH	pCO_2	pO_2
C	7.31±0.16	33.6±7.6	113.3±23
LAP	7.30±0.1	39.7±10	95±31
PNP	7.16±0.16**	47±9.7	102±15
PNP/CLP	6.8±0.25*	86.5±41.3*	82.3±15§
LAP/CLP	7.27±0.11	48.3±9.4	122.3±19

*P<0,01 vs C, LAP, PNP, LAP/CLP; **p<0.01 vs C, LAP; § p<0,05 vs C, PNP/CLP

Intraperitoneal exsudate gas analysis revealed acidosis in the PNP group, and the difference was significant when compared with C and LAP groups (p<0.01). However, the septic rats subjected to CO_2 pneumoperitoneum (PNP/CLP) developed a profound intraperitoneal acidosis, as a consequence of pCO_2 significantly higher (p<0.01) than in all other groups (Table 2). In contrast, the pO_2 was significantly lower than in C and LAP/CLP groups (p<0.05).

While subperitoneal exsudate pH (6.5±0.05) following laparoscopic CLP using CO_2 (PNP/CLP) was significantly lower (p<0.01) than in C, LAP and LAP/CLP rats, the difference did not reach statistical significance when compared with the acidotic pH (6.7±0.08) of PNP group (Table 3). The CO_2 pneumoperitoneum produced a significant increase in pCO_2 in the subperitoneal exsudate, when compared with all the other groups (p<0.01).

Table 3 – Subperitoneal exsudate gas parameters

GROUP	pH	pCO_2	pO_2
C	7.16±0.2	21±8	146±12
LAP	7.0±0.2	18±9.6	161±20
PNP	6.7±0.08	15±4.4	130±19
PNP/CLP	6.5±0.05*	46±3.8*	122±8.9*
LAP/CLP	7.16±0.1	24±9.8**	165±10.8**

*p<0,01 vs C, LAP, LAP/CLP; **p<0,01 vs PNP/CLP

Leukocytes - White blood cell count in pneumoperitoneum and laparoscopic CLP groups was similar to those of controls (Table 4). Significantly reduced white cell counts were observed following laparoscopic CLP compared to open CLP (p<0.01)

No significant difference in blood neutrophil and lymphocyte count was found among control, LAP, PNP and PNP/CLP rats. Nevertheless, the LAP/CLP rats had a significant increase in blood neutrophils when compared with controls (p<0.05), as can be observed in Table 4. The PNP/CLP and LAP/CLP procedures produced a significant reduction in lymphocyte count, compared with controls (p<0.05). Additionally, the reduction in lymphocytes was significantly higher in open CLP (LAP/CLP) rats than in PNP/CLP (p<0.05). There was a significant reduction in eosinophil count in PNP, LAP/CLP and

PNP/CLP, compared with controls ($p<0.05$). The decrease in LAP/CLP eosinophil was greater than in PNP/CLP rats.

Table 4. Leukocyte blood counts after laparotomy, pneumoperitoneum and cecal ligation and puncture.

LEUCOCYTES	C	LAP	PNP	PNP/CLP	LAP/CLP
WBC (K/μL)	6.2±0.6∫	8.8±0.9ф	4.8±0.6	4.7±0.6†	10.4±1.9
Neutrophil	51.7±12	49.1±12	51.3±9.5	60.8±11	78.2±8*
Lymphocyte	32.5±10	40±14	36.4±9	23±4.8**	11.7±3
Eosinophils	4±0.5	3.9±0.3	1.7±0.06§	2±0.08	0.8±0.01*

C, control; LAP, laparotomy; PNP, pneumoperitoneum; PNP/CLP, pneumoperitoneum/cecum ligation and puncture; LAP/CLP, laparotomy/ cecum ligation and puncture; WBC, white blood count.
∫ $p<0.01$ vs LAP, LAP/CLP; ф $p<0.01$ vs PNP, PNP/CLP; † $p<0.01$ vs LAP/CLP; *$p<0.01$ vs C, LAP, PNP, PNP/CLP; **$p<0.01$ vs LAP,PNP; §$p<0.05$ vs C,LAP, LAP/CLP.

Discussion

Because laparoscopic surgery is increasingly used for treating peritonitis and other septic states, a theoretic concern is related to the hypothesis that CO_2 pneumoperitoneum may increace bacteremia with adverse effects for the patient. Some studies have reported technical fiseability of laparoscopic appendectomy, perforated peptic ulcer, perforated diverticulitis and other septic surgical situations[14,15,16]. They are small studies with low numbers of included patients, that can not give strong evidence about improved safety of laparoscopic surgery regarding septicemia. Study in rats showed that pneumoperitoneum causes intestinal ischemia with oxygen free radical production and bacterial translocation, related to mechanical pressure of CO_2[17]. Other studies focused on whether a pneumoperitoneum amplifies the extent and severity of peritonitis or of bacteremia in various animal models[18,19,20,21]. Findings from these investigations are controversial. Whereas some authors reported no increase in bacteremia, intraperitoneal abscess formation, or correlates of sepsis, others reported increased bacterial translocation and severity of peritonitis and sepsis.

Laparoscopic surgical technique requires the maintenance of a continuous positive intraperitoneal pressure in patients (approximately 10-15 mmHg) for visualization and manipulation of the viscera. In cases of peritonitis, viable bacteria and bacterial byproducts (including endotoxin) exist free in the peritoneal cavity. Positive intraperitoneal pressure may increase bacteraemia and endotoxaemia, and thus may worsen clinical sepsis. As experience with laparoscopic surgery increases, its use in more debilitated and critically ill patients is being reported[22]. These patients often have sepsis and suffer from diffuse peritonitis of unclear aetiology, usually the result of a perforated viscera. It has not been clear whether a laparoscopic approach worsens the septic state or whether minimally invasive surgery is beneficial in these critically ill patients.

Experimental model - Several animal models of peritoneal sepsis have been described varying in many respects, including the specific animal utilized and the method of sepsis[20,23,24,25]. The model of creating sepsis used in this study was adapted from the work of Hanly et al[26].

The convenience of the model of laparoscopic CLP in rats, used in the present experiment, is two-fold. First, the use of a septic animal model magnifies the stress induced by a surgical procedure to more clearly delineate the modifying effects of laparoscopy on the inflammatory response. Second, the combined stressors of bacterial contamination of
the peritoneal cavity and bowel ischemia present following laparoscopic CLP provide an environment analogous to clinical situations in which laparoscopy is used to aid in the diagnosis and treatment of patients with peritonitis. The model used in this study presupposes that CLP caused sepsis and that the injury caused by CLP was equivalent between groups. We established the presence or absence of sepsis in rats by evaluating each rat for the presence or absence of periorbital dark halo, the presence or absence of piloerection and normal or decreased activity. All 12 rats that were subjected to CLP were identified as having clinical sepsis, and all 20 rats considered control, or that had received other procedures, were identified as not having sepsis.

Cytokines - In the present study we observed a significant decrease of TNFα, IL-1β and IL-6 in rats subjected to CO_2 pneumoperitoneum with and without sepsis, and these findings coincided with acidosis in arterial blood, in intraperitoneal and subperitoneal exsudates. These data are in agreement with other investigators, who showed inhibition of human peritoneal macrophage cytokine production when these cells were incubated in an acidic extracellular environment, lowered the intracellular pH and attenuated cytokine release[27]. Similarly, Carozzi et al[28] showed decreased spontaneous release of IL-1, IL-6, IL-8, and TNF when incubations were performed in pH 5.5 medium compared to much higher cytokine levels from cells incubated in medium with a pH of 7.4. In the present study the PNP and PNP/CLP rats exhibited arterial, intraperitoneal and subperitoneal acidosis, suggesting intracellular acidosis. West et al have proposed relative intracellular acidosis as the mechanism by which the decrease in cytokines is exerted[29]. Redmond et al[30] showed that circulating monocytes obtained from patients after laparoscopic cholecystectomy exhibited reduced TNF release compared to those from patients who had open cholecystectomy. They also reported that peritoneal macrophages derived from animals undergoing CO_2 laparoscopy released less TNF in response to lipopolyssacharides (LPS) than those undergoing air laparoscopy[31]. West et al[32] have shown that murine peritoneal macrophages exposed to CO2 in vitro exhibit inhibition of LPS-stimulated IL-1 and TNF cytokine release, suggesting that this effect is related to the influence of the CO2 environment. These findings contribute to explain the reduction of cytokine release when animals and patients are operated under effect of CO_2 pneumoperitoneum.

Whereas these effects might be considered beneficial from the standpoint of inflammation following elective surgery, one experimental study suggests that CO_2 induced immunossuppression might be deleterious in the setting of infection[13]. The clinical significance of these findings remains unknown. In studies where laparoscopy was compared to open surgery for peritoneal infection such as appendicitis, there was no clear augmentation of infectious complications associated with the use of CO_2 pneumoperitoneum[33].

Gas analysis - The effect of pneumoperitoneum on hemodynamics and blood gas variables has been studied extensively in nonseptic animals[34], and to

a lesser degree in acute models of sepsis. In the present study, pneumoperitoneum alone and associated with CLP sepsis induced alterations of the acid-base balance, such as fall of pH, and elevation of pCO_2 , in arterial blood, intraperitoneal and subperitoneal exsudate, without correlation to pO_2. The decrease in pH, that were more accentuated with CLP, was similar to what has been reported[25]. These findings were substantiated by other investigators, who found that intraabdominal pH diminishes with application of a CO_2 pneumoperitoneum. CO_2 used as an insufflation gas appeared to lower peritoneal, blood, and subcutaneous pH more than helium, which induced smaller changes[35]. Gandara et al[36] stated that, in addition to CO_2 absorption, this might be a phenomenon of tissue hypoperfusion. Nevertheless, in our study pO_2 was not affected by pneumoperitoneum in septic and non septic rats. This finding can be explained by the fact that pneumoperitoneum was performed with 3 mmHg pressure, sufficient to keep a normal and spontaneous respiration in rats. This pressure was used in rats by other investigators[37]. By the way, Kuntz et al[35]. showed that, after insufflation with CO_2, intraperitoneal pH was inversely related to the intraabdominal pressure.

Leukocytes - All rats subjected to CLP were found at autopsy having darkish, foul-smelling peritoneal fluid consistent with gross fecal contamination of the abdominal cavity. In the present study the total white cell count and circulating neutrophil were significantly lower following laparoscopic CLP using CO_2 than following LAP/CLP. Laparotomic CLP produced a significant reduction in lymphocyte count compared to laparoscopic CLP using CO2, corroborating with data from other authors[37]. Another work has shown a profound drop in white blood cells in animals subjected to laparotomy or pneumoperitoneum under intraperitoneal inoculation of *Escherichia coli,* without difference between them. These data suggest that some results are conflicting, but there is a tendency to leucopenia and lymphopenia after laparoscopic CLP.

Conclusions- In conclusion, this study demonstrated that the CO_2 pneumoperitoneum reduced the inflammatory response in an animal model of peritonitis with respect to intraperitoneal cytokines, white blood cell count and clinical correlates of sepsis. The pneumoperitoneum produced hypercarbic acidosis in septic rats.

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3.2. Artigo II - Total gastrectomy with substitution of stomach by jejunal pouch with and without duodenal passage. Study in rats[1].

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Total gastrectomy with substitution of stomach by jejunal pouch with and without duodenal passage. Study in rats[1].

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ABSTRACT - **Purpose**: A comparison was done between the F. Paulino jejunal pouch (FP) and a jejunal pouch (JP) as esophagus-duodenum interpositional graft, for replacing the stomach after total gastrectomy. It was investigated the effect of the two procedures on esophagus histology, nutritional state and serum gastrin in rats. **Methods:** Male Wistar rats weighing 282±17g were randomly submitted to sham operation (S), FP and JP after total gastrectomy. After eight weeks the rats were killed with overdose of anesthetic and tissue was taken from the distal esophagus for histology. Serum levels of total proteins, albumin, iron, transferring, folate, cobalamine, calcium, as well as serum gastrin were determined. Survival was considered. **Results:** Fourty six rats were operated and thirty survived for eight weeks. Five (33.3%) died after FP and 11 (52.3%) after JP ($p<0.05$). Postoperative esophagitis occurred in 6 JP rats. At 8th week, no difference was observed on body weight when compared FP and JP rats ($p>0.05$). The JP rats had a significant decrease in serum albumin, glucose, transferrin, iron, folate and calcium, compared to sham ($p<0.05$). Serum gastrin, iron and calcium were significantly higher in JP rats than in FP rats ($p<0.05$). In FP rats, transferrin and cobalamine showed significant decrease comparing the preoperative with 8th week levels ($p<0.05$). **Conclusion**: F. Paulino pouch in rats had lower mortality than JP, and esophagitis was not detected in it. JP rats had serum gastrin, iron and calcium unaffected, possibly because of preservation of duodenal passage.
KEY WORDS – Total gastrectomy. Jejunal pouch. Nutrition. Gastrin. Reflux.

Introduction

There are more than 50 described operations for intestinal reconstruction following total gastrectomy[1]. However, the optimal reconstruction of the gastrointestinal tract following total gastrectomy has not been conclusively identified. Patient quality of life will be dependent on the severity of symptoms that develop postoperatively. The optimal reconstruction should be designed to function in a manner akin to the non-operated gut. The questions that have to be investigated are how to keep nutritional status, determine the benefit of preserving the duodenal food passage as well as the repercussion on the level of gastrinemia, in order to identify which procedures may be advantageous for the patient. Clinical trials comparing various methods of intestinal reconstruction following total gastrectomy have been reported[2]. Unfortunately, there has been no uniform operation performed and several different outcome variables have been compared. The search for a superior method of reconstruction has been hampered historically by the reliance on retrospective analyses of small groups of patients[1]. Actually, there is no general agreement with regard to the ideal reconstruction type after total gastrectomy. The importance of the duodenal passage[3], and the need for pouch reconstruction[2].
The objective of the present study was to examine the benefits and technique of a distal isoperistaltic jejunojejunal stomach replacement pouch (Pernando Paulino pouch) versus interposition of jejunal pouch. Which technique offer the best preconditions related to the importance of the duodenal passage and determine physiologic regulation of postoperative nutrition, mortality, reflux and serum gastrin in rats.

Methods

Male Wistar rats weighing 282±17g were used for experiments. Rats were housed under controlled conditions of illumination (12/12 hours light/dark cycle), humidity (60–70%), and temperature (21°C). The International guidelines for the care and use of laboratory animals were followed throughout the study.

Surgical Procedures
a)Sham: (S) Sham operation was performed by 3 cm midline laparotomy under ketamine hydrochloride (100 mg/kg) and xylazine (15 mg/kg) anesthesia. The stomach and the intestine were covered with saline-moistened gauze for 40 minutes, which corresponds to the time period required for the other surgical procedures.
b) Fernando Paulino jejunal pouch: (FP) The total gastrectomy was done and the jejunum was divided 10 cm distal to the ligament of Treitz. The distal end of jejunum was anastomosed with the transected esophagus using single stitches (6-0 polipropilene). A jejujojejunal pouch was done,as seen in figure 1. The anastomosis were performed by using a (9x) binocular microscope (DF Vasconcelos®, Brazil).

Figure 1. Isoperistaltic Fernando Paulino pouch.

c) Jejunal pouch interposition graft: (JP) After total gastrectomy the jejunum was divided 5 cm distal to the ligament of Treitz. The digestive reconstruction method included interposition of a jejunal pouch reservoir between the esophagus and duodenum, preserving the duodenal passage (Figure 2).

Figure 2. Jejunal pouch interposition graft.

After the operations the rats had an infusion of Ringer 10ml/Kg intraperitoneal and free access to oral glucose 10% was permitted. Rats resumed to normal diet on third postoperative day. If some animal died before the 60th postoperative day, it was substituted in order to complete 10 rats in each group and the mortality was computed. They were weighed on the same scale each two weeks. A recovery period of eight weeks was allowed for all operated animals before the following experiments were commenced.

Histological procedures

After eight weeks the rats were killed with overdose of anesthetic and tissue was taken from the distal esophagus (1 cm distant to the anastomosis) for investigation with regard to esophagitis. They were fixed in 10% formalin,

embedded in paraffin, cut at 4μm, and stained with hematoxylin and eosin. The following parameters were considered: 1- loss of surface epithelium; 2- neutrophil infiltration; 3 - increased height of the basal cell layer of the squamous epithelium; 4 - increased depth of the papillae. Diagnosis of esophagitis was positive when 2 or more of these parameters were present. Duodenal mucosa was examined in regard to height of cripts, celularity and depth of papillae.

Laboratory tests
Laboratory measurements were performed before and eight weeks after the operations. Serum levels of total proteins, albumin, iron, transferring, folate, cobalamine, and calcium were determined with an autoanalyzer (Weiner Lab BT Plus 3000). Gastrinemia was measured by a double-antibody liquid phase radioimmunoassay.

Statistical analysis
Datas were analysed by one way variance ANOVA complemented with Newman-Keuls tests. Differences were considered significant at $p<0.05$ in the two-tailed tests.

Results

Forty six rats were operated. Thirty survived for eight weeks. Five (33.3%) died soon in the postoperative period in the FP group, and 11 (52.3%) of group JP died before the fourth week (Table 1). The difference in mortality was significant ($p<0.05$).
Postoperative esophagitis occurred in 6 survived rats of the group JP. Loss of surface epithelium, neutrophil infiltration and, increased depth of the papillae were found in 2 rats of this group. Increased height of the basal cell layer of the squamous epithelium and neutrophil infiltration were found in 4 rats. The FP rats had low increasing in height of the basal cell layer, and the sham rats had no signs of histological esophagitis. In group FP it was observed atrophy of duodenal mucosa and, the JP rats showed no mucosal pathological signs.

Table 1 – Operative mortality

Groups	Operated rats (n)	Mortality n(%)	Survived after 8 weeks
FP	15	5 (33.3%)	10
JP	21	11(52.3%)*	10
Sham	10	0 (0.0%)	10
Total	46	16 (34.7%)	30

* $p<0.05$ compared to FP group. Values are expressed as mean±SEM; FP, Fernando Paulino pouch; JP, jejunal pouch interposition graft.

The weight evolution showed a significant weight reduction on the first four weeks in group FP, and a partial weight gain until the 8th week. In JP rats the weight reduction occured throut the 5th week, and they stabilized in the ^{8th} week (Figure 3).

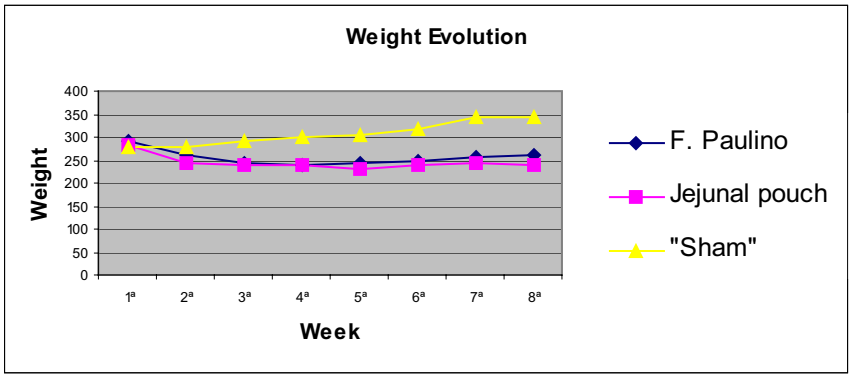

Figure 3 – Weight evolution of rats subjected to total gastrectomy and Fernando Paulino pouch, jejunal pouch and sham. No difference was observed between FP and JP groups (p>0,05).

The JP interposition graft was associated with a significant decrease in serum albumin, glucose, transferrin, folate and cobalamine concentration in the 8th postoperative week, when compared to sham operated rats (p<0.05); calcium and iron was unaffected (table 2). JP rats in the preoperative period, as compared to 8th week, displayed a significant decrease in serum albumin, glucose, transferrin, folate and cobalamine (p<0.05). Glucose and cobalamine serum concentrations showed significantly reduced in FP rats when compared to S rats in 60th postoperative day (p<0.05). In FP rats, only transferrin showed significant decrease comparing the preoperative with 8th week postoperative levels (p<0.05). The serum gastrin levels showed significantly reduced in FP rats compared to JP and sham rats (p<0.05).

Table 2 – Laboratory findings at preoperative and eight weeks.

Laboratory parameter	**Group**	**Preoperative**	**8th week**
Albumin (g/dL)	Sham	3.6±0.5	3.8±0.3
	FP	3.4±0.2	3.1±0.2
	JP	3.4±0.4**	2.7±0.3*
Glucose (mg/dL)	Sham	102±13.1	99±6.3
	FP	96±10.3	86±23.7*
	JP	100±14.3**	80±12.1*
Transferrin (mg/dl)	Sham	236±20.4	229±24.1
	FP	224±22.8§	160±15**
	JP	231±19**	163±21.3*
Iron (µg/dL)	Sham	288±13	286±22
	FP	277±12.6	223±14.1
	JP	268±12**	279±20.2*

Folate (ng/ml)	Sham	8.5±3.1	8.2±1.9
	FP	8.1±2.0	7.5±2.5
	JP	8.1±2.4**	7.2±0.7*
Cobalamine (pmol/L)	Sham	221±18	214±20.4
	FP	234±22§	163±14*
	JP	219±23.2**	161±12.7*
Calcium (mg/dL)	Sham	8.4±0.5	9.7±1.8
	FP	9.1±0.8	6.6±3.3
	JP	8.9±1.6**	8.5±2.0*
Gastrin (pg/mL)	Sham	128.5±7.8	133.7±10.2
	FP	122.3±9.0**	17.4±3.1*
	JP	117.8±10.4**	86.4±8.2*,§

Values are expressed as means±SEM; FP, Fernando Paulino pouch; JP, jejunal pouch interposition graft.
* $p<0.05$ compared to sham 8th week; § $p<0.05$ compared to FP 8th week; ** $p<0.05$ compared to JP 8th week.

Discussion

It has been hypothesised that passage of food across the duodenum, resulting in the mixture of chyme with biliary and pancreatic secretions, aids in digestion, absorption, and the stimulation of the remaining intestinal tract[4,5]. These processes should result in better calcium and iron absorption with improved lipid and protein digestion[7]. In fact, in the present study calcium and iron showed high serum levels when compared the pouch with duodenal passage to interpositional pouch. The formation of an appropriate replacement gastric reservoir, to simulate pre-operative gastric volume, is considered important. Construction of an enteric pouch is thought to enable the patient to consume larger, more customary, and satisfying meals[4,6]. A pouch should therefore improve the patients quality of life, allow them to ingest more calories, and help to prevent malabsorption and weight loss. Compared to sham, weight loss occurred in the two pouches tested in our study, and no difference was observed between them. Paulino and Roselli[8] reported, in 1973, notably satisfying results in three patients with a distal jejunojejunal pouch, tested in the present work. They described the use of either an isoperistaltic or antiperistaltic side-to-side attachment of the proximal afferent to the efferent jejunum, at the Roux-en-Y level, together with an end-to-end esophagojejunal anastomosis. The isoperistaltic technique was used in the present study.
The optimal reconstruction protocol after total gastrectomy is still a matter of debate. Given the decreasing morbidity and mortality rates after total gastrectomy[9,10,17], this issue is gaining even more importance. Pouch reconstructions are developed to create a larger reservoir with a better reflux barrier; of all the reconstruction types without restoration of the duodenal passage, are used most often. An alternative is the interposition of a jejunal loop with reestablishment of the duodenal passage. Preservation of the duodenal passage should result in better physiologic enrichment of the chyme

with bile and pancreatic juice and better physiologic regulation of gastrointestinal hormones, thereby offering substantial advantages[4]. The ideal reconstruction should supply the patient with a sufficiently large reservoir to accommodate more extensive meals. It should also act as a reflux barrier to avoid reflux esophagitis and should enable optimum utilization of the administered substrates. These practical demands are theoretically best served by the formation of a pouch with preservation of the duodenal passage, as that described by Nakayama[16].

Advantages of pouch reconstructions[9,10], compared to the Roux-en-Y reconstruction and the advantages of restoration of the duodenal passage[11] have been repeatedly described, but definitive, statistically significant proof of the superiority of this method has not yet been presented in aprospective randomized study. Total gastrectomy patients suffer from a weight loss of 15% to 20% [12], which is less if the duodenal passage is preserved[13]. In the present work the levels of serum iron and calcium were preserved in the JP rats, the group where the duodenal passage was preserved and the iron levels showed similar to the sham group As the iron and calcium is absorbed in duodenum, possibly this physiologic characteristic possibly turned this fact possible. In present study no difference was observed in the weight loss when compared the reconstruction tested.

After total gastrectomy iron levels are low in up to 90% of patients[14]. Blood glucose regulation is disturbed after gastrectomy[12,15]. Pathologic glucose tolerance develops if the duodenal passage is eliminated. Glucose was unaffected in FP and JP rats of the present study. Alkaline esophagitis occurs after any method of postgastrectomy reconstruction that allows reflux of bile and pancreatic secretions into the distal part of the esophagus. A Roux-en-Y esophagojejunostomy eliminates esophagitis if the length of the jejunum, between the esophagealenteric and distal jejunojejunal anastomoses, is at least 40 to 45 cm . In fact, in the present study F. Paulino procedure eliminated esophagitis, because the referred anastomosis was very distant to esophagus in our experiment.

The gastric antrum is the richest source of gastrin. It secreted by G cells and has been localized by immunohistochemical technique[18]. The intestinal gastrin source is highest in the proximal duodenum and there is evidence for release of gastrin from it in response to a meal[19]. The total gastrin concentration is considerably less in duodenum then it is in the antrum, gradually decreasing toward the distal part of the small intestine[20]. Our study showed that JP rats with duodenal food passage had serum gastrin higher than FPO group, and the duodenal mucosa was near normal when compared to sham rats. In JP rats is was observed duodenal wall atrophy. Gastric mucosa from the parietal cell region of man and rats have been grown in tissue culture with and without the addition of pentagastrin, proving the trophical effects of gastrin[21].

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RESUMO
Objetivo: Estudo comparativo foi realizado entre a bolsa jejunal de Fernando Paulino (FP) e uma bolsa jejunal (JP) interposta entre o esôfago e duodeno, para substituir o estômago após gastrectomia . Foi investigado o efeito dos dois procedimentos na histologia do esôfago, estado nutricional e gastrinemia sérica em ratos. **Métodos**: Quarenta e seis ratos Wistar pesando 282±17g foram aleatoriamente submetidos a *sham operation* (S), FP e JP após gastectomia total. Decorridas 8 semanas, foi colhido sangue por punção cardíaca para dosagem de proteínas totais, albumina, ferro, transferrina, folato, cobalamina, calcio, e gastrina. Os animais receberam dose letal de anestésico e tecido do esôfago terminal foi retirado para histologia. Foi observada a mortalidade operatória dos animais. **Resultados:** Quarenta e seis ratos foram operados e 30 sobreviveram por 8 semanas. Cinco (33,3 %) morreram após FP e 11 (52,3%) após JP ($p<0.05$). Esophagitis pós-operatória ocorreu em 6 ratos JP. Na 8ª semana o peso corporal foi maior nosratos submetidos a FP do que JP ($p>0.05$). Os ratos submetidos a JP tiveram uma diminuição significativa na albumina, glucose, transferrina, ferro, folato e cálcio, comparado com o *sham* ($p<0.05$). Os níveis de gastrina sérica, ferro e calcio mostraram-se significantemente maiores nos ratos submetidos a JP do que nos FP ($p<0.05$). Nos ratos FP a transferrina e a cobalamina estiveram significantemente diminuídas comparando-se os níveis do pré-operatório com a 8ª semana ($p<0.05$). **Conclusão**: A bolsa jejunal de F. Paulino, em ratos, resultou em mortalidade operatória e incidência de esofagite de refluxo menor do que a interposição de JP. A JP não afetou a dosagem sérica de gastrina, ferro e cálcio, provavelmente devido à preservação da passagem dos alimentos pelo duodeno.
DESCRITORES: Gastrectomy total. Bolsa jejunal. Nutrição. Gastrina. Refluxo.

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3.3.Artigo III: ASSOCIATION OF ADVANCED GASTRIC CARCINOMA WITH *Helicobacter pylori* INFECTION

Submetido para publicação em Arquivos de Gastroenterologia (Indexada no MEDLINE)

ASSOCIATION OF ADVANCED GASTRIC CARCINOMA WITH *Helicobacter pylori* INFECTION

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ABSTRACT - *Background* - There is substantial evidence that infection with *Helicobacter pylori (H pylory)* plays a role in the development of gastric cancer and that it is rarely found in gastric biopsy of atrophic gastritis and gastric cancer. On advanced gastric tumors, the bacteria can be lost from the stomach. ***Objectives*** - To analyze the incidence of *H pylori* in operated advanced gastric carcinomas and adjacent non-tumor tissues, their morphological features, localization, relationships between the infection and clinical-pathologic characteristics of advanced gastric carcinomas**.** ***Methods*** - A prospective controlled study enrolled 56 patients from Hospital Universitário-UFRN with advanced gastric cancer treated from 2000 february to 2003 march. Immediately after gastrectomy, the resected stomach was opened and several mucosal biopsy samples were taken from the gastric tumor and from the adjacent mucosa within 4 cm distance from the tumor margin. Tissue sections were stained with hematoxyllin and eosin. Lauren's classification for gastric cancer was used. The *H pylori* infection status was assessed by the urease rapid test, IgG by ELISA and histopathological evaluation by Giemsa staining. *H pylori* infected patients were treated with omeprazole, clarithromycin and

amoxicillin for 7 days. Follow-up endoscopy and serology were performed 6 months after treatment to determine successful eradication of *H pylori*. Thereafter, follow-up endoscopies were scheduled annually. ***Results*** - Thirty-four tumors (60.7%) were intestinal-type and 22 (39.3%) diffuse type carcinomas. In adjacent non-tumor gastric mucosa, chronic gastritis were found in 53 cases (94.6%) and atrophic mucosa in 36 patients (64.3%). All the patients with atrophic mucosa were *H pylori* positive. When examined by Giemsa and urease test, *H pylori* positive rate in tumor tissue of intestinal type carcinomas was higher than that in diffuse carcinomas (OR=4.01; 95% CI 1.2-12.5; p=0.03). In tumor tissues, 34 (60.7%) cases with *H pylori*-positive in gastric carcinomas were detected by Giemsa method. *H pylori* was observed in 30 of 56 cases (53.5%) in tissues 4 cm adjacent to tumors. This difference was not significant (OR=0.74; 95% CI 0.3- 1.5; p=0.56). Eradication of *H pylori* led to a complete negativity on the 12th postoperative month. ***Conclusions*** – The data suggest a high prevalence of *H pylori* in tumor tissue of gastric advanced carcinomas and in adjacent normal mucosa of operated stomachs. The association of *H pylori* with intestinal-type was higher than with diffuse-type carcinoma.
HEADINGS **–** Gastric cancer. *Helicobacter pylori*. Advanced carcinoma. Etiology.

INTRODUCTION

Gastric carcinoma is one of the most common human malignant cancers in the world. There is substantial evidence that infection with the gastric bacterium *Helicobacter pylori* plays a role in the etiology of gastric cancer[39]. The International Agency for Research on Cancer, sponsored by the World Health Organization, has categorized *H pylori* infection as a definite human carcinogen since 1994[14]. Some years after that decision, the causal role of *H pylori* in gastric carcinogenesis is still poorly understood[9,13]. The magnitude of the risk of gastric cancer associated with infection remains unclear and there have been suggestions that this risk varies with sex[11,24], age[23], and the histological subtype of the cancer[26].

There is evidence that *H pylori* is rarely found in gastric biopsy specimens from individuals with atrophic gastritis, gastric cancer, intestinal metaplasia, and that with the development of advanced gastric disease the bacteria can be lost from the stomach[15].

With loss of infection, the level of circulating anti-*H pylori* antibodies will fall, so that patients with gastric cancer may be *H pylori* seronegative even though they have been infected in the past[8]. Although the etiopathological mechanism of human gastric cancer remains unclear, most researchers believe that the pathogenesis of human gastric cancer is a multifactorial and multistage process[12,18,21]. Epidemiological and histopathological studies have shown that *H pylori* infection is closely associated with gastric carcinogenesis[10,37].

In the present study, the prevalence of *H pylori* infection in gastric carcinomas and adjacent mucosa from operated patients was estimated. Both morphological features and localization of *H pylori* in gastric carcinomas and adjacent non-tumor tissues were demonstrated. Relationships between *H pylori*

infection and the clinical-pathologic characteristics of gastric carcinomas were analyzed.

METHOD

The prospective controlled study enrolled 56 patients from Hospital Universitário-UFRN with advanced gastric cancer according to the TNM classification, treated from 2000 february to 2003 march. Demographic data are expressed in Table 1. Patients with chronic diseases, immunossupressed, using non steroid anti-inflammatory, previous radiotherapy/chemotherapy and H2 blockers were excluded. All patients were subjected to gastrectomy. After resection, the greater curvature of the stomach was opened and several (usually 12) mucosal biopsy samples were taken from the gastric tumor, and the adjacent macroscopically non-tumorous mucosa within 4 cm distance from the tumor margin. For morphological analysis, tissue sections were routinely stained with hematoxyllin and eosin. Adjacent non-tumor tissue was examined for diagnosis of atrophy of mucosa and chronic gastritis. The histological typing of gastric cancer was assessed according to Lauren's classification[17]. The *H pylori* infection status was assessed by the urease rapid test (Gastroteste kit). *H pylori* IgG antibody in plasma was measured by an enzyme-linked immunosorbent assay (ELISA), using commercially available kit Cobas Core II (Roche). A cut off value of > 7.5 U was taken to categorize samples as positive, as recommended by the manufacturer. For histopathological evaluation of the *H pylori* colonization, the specimens from tumor tissue and adjacent mucosa were loaded into 1% formalin and routinely screened with microscope (Giemsa staining).

For those patients infected with *H pylori*, treatment was performed after the 30th postoperative day. Patients received omeprazole 2 × 20 mg, clarithromycin 2 × 500 mg, and amoxicillin 2 × 1000 mg given before breakfast and before dinner for 7 days. The first follow-up endoscopy was performed 6 months after treatment to determine successful eradication of *H pylori* and tumor recurrence. Thereafter, follow-up endoscopies were scheduled annually.

All patients gave an informed consent before the surgical procedures. The study was conducted in accordance with the Declaration of Helsinki and the 196/96 Resolution from Conselho Nacional de Saúde-Brazil and was approved by the Ethics on Research Committee of the Federal University of Rio Grande do Norte, RN, Brazil (Protocol 261.01).

For statistical analysis of a difference between proportions, Odds Ratios (OR) and 95% confidence interval (95% CI) were calculated for the association between *H pylori* and GC. *Chi*-square tests were used to compare categorical variables. $P<0.05$ was considered statistically significant.

RESULTS

Thirty-four tumors (60.7%) were classified as intestinal-type, and the remaining 22 (39.3%), as diffuse type carcinomas. When the cancer was separated according to the histological type, the magnitude of the association with *H pylori* infection was higher in intestinal than in diffuse-type (Table 2). Statistically significant differences were found between these groups, mainly

when the diagnosis was performed by Giemsa staining and urease rapid test ($p<0.05$). Histopathological changes of adjacent non-tumor gastric mucosa were observed; chronic gastritis was found in 53 cases (94.6%) and atrophic mucosa in 36 patients (64.3%). All the patients with accentuated reduction in the epithelial thickness (atrophic mucosa) were *H pylori* positive when assessed by Giemsa and urease test.

Helicobacter pylori in tumor tissue

Giemsa staining showed 34 positive cases (60.7%) of tissue sections carrying bacterial bodies of *H pylori* from 56 gastric carcinomas, and the positive rate was lower than that detected by ELISA method in 36 cases (64.3%). The urease rapid test detected *H pylori* in tumor tissue of 34 patients (60,7%). The differences among these data were not significant (OR=0,85; 95% CI 0.3-1.8; p=0.84). When detected by Giemsa, *H pylori* positive rate in intestinal type carcinomas was higher than that in diffuse carcinomas (OR=4.01; 95% CI 1.2-12.5; p=0.03) (Table 2). The difference was also significant comparing *H pylori* positive in intestinal and diffuse carcinomas by rapid urease test (OR=4.8; 95% CI 1.3-17.1; p=0.02). In diffuse carcinomas**,** *H pylori* was predominantly negative when Giemsa staining and urease rapid test were used (Table 2).

Helicobacter pylori in non-tumor tissue

In tumor tissues, some bacteria were found. In 34 (60.7%) cases with *H pylori*-positive gastric carcinomas, detected by Giemsa method, bacterial bodies of *H pylori* were observed in the mucus and epithelial cells. They were observed in 30 of 56 cases (53.5%) in the glands and mucous pool of normal tissues 4 cm adjacent to tumors. So, *H pylori* microscopic positive rate in non-tumor sites was lower than that in tumor (60,7%) sites, but this difference was not significant (OR=0.74; 95% CI 0.3- 1.5; p=0.56).

ELISA antibody and urease test after eradication therapy

To examine the effect of *H pylori* treatment on antibody expression and urease rapid test, a total of 40 patients were followed endoscopically (16 were lost of follow up). We analysed the *H pylori* infected gastric mucosa obtained from gastric cancer and adjacent non-tumor tissue, from patients before and after *H pylori* eradication therapy. Six months later, only two patients had IgG/ELISA and urease positive tests 2/36 (5%) and the treatment was repeated. The second treatment of *H pylori* led to a complete negativity of IgG/ELISA and urease test on the 12^{th} postoperative month. On the second follow up year, tumor recurrence occurred in five patients with diffuse carcinomas, whose *H pylori* tests had been negative after 6 and 12 postoperative months. These patients died four months later.

DISCUSSION

The epidemiology of *H pylori* infection has been studied in the Brazilian population. Rocha et al[29], using indirect immunofluorescence, detected a prevalence of 62.1% *H pylori* infection in asymptomatic Brazilian blood donors in an urban area. A prevalence of 84.7% *H pylori* infection in adults in a rural area of a central region of Brazil was also reported[33]. Thus, the prevalence is highest in developing regions, including all the countries of Latin America. Around the world, the prevalence of *H pylori* infection ranges from 20% to over 90% in adult populations[28]. It has been postulated that transmission decreases as sanitation improves[1]. Within countries, *H pylori* infection is linked to low socioeconomic status, residential conditions and migration from high prevalence regions[3,32].

Histological studies have reported the association between *H pylori* infection and gastric cancer[4,12,22,26]. However, the results have not been always consistent; higher rates of serologically and histologically detected *H pylori* positivity have been reported for early stage cancer than for advanced gastric cancer[4]. Different from what could be expected, in the present study all the patients had advanced cancer and the prevalence of positive *H pylori* in tumor tissue was 60.7%, as detected by Giemsa staining and by ELISA serology (64.3%). The high frequency of *H pylori* infection in gastric cancer tissue may be one of the carcinogenic factors in our patients. The prevalence of *H pylori* in non-tumor tissue, 4 cm adjacent to gastric cancer, was not different from that detected in tumor tissue. Atrophy of the gastric mucosa adjacent to tumor tissue was observed in all patients with *H pylori* positive gastric carcinomas, with intestinal or diffuse types. This finding, associated with the high prevalence of chronic gastritis in adjacent tumor tissue (94.6%), may be a predetermining condition in the carcinogenesis of the gastric tumor of our patients. Ueruma et al[36] reported that subjects with severe gastric atrophy, corpus predominant gastritis, or intestinal metaplasia had an increased risk for gastric cancer. Another study confirmed that gastric atrophy status was essential for cancer development[40].

According to Correa's[6] model of gastric carcinogenesis, continuous exposure to irritants of the gastric mucosa produces repeated episodes of superficial gastritis**.** When this occurs in patients with nutritional deficits, a degenerative sequential process causes atrophic gastritis, intestinal metaplasia, dysplasia and, ultimately, carcinoma. Although the precise role of *H pylori* in this sequence remains unclear, it may be considered an agent that causes chronic inflammation of the gastric mucosa. Histological studies have described a corpus-dominant pattern of mucosal inflammation, which is found not only in most *H pylori* infected gastric cancer patients irrespective of the clinical stage[20], but also in healthy relatives of gastric cancer patients[19]. Based on histological studies, patients with a corpus-dominant *H pylori* gastritis have about 9-fold increased risk for gastric cancer[22].

Although cancer development is a multifactorial process[7], *H pylori* infection increases the risk of gastric cancer. Mongolian gerbils were orally inoculated and infected with *H pylori*, which induced gastric carcinomas located in the pyloric region. After the 26th week, severe active chronic gastritis, ulcers, and intestinal metaplasia could be observed in the infected animals. After the 62nd week, adenocarcinoma had been developed in the pyloric region of 37%

(10/27) of the infected animals. It was found that adenocarcinoma development seemed to be closely related to intestinal metaplasia[38]. In our study the presence of *H pylori* in tumor tissue with intestinal-type gastric adenocarcinoma occurred more frequently than in the diffuse-type, and the difference was significant. In the diffuse-type carcinoma the *H pylori* was predominantly negative when the Giemsa and urease test were used. These data differ from those published by other authors[5,27].

"The Maastricht Consensus Report" recommends *H pylori* eradication therapy following early resection for gastric cancer[34]. There are some data showing that *H pylori* eradication is associated with a decrease in the recurrence rate in patients with early gastric cancer that is resected endoscopically[30,35] Some reports emphasize the importance of *H pylori* treatment at a young age. These studies conclude that *H pylori* eradication is also useful for the prevention of new cancer development from high-risk mucosa for gastric cancer[2,16,31]. These recommendations are in agreement with the management adopted and the results of the present study. All the patients operated with gastric cancer were treated for *H pylori* infection, when it was present. All of them were *H pylori* negative after one year of follow up and no recurrence of cancer was observed. After two years, endoscopy showed recurrence in five patients, with *H pylori* negative tests. These recurrences may be explained by the fact that all the patients were operated for advanced gastric carcinomas, with poor prognosis. These results suggest that *H. pylori* eradication, even in advanced tumors, may reduce gastric cancer recurrence.

As suggested by an economical analysis based on the United States data, a screen-and-treat strategy for *H pylori* infection, even under conservative assumptions, may be a cost-effective strategy for gastric cancer prevention comparable to the costs of breast mammography screening programs[25].

CONCLUSIONS: The data of the present study suggest a high prevalence of *H pylori* in tumor tissue of gastric advanced carcinomas and in adjacent normal mucosa of operated stomachs. An association of *H pylori* with intestinal-type carcinoma was observed and it was significantly higher than that with diffuse-type carcinoma.

Araújo-Filho I, Brandão-Neto J, Egito EST, Rezende AA, Pinheiro LAM, Medeiros AC. Association of advanced gastric carcinoma with *Helicobacter pylori* infection. Arq Gastroenterol

RESUMO - Racional - Existe evidência de que a infecção pelo *Helicobacter pylori* desempenha um papel importante na etiologia do câncer gástrico e que é raramente encontrado em biópsias de gastrite atrófica e em tecido tumoral de câncer do estômago. Com a evolução para câncer gástrico avançado, a bactéria tende a desaparecer do tecido tumoral. ***Objetivos*** - Analisar a prevalência do *H pylori* em peças operatórias de carcinomas gástricos avançados e no tecido adjacente aos tumores. Avaliar a relação entre a infecção e as características clinico-patológicas dos carcinomas gástricos. ***Método*** **-** Estudo prospectivo controlado incluiu 56 pacientes operados no Hospital Universitário-UFRN com câncer gástrico avançado, entre fevereiro de 2000 e março de 2003. Imediatamente após a gastrectomia, a peça opeatória foi aberta e foram feitas várias biópsias do tecido neoplásico e da mucosa adjacente a 4 cm da margem tumoral. Os tecidos formam processados e

corados pela hematoxilina/eosina. Foi usada a classificação de Lauren para carcinoma gástrico. A infecção pelo *H pylori* foi diagnosticada pelo teste da urease, dosagem de IgG por ELISA e histopatologia com coloração Giemsa. Os pacientes infectados pelo *H pylori* foram tratados com omeprazol, claritromicina e amoxicilina por 7 dias. Após 6 meses, 1 ano e 2 anos, foi feito seguimento utilizando endoscopia, dosagem de IgG e teste da urease para avaliar o sucesso da erradicação do *H pylori* e recidiva do tumor. ***Resultados -*** O carcinoma tipo intestinal ocorreu em 34 (60.7%) pacientes e 22 (39.3%) foram acometidos de carcinoma difuso. No tecido adjacente não tumoral a gastrite crônica foi observada em 53 casos (94.6%) e mucosa atrófica em 36 pacientes (64.3%), todos *H pylori* positivos. Exames pelo Giemsa e teste da urease revelaram maior prevalência de *H pylori* positivo no tecido tumoral do carcinoma tipo intestinal do que no tipo difuso (OR=4.01; 95% CI 1.2-12.5; p=0.03). Quando foi comparada a presença de *H pylori* no tecido tumoral (60.7%) com a que ocorreu na mucosa gástrica adjacente ao carcinoma (53%), diferença foi insignificante (OR=0.74; 95% CI 0.3- 1.5; p=0.56) A erradicação do *H pylori* resultou em negatividade completa no segundo ano de seguimento. ***Conclusões –*** Os dados sugerem presença significante de *H pylori* no tecido tumoral de carcinoma gástrico avançado e na mucosa adjacente de peças operatórias. A associação de *H pylori* com carcinoma tipo intestinal foi maior do que com o tipo difuso.

DESCRITORES – Câncer gástrico. *Helicobacter pylori*. Carcinoma avançado. Etiologia.

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Table 1. - Demographic data of patients operated with gastric cancer.

NUMBER	56
Male/female	38/18
Mean age (year)	62
Age range (year)	21 – 78
H. pylori infection rate (%)	60.7

Table 2 - *H pylori* infection detected by ELISA, Giemsa and urease test. Comparison with histological types of gastric carcinoma (tumor tissue).

Histological types	Total cases	ELISA		Giemsa		Urease test	
		P	N	P	N	P	N
Intestinal	34	20	14	25*	9	26**	8
Diffuse	22	16	6	9	13	8	14
TOTAL	56	36	20	34	22	34	22

P, positive; N, negative.
*OR=4.01compared to diffuse GC. **OR=4.8 compared to diffuse GC.

4 COMENTÁRIOS, CRÍTICAS E CONCLUSÕES

As vantagens clínicas da cirurgia laparoscópica têm provocado o uso amplo em um número cada vez maior de intervenções cirúrgicas, e estimulado a investigação das bases fisiopatológicas da cirurgia laparoscópica em comparação com a aberta.

Dado o crescente volume de estudos em animais e clínicos[4,8,10,12,14], sugerindo que o CO_2 insuflado na cavidade peritoneal influencia o resultado da cirurgia laparoscópica, seria imprudente apenas atribuir as vantagens da laparoscopia à lesão tecidual mínima secundária às incisões mínimas. Considerando que os cirurgiões continuam a realizar operações laparoscópicas cada vez mais longas e complexas, com dissecções extensas, intensa manipulação e descolamento de tecidos, o grau de lesão atribuível ao tamanho das incisões torna-se relativamente menos importante. Assim sendo, a magnitude das operações laparoscópicas está concorrendo em muitos casos, com o tamanho das incisões nas operações abertas. Pode-se dizer que a diferença entre elas passa a ser predominantemente a fisiopatologia do pneumoperitônio com CO_2.

Partindo do princípio de que o CO_2 absorvido durante a cirurgia laparoscópica pode afetar o balanço ácido-base e muitos modelos experimentais de estudo nesta linha em roedores empregam sistema de anestesia que não requer ventilação mecânica, julgamos importante utilizar o mesmo modelo para confrontar nossos achados com os de outros investigadores. Foi utilizado o modelo de sepse abdominal com ligadura e perfuração do ceco, em detrimento de outros modelos que usam injeção de lipopolissacárides, com a intenção de produzir sepse clinicamente relevante, por via laparoscópica. O estresse cumulativo provocado pelo acesso laparoscópico e a contaminação bacteriana do peritônio, provocada pela ligadura e perfuração do ceco, serviu para simular a real situação da cirurgia laparoscópica do cólon em vigência de sepse. A validade deste modelo tem sido testada por outros autores[63].

No presente estudo foi possível determinar que o pneumoperitônio com CO_2 produziu significantes alterações no balanço ácido-base, tanto no compartimento intra-arterial, intraperitoneal, quanto no espaço subperitoneal. A

observação de diminuição de pH e aumento da pCO_2 no sangue arterial e nos exsudatos intraperitoneal e subperitoneal representou a condição de acidose hipercárbica. Como foi observada a diminuição de pH nos exsudatos intraperitoneal e subperitoneal, cujos espaços são separados pela tênue membrana do peritônio, pode-se inferir que uma acidose intracelular também deve ter ocorrido nos animais estudados. De fato, a hipótese de que o maior componente da repercussão da cirurgia laparoscópica na reação inflamatória está relacionado com o pneumoperitônio com CO_2, tem sido estudada, partindo do princípio de que o CO_2 difunde-se rapidamente nas células e produz acidificação intracelular. Swallow et al(1993)[64] demonstraram que a acidificação intracelular causa uma inibição da produção de superóxido pH dependente, em macrófagos intraperitoneais. Um outro estudo observou que em pH maior do que 7, a liberação de FNT foi comparável com a liberação ocorrida nas células controles sob incubação. Entretanto, em pH <6,5 a inibição do FNT foi a mesma ocorrida em células expostas ao CO_2. Esses dados sugeriram portanto, que a acidificação citoplásmica induzida pela exposição ao CO_2 foi suficiente para reduzir a liberação de FNT. No estudo aqui relatado foi observada a redução de FNT, IL-1α e IL-6 nos animais submetidos ao pneumoperitônio, com e sem infecção, corroborando com as informações da literatura. Nossos achados falam a favor de correlação entre insuflação de CO_2 na cavidade peritoneal, acidificação de exsudatos/sangue arterial e redução da expressão de citocinas intraperitoneais.

As informações colhidas da literatura sugerem que a cirurgia laparoscópica resulta em menor lesão tecidual e menor resposta inflamatória do que a cirurgia aberta, apesar do fato de que o pneumoperitônio é considerado um procedimento agressivo, com resposta neuroendócrina que se assemelha à resposta à cirurgia aberta[65]. No modelo experimental utilizado no presente trabalho ficou evidenciado que a resposta imune, determinada pela expressão de citocinas pró-inflamatórias, foi melhor preservada após os procedimentos realizados com pneumoperitônio, do que com a cavidade abdominal aberta. Outros trabalhos anteriormente publicados confirmam esses achados[20,21,66].

A resposta imune reduzida na cirurgia aberta está associada ao grau de lesão tecidual, que facilita a proliferação bacteriana e peritonite, seguida de

maior liberação de citocinas. Fator importante a ser considerado é o CO_2 que, segundo alguns estudos, pode atuar como um agente bacteriostático[67,68], previne o contato do peritônio com lipopolissacárides existentes no ar e no meio ambiente, reduzindo a resposta inflamatória no peritôneo[14]. A partir dos resultados do presente trabalho, algumas informações relevantes foram captadas: o modelo experimental de sepse abdominal por via laparoscópica mostrou-se factível e compatível com trabalhos semelhantes anteriormente publicados; a cirurgia laparoscópica utilizando pneumoperitônio com CO_2 provocou menor reação inflamatória, menor repercussão imunológica e uma tendência a acidose, associadas à insuflação de CO_2 na cavidade peritoneal, quando comparada com a cirurgia aberta.

Muitos outros aspectos necessitam ser estudados a respeito da cirurgia laparoscópica, com o objetivo de explicar as alterações decorrentes do pneumoperitônio com CO_2, como por exemplo as alterações no nível molecular. Devem ser buscados dados preditivos que possam guiar a indicação da cirurgia laparoscópica para situações ou grupos especiais de pacientes.

Perspectivas de trabalhos na mesma linha de pesquisa

Como perspectivas de estudos posteriores, algumas perguntas podem ser respondidas em trabalhos futuros relacionados com a cirurgia laparoscópica, como: que pacientes podem estar particularmente em alto risco de isquemia intestinal fatal? Até que ponto a cirurgia laparoscópica pode levar a uma instabilidade hemodinâmica preocupante em pacientes susceptíveis? Na sepse grave e no politraumatizado, que fatores devem ser levados em consideração para beneficiar os doentes na indicação e na contra-indicação da cirurgia laparoscópica? Na área obstétrica, quais os riscos para o binômio mãe/feto?

Conclusões: A partir do modelo experimental utilizado no presente trabalho podem ser postulados os seguintes pontos como conclusões:

1. O pneumoperitônio com CO2, na presença de peritonite, reduziu a reação inflamatória sistêmica representada pela atenuação dos níveis de citocinas em exsudato peritoneal.

2. Na vigência de peritonite, o pneumoperitônio com CO_2 em ratos provocou diminuição significante no pH do sangue arterial, do exsudato intraperitoneal e subperitoneal, correlacionada com aumento da pCO_2.
3. O pneumoperitônio com CO_2 foi responsável por maior número de linfócitos e redução de neutrófilos no sangue periférico, em níveis significantes, em relação ao controle e ao grupo laparotomia.

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(Relativas aos textos da introdução, revisão da literatura, comentários e críticas)

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Abstract

Laparoscopic surgery is associated with reduced surgical trauma, and less acute phase response, as compared with open surgery. Cytokines are important regulators of the biological response to surgical and anesthetic stress. The aim of this study was to determine if CO_2 pneumoperitoneum would change cytokine expression, gas parameters and leukocyte count in septic rats. **Methods:** Wistar rats were randomly assigned to five groups: control (anesthesia only), laparotomy, CO_2 pneumoperitoneum, cecum ligation and puncture by laparotomy, and laparoscopic cecum ligation and puncture. After 30 min of the procedures, arterial blood samples were obtained to determine leukocytes subpopulations by hemocytometer. TNFα, IL-1β, IL-6 were determined in intraperitoneal fluid (by ELISA). Gas parameters were measured on arterial blood, intraperitoneal and subperitoneal exsudates. **Results:** Peritoneal TNFα, IL-1β and IL-6 concentrations were lower in pneumoperitoneum rats than in all other groups ($p<0.05$). TNFα, IL-1β and IL-6 expression was lower in the laparoscopic than in laparotomic sepsis ($p<0.05$). Rats from laparoscopic cecum ligation and puncture group developed significant hypercarbic acidosis in blood and subperitoneal fluid when compared to open procedure group. Total white blood cells and lymphocytes were significantly lower in laparoscopic cecum ligation and puncture rats than in the laparotomic ($p<0.01$). Nevertheless, the laparotomic cecum ligation rats had a significant increase in blood neutrophils and eosinophils when compared with controls ($p<0.05$). **Conclusions:** This study demonstrates that the CO_2 pneumoperitoneum reduced the inflammatory and immune response in an animal model of peritonitis with respect to intraperitoneal cytokines, white blood

cell count and clinical correlates of sepsis. The pneumoperitoneum produced hypercarbic acidosis in septic animals.

Key words: Pneumoperitoneum. Carbonic gás. Sepsis. Acidosis. Leucocytes.

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