Complications after intestinal resection in Crohn’s disease: laparoscopic versus conventional approach☆

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Background: significant advances in medical therapy for Crohn’s disease (CD) occurred in the last 12 years, mainly due to the introduction of anti-TNF therapy. Laparoscopic colorectal surgery represented the most important advance on surgical treatment in the management of CD, as it also had developed in the treatment of other conditions. There is a tendency for lower complication rates after laparoscopic bowel resections as compared to open surgery. The aim of this study was to analyze and compare the complication rates after bowel resections for CD between the two approaches in a Brazilian case series.

Methods: this was a retrospective longitudinal study, including CD patients submitted to bowel resections from a single Brazilian Inflammatory Bowel Diseases (IBD) referral center, treated between January 2008 and June 2012 with laparoscopic approach (LA) or conventional approach (CA).

Variables analyzed: age at surgery, gender, Montreal classification, smoking, concomitant medication, type of surgery, surgical approach, presence and type of complication up to 30 days after the procedures. Readmission and reoperation rates, as well as mortality, were also analyzed. Patients were allocated in two groups regarding the type of procedure (LA or CA), and complication rates and characteristics were compared. Statistical analysis was performed with Mann-Whitney test (quantitative variables) and chi-square test (qualitative variables), with p < 0.05 considered significant.

Results: a total of 46 patients (25 men) were included (16 submitted to LA), with mean age of 38.1 (± 12.7) years. The groups were considered homogeneous according to age, gender, CD location, perianal disease and concomitant medications. There were more patients with fistulizing CD on the CA group (p = 0.029). The most common procedure performed was ileocolic resection on both groups (56.7% of the CA and 75% of the LA patients – p = 0.566). Overall, total complications (surgical and medical, including minor and major issues) occurred in 60% (18/30) of the CA group and 12.5% (2/16) of the LA group (p = 0.002). Wound infection was the most frequent complication (10/30 on CA and 1/16 on the LA groups). There were 3 deaths in the CA group. Specific analysis of each complication did not demonstrate
Palavras-chave:
Doença de Crohn
Cirurgia laparoscópica
Complicações

Complicações após ressecções intestinais na doença de Crohn: há diferenças entre as vias convencional e videolaparoscópica?

Resumo

Introdução: avanços significativos no tratamento medicamentoso da doença de Crohn (DC) ocorreram nos últimos 12 anos, principalmente após a introdução da terapia anti-TNF. A cirurgia laparoscópica colorretal representa um dos maiores progressos no tratamento cirúrgico minimamente invasivo da DC, bem como no manejo de outras afecções. Existe uma tendência a menores taxas de complicações com as ressecções intestinais laparoscópicas quando comparadas a cirurgia convencional. O objetivo deste estudo foi analisar e comparar as taxas de complicações após ressecções intestinais na DC entre as duas vias de acesso em uma série de casos brasileira.

Método: estudo retrospectivo longitudinal, incluindo pacientes com DC submetidos a ressecções intestinais em um centro de referência brasileiro em doença inflamatória intestinal (DII), tratados entre janeiro de 2008 e junho de 2012, com cirurgia laparoscópica (CL) ou cirurgia convencional (CC). As variáveis analisadas foram: idade no momento da cirurgia, gênero, classificação de Montreal, tabagismo, medicações concomitantes, tipo de cirurgia, via de acesso, presença e tipo de complicações em até 30 dias do procedimento. Readmissão, reoperações e mortalidade também foram avaliadas. Os pacientes foram alocados em dois grupos de acordo com a via de acesso (CL ou CC), sendo as taxas de complicações comparadas entre si. Análise estatística foi realizada pelos testes de Mann-Whitney (variáveis quantitativas) e chi-quadrado (qualitativas), com p<0.05 considerado significativo.

Resultados: No total, 46 pacientes (25 homens) foram incluídos (16 operados por CL), com média de idade de 38,1 (± 12,7) anos. Os grupos foram considerados homogêneos em relação a idade, gênero, localização da doença, DC perianal e medicações concomitantes. Houve predomínio da forma fistulizante no grupo de cirurgias abertas (p = 0.029). O procedimento mais realizado foi a ileocolectomia direita em ambos os grupos (56,7% na CC e 75% na CL – p = 0.566). No total, complicações pós-operatórias (cirúrgicas e clínicas) ocorreram em 60% (18/30) dos casos após CC e 12,5% (2/16) após a CL (p = 0.002). Infecção da ferida operatória foi a complicação mais frequente (10/30 na CC e 1/16 na CL). Ocorreram 3 óbitos no grupo da CC. Em análise individualizada das complicações, não houve diferença entre os grupos em relação a sepse abdominal, infecções urinárias, pneumonia, reinternações, reoperações e óbitos (p=0.074).

Conclusões: houve maior taxa de complicações nos pacientes operados por CC comparado a CL. A seleção de casos para cirurgia laparoscópica provavelmente contribuiu para este resultado, havendo uma tendência a utilização da cirurgia convencional nos casos mais graves, geralmente com DC abdominal fistulizante. A CL parece ser a via de acesso recomendada na maioria dos casos de DC não-complicada.

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Introduction

Despite recent advances in medical therapy for Crohn’s disease (CD), especially with the development and wider acceptance of biological therapy, surgical treatment with intestinal resection still has a key role in the management of these patients. In addition to being incurable, CD affects mainly young individuals, which determines the need for treatment in the long term, making it difficult to achieve total disease control. It is estimated that approximately 70 to 90% of patients will require some form of surgical treatment throughout their lifetime.1,2

The role of laparoscopic surgery in the management of colorectal diseases is well established, with numerous benefits in both benign and oncologic resections.3 However, laparoscopic surgery in CD has been shown to be challenging due to the inflammatory, transmural and recurrent characteristics of this condition.4-7 The presence of active inflammation, mes-
entery infiltration and friability, presence of fistulae, adhesions and concomitant immunosuppression can significantly complicate a procedure that already has intrinsic difficulties even when using the conventional approach.\textsuperscript{14}

In spite of these characteristics, laparoscopic surgery has been increasingly performed in the management of CD, especially in primary ileocolic disease.\textsuperscript{7} Recent studies have demonstrated that this is a feasible, safe, effective procedure, which shows benefits in postoperative recovery, even in the presence of abscesses and intestinal fistulae.\textsuperscript{5,7} However, careful preoperative evaluation is required to predict the occurrence of complications in these situations.\textsuperscript{8}

In Brazil, there are few data on the actual role of laparoscopic surgery in CD, as well as on the complication rates when compared to procedures performed by laparotomy. The indication of laparoscopy in the treatment of inflammatory bowel diseases (IBD) is not common in our country. In a national survey published in 2008, whereas benign diseases represented 49.6% of the operated patients, CD represented only 2% of these indications.\textsuperscript{9}

Due to the scarcity of data on the subject in our country, the objective of this study was to analyze the rates of postoperative complications in patients with CD submitted to bowel resection surgery, comparing the data between the laparoscopic (LA) and conventional (CA) approaches.

Method

This study was approved by the Human Research Ethics Committee of the Pontifícia Universidade Católica do Paraná (PUCPR), under protocol number 14201613.0.0000.0020/2013 through the Brazil platform system.\textsuperscript{10}

This was a retrospective, longitudinal study carried out in a single referral center for the treatment of IBD in southern Brazil. All patients with CD consecutively submitted to abdominal operations with intestinal resection between January 2008 and August 2012 were analyzed, regardless of the approach used, either laparoscopic (LA) or conventional (CA).

Inclusion criteria were: patients between 18 and 65 years of age, with CD, regardless of the medication used prior to the procedures, submitted to abdominal surgery with resection of any intestinal segment (small intestine, colon or rectum), operated during the evaluation period. Exclusion criteria were: patients undergoing abdominal surgery for CD without bowel resection (deviation stoma, stricturoplasty or diagnostic laparoscopy), patients with ulcerative colitis (UC) or indeterminate form of IBD.

There was no sample size calculation for this study, which was carried out with a convenience sample, based on the actual number of patients consecutively submitted to surgery during the study period.

Data were collected by reviewing electronic medical charts and the filling out of a protocol previously established for the service. The analyzed variables included: demographics (gender, age), Montreal classification, smoking, concomitant medications prior to the procedure, surgical approach used (LA or CA), type of surgery, presence and type of complication found.

Patients were distributed according to the surgical approach used in the two study groups, laparoscopic approach (LA) and conventional approach (CA). The characteristics of each group were compared in an attempt to find homogeneity between them. Early postoperative complications (occurring up to 30 days after surgery) were described and compared between the groups. Any situation that deviated from a normal postoperative period requiring medical, surgical, endoscopic treatment or radiological intervention was considered a complication. Among these, we evaluated the presence of abdominal sepsis, anastomotic dehiscence, fistulas, abscesses, surgical wound infection, reoperation, pneumonia, urinary tract infection, pancreatitis, central catheter infection, CD flare, readmission or death, among others.

Laparoscopic procedures were performed under general anesthesia, according to the colorectal surgery unit standardization, using four ports (2 of 5 and 2 of 10 mm), priority approach from medial to lateral, and the performance of anastomoses according to the location of the resected area. Preference was given to extra-corporeal and mechanical anastomosis. Some surgical procedures were performed with the aid of a harmonic scalpel.

Conventional procedures were performed under general anesthesia associated with epidural catheter with median laparotomy.

The dissection of the affected areas was performed lateral to medially and anastomoses were preferably mechanical.

There was no randomization concerning the type of approach and the selection of the latter for certain cases was made by the attending physician. Patients were not all operated by the same surgeon, as the colorectal surgery unit is a teaching hospital, with the participation of fellow physicians.

The study hypothesis was that there would be a lower rate of complications in patients submitted to LA when compared to the CA. Statistical analysis was performed using the Mann-Whitney test (quantitative variables) and chi-square test (qualitative), with p < 0.05 considered statistically significant.

Results

Forty-eight patients with CD underwent abdominal operations during the study period. Two cases were excluded for not undergoing a bowel resection procedure (one patient underwent multiple stricturoplasies and another derivative loop ileostomy for severe perianal CD). Of the 46 patients included, 16 (34.8%) were submitted to the LA and 30 (65.2%) to CA.

Clinical characteristics and medications used by patients at the time of the procedures are detailed in Table 1. It was observed that patients submitted to the LA showed a trend to shorter mean duration of CD than the group submitted to the CA (53.56 × 96.87 months), in absolute numbers. Another noteworthy fact was a trend to greater use of biological agents in the LA group (10/16 patients, 62.5%). However, there was no significant difference between the groups regarding these and the remaining variables and they were considered homogeneous.

Regarding the Montreal classification, the results are summarized in Table 2. There was no statistical difference regarding age at diagnosis (A), location of CD (L) and presence of perianal disease (p) between the groups. There was a trend to a higher proportion of patients with ileocolic location (L3) in
the CA group and of ileal location (L1) in the group of patients submitted to the LA, however without statistically significant difference.

According to the behavior of CD, patients submitted to the LA had predominantly stenotic disease (B2 – 68.8%) when compared to the group submitted to the CA, where penetrating injuries (B3 – 56.7%) were more prevalent, with significant statistical difference ($p = 0.029$).

The main surgical indications in patients submitted to the CA were: intestinal fistulae (n = 18), stenosis (n = 17), and intra-abdominal abscesses (n = 3). In the group submitted to the LA, they were stenosis (n = 14), abdominal fistulae (n = 4) and clinical failure (n = 1). It is noteworthy the fact that some patients had more than one surgical indication.

The most often performed surgical procedures were isolated right ileocolectomy (13 × 11) and segmental bowel resection (5 × 1) in the CA and LA groups, respectively. Other procedures such as right ileocolectomy and associations (stricturoplasty, enterectomy or segmental sigmoidectomy), left colectomy, rectosigmoidectomy, subtotal colecetomy and total proctocolectomy were also carried out at smaller numbers.

Regarding postoperative complications in up to 30 days after the procedures, 18 patients (60%) of the CA group had some type of complication, compared with only 2 patients (12.5%) in the LA group ($p = 0002$) (Fig. 1). Table 3 shows the frequency and type of complication according to the surgical access approach. Despite the higher number of complications in the CA group, there was no statistical difference between the two groups regarding abdominal sepsis, surgical wound abscess, presence of urinary tract infection, pneumonia, readmissions, reoperations and deaths ($p = 0.074$) when compared individually. There were cases where patients had more than one complication.

Discussion

Some studies have demonstrated the feasibility and safety of laparoscopy surgery in CD. This access approach has been increasingly used in the management of benign and malignant colorectal diseases, showing advantages when compared to conventional surgery. The classic benefits of laparoscopy, such as better cosmetic results, less postoperative pain and faster recovery, are also observed in CD. However, some doubts still remain, especially regarding surgical complications after the operation.

Table 1 – Demographic data, clinical characteristics and current medications in the two groups of patients. Statistical analysis was performed using the Mann-Whitney and chi-square tests ($p < 0.05$).

<table>
<thead>
<tr>
<th></th>
<th>CA (n = 30)</th>
<th>LA (n = 16)</th>
<th>p value&lt; (0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>38.13</td>
<td>38.25</td>
<td>0.686</td>
</tr>
<tr>
<td>Gender M/F</td>
<td>15/15</td>
<td>10/6</td>
<td></td>
</tr>
<tr>
<td>Mean duration of CD (months)</td>
<td>96.87</td>
<td>53.56</td>
<td>0.235</td>
</tr>
<tr>
<td>Smoker</td>
<td>2/30 (6.7%)</td>
<td>1/16 (6.2%)</td>
<td>0.957</td>
</tr>
<tr>
<td>Current medications</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>8 (26.7%)</td>
<td>4 (25%)</td>
<td>0.902</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>22 (73.3%)</td>
<td>10 (62.5%)</td>
<td>0.447</td>
</tr>
<tr>
<td>Biological agents</td>
<td>11 (36.7%)</td>
<td>10 (62.5%)</td>
<td>0.094</td>
</tr>
</tbody>
</table>

CA, conventional approach; LA, laparoscopic approach.

Table 2 – Montreal classification in the analyzed groups. Statistical analysis was performed using the chi-square test ($p < 0.05$).

<table>
<thead>
<tr>
<th></th>
<th>CA n = 30</th>
<th>LA n = 16</th>
<th>p value&lt; (0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>3 (10%)</td>
<td>3 (18.8%)</td>
<td>0.694</td>
</tr>
<tr>
<td>A2</td>
<td>18 (60%)</td>
<td>9 (56.2%)</td>
<td></td>
</tr>
<tr>
<td>A3</td>
<td>9 (30%)</td>
<td>4 (25%)</td>
<td></td>
</tr>
<tr>
<td>L1</td>
<td>9 (30%)</td>
<td>10 (62.5%)</td>
<td>0.095</td>
</tr>
<tr>
<td>L2</td>
<td>5 (16.7%)</td>
<td>2 (12.5%)</td>
<td></td>
</tr>
<tr>
<td>L3</td>
<td>15 (53.3%)</td>
<td>4 (25%)</td>
<td></td>
</tr>
<tr>
<td>L4</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>4 (13.3%)</td>
<td>2 (12.5%)</td>
<td>0.029&lt;</td>
</tr>
<tr>
<td>B2</td>
<td>9 (30%)</td>
<td>11(68.8%)</td>
<td></td>
</tr>
<tr>
<td>B3</td>
<td>17 (56.7%)</td>
<td>3 (18.8%)</td>
<td></td>
</tr>
<tr>
<td>Perianal (p)</td>
<td>14 (46.7%)</td>
<td>4 (25%)</td>
<td>0.152</td>
</tr>
</tbody>
</table>

*Statistically significant difference. CA, conventional approach; LA, laparoscopic approach.

Fig. 1 – Complication rates in the two groups. Statistical analysis was performed using the Chi-square test ($p < 0.05$).

Table 3 – Early postoperative complications between the two groups. Statistical analysis was performed using the chi-square test ($p < 0.05$).

<table>
<thead>
<tr>
<th></th>
<th>CA n = 30</th>
<th>LA n = 16</th>
<th>p value&lt; (0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anastomosis dehiscence</td>
<td>1 (3.33%)</td>
<td>0 (0%)</td>
<td>0.074</td>
</tr>
<tr>
<td>Abdominal sepsis</td>
<td>7 (23.3%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Wound abscess</td>
<td>10 (33.3%)</td>
<td>1 (6.25%)</td>
<td></td>
</tr>
<tr>
<td>Intestinal obstruction</td>
<td>1 (3.33%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>ITU</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>3 (10%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Readmissions</td>
<td>7 (23.3%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Reoperations</td>
<td>6 (20%)</td>
<td>1 (6.25%)</td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>3 (10%)</td>
<td>0 (0%)</td>
<td>0.191</td>
</tr>
<tr>
<td>Others</td>
<td>8 (26.6%)</td>
<td>1 (6.25%)</td>
<td></td>
</tr>
</tbody>
</table>

CA, conventional approach; LA, laparoscopic approach.
procedures. Would the LA be safe in intestinal resections in CD? What is the real learning curve necessary to perform these procedures through the minimally invasive technique?

Two meta-analyses compared the laparoscopic access with conventional surgery in primary ileocolic resections in patients with CD, with substantial advantages in favor of laparoscopy regarding postoperative ileus recovery, length of hospital stay and postoperative complications.11,13 Even in recurrent disease or CD complicated by fistulæ and abscesses, when performed by experienced surgeons, ileocolic resections by laparoscopic approach seems to be safe and feasible, without increasing the rates of postoperative complications.13

Clearly, more complex cases require a longer learning curve, more experience and use of last-generation equipment such as sealants and harmonic scalpel, for adequate safety. This is mainly necessary in more advanced cases, with the occurrence of mesentery thickening, inflammatory masses and internal fistulas.

In our country, despite the increasing use of laparoscopy in colorectal diseases, only a small portion is performed in patients with CD.9 The probable explanation for this fact may be based on two factors. First, with the increasing use of biological therapy by medical experts, the need for surgery may be decreasing over time. Furthermore, inadequate management often noticed in patients with CD may delay surgical indication; this will result in patients coming to the surgeons as potentially complicated cases, which would limit the use of laparoscopy in these situations due to the natural selection of cases.

In the present study, there was a predominance in the CA use (65.2%) compared to the LA (34.8%) considering the total number of patients. In addition to the approach indication being dependent on the surgeon’s experience, one of the factors that contributed to this difference was patient selection for the LA. The presence of previous abdominal surgery and obesity, added to the complexity of most cases, the advance and natural evolution of CD to stenoses, fistulæ and masses are factors that hinder the laparoscopic procedure even in the hands of experienced surgeons. For this reason, there was a greater tendency for the indication of the CA in these cases and the LA in less complex cases.

The clinical and demographic characteristics were similar in both groups. However, although there was no statistical significance, the duration of CD was higher in patients submitted to the CA (96.8 months), which shows that patients with longer time of diagnosis probably had more complicated CD.

There was no statistical difference regarding the medications used during surgery, although there was a trend to greater use of anti-TNF agents in the LA group. This fact seems to have no justification that can be interpreted.

The Montreal classification was similar regarding age at diagnosis (A), disease location (L) and the presence of perianal disease (p) between the two study groups. However, the phenotypic behavior of CD was different, with a predominance of stenotic disease in patients submitted to LA (68.8%) and penetrating in those submitted to the CA (56.7%), with statistical significance. What justifies these findings is once again the selection of cases for surgical access approach.

Penetrating lesions, represented by fistulæ (enteroenteric, enteroctaneous and enterocolonic) are severe complications that considerably hinder the surgical procedure. The inflammatory process intensification, formation of adhesions and masses in these situations often make the laparoscopic approach unfeasible. Due to the severity and technical difficulties, there was a greater number of cases with penetrating disease submitted to the CA, compared to the LA group. The most common surgical indications were intestinal fistulæ (n = 18) and intra-abdominal abscesses (n = 17) in the CA group, a fact that corroborates the use of this approach in patients with more aggressive and complicated disease. In patients submitted to LA, the presence of stenosis was the most common feature (n = 14) and to a lesser extent, intestinal fistulæ (n = 4). This fact also proves that case selection had great influence on our results.

Although many patients had more than one indication for surgery, these data differ from recent studies that evaluated the use of LA. Soop et al., in a prospective study of 109 patients undergoing laparoscopic ileocolic resection, observed as the main surgical indication clinical failure (63%), followed by fibrostenotic disease (27%), in addition to fistulæ and abscesses (6% and 4% respectively).14 Fichera et al., also in a prospective study of patients with ileocolic CD, reported failure to medical therapy as the main surgical indication using open surgery (45/87), whereas for the laparoscopic approach it was the presence of obstructive lesions (36/59). These data on laparoscopic surgery indication can be compared with those of the present study, in spite of the different methodology used.15

The most often performed operation was isolated right ileocolectomy in both groups (CA = 13 vs. LA = 11). Associated procedures, as well as enterectomies and colectomies, were also carried out in a smaller number of patients. CD of the terminal ileum and right colon occurs in over 55% of cases and most of these patients will require surgical resection during the course of the disease.17 Therefore, ileocolic resections are currently the most often performed operations and the ones with the most benefits using laparoscopic surgery, especially in primary CD, in the present study and in several reviewed studies.5,6,11,12,14,16–19

Early postoperative complications (within up to 30 days) were significantly higher in patients submitted to the CA (60%). Only 2 patients in the LA group had some type of complication (12.5%). This significant difference can be explained by some aspects in the selection of the surgical method used and the variables analyzed by the chosen methodology. Clearly, patients with more severe and more complicated CD were submitted to the CA, which explains the higher incidence of complications.

Any situation that deviated from a normal postoperative period requiring medical, surgical, endoscopic treatment or radiological intervention was considered a postoperative complication.

Therefore, major complications were reported related to surgery (dehiscence, fistulæ, wound infection, for instance), as well as alterations often secondary to patient exposure to hospital admission and procedures performed during this period (urinary tract infection, central catheter infection, drug-induced pancreatitis, perianal abscess), without associations to the surgical procedure itself. The need for readmission, reoperation and death within 30 days of surgery were also included as complications. Clearly, this range of situations increased complication rates, especially in the CA group.
The approach selection differed according to the surgeon's experience, case severity and presence of comorbidities. There was a trend toward the use of the CA in patients considered to be severe cases, with multiple abdominal surgeries, obesity, severe fistulizing CD or presence of large abdominal masses. For the same reason, there were three deaths among patients operated by the CA. The LA in these cases, in addition to not yielding the same benefits as the primary ileocolic resections, can increase the rate of complications related to the procedure even when performed by experienced surgeons.

Thus, despite the discrepancy in our results, these data should be interpreted with caution due to the methodological bias of case selection and convenience sample. The LA seems to actually show more benefits than the CA, especially in primary ileocolic disease, although some studies have demonstrated its feasibility in recurrent and colonic CD. However, patient selection for this procedure must be carefully performed in order to detect the ideal candidate and offer the benefits of a minimally invasive surgery.

Despite the large difference found in the overall rates of complications between the groups, individual analysis of the complications among them showed no significant statistical difference. With a larger sample, this difference would tend to occur in some of the complications analyzed.

This study has clear limitations, as previously explained. The retrospective methodology, associated with the relatively low number of patients analyzed may have brought some bias to the analysis. Clearly, the selection of cases performed by the assistant surgeon, choosing less complicated cases for the laparoscopic approach, seems to have a direct association with the low number of complications found in this group of patients. In addition, patients were not operated by the same surgeon, due to the fact that the institution is a teaching hospital with active medical fellowship program.

In summary, in this retrospective series of CD patients submitted to bowel resections, patients submitted to the LA had lower rates of complications than the ones submitted to the CA. This fact can be explained by the initial, carefully performed selection of these patients. It is expected that this study can contribute as a stimulus to perform a prospective study in our country with a larger sample size, to achieve more solid conclusions on the subject.

Conflicts of interest

The authors declare no conflicts of interest.

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