



# Isoperistaltic versus antiperistaltic ileocolic anastomosis. Does it really matter? Results from a randomised clinical trial (ISOVANTI)

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## Abstract

**Background** Right hemicolectomy is a very common surgery. Many studies compare different options for laparoscopic ileocolic anastomoses: intra- or extracorporeal; handsewn or stapled; side-to-side or end-to-side. However, there are no studies about the influence that peristalsis could have on this anastomosis. The aim of this study is to compare safety and feasibility of isoperistaltic and antiperistaltic anastomosis in terms of postoperative morbidity and mortality between both groups. The secondary endpoint is to compare long-term functional outcomes (chronic diarrhoea) and quality of life (GIQLI questionnaire) after a 1-year follow-up period.

**Methods** A double-blind, randomised, prospective trial in patients undergoing scheduled surgery for right colon cancer with laparoscopic right hemicolectomy and isoperistaltic (ISO) or antiperistaltic (ANTI) ileocolic anastomoses.

**Results** Hundred and eight patients were included in the study. Patients were randomised either to isoperistaltic or antiperistaltic configuration (54 ISO/ANTI). No significant differences in baseline variables were found. No differences in surgical time (130 [120–150] min ISO vs. 140 [127–160] ANTI,  $p=0.481$ ), nor in anastomotic time (19 [17–22] vs. 20 [16–25],  $p=0.207$ ) and nor in postoperative complications: 37.0% ISO versus 40.7% ANTI, ( $p=0.693$ ) were found. There were no differences in postoperative ileus ( $p=0.112$ ) nor in anastomotic leakage (3.7% vs. 5.56%,  $p=1.00$ ). Differences in “time to first flatus” and “time to first deposition” were found in favour of the antiperistaltic group ( $p=0.004$  and  $p=0.017$ ). Anastomotic configuration did not influence hospital stay (3 days [2–6] isoperistaltic vs. 3 [2–4] antiperistaltic,  $p=0.236$ ). During follow-up, there were no differences between the two groups at 1, 6 and 12 months ( $p=0.154$ ,  $p=0.498$  and  $p=0.683$ ), nor in chronic diarrhoea rates in GIQLI scores (24% ISO vs. 31.4% ANTI,  $p=0.541$ ).

**Conclusions** The isoperistaltic and antiperistaltic ileocolic anastomosis present similar results in terms of performance, safety and functionality. However, further studies must be carried out in order to assess relationship between postoperative ileus and anastomosis configuration.

**Trial registration** Randomised Clinical trial (Identifier: NCT02309931).

**Keywords** Isoperistaltic · Antiperistaltic · Ileocolic anastomosis · Ileocaecal valve · Right hemicolectomy · Intracorporeal anastomosis

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Noelia Ibáñez and Jesús Abrisqueta contributed equally to this work.

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This paper is based on a previous communication to the Spanish National Coloproctology Meeting and it won the first price as best congress' communication.

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Colon cancer is one of the most common health problems in developed countries [1]. Right side hemicolon tumours represent about 30% of all colorectal cancers [2]. Despite oncologic medical treatments, surgery is still the best option for these patients. In the last decade, laparoscopic approach has been one of the most important advances in colorectal

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surgery. Several studies have shown its advantages over conventional surgery and as equally viable as open surgery [3–5]. However, laparoscopic right hemicolectomy is considered as a special case due to anatomical variations in its vascularisation, the proximity of critical structures such as the duodenum, the removal of the ileocaecal valve and the need of an anastomosis to recover digestive transit [6–8].

Numerous articles highlight the importance and complexity of ileocolic anastomosis [8, 9]. In recent years, multiple variations in surgical technique have been detailed. Ileocolic anastomosis can be carried out intra- or extracorporeally, side-to-side or end-to-side, stapled or handsewn [10]. Many papers try to standardise surgical technique and discuss these possibilities with controversial results. We can affirm that surgical technique is not yet standardised [3, 7, 11].

Today, it is unknown if iso- or antiperistaltic configuration has any influence over ileocolic anastomosis in terms of postoperative complications and quality of life. In favour of isoperistaltic configuration, some articles affirm that it has advantages in other locations such as the oesophagus, stomach or hepatobiliary tract [12]. On the other hand, it has been previously published that antiperistaltic configuration could avoid the mesentery twist that takes place in isoperistaltic anastomosis. This way, antiperistaltic configuration could diminish postoperative ileus [13].

Regarding quality of life, it is believed that losing the ileocaecal valve has no consequence for healthy patients without short bowel syndrome or inflammatory bowel disease. Despite this fact, Folaranmi et al. [14] described rates of more than 25% in chronic diarrhoea after the removal of the ileocaecal valve in paediatric population. Actually, there are no studies to evaluate functional effects of any of the peristaltic possibilities on ileocaecal anastomosis in adults.

In view of this uncertainty, we designed a randomised trial to evaluate the effects of iso- and antiperistaltic anastomosis after performing a right hemicolectomy. The primary endpoint was to evaluate early and late postoperative complications in both groups, and the secondary endpoint

was to assess long-term quality of life. Our hypothesis was that antiperistaltic configuration is as safe and feasible as isoperistaltic configuration.

## Methods

### Study design

The study was designed as a single-centre, double-blind, randomised controlled trial. Patients were randomised into two groups: those with isoperistaltic side-to-side anastomosis and those with antiperistaltic side-to-side anastomosis. The study protocol adhered to the Helsinki Declaration and was approved by the Hospital Ethics Committee. The study was registered at ClinicalTrials.gov (Identifier: NCT02309931) and adhered to the CONSORT 2010 criteria for RCTs. The original study protocol has previously been published [15].

### Patients

All patients aged  $\geq 18$  years and referred to elective right laparoscopic hemicolectomy were screened for inclusion criteria (Table 1). After inclusion, all patients answered gastrointestinal quality of life index (GIQLI) prior to surgery [16].

### Surgery

All patients underwent elective surgery performed by surgeons with vast experience in colorectal laparoscopic surgery. Patients were placed under general anaesthesia with endotracheal intubation following preoperative antibiotic and antithrombotic prophylaxis and bladder catheterisation. No mechanical or oral antibiotic bowel preparation was done. Patients were placed supine in the Lloyd-Davies position and a right laparoscopic hemicolectomy was carried out with intracorporeal anastomosis. Surgical technique has previously been detailed in the full study protocol [15]. Iso

**Table 1** Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Patients indicated for elective right hemicolectomy due to right-sided colon cancer	Inability to consent
Age $\geq 18$ years	Intestinal bowel disease like Crohn's disease or ulcerative colitis
Informed consent	Previous abdominal surgery with bowel resection
Complete preoperative cancer staging	Malabsorption syndromes
Tumour characteristics	Non-laparoscopic surgery
Cecum, ascending, hepatic angle or transverse colon tumour confirmed by endoscopic biopsy	
Radiologically resectable tumours	
Absence of vascular, nervous or bone infiltration	
Absence of peritoneal carcinomatosis	

and antiperistaltic anastomosis were carried out following the same technique. After transection of the ileum and colon with a stapler (Endo GIA), the anastomosis is fashioned. Two enterotomies are created in the distal ileum and colon. A side-to-side anastomosis is created with a 60-mm linear-endostapler and the enterotomy is closed with continuous suture. In case of isoperistaltic anastomosis, ileal enterotomy is performed at least 2 cm from the stapled line. In antiperistaltic anastomosis, the distance from the stapled line was at least 8 cm to avoid distal ischaemia. The specimen was extracted through a Pfannenstiel incision, except in patients who had undergone a previous laparotomy, in which case the laparotomy site was used.

During the postoperative period a fast-track protocol was applied, consisting of early mobilisation, urinary catheter removal and oral tolerance from day 1. Discharge criteria were satisfactory oral tolerance, one deposition, accurate pain control and absence of fever.

Follow-up after hospital discharge was carried out by the Coloproctological Unit (and Oncology Service when necessary) at 1, 6 and 12 months. During follow-up visits, besides routine physical examination and complementary studies, patients were questioned for the Gastrointestinal Quality Life Index test (GIQLI) [15].

## Study outcome

### Primary endpoint

The primary endpoint was to compare short-term (30 days after surgery) and long-term (during follow-ups 3–12 months) complications between both groups. Short-term complications were grouped using the Clavien–Dindo classification [17]. The complications considered were perforation, ischaemia, anastomotic leakage (clinical or diagnosed with a CT scan), surgical wound infection, fever, postoperative ileus, bleeding, death and other medical complications such as pulmonary thromboembolism, heart attack, pneumonia. Regarding long-term complications, bowel obstruction/constipation, ventral hernia and chronic diarrhoea were taken into account. The criteria for chronic diarrhoea were more than 3 liquid/semiliquid stools per day during more than 4 weeks.

Secondarily, the intra and postoperative results of both groups were also compared in terms of total operating time, anastomosis time, time to first satisfactory oral intake, time to first flatus, time to first stool and hospital stay.

### Secondary endpoint

To evaluate quality of life, patients were interviewed at 1, 6 and 12 months after surgery using Gastrointestinal Quality of Life Index tests (GIQLI).

## Sample size

Due to the lack of comparative studies between iso- and antiperistaltic anastomosis, sample size was calculated using Chang et al. [18] study on a series of 243 patients with antiperistaltic anastomosis. Short-term complication rates in this study were 6.2%. For isoperistaltic anastomosis, we took into account Kornmann et al. [19] study on a series of 162 patients with isoperistaltic anastomosis and its complication rate (24.1%). We refrained from using the complication rate of laparoscopic right hemicolectomy with isoperistaltic anastomosis in our centre, because when the ISOVANTI trial protocol was done, we had not published it yet. However, it was very similar to Kornmann's study (24.54% vs. 24.1%) so we decided to use it instead of ours. An alpha risk of 0.05%, confidence level of 95% and 80% power were considered to calculate sample size. Using Epidat 4.0 software, we obtained a sample size of 98 patients (49 per group). Given a 10% of patient loss during follow-up, the authors considered a size of 108 patients enough to analyse the study endpoints.

## Randomisation and blinding

Randomisation was carried out with Epidat 4.0 software. Patients were randomised using 1:1 ratio. Patient allocation was revealed to the surgeon after randomisation by one of the investigators using a written form, which was destroyed immediately after the unveiling. Patients were not informed about which anastomosis technique would be used during surgery. In order to keep the study data confidential, a different surgeon (from the Colorectal Unit) followed patients during the postoperative period; data collection was administered by a different surgeon from those who performed surgery. Moreover, GIQLI questionnaires were assisted by our Clinical Nurse Specialist during follow-up visits, and statistical analysis was done by an independent statistician.

## Statistical analysis

IBM SPSS Statistics v19 for Windows was used. For qualitative variables, Chi test was used (or Fisher test when necessary). For quantitative variables, *t* Student test or its counterpart non-parametric were used. ANOVA test was also used when required. A  $p < 0.05$  value was considered as significant in all cases.

## Results

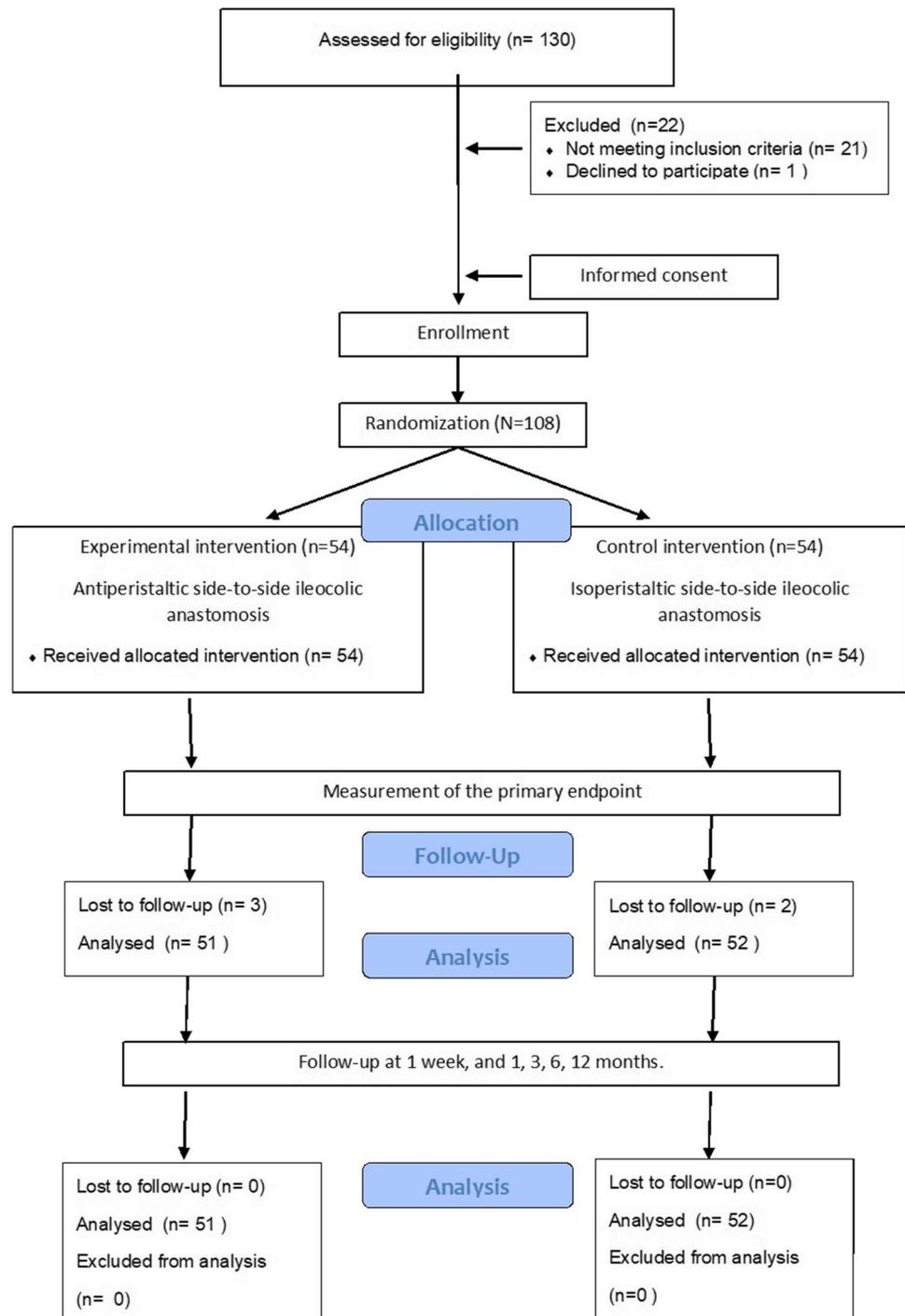
A total of 130 patients have been evaluated for inclusion in the study from November 2014 to November 2016 (Fig. 1). All patients were included consecutively until reaching the sample size.

Most patients were diagnosed during regional colorectal cancer screening program (33% in isoperistaltic group and 25.9% in the antiperistaltic group). No patient received neoadjuvant treatment despite being diagnosed with synchronous metastases (seven patients in the isoperistaltic group and two in the antiperistaltic group). Forty-four percent of patients in both groups had undergone surgery previously. The most frequent previous surgery was appendectomy (nine

patients in the isoperistaltic group and five in the antiperistaltic group), followed by cholecystectomy (five patients in the isoperistaltic group and six patients in the antiperistaltic group). No differences were found in demographic variables between the groups (Table 2).

Table 3 shows intraoperative variables. No significant differences were found between the groups regarding the kind of resection done ( $p=0.479$  y  $p=0.102$ , respectively).

Fig. 1 CONSORT diagram



Surgery was performed by surgeons with a wide experience in laparoscopic colorectal surgery (J.L., Q.H. and J.A.). The conversion rate was 3.7% in the isoperistaltic group and 5.7% in the antiperistaltic group. There were no statistically significant differences between the two groups ( $p=0.500$ ).

Total operative time (130 min. in isoperistaltic vs. 140 min in antiperistaltic) and anastomotic time (19 min in isoperistaltic vs. 20 min in antiperistaltic) were similar for both groups ( $p=0.481$  y  $p=0.207$ , respectively).

The main postoperative variables are shown in Table 4. Despite the fact that postoperative ileus was the most frequent complication in the isoperistaltic group (14.8% of patients,  $n=8$  vs. 5.6%,  $n=3$  in the antiperistaltic group), no differences were found in the global complication rates between the groups ( $p=0.693$ ). Also, there were no differences in the requirements of nasogastric tube for this complication (nine patients in isoperistaltic group vs. five in the antiperistaltic one,  $p=0.252$ ) nor in parenteral nutrition use (six patients in isoperistaltic group vs. five in antiperistaltic anastomosis,  $p=0.507$ ). No differences were found in complications after applying Clavien–Dindo's classification (C–D) ( $p=0.381$ ) and most patients were C–D-I and II (27.8% in the isoperistaltic group and 33.4 in

**Table 3** Surgical variables

	Isoperistaltic ( $n=54$ )	Antiperistaltic ( $n=54$ )	<i>p</i>
Tumour localisation			0.479
Caecal	19 (35.2%)	23 (42.6%)	
Ascending colon	17 (31.5%)	9 (16.7%)	
Hepatic flexure	6 (11.1%)	19 (35.2%)	
Proximal transverse colon	12 (22.2%)	3 (5.6%)	
Conversion	2 (3.7%)	3 (5.7%)	0.500
Cause of conversion			0.717
Infiltration	2 (3.7%)	1 (1.9%)	
CO <sub>2</sub> patient's intolerance	0	1 (1.9%)	
Bleeding	0	1 (1.9%)	
Total operating time <sup>a</sup>	130 [120–150]	140 [127–160]	0.481
Anastomotic time <sup>a</sup>	19 [17–22]	20 [16–25]	0.207
Abdominal incision			0.469
Median laparotomy	21 (38.9%)	18 (33.3%)	
Pfannenstiel	27 (50%)	35 (64.8%)	
Other	6 (11.2%)	1 (1.9%)	

<sup>a</sup>Minutes

**Table 2** Demographic variables

	Isoperistaltic ( $n=54$ )	Antiperistaltic ( $n=54$ )	<i>p</i>
Age <sup>a</sup>	68.2 ± 10.8	68.8 ± 10.3	0.765
Sex			
Male	36 (66.6%)	33 (61.1%)	0.548
Female	18 (33.3%)	21 (38.9%)	
BMI <sup>b,c</sup>	27.28 [25.07–29.79]	27.16 [23.98–31.31]	0.336
ASA			0.446
I	1 (1.9%)	4 (7.4%)	
II	30 (55.5%)	25 (46.2%)	
III	20 (37.0%)	23 (42.5%)	
IV	3 (5.5%)	2 (3.7%)	
Albumin levels <sup>b,d</sup>	4.20 [3.57–4.70]	4.25 [3.95–4.50]	0.700
CEA blood levels <sup>b,e</sup>	2.35 [1.60–3.95]	3.05 [2.00–4.00]	0.248
Smokers			0.755
Yes	16 (29.6%)	16 (29.6%)	
No	25 (46.2%)	28 (51.8%)	
Ex-smokers	13 (24.0%)	10 (18.5%)	
Previous abdominal surgery (yes/no)	24 (44.4%)/30 (55.5%)	24 (44.4%)/30 (55.5%)	1.000
Previous comorbidities (yes/no)	47 (87.0%)/7 (12.9%)	39 (72.2%)/15 (27.7%)	0.056
Previous cancer (yes/no)	13 (24.0%)/41 (75.9%)	12 (22.2%)/42 (77.7%)	0.820
Immunosuppressive treatment (yes/no)	3 (5.5%)/51 (94.4%)	5 (9.2%)/49 (90.7%)	0.358

<sup>a</sup>Years (DS)

<sup>b</sup>Median [IQR]

<sup>c</sup>Body mass index (kg/m<sup>2</sup>)

<sup>d</sup>g/dL

<sup>e</sup>ng/mL

**Table 4** Postoperative variables

	Isoperistaltic (n = 54)	Antiperistaltic (n = 54)	p
Complications	20 (37.0%)	22 (40.7%)	0.693
Type of complication			0.473
Paralytic ileus	8 (14.8%)	3 (5.6%)	
Wound infection	4 (7.4%)	9 (16.7%)	
Anastomotic leakage	2 (3.7%)	3 (5.6%)	
Pneumonia	1 (1.9%)	1 (1.9%)	
Fever	1 (1.9%)	1 (1.9%)	
Heart attack	1 (1.9%)	0	
Renal failure	1 (1.9%)	0	
Bleeding	1 (1.9%)	2 (3.7%)	
Other	1 (1.9%)	3 (5.6%)	
Nasogastric tube	9 (16.7%)	5 (9.3%)	0.252
Parenteral nutrition	6 (11.1%)	4 (7.4%)	0.507
Reoperation	4 (7.4%)	3 (5.6%)	0.500
First flatus <sup>a</sup>	2 [1–3]	1 [1–2]	0.004
First stool <sup>a</sup>	2 [2–4]	2 [2–3]	0.017
Bowel transit recovery <sup>a,b</sup>	2 [1.5–3.5]	1.5 [1.5–2.12]	0.016
Satisfactory oral tolerance <sup>a</sup>	1 [1–1.5]	1 [1–1]	0.473
Hospital stay <sup>a</sup>	3 [2–6]	3 [2–4]	0.236
Hospital re-admission	6 (11.1%)	7 (13.0%)	0.767
Mortality	3 (5.6%)	2 (3.7%)	0.500

<sup>a</sup>Days [IQR]<sup>b</sup>Calculated as the mean between first flatus and first stool

the antiperistaltic group). In the isoperistaltic group, three patients died during the immediate postoperative time: one patient due to anastomotic leakage, one due to a heart attack and another from respiratory insufficiency due to worsening of a respiratory pre-condition. In the antiperistaltic group, two patients died due to anastomotic leakage.

**Table 5** Follow-up results

	Isoperistaltic (n = 51)	Antiperistaltic (n = 52)	p
Ventral hernia	5 (9.2%)	3 (5.5%)	0.358
Bowel obstruction	1 (1.9%)	0	0.500
Adjuvant treatment	13 (24.0%)	13 (24.0%)	1.000
Metastasis surgery	3 (5.5%)	1 (1.9%)	0.280
Exitus	3 (5.5%)	2 (3.7%)	0.507
Diarrhoea <sup>a</sup>	10 (18.5%)	16 (29.6%)	0.349
Chronic diarrhea (after 1 year)	13 (24.0%)	17 (31.4%)	0.541
GIQLI score	97.80 ± 19.50	95.12 ± 19.31	0.484
GIQLI score (1 month after surgery)	102.19 ± 18.55	96.52 ± 20.37	0.154
GIQLI score (6 months after surgery)	104.29 ± 20.68	101.13 ± 20.02	0.498
GIQLI score (1 year after surgery)	108.92 ± 17.55	107.43 ± 18.48	0.683

<sup>a</sup>> 3 Stools/day<sup>b</sup>3 Stools/day during > 4 weeks

Regarding functional results, the antiperistaltic group showed better results than the isoperistaltic group with less time to first flatus (1 [1–2] days vs. 2 [1–3] days in the isoperistaltic), less time to first stool (2 [2–3] days vs. 2 [2–4] days in isoperistaltic) and shorter time to satisfactory oral intake (1.5 [1.5–2.12] vs. 2 [1.5–3.5] in isoperistaltic) with statistically significant differences in all cases ( $p=0.004$ ,  $p=0.017$  y  $p=0.016$ ). However, this fact did not reduce hospital stay and there were no differences between both groups (3 [2–6] days in isoperistaltic group vs. 2 [2–4] days in antiperistaltic,  $p=0.236$ ).

TNM staging was similar between the two groups ( $p=0.163$ ) No differences were found between the groups in surgical specimen length ( $33.53 \pm 9.36$  cm in isoperistaltic group vs.  $32.07 \pm 11.65$  cm in the antiperistaltic group,  $p=0.474$ ), nor in ileal or colonic length in isolation ( $p=0.121$  and  $p=0.849$ , respectively).

Finally, Table 5 shows follow-up and quality of life variables. Although no differences were found ( $p=0.349$ ), more patients in the antiperistaltic group recounted diarrhoea at least once during the follow-up interviews ( $n=16$ , 29.6% vs.  $n=10$ , 18.5% in the antiperistaltic). This finding remained after 1 year from surgery, although differences were not found this time either ( $p=0.541$ ).

Patients from both groups showed an important improvement in their quality of life parameters post-surgery and with significant differences when compared to their pre-surgery interview results ( $p<0.001$ ), without differences between the groups ( $p=0.635$ ). However, there were no differences in quality of life between groups at 1 month ( $p=0.187$ ), 6 months (0.498) or a year after surgery ( $p=0.683$ ).

## Discussion

ISOVANTI trial is the only prospective randomised trial comparing iso- and antiperistaltic anastomosis after a right-sided hemicolectomy. No differences were found between the groups in terms of short- and long-term complications or in quality of life test results.

Several articles highlight the necessity of giving more importance to this anastomosis because of the hypothetical influence it could have on post-surgical intestinal functionality [8, 14, 20, 21]. One article published in 2005 on right hemicolectomy surgical technique with side-to-side anastomosis concluded that isoperistaltic configuration had advantages in other gastrointestinal locations, but there were no studies on ileocolic anastomosis configuration for healthy adult patients without a history of inflammatory bowel disease [12].

In Tarta's review in 2013 on intracorporeal anastomosis, anastomotic configuration was one of the factors considered for the analysis of leakage risk factors. Their results were similar to those in this study. However, the reviewed studies used a different surgical technique depending on the type of anastomotic configuration. In fact, in 75% of the studies on antiperistaltic anastomosis, the anastomoses were all stapled; while for isoperistaltic anastomosis, all were stapled-handsewn [22]. In ISOVANTI trial, the surgical technique was the same in all patients to make both groups more homogeneous.

There is only one other study comparing both anastomotic configurations, with a small sample size and in which different locations are mixed (not only the ileocolic anastomosis). It has important differences regarding the baseline variables, which have already been described as risk factors for ileocolic anastomosis leakage [2, 23]. In that study, it is not possible to obtain clear conclusions.

Although some authors defend that antiperistaltic configuration diminishes the mesentery twist [22], others point out exactly the opposite, arguing that this bowel loop disposition is more difficult and needs greater intestinal mobilisation [9]. In our study, both modalities showed comparable conversion rates, operative and anastomotic times without statistically significant differences. Surgical specimen lengths were similar, so it is probable that any previously described difficulties are due to an extracorporeal anastomosis creation. The intracorporeal anastomosis performance allows proper bowel mesentery visualisation during the anastomotic process and also avoids torsion and traction [24].

Regarding postoperative complications, rates for both groups are similar to previously published studies if we take into account that they include surgical site infection [2, 19]. These results follow the ones published by Tarta

et al. but differ from the ones of Matsuda et al., which had to be interrupted sooner than planned because of detecting higher morbidity in isoperistaltic group. Interestingly, the isoperistaltic group had a higher postoperative ileus rate in our study. Some studies claim that postoperative ileus after losing ileocaecal valve has a close relation with coloileal reflux and secondarily to small intestinal bacterial overgrowth (SIBO) [25, 26]. It is possible that antiperistaltic anastomosis could act like a functional pseudovalvular mechanism diminishing ileocaecal reflux and postoperative ileus. This theory could explain the earlier recovery of intestinal transit in the antiperistaltic group. Concerning quality of life results, our study shows chronic diarrhoea rates in both groups similar to the ones published in children (higher than 25%) [14]. Although we did not find any significant differences, a greater number of patients suffered diarrhoea after surgery and chronic diarrhoea after 1 year in the antiperistaltic group. The findings agree with the theory of "the functional pseudovalvular mechanism" previously described for antiperistaltic anastomosis. Taking into account both outcomes, patients with antiperistaltic anastomosis seem to have a shorter intestinal transit time than those with isoperistaltic anastomosis. Nevertheless, functional studies are needed to confirm this hypothesis.

Regarding gastrointestinal quality of life tests results, differences were found between scores when comparing pre-surgical results and those obtained after 1 year from surgery. According to other recently published articles, patients who underwent surgery for malignant pathologies tended to show better results in quality of life tests [27–29]. However, the presence of chronic diarrhoea has no correlation with worse quality of life result in the antiperistaltic group.

## Conclusion

In conclusion, iso- or antiperistaltic configuration does not modify postoperative complication rates in patients with right hemicolectomy. On long-term results, it seems to be a shorter intestinal transit time in the antiperistaltic group, and a non-statistically significant tendency to a higher chronic diarrhoea rate which does not mean worse quality of life.

**Author contributions** Conception and design: NI, JA, JL. Acquisition of a substantial portion of data: NI, JA, MDR, QH. Analysis and interpretation of data: NI, JA, MDR. Drafting of the manuscript: NI, JA, JL. Critical revision of the manuscript for important intellectual content: JL, PP. Statistical expertise: NI, JL. Obtaining funding for this project or study: no specific funding was used for this study. Supervision: JA, JL, PP. Final approval of the version to be published: NI, JA, JL, MDR, QH, PP.

## Compliance with ethical standards

**Disclosures** Noelia Ibáñez, Jesús Abrisqueta, Juan Luján, Quiteria Hernández, María Dolores Rufete and Pascual Parrilla have no conflicts of interest or financial ties to disclose.

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