



Robotic versus laparoscopic right colectomy within a systematic ERAS protocol: a propensity-weighted analysis

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Abstract

The purpose of this study is to compare the early postoperative and pathological outcomes of robotic right colectomy (RRC) to those of laparoscopic right colectomy (LRC) with intracorporeal anastomosis (IA) within the systematic application of an enhanced recovery after surgery (ERAS) program. A single-institution prospective database of patients who underwent elective RRC or LRC with IA for neoplastic lesions between April 2010 and June 2018 was retrospectively reviewed. The patients' demographic characteristics, and perioperative and pathological outcomes were analyzed. Propensity-weighted analysis was employed to address potential selection biases of treatment allocation. A total of 216 patients (46 RRC, 170 LRC) were included. RRC demonstrated a significantly longer operative time (mean 242.43 min, SD 47.51) compared to LRC (mean 187.60 min, SD 56.60) ($p=0.001$), confirmed by the propensity-weighted analysis (Coefficient 50.65; $p<0.001$). Conversion rate between the two groups was comparable ($p=0.99$). Median length of hospital stay (LOS) was the same in the RRC and the LRC group (4 days, $p=0.35$). Readmission rate within 30 days in the RRC and LRC group was 2.2% and 2.4%, respectively ($p=0.99$). Overall 30-day morbidity and 30-day mortality was 32.6% versus 27.1% ($p=0.46$), and 0% versus 1.2% ($p=0.99$) in the robotic and laparoscopic groups, respectively. No difference was found in the number of harvested lymph nodes ($p=0.75$). In an ERAS environment, without the bias of mixed techniques of anastomosis, RRC had similar postoperative and pathological outcomes compared to the laparoscopic approach, but was associated with a longer operative time.

Keywords Right colectomy · Laparoscopic · Robotic · Enhanced recovery after surgery

Introduction

The first laparoscopic right colectomy (LRC) was performed in 1990 [1], and since then, it has been increasingly adopted for both benign and malignant diseases. This minimally invasive (MI) approach provides faster recovery with earlier return to normal bowel function, shorter length of hospital stay (LOS), and lower postoperative morbidity compared with open surgery, with similar oncological outcomes [2–5]. However, laparoscopy has well-known drawbacks, such as

tremor, loss of a three-dimensional view, poor ergonomics, fixed tips, and limited movement dexterity.

These laparoscopy-related limitations could be a disadvantage, especially when performing an intracorporeal anastomosis (IA) [6, 7]. Indeed, although the choice between IA and extracorporeal anastomosis (EA) for right colectomy is still controversial, some advantages have recently been reported including a better postoperative recovery for IA [8, 9]. However, in the laparoscopic setting, most surgeons prefer to perform EA, because of the significant technical challenges of IA and advanced skills required.

The introduction of robotic surgery, thanks to its undoubted technical advantages [10], provided high-precision dissection and suturing. In right colectomy, an IA seems more easily performable with the aid of robotic wrists [11]. Since the first robotic colectomy was reported in 2002 [12], robotic right colectomy (RRC) is being increasingly adopted and has proven to be both safe and feasible [13]. However,

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its application is nowadays still debated due to the absence of sound evidence in the literature [14, 15], apart from the increased operative time and costs reported for the robotic system [16, 17]. Moreover, to date, a few comparative studies have analyzed the robotic versus laparoscopic approach with IA [14, 15, 18–20].

Alongside surgical development, over the past 2 decades, the enhanced recovery after surgery (ERAS) program has been applied to colorectal surgery [21, 22]. It includes evidence-based items designed to reduce perioperative stress, maintain postoperative physiological function, and accelerate recovery after open and MI colorectal surgery [23]. Furthermore, when feasible, the use of a MI approach (be it laparoscopic or robotic) has been strongly recommended over open surgery [24], on the basis of high-quality evidence [25–27] to guarantee the patient's optimal perioperative care.

At our institution, the laparoscopic approach with IA currently represents the standard treatment for right-colon diseases, and since 2013, the da Vinci[®] Si[™] System (Intuitive Surgical Inc., Sunnyvale, CA) has been employed, as well, following the same technique. Furthermore, since April 2010, an ERAS protocol has been systematically implemented on colorectal surgery, irrespective of the type of approach.

Given this background, the purpose of this study is to compare the short-term outcomes of RRC and LRC with IA within the systematic application of an ERAS program.

Materials and methods

A retrospective analysis of our institutional database of prospectively collected data was conducted. All patients who underwent elective MI right colectomy with IA for benign and malignant neoplastic lesions between April 2010 and June 2018 at Santa Croce e Carle Hospital (Cuneo, Italy) were included in the study. While LRC has been performed routinely since 2010, RRC has been carried out only since 2013, when robotic technology was implemented in our Department. Patients with distant metastasis on preoperative evaluation requiring other major surgical procedures (i.e., hepatectomies or multivisceral resections) were excluded.

Patients' demographic characteristics were analyzed including age, gender, body mass index (BMI), American Society of Anesthesiologists (ASA) score, history of previous abdominal surgery (laparoscopy or laparotomy), comorbidities, and lesion location (i.e., caecum, ascending colon, hepatic flexure, or proximal transverse colon).

Perioperative and pathological outcomes included: total operative time, conversion rate to open surgery (defined as any unplanned abdominal incision for any procedure rather than just specimen extraction), oral intake within first postoperative day, time to first flatus, postoperative ileus (defined

as nasogastric tube insertion) [28], LOS, readmission rate, 30-day postoperative morbidity [according to the Clavien–Dindo (CD) classification scale] [29], American Joint Committee on Cancer (AJCC) staging, number of retrieved lymph nodes, and rate of more than 13 lymph nodes harvested (as a surrogate endpoint of radical surgery) [30].

The choice of the robotic technique was based solely on availability of the robotic system and the surgeon. Four well-versed surgeons experienced in MI colorectal surgery performed all of the procedures.

All patients were treated according to a standardized ERAS protocol [24] that can be briefly summarized as follows:

- *Preoperative phase* An informative booklet is routinely given to the patient and caregivers, mechanical bowel preparation is never used, carbohydrate overload is administered 3 h before surgery, and usually, no premedication is given, thromboprophylaxis and cephazoline 2 g plus metronidazole 500 mg i.v. are administered 12 h and 1 h before surgery respectively.
- *Intraoperative phase* The epidural catheter is the preferred analgesic regimen to avoid opioids, the anesthetic protocol is standardized using a careful fluid balance, and maintenance of normothermia is ensured.
- *Postoperative phase* The nasogastric tube is removed at the end of surgery, a free fluid oral intake is allowed 6 h after the procedure, and a semisolid diet starts from the same day, early mobilization, starting 4 h postoperatively, is strongly encouraged, intravenous fluid therapy is stopped the day after surgery, and the urinary catheter is also removed, the epidural catheter is removed on postoperative day 2.

The discharge criteria include the absence of complications, a correct oral intake, passing of flatus, good pain control using oral painkillers, and patient confidence to go home.

Surgical technique

In our department, the principles of surgical procedure are identical for RRC and LRC, except for the trocar placement. In both approaches, a 12 mm Hg pneumoperitoneum is obtained with a Veress needle at the Palmer's point.

In LRC, a 12-mm 30° camera port is placed in the left transverse umbilical line. Under direct vision, three laparoscopic ports (two 5 mm and one 15 mm) are inserted along an imaginary curvilinear line from the epigastric region to the left lower quadrant of the abdomen (Fig. 1a). In RRC, a 12-mm 30° camera port is placed in the left transverse umbilical line. Following an imaginary curvilinear line from the left upper quadrant to the right lower quadrant of the

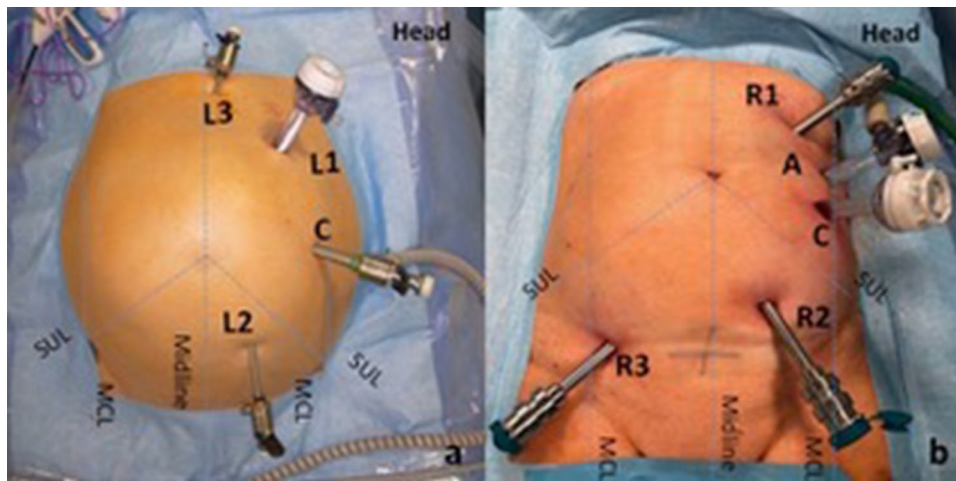


Fig. 1 Trocar layout for laparoscopic and robotic right colectomy. **a** Trocar layout for laparoscopic right colectomy. L1, 10–15 mm laparoscopic port for radiofrequency device, linear stapler, and needle holder; L2, 5 mm laparoscopic port for grasper, irrigation/suction probe; L3, 5 mm laparoscopic port for the assistant. **b** Trocar Lay-

out for da Vinci® Si™ right colectomy. A, assistant port; R1, robotic arm 1 for cautery hook and needle holder; R2, robotic arm 2 for bipolar forceps; R3, robotic arm 3 for Cadie's fenestrated forceps. *SUL* spino umbilical line, *MCL* midclavicular line, *C* camera port

abdomen, three 8 mm robotic ports and an additional 15 mm assistant port are inserted (Fig. 1b). The cart is docked at the patient's right side.

For both MI approaches, slight modifications of trocar position might occur according to the tumor site (cecal/ascending/flexure or proximal transverse colon).

The patient is placed in a lithotomy position, with a slight Trendelenburg (10°) and left tilt (10° – 12°).

After the exploration of the abdominal cavity to rule out peritoneal seeding and liver metastasis, the small bowel is positioned in the left lower abdomen to expose the surgical field.

A medial-to-lateral approach is progressively carried out: the parietal peritoneum is incised below the ileo-colic vessels identifying Gerota's and Toldt's fascias up to the third portion of the duodenum. The vascular dissection is performed along the right border of the superior mesenteric vein: the ileo-colic, the right colic (if present), and the right branch or the main trunk of the middle colic vessels are divided at their origin.

Colo-epiploic detachment and gastrocolic ligament division are followed by a complete mobilization of the hepatic flexure. The transverse colon and ileus (10 cm from the ileocecal valve) are transected by a linear stapler. Then, the right-colon detachment is completed and the specimen is inserted into an endo bag and placed in the Douglas pouch.

Thus, the IA is performed as follows: the ileal loop and the transverse colon are aligned by means of the application of two stay sutures. The cautery hook creates the enterotomies on both the ileal and colonic loop, through which a 60 mm linear stapler is introduced to fashion a side-to-side mechanical isoperistaltic anastomosis. The enterotomies

are then closed with continuous monofilament absorbable suture.

The mesenteric defect is always closed with a barbed suture. Finally, the surgical specimen is extracted through a mini-Pfannenstiel incision. No abdominal drain is routinely left in place.

Statistical analysis

The distribution of continuous variables was evaluated using the one-sample Kolmogorov–Smirnov test. Continuous variables are presented as mean and standard deviation or as median and range of values for data with a normal distribution. Categorical variables are expressed as numbers and proportions. Univariate comparisons were performed using the independent *t* test for normally distributed data. The Chi-squared test was used for categorical data and Fisher's exact test was used when the minimum cell size requirements for the Chi-squared were not satisfied.

Propensity-weighted analysis was employed to address potential selection biases of treatment allocation related to the retrospective analysis of our institutional prospectively maintained database. The ultimate purpose of using propensity-weighted analysis is to balance the treatment groups on the observed covariates. The probability of a patient undergoing RRC (propensity score) was generated by a non-parsimonious logistic regression model. Regressors included the following preoperative data: gender, age, BMI, previous laparoscopy, previous laparotomy, diabetes mellitus, cardiovascular disease, pulmonary disease, nephrological disease, neurological disease, ASA score, and lesion location. Adjustment for confounding was carried out by

weighting regression modeling with propensity scores. The key of this balancing method is the creation of weights based on propensity scores. The inverse-probability-of-treatment-weight (IPTW) was defined as the inverse of the probability of receiving the treatment that the patient actually received (also known as the “average treatment effect on the treated”). The endpoints of the analysis were then studied with multivariate logistic regressions and the Cox model, which were weighted for the IPTW (IPTW-LR and IPTW-Cox, respectively).

Propensity-weighted analysis has the advantage of using all the subjects in the two treatments groups for the outcome analysis compared to propensity score matching.

Two-sided statistics were performed with a significance level of 0.05. For all of the analyses, we used R language [R 3.6.0; R Development Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org/>] and IBM SPSS Statistics Version 25, [2017].

Results

Overall, a total of 216 consecutive patients (46 RRC, 170 LRC) were included in the study. Regarding the demographic data, there were no significant differences between the two groups (Table 1), except for the prevalence of pulmonary disease, which was higher in the LRC group (13.5% vs 0%, $p=0.005$).

Perioperative outcomes are listed in Table 2. IA was performed in all the patients in the two groups. RRC demonstrated a significantly longer total operative time (mean 242.43 min, SD 47.51) compared to LRC (mean 187.60 min, SD 56.60) ($p=0.001$). Only one conversion to open surgery was recorded in the RRC and six in the LRC series, with no significant difference ($p=0.99$). Conversions were due to the presence of extensive intra-abdominal adhesions in four patients, local extension of the neoplasm in two patients, and intolerance to pneumoperitoneum in one patient.

Postoperative outcomes including oral intake within the first postoperative day, time to first flatus, and postoperative ileus did not differ between the two groups. LOS was similar in the RRC (median 4, range 3–18) and the LRC groups (median 4, range 2–40) ($p=0.35$). Readmission rate within 30 days was 2.2% and 2.4% in the RRC and LRC groups ($p=0.99$), respectively.

No intraoperative complications occurred in either series. Overall 30-day morbidity was 32.6% in RRC group and 27.1% in LRC group ($p=0.46$). Considering only CD III–IV, no difference was found (2.2% vs 3.5% in RRC and LRC, respectively) ($p=0.99$). Two patients, one in each series, required a radiological drainage because of

Table 1 Baseline preoperative characteristics of included patients

Variable	RRC <i>n</i> = 46	LRC <i>n</i> = 170	<i>p</i> value
Age, mean (SD), years	68.70 (9.2)	71.92 (10.1)	0.52
Male gender, <i>n</i> (%)	22 (47.8)	96 (56.5)	0.32
BMI, mean (SD)	26.05 (4.05)	25.52 (4.07)	0.43
ASA, <i>n</i> (%)			0.09
1	11 (23.9)	17 (10)	
2	19 (41.3)	87 (51.2)	
3	15 (32.6)	63 (37.1)	
4	1 (2.2)	3 (1.7)	
Comorbidities, <i>n</i> (%)			
Hypertension	21 (45.7)	71 (41.8)	0.74
Diabetes	3 (6.5)	20 (11.8)	0.42
Cardiovascular disease	8 (17.4)	42 (24.7)	0.33
Pulmonary disease	0 (0)	23 (13.5)	0.005
Nephrological disease	2 (4.3)	14 (8.2)	0.53
Neurological disease	5 (10.9)	14 (8.2)	0.56
Previous abdominal surgery, <i>n</i> (%)			
Laparoscopy	3 (6.5)	17 (10)	0.58
Laparotomy	12 (26.1)	61 (35.9)	0.29
Lesion location, <i>n</i> (%)			0.10
Caecum	14 (30.4)	61 (35.9)	
Ascending colon	23 (50)	78 (45.9)	
Hepatic flexure	8 (17.4)	14 (8.2)	
Proximal transverse colon	1 (2.2)	17 (10)	

The bold value is used to underline statistically significant data

RRC robotic right colectomy, LRC laparoscopic right colectomy, SD standard deviation, BMI body mass index, ASA American Society of Anesthesiologists

an abdominal collection (CD IIIa). In the laparoscopic group, five patients needed surgical reintervention (CD IIIb) due to anastomotic leaks (4 cases) and colonic stump ischemia (1 case). No surgery was required in the RRC series. 30-day mortality occurred in 2 patients (1.2%) of the LRC group as a consequence of anastomotic failure.

Pathological outcomes are reported in Table 3. The mean number of harvested lymph nodes was 19.41 versus 19.91 in the robotic and laparoscopic groups, respectively ($p=0.75$). At least 13 or more lymph nodes were retrieved in 87% of RRC and 78.6% of LRC ($p=0.29$). Finally, specimen length was 31.7 cm in the RRC and 28.8 cm in the LRC series ($p=0.06$).

Tables 4, 5 report the outcomes of regression models (unadjusted and IPTW adjusted) with propensity-weighted analysis for dichotomous and continuous variables, respectively. The analysis confirmed that the use of the robotic platform was associated with a significant longer operative time (Coefficient 50.65; 95% CI 37.2–64.1, $p<0.001$). No difference between the two study groups regarding the

Table 2 Comparison of outcomes between two groups

Outcome	RRC <i>n</i> = 46	LRC <i>n</i> = 170	<i>p</i> value
Total operative time, mean (SD), min	242.43 (47.51)	187.60 (56.60)	0.001
Conversion, <i>n</i> (%)	1 (2.2)	6 (3.5)	0.99
Oral intake within first postoperative day, <i>n</i> (%)	44 (95.7)	159 (94.6)	0.99
Time to first flatus, mean (SD), days	1.65 (1.04)	1.61 (0.83)	0.75
Nasogastric tube replacement, <i>n</i> (%)	6 (13)	7 (4.1)	0.07
Length of hospital stay, median (range), days	4 (3–18)	4 (2–40)	0.35
Readmission, <i>n</i> (%)	1 (2.2)	4 (2.4)	0.99
Overall 30-day morbidity (Clavien–Dindo I–IV), <i>n</i> (%)	15 (32.6)	46 (27.1)	0.46
Major morbidity (Clavien–Dindo III–IV), <i>n</i> (%)	1 (2.2)	6 (3.5)	0.99
30-day mortality, <i>n</i> (%)	0 (0)	2 (1.2)	0.99

The bold value is used to underline statistically significant data

RRC robotic right colectomy, LRC laparoscopic right colectomy, SD standard deviation

Table 3 Postoperative pathological characteristics of included patients

Pathological stage	RRC <i>n</i> = 46	LRC <i>n</i> = 170	<i>p</i> value
Benign polyps not suitable for endoscopic removal, <i>n</i> (%)	3 (6.5)	7 (4.1)	0.45
T, <i>n</i> (%) ^a			
Tis	1 (2.4)	2 (1.2)	0.65
T1	6 (13.9)	17 (10.4)	
T2	5 (11.6)	31 (19.1)	
T3	26 (60.5)	87 (53.4)	
T4	5 (11.6)	26 (15.9)	
N, <i>n</i> (%) ^a			0.32
N0	24 (55.8)	111 (68.1)	
N1	12 (27.9)	34 (20.8)	
N2	7 (16.3)	18 (11.1)	
M, <i>n</i> (%) ^a			0.12
M0	43 (100)	152 (93.2)	
M1	0 (0)	11 (6.8)	
Specimen length, mean (SD), cm	31.7 (9.1)	28.8 (8.9)	0.06
Number of retrieved lymph nodes, mean (SD)	19.41 (6.84)	19.91 (8.22)	0.75
Number of retrieved lymph nodes > 13, <i>n</i> (%)	40 (87)	132 (78.6)	0.29

RRC robotic right colectomy, LRC laparoscopic right colectomy

^aAccording to TNM 8th edition. % are referred to patients with malignant histology

Table 4 Coefficients derived by logistic regression model in univariate unadjusted cohort and after multivariate with propensity-weighted analysis for continuous variables

Outcome	Unadjusted coefficient	<i>p</i> value	Adjusted coefficient	<i>p</i> value
Operative time	54.8 (36.9 to 72.8)	< 0.001	50.65 (37.2 to 64.1)	< 0.001
Length of hospital stay	−0.05 (−1.22 to 1.13)	0.94	0.16 (−0.79 to 1.10)	0.74
Number of retrieved lymph nodes	−0.50 (−3.10 to 2.11)	0.71	−0.94 (−2.89 to 1.01)	0.34

The bold values are used to underline statistically significant data

Table 5 ORs derived by logistic regression model in univariate unadjusted cohort and after multivariate with propensity-weighted analysis for dichotomous variables

Outcome	Unadjusted OR	<i>p</i> value	Adjusted OR	<i>p</i> value
Conversion	0.61 (0.07–5.18)	0.65	0.85 (0.06–12.32)	0.90
Readmission	0.92 (0.10–8.46)	0.94	37.24 (0.01–112)	0.28
Overall 30-day morbidity (Clavien–Dindo I–IV)	1.30 (0.65–2.64)	0.46	1.02 (0.62–1.68)	0.71
Major morbidity (Clavien–Dindo III–IV)	0.61 (0.07–5.18)	0.65	0.75 (0.05–12.80)	0.84
30-days mortality, <i>n</i> (%)	–	–	–	–
Number of retrieved lymph nodes > 13	1.8 (0.71–4.63)	0.21	1.02 (0.27–3.89)	0.97

OR odds ratio

other intraoperative, postoperative, and pathological outcomes was pointed out.

Discussion

In this monocentric study, we showed the safety and the efficacy of robotic and laparoscopic right colectomy with IA, underlining the combined synergic effects of MI surgery within a systematic application of an ERAS protocol. Short-term outcomes and pathological results were comparable between the two groups, except for a longer operative time by a mean of 55 min for the robotic approach, as confirmed by propensity-weighted analysis.

The majority of the most recent reported studies comparing RRC and LRC highlighted RRC as a more time-consuming procedure [11, 13, 14, 31], even considering the adoption of IA in both cohorts. One of the main reasons might be related to the docking time and to the learning curve [32].

In our experience, a longer operative time for RRC did not affect either the time to first flatus (1.65 days compared to 1.61 days for the laparoscopic group) nor the LOS (median 4 days in both groups). Our results were consistent with the findings of the largest previous study comparing LRC and RRC with IA [18]. However, in our experience, the LOS for both groups was significantly lower (4 days) than the one reported in the latter study (7 days in RRC and 8 days in LRC). In addition, our median LOS was shorter than the median LOS (8 days) reported by PNE-AGENAS (Piano Nazionale Esiti—Agenzia Nazionale Servizi Sanitari), an Italian nationwide database [33]: this might be probably due to the combination of the minimal surgical trauma with IA and our standardized and systematic implementation of the ERAS protocol, rather than to the actual type of MI surgical approach.

However, being more challenging from a technical perspective and without strong evidence of superiority over EA [8], IA is not universally performed during laparoscopic right colectomy. The introduction of robotic surgery has overcome this technical limitation making IA fashioning reproducible, just as in open surgery. At present, in most studies, RRC with IA is compared to LRC with EA [13, 16, 32], thus leading to a considerable bias when interpreting the results.

The ERAS protocol has proven to enhance the advantages of MI colorectal surgery, reducing surgical stress response and postoperative recovery [34, 35], as confirmed by our results. Dealing with ERAS application, a key point is represented by the compliance to the program, which is correlated with LOS reduction and overall complication rate. Nelson et al. [36] underlined a higher compliance when the MI approach is adopted. MI surgery independently has the capacity to reduce LOS and complications without increasing mortality and readmission, which are also the ultimate goals of an ERAS program, thus achieving a synergic effect.

In line with most previous studies [19, 37] and a recent meta-analysis [32], in our series, the overall 30-day morbidity did not differ significantly in the two groups. Similarly, the major complication rate was comparable between the groups ($p = 0.99$). Some authors argue that performing anastomosis in the abdominal cavity could result in an increased intra-abdominal infection rate [38], but this theory is not supported by our findings.

A common idea in the surgical community is that the ERAS pathway reduces LOS while increasing the hospital readmission rate, although this is not confirmed by the available literature [34]. Similarly, in our study, readmission rate was low and comparable between the two groups, meaning that the ERAS program could be considered safe and effective also in right-colon MI surgery.

Concerning the pathological outcomes, the number of retrieved lymph nodes is considered a landmark of adequate oncological surgery. Two pivotal meta-analyses seem to lead to different results: Rondelli et al. [16] found no difference between RRC and LRC in term of number of harvested lymph nodes, while Solaini et al. [13] highlighted a tendency to a higher number of harvested lymph nodes in the RRC group. One possible explanation of this latter outcome might be the high dexterity and extremely accurate view of the operating field, which distinguish robotic surgery. In our study, we followed the same principles of lymphadenectomy for RRC and LRC and we found no difference between both series. Probably, robotic surgery could advantage surgeons in performing more complex procedures like complete mesocolic excision [39]. However, though some have advocated complete mesocolic excision to ensure satisfactory

clearance, its impact on improved long-term oncological outcomes has not been proven so far [40] and we do not perform it routinely in right colectomy.

To the best of our knowledge, this is the largest monocentric study in the literature that specifically compares RRC with LRC both with systematic IA and within an ERAS protocol.

Limitations of this study include its retrospective nature, with all its inherent biases, especially in the choice of surgical approach. We nevertheless tried to minimize these biases by the use of the propensity-weighted analysis. An additional drawback of this study is the lack of a cost analysis that is a crucial issue in our health care system, considering that robotic colorectal surgery costs are approximately two-to-threefold greater than the conventional laparoscopic surgery [41]. Finally, a more consistent series of patients might be needed to achieve more sound outcomes.

In conclusion, in our experience, robotic and laparoscopic right colectomy provided similar efficacy and safety, suggesting that the synergic combination of a standardized surgical MI technique with ERAS implementation may have a major impact on the postoperative outcomes.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in this study.

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