



Carnoy solution versus GEWF solution for lymph node revealing in colorectal cancer: a randomized controlled trial

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Accepted: 17 October 2019 / Published online: 15 November 2019
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Abstract

Purpose This study aimed to compare the performance of two lymph node revealing solutions.

Methods This randomized clinical trial (NTC02704988) investigated patients with colon or rectal cancer who underwent surgical resection with D2 lymphadenectomy. Specimens submitted for conventional pathological examination were randomly assigned for additional fixation with Carnoy or GEWF solution, and dissection was performed to examine the missed lymph nodes. The number of lymph nodes retrieved, additional identified metastatic lymph nodes, lymph node upstaging, and complementary indication of adjuvant therapy were investigated.

Results The number of lymph nodes retrieved was significantly higher with the use of lymph node revealing solutions than with the conventional method in colon cancer (GEWF: 29.5 vs 27; $p < 0.001$; Carnoy: 27.7 vs 25.2; $p < 0.001$) and rectal cancer (GEWF: 25.8 vs 23.6; $p < 0.001$; Carnoy: 23.1 vs 20.8; $p < 0.001$). There were no differences between the solutions and conventional examination with respect to the median number of additional metastatic lymph nodes identified (0 in all arms), the number of patients with lymph node upstaging (colon cancer: 1 in the Carnoy arm, 0 in the GEWF arm; rectal cancer: 1 in the GEWF arm, 0 in the Carnoy arm), or the number of patients with complementary indication of adjuvant therapy (colon cancer: 1 in the Carnoy arm, 0 in the GEWF arm; rectal cancer: 0 in both arms).

Conclusion Despite the higher number of lymph nodes retrieved, neither solution resulted in significant changes in patient staging or treatment. Both solutions exhibited equal performance with respect to all outcomes.

Trial registration NTC02704988

Keywords Randomized controlled trial · Colorectal cancer · Lymph node · Lymphadenectomy · Lymph node revealing solution

Introduction

Lymph node (LN) metastasis is a cornerstone parameter for staging and decision-making in colorectal cancer (CRC). The conventional method of LN retrieval by visual searching and palpation of the perivisceral fat tissue is laborious, time-consuming, and depends on the pathologist's diligence. LN-revealing solutions (LNRSs) are a simple, safe, low-cost alternative to facilitate this task [1, 2]. LNRSs dehydrate the mesenteric fat and improve the visibility of the lymphatic tissue [3, 4]. Despite the significant increase in the number of LNs retrieved, to date, few well-designed studies have evaluated the impact of routine employment of LNRSs in clinical practice, and there is no consensus as to the best solution [5–8]. We aimed to compare the performance of the two most commonly used LNRSs (Carnoy and GEWF) with respect to the number of LNs retrieved, number of additional metastatic LNs identified, and proportion of patients with LN upstaging and complementary indication of adjuvant therapy.

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Materials and methods

Study design

Patients with colon cancer (CC) and rectal cancer (RC) were randomized into two independent groups, and specimens submitted to conventional LN examination underwent additional fixation with either Carnoy or GEWF solution and dissection to find any missed LNs. Randomization was performed at a 1:1 ratio. Surgeons and patients remained blinded during the procedure. Pathologists could not be blinded because of the typical odor of the solutions.

Study setting and participants

This study was performed in a private reference cancer center in Porto Alegre, Brazil between March 2015 and February 2018. Patients aged ≥ 18 years with adenocarcinoma of the colon or rectum were considered eligible. Exclusion criteria were synchronous CRC, multivisceral or extended colorectal resection, extended lymphadenectomy, inflammatory bowel disease, and previous colorectal surgery on the segment affected by the tumor. All surgical procedures were performed by one board certified colorectal surgeon with > 10 years of experience. To homogenize the specimens, only D2 lymphadenectomies were included. One pathology assistant with > 10 years of experience dissected the LNs from the specimens. Histological examinations were performed by two board certified pathologists with > 7 years of experience. This trial is registered with [ClinicalTrials.gov](https://www.clinicaltrials.gov/ct2/show/study/NCT02704988), NTC02704988.

Pathological examination

Initially, the specimens were longitudinally opened, rinsed, and submerged in 10% neutral buffered formalin for 48 h. Pathological examination was performed according to the College of American Pathologists protocol. The perivisceral fat was dissected from the intestine with scissors and submitted to serial sectioning in order to perform the conventional method of LN searching. All retrieved nodular structures were embedded in a block of paraffin and sent for microscopic examination. Each identified LN was counted. Sections cut at 4 μm were stained with hematoxylin-eosin. The examination for additional LNs included three steps: (1) specimen submersion in one of the LNRSs for 48 h; (2) additional searching for missed LNs by visual and palpatory examination over 40 min; and (3) histological examination of all additional nodular structures identified. LNs were counted only when they were confirmed microscopically.

Outcomes

The primary endpoint was the total number of LNs retrieved after the use of LNRSs in comparison to that identified by the conventional method. Secondary endpoints were the number of additional metastatic LNs identified, the number of patients with LN upstaging, and the number of patients who received indication of adjuvant therapy owing to the use of LNRSs. With the aim to detect a difference of one additional LN (standard deviation ± 0.7) [9] between the intervention arms, we estimated a minimum of 66 patients per group to obtain statistical power of 80% in detecting this difference at the 5% level. The size calculation was the same for both groups, totaling a sample size of at least 132 patients for the whole study.

Results

Of the 181 randomized patients, 7 in the CC group and 1 in the RC group were excluded. Therefore, a total of 173 patients were included in the analysis: 107 in the CC group (56 in the GEWF arm and 51 in the Carnoy arm) and 66 in the RC group (33 in each arm) (Fig. 1). Patients' baseline characteristics were similar in both the GEWF and Carnoy groups. The mean number of LNs retrieved with conventional examination before and during this trial did not differ, both for CC (24.4 ± 10.9 vs 26.1 ± 10.8 ; $p = 0.27$) and for RC (20.2 ± 9.6 vs 22.2 ± 7.9 ; $p = 0.31$). Only 5 patients (4.7%) in the CC group (3 in the GEWF arm and 2 in the Carnoy arm) and 1 patient (1.5%) in the RC group (Carnoy arm) had fewer than 12 LNs identified in the conventional examination.

The mean total number of LNs retrieved was significantly higher with GEWF and Carnoy solutions than with the conventional method in the CC group (GEWF arm: 29.5 vs 27; $p < 0.001$; Carnoy arm: 27.7 vs 25.2; $p < 0.001$) and the RC group (GEWF arm: 25.8 vs 23.6; $p < 0.001$; Carnoy arm: 23.1 vs 20.8; $p < 0.001$). No differences were observed in the median number of additional metastatic LNs, the proportion of patients with LN upstaging, or the proportion of patients with indication of adjuvant chemotherapy because of the intervention in either group (Table 1).

Overall, 1512 and 1283 LNs were retrieved by conventional examination of CC patients in the GEWF and Carnoy arms, respectively. One hundred forty-one and 127 additional LNs were identified after fixation with GEWF and Carnoy solutions, respectively ($p = 0.65$). No difference was observed between the number of additional LNs retrieved in the GEWF and Carnoy arms in the RC group (70 and 76, respectively) of the 778 and 690 LNs identified at the conventional examination, respectively ($p = 0.23$). Moreover, neither solution presented superior performance with respect to the overall number of additional metastatic LNs retrieved in the CC group (GEWF vs Carnoy: 5 vs 1; $p = 0.22$) or the RC group (GEWF vs

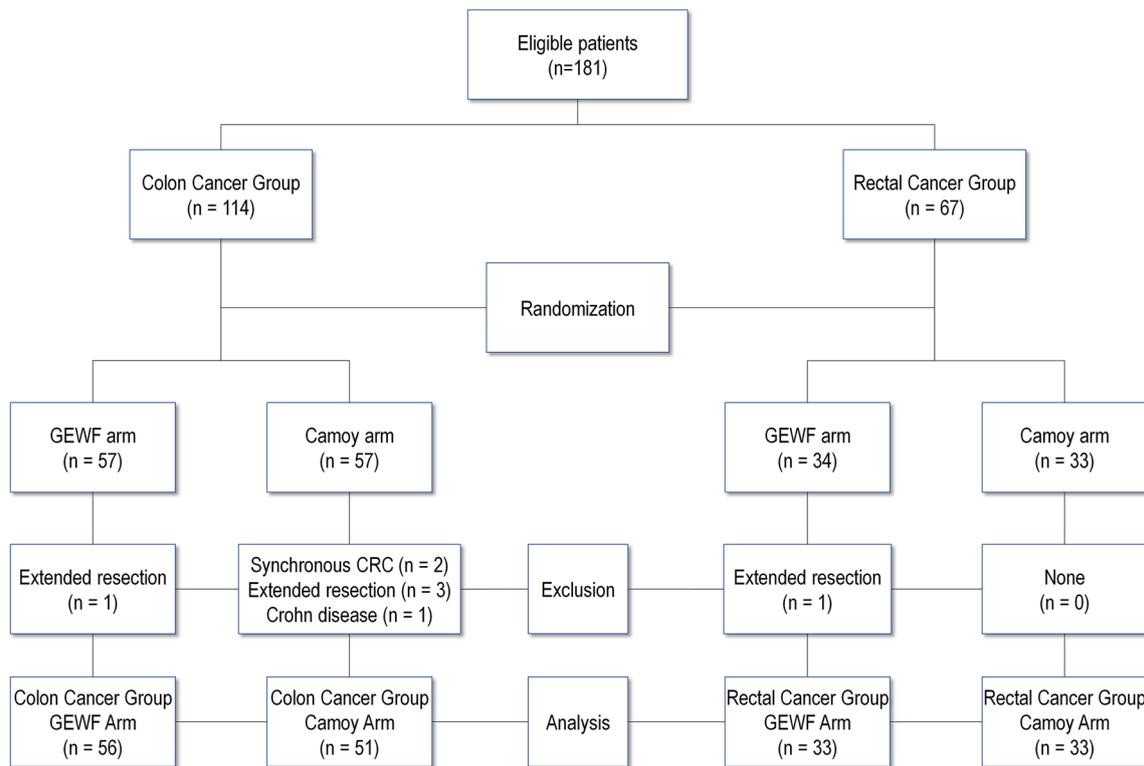


Fig. 1 Randomization and analysis flow diagram

Camoy: 2 vs 0; $p = 0.24$). No differences between the two arms were observed in the proportion of patients with less than 12 LNs, additional LNs, additional metastatic LNs, LN upstaging, or indication of adjuvant chemotherapy. It should be noted that 99.3% and 100% of additional LNs retrieved measured ≤ 5 mm in the CC and RC groups, respectively.

Five patients had additional metastatic LNs detected by LNRSs. Four already had LN metastases identified by conventional examination and had N2b stage disease. Therefore, this complementary finding did not impact disease staging or treatment strategy. Of note, only 1 patient, a 64-year-old woman with node-negative left CC, had one

Table 1 Lymph node-related data before and after the use of lymph node revealing solutions in patients with colon and rectal cancer

Colon cancer	GEWF (n = 56)			p	Carnoy (n = 51)		
	Before	After			Before	After	p
Number of:							
LNs, mean (\pm SD)	27 (10.6)	29.5 (10.9)	< 0.001	25.2	27.7 (11.7)	< 0.001	
+LNs, median (IQR 25th–75th)	0 (0–1.8)	0 (0–1.8)	0.180	(11.1) 0 (0–2)	0 (0–2)	0.32	
Patients:							
pN+, n (%)	19 (33.9)	19 (33.9)	> 0.99	18 (35.3)	19 (37.3)	> 0.99	
With CT indication, n (%)	19 (33.9)	19 (33.9)	> 0.99	18 (35.3)	19 (37.3)	> 0.99	
Rectal cancer	GEWF (n = 33)			p	Carnoy (n = 33)		
	Before	After			Before	After	p
Number of:							
LNs, mean (\pm SD)	23.6	25.7 (8)	< 0.001	20.9 (8)	23.2 (9.1)	< 0.001	
+LNs, median (IQR 25th–75th)	(7.7) 0 (0–1.5)	0 (0–2.5)	0.07	0 (0–0)	0 (0–0.5)	0.32	
Patients:							
pN+, n (%)	12 (36.4)	12 (36.4)	> 0.99	7 (21.2)	7 (21.2)	> 0.99	
With CT indication, n (%)	12 (36.4)	12 (36.4)	> 0.99	7 (21.2)	7 (21.2)	> 0.99	

LNs, lymph nodes; SD, standard deviation; +LNs, metastatic lymph nodes; IQR, interquartile range; pN+, presence of LN metastasis; CT, chemotherapy

additional metastatic LN detected with LNRSs. She was diagnosed with a sole micrometastasis and received adjuvant chemotherapy.

Discussion

In this study, the routine use of LNRSs significantly increased the number of LNs retrieved from CC and RC specimens compared to the conventional method. GEWF and Carnoy solutions had similar performance with respect to the number of additional LNs retrieved in CC and RC and the number of metastatic LNs identified. Neither solution resulted in significant changes in pathological staging or complementary indication of adjuvant therapy. Therefore, the question is not which solution must be used, but which patients benefit and under what conditions. In our trial, LN upstaging was rarely observed. Considering that most of these patients already had advanced LN metastasis (N2) by conventional examination, the routine use of LNRSs did not add any relevant information with respect to prognosis or treatment, and therefore could have been avoided.

In the literature, LN upstaging is reported in 2.4 to 33% of patients whose specimens undergo an LN revealing protocol. However, several investigations had a questionable quality of LN search by conventional examination, i.e., fewer than 12 LNs retrieved [6–8, 10, 11], which favored the occurrence of LN upstaging with the use of LNRSs. Less than 4% of patients in our trial had an insufficient number of LNs retrieved by conventional examination. This rate reflects the high quality of surgeries and pathological examinations performed and is probably responsible for the small number of metastatic LNs identified and the low rate of LN upstaging observed. In the clinical point of view, these findings raise doubt on the benefit of routine use of LNRSs in hospitals with such a high level of performance. However, renouncing the routine use of LNRSs cannot be generalized for hospitals in which an inadequate number of LNs is dissected. Only 1 of 173 patients analyzed in this trial was directly benefited by LN upstaging and consequent administration of adjuvant chemotherapy. Although upstaging was uncommon, the clinical relevance of this finding must be valued and analyzed from a cost-benefit perspective.

In the present study, 99.5% (411/413) of the additional LNs harvested were < 5 mm. Small LNs (< 5 mm) are difficult to recognize during dissection of mesenteric tissue fixed with formalin, mainly in specimens of RC treated with neoadjuvant chemoradiotherapy [1, 11]. Small LNs are relevant because they may harbor micrometastases (0.2–2 mm) and even macrometastases (> 2 mm), both of which are associated with worse survival [12].

Conclusion

GEWF and Carnoy solutions exhibited similar performance in the search for additional LNs in patients with CRC. Despite the increase in the LN count, neither solution resulted in significant changes in patients' prognosis, staging, or treatment.

Acknowledgments The authors thank Daniela Benzano Bumaguin for her help in the statistical analysis and Maria Francisca Torres Lopes and Márcio Machado for the pathological examination of surgical specimens.

Author contributions Tiago L. Ghezzi: study conception and design, acquisition and interpretation of data, statistical analysis, manuscript writing, critical revision, final approval, and agreement to be accountable for all aspects of the work; Márcia P. Pereira: study conception and design, acquisition and interpretation of data, critical revision, final approval, and agreement to be accountable for all aspects of the work; Oly C. Corleta: study conception and design, acquisition and interpretation of data, critical revision, final approval, and agreement to be accountable for all aspects of the work; Antonio N. Kalil: study conception and design, acquisition and interpretation of data, manuscript writing, critical revision, final approval, and agreement to be accountable for all aspects of the work.

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