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A case–control study of using carbon nanoparticles to trace decision-making lymph nodes around inferior mesenteric artery in rectal cancer

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Abstract

Background Accurate identification of lymph nodes localized around inferior mesenteric artery (IMA), with or without metastasis, is of crucial importance for surgeons when dissecting D2 or D3 lymph nodes in patients with rectal cancer (RC). The following study evaluates whether carbon nanoparticles can be used for detection of decision-making lymph nodes (DLNs) in station 253 lymph nodes found around IMA during RC surgery.

Methods A total of 66 patients with rectal adenocarcinomas were recruited between January 2014 and August 2017. Patients were divided into carbon nanoparticle (CN) group and control (CL) group; for the CN group, 1 ml nanoparticles were endoscopically injected into submucosal layer of primary tumor 1 day before surgery. DLNs were defined as black-dyed nodes in CN group or macroscopic lymph nodes in CL group localized along the IMA, proximal to the origin of the left colic artery. D3 lymph nodes were dissected using laparoscopic radical resection, and then examined using pathological approach. Intra-operative and post-operative data were compared between the two groups.

Results In CN group, black-dyed DLNs were easily found under laparoscopy; the median number of DLNs was 3 (range 1–9). In CL group, the median number of DLNs was 0 (range 0–3). Consistency between intra-operative DLNs and post-operative station 253 nodes were confirmed by pathological examination. Significant higher number of DLNs in station 253 nodes (2.91 ± 2.47 vs 0.58 ± 0.75, $p < 0.001$), number of station 251 nodes (12.85 ± 8.99 vs 8.09 ± 5.85, $p = 0.014$), number of station 253 nodes (5.21 ± 5.26 vs 3.15 ± 2.32, $p = 0.045$), and the number of total lymph nodes (24.06 ± 13.20 vs 16.21 ± 9.09, $p = 0.007$) were found in the CN group compared to CL group.

Conclusions Carbon nanoparticles are useful for identifying DLNs in station 253 LNs around IMA in RC. It is not necessary to perform D3 lymph node dissection if there are no intra-operative DLNs metastases in RC.

Keywords Carbon nanoparticles · Inferior mesenteric artery · Station 253 nodes · Lymph node dissection · Rectal cancer

Successful lymph node recovery relies on close collaboration between pathologists and surgeons. The ideal surgical approach for dissecting D2 or D3 lymph nodes in rectal cancer (RC) patients remains debatable [1–3]. Several studies have suggested that removing lymph node around the root of the inferior mesenteric artery (IMA) leads to a higher successful rate due to better long-term survival and a more precise staging owing to more extensive lymph node clearance in RC [4–6]. On the contrary, Kobayashi et al. have indicated that it takes longer time to perform D3 lymph node dissection in RC patients. In their study, D2 lymph node dissection in RC patients with lower BMI was performed by less experienced surgeons [7]. Similarly, Liang et al. have suggested that D3 lymph node dissection is technically demanding, and that learning curve for laparoscopic D3 lymph node dissection requires minimally 20 procedures [8]. Currently, it was still controversial about D2 or D3 lymph node dissection in RC surgery.

According to Japanese Society for Cancer of the Colon and Rectum (JSCCR) [9], station 253 lymph nodes are those

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nodes that lie along the IMA, proximally to the origin of the left colic artery, and the metastasis rate in those nodes is 0.3–8.6% [2]. Lymph node involvement is a major prognostic factor for survival after RC surgery, which advocates the importance of pathologic examination of 12 or more nodes [10], especially in stage II RC [11]. Therefore, it is necessary to harvest more station 253 nodes for the better long-term survival and the more precise staging. Also, D3 lymph node dissection has significant survivals benefits for patients with metastatic station 253 nodes [6, 12–14]. Unfortunately, 253 nodes are extremely difficult to localize. Consequently, the aim of this study was to clearly show station 253 nodes intra-operatively.

Over the last decade, we have witnessed a rapid development of nanotechnology. Carbon nanoparticles have been used as a lymph node tracer in different kinds of surgeries [15–18]. Due to their appropriate size (average diameter of 150 nm), these nanoparticles are high selectively permeable, i.e., they are able to pass through the lymphatic vessels rather than blood capillaries. After reaching the tumor site, carbon nanoparticles are rapidly engulfed by macrophages. The particles then enter the lymphatic vessels and accumulate in the lymph nodes, thus staining them black, and allowing surgeons to localize them. This approach has been successfully used for colorectal cancer, breast cancers, thyroid cancers, and gastric cancer surgeries [15–21]. In our previous study, we have confirmed that more accurate N staging and more precise oncologic prognosis assessment were achieved for patients with RC after neoadjuvant chemoradiotherapy, by increasing the number of lymph nodes counted using carbon nanoparticles [16]. Based on our previous results, we defined decision-making lymph nodes (DLNs) as nodes that were black-dyed by carbon nanoparticles in station 253 nodes around IMA in RC, which in turn help surgeons to accurately perform D2 or D3 lymph node dissections. We further hypothesized that carbon nanoparticles can clearly show DLNs in station 253 nodes around IMA in RC. In addition, to the best of our knowledge, this is the first study that investigated the use of carbon nanoparticles for 253 nodes detection.

Materials and methods

Patients

A case–control study was performed between January, 2014 and August, 2017. Inclusion criteria were as follows: 18–70 years of age, Body Mass Index (BMI) less than 30, American Society of Anesthesiologists (ASA) class 1–3, single RC, rectal adenocarcinomas confirmed pathologically by endoscopic biopsy, and planned radical resection. Exclusion criteria were previous abdominal surgery, pregnant woman, emergency patients with obstruction or perforation, T4b cancer evaluated by CT or MRI or endoscopic ultrasonography, pelvic or distant metastasis, and T1 cancer planned local excision. Sixty-six patients were enrolled in this study. Patients were divided into carbon nanoparticle (CN) group and control (CL) group and matched with 1:1 manner based on age (±3), gender, BMI (±2), tumor location, pre-operative tumor stage, and those with or without neoadjuvant therapy. Thirty-three patients from CN group were endoscopically injected carbon nanoparticles into submucosal layer of primary tumor 1 day before surgery, and then were compared with 33 control patients. This study was approved by institutional review board. Written consent was obtained from all patients.

Methods

Patients in the CN group received 1 ml carbon nanoparticle suspension, which were endoscopically injected into submucosal layer at four points, around the site of primary tumor 1 day before surgery. The concentration of the carbon nanoparticles in this study was 1%, 1 ml (50 mg) carbon nanoparticle suspension with 5 ml saline were endoscopically injected into submucosal layer of the primary tumor. All patients were then subjected to laparoscopic radical resection with D3 lymph node dissection. We defined DLNs in CN group as nodes that were black-dyed by carbon nanoparticles localized along the IMA, proximal to the origin of the left colic artery. In CL group, DLNs were defined as macroscopic lymph nodes localized along the IMA. During surgery, the IMA was skeletonized and DLNs around IMA were taken intra-operatively by experienced surgeon. These DLNs were subjected to intra-operative frozen pathological examination so as to confirm whether tumor metastasis was present. Consequently, lymph nodes were taken out and sent to pathological lab for further examination. Intra-operative and post-operative data were recorded and they included the following: case number of positive DLNs, mean number of DLNs, mean number of harvested station 251 nodes, mean number of positive station 251 nodes, mean number of harvested station 252 nodes, mean number of positive station 252 nodes, mean number of harvested station 253 nodes, mean number of positive station 253 nodes, mean number of total harvested lymph nodes, mean number of positive total lymph nodes, operative time, blood loss, hospital stay, and complications. Station 251 nodes were defined lymph nodes that on the wall of the rectum and those along the superior rectal artery up to the level of Sudeck's point. Station 252 nodes were defined lymph nodes that lie along the IMA from distal to the origin of the left colic artery to the bifurcation of the superior rectal artery. Station 253 nodes were defined lymph nodes that lie along the IMA proximal to the origin of the left colic artery [9]. These data were compared

between CN group and CL group. A teaching program for surgeons to detect DLNs included (1) materials preparation: 1 ml (50 mg) carbon nanoparticle suspension, 5 ml normal saline, and 5 ml syringe; (2) endoscopical submucosal injection: 1 day before surgery, 1 ml carbon nanoparticle suspension with 5 ml saline were endoscopically injected into submucosal layer at four points around the site of the primary tumor; (3) laparoscopy exploration and DLNs dissection: DLNs were black-dyed during laparoscopy exploration, these DLNs were dissected; (4) intra-operative frozen: DLNs were subjected to intra-operative frozen pathological examination; (5) D3 lymph node dissection: laparoscopic radical resection with D3 lymph node dissection was performed.

Statistical analysis

Data are presented as mean (± SD) for continuous variables and as frequency (%) for categorical variables. Differences between groups were compared by the Mann–Whitney *U* test for continuous variables, and the Chi-square test or Fisher's exact test for categorical variables. A p value < 0.05 was considered statistically significant. All statistical analyses were performed with SPSS version 20.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

A total of 66 patients (33 in the CN group and 33 in the CL group) with rectal adenocarcinomas were recruited in this study. All patients underwent laparoscopic radical resection with D3 lymph node dissection. Similar baseline and tumor characteristics were found between groups (Table 1).

In CN group, black-dyed DLNs were easily found under laparoscopy; the median number of DLNs was 3 (range 1–9). In CL group, the median number of DLNs was 0 (range 0–3). The localizations of primary tumor and its surrounding lymph nodes were easily identified in CN group compared to CL group (Fig. 1A–D). Laparoscopic radical resection with D3 lymph node dissection was performed by experienced surgeon and hypogastric nerve was protected. The specimen was opened after surgery and carbon nanoparticles were observed around the RC (Fig. 1E) compared to control group (Fig. 1F). Subsequently, station 251, 252, 253 nodes were taken out and underwent pathological examination both two group (Fig. 1G, H).

In CN group, intra-operative frozen results of DLNs black-dyed by carbon nanoparticles were consistent with post-operative pathological results of paraffin section of station 253 nodes. When intra-operative frozen pathological examination showed no tumor metastasis in DLNs, the following pathological results of paraffin section also showed no tumor metastasis in station 253 nodes after surgery. Intra-operative and post-operative data in CN group and CL group were shown in Table 2. Intra-operatively, significantly higher number of DLNs were harvested in CN group compared to CL group (2.91 ± 2.47 vs 0.58 ± 0.75, $p < 0.001$). Post-operatively, significantly higher number of station 251 nodes (12.85 ± 8.99 vs 8.09 ± 5.85, $p = 0.014$) and 253 nodes (5.21 ± 5.26 vs 3.15 ± 2.32, $p = 0.045$) were harvested in CN group compared to CL group. In addition, significantly higher total number of lymph nodes were harvested in CN group compared to CL group (24.06 ± 13.20 vs 16.21 ± 9.09, $p = 0.007$). Case number of positive DLNs, positive station 251 nodes, positive station 252 nodes, positive station 253 nodes, and total positive lymph nodes were not significantly different between the two groups. Furthermore, no significant difference in operative time, blood loss, post-operative hospital length of stay, post-operative complications were found between the two groups; post-operative complications included anastomotic leakage, anastomotic hemorrhage,

Table 1 Baseline characteristics of the two groups of participants

Characteristic	CN group ($n = 33$)	CL group ($n = 33$)	p
Age (years)	57.2 ± 9.4	57.8 ± 9.7	0.798
Gender			1
Male	26 (78.8)	26 (78.8)	
Female	7 (21.2)	7 (21.2)	
BMI (kg/m^2)	23.3 ± 3.6	22.8 ± 3.4	0.555
Tumor location			1
Lower (< 5 cm)	3 (9.1)	3 (9.1)	
Middle (5–10 cm)	17(51.5)	17 (51.5)	
Upper (10–15 cm)	13 (39.4)	13 (39.4)	
Pre-operative stage I/II/III/IV	7/19/7/0	7/19/7/0	1
Neoadjuvant therapy			1
Yes	12 (36.4)	12 (36.4)	
No	21 (63.6)	21 (63.6)	

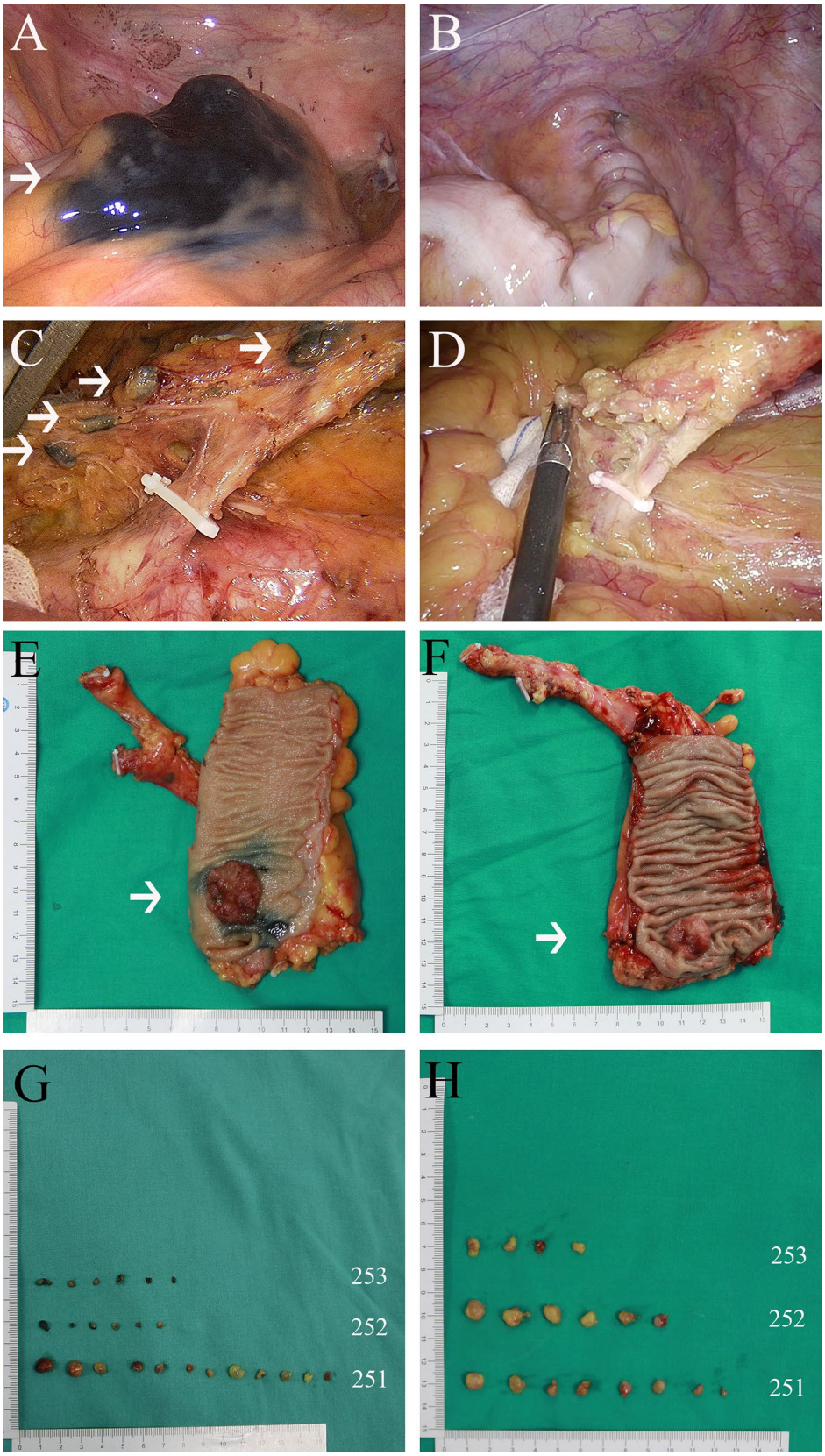

Fig. 1 Comparisons of carbon nanoparticle (CN) group with control (CL) group. **A** The localizations of primary tumor in CN group. **B** Primary tumor without carbon nanoparticle injection in CL group. **C** Decision-making lymph nodes (DLNs) around inferior mesenteric artery were black-dyed by carbon nanoparticles under laparoscopy in CN group. The arrows showed black-dyed DLNs. **D** Lymph nodes around inferior mesenteric artery in CL group. **E** Ex vivo images of rectal cancer specimens in CN group. **F** Ex vivo images of rectal cancer specimens in CL group. **G** Station 251, 252, and 253 nodes were collected in CN group. **H** Station 251, 252, and 253 nodes were collected in CL group

Table 2 Intra-operative and post-operative data in CN group and CL group

Variable	CN group ($n=33$)	CL group ($n=33$)	p
Mean no. of DLNs	2.91 ± 2.47	0.58 ± 0.75	< 0.001
Case number of positive DLNs, n (%)	2 (6.06%)	0	0.245
Operative time (min)	187.82 ± 70.68	170.67 ± 55.53	0.277
Blood loss (ml)	52.88 ± 32.14	55.30 ± 42.57	0.795
Mean no. of station 251 nodes harvested	12.85 ± 8.99	8.09 ± 5.85	0.014
Mean no. of positive station 251 nodes	0.73 ± 1.79	0.27 ± 0.76	0.187
Mean no. of station 252 nodes harvested	6 ± 3.69	4.97 ± 4.25	0.297
Mean no. of positive station 252 nodes	0.27 ± 0.98	0.09 ± 0.38	0.326
Mean no. of station 253 nodes harvested	5.21 ± 5.26	3.15 ± 2.32	0.045
Mean no. of positive station 253 nodes	0.18 ± 0.88	0 ± 0	0.245
Mean no. of total lymph nodes harvested	24.06 ± 13.20	16.21 ± 9.09	0.007
Mean no. of total positive lymph nodes	1.00 ± 2.72	0.55 ± 1.87	0.432
Post-operative hospital stay (day)	7.3 ± 2.34	7.76 ± 1.90	0.390

Table 3 Post-operative complications in CN group and CL group

Complications	CN group ($n=33$)	CL group ($n=33$)	p
Total, n (%)	3 (9.09)	5 (15.15)	0.451
Anastomotic leakage	1	1	
Anastomotic hemorrhage	1	0	
Ileus	0	1	
Lymphorrhagia	1	0	
Perianal abscess	0	1	
Pneumonia	0	1	
Incisional infection	0	0	

ileus, lymphorrhagia, perianal abscess, pneumonia, and incisional infection (Table 3).

Discussion

According to existing data, station 253 nodes positive rate in RC has shown to be 0.3–8.6% [2], and thus most surgeons still face the same clinical problems with reference to rectal patients, i.e., whether they need to receive D2 or D3 lymph node dissection. Even though several previous studies have shown poor survival in patients with station 253 nodes metastasis [6, 12–14], identification of metastatic station 253 node still remains an important issue. In the present study, we defined decision-making lymph nodes (DLNs) as nodes that were black-dyed by carbon nanoparticles in station 253 nodes around IMA in RC, which was similar to sentinel lymph nodes (SLNs) in the management of various cancers. Therefore, DLNs biopsy could be very helpful in selection of D2 or D3 lymph node dissection in RC. Currently, most of surgeons choose to perform D3 lymph node dissection in RC due to lack of specific DLNs tracers, which not only increase the difficulty of operation but also lead to unnecessary lymph node dissections. So far, numerous studies have proposed various methods for lymph node detection. In 2007, Ishikawa et al. [22] have used an infrared electronic endoscopy system (IREE) with indocyanine green (ICG) injection in patients with gastric cancer; the accuracy of their technology was 94% in 16 cases, with just one false-negative case. However, this method required the use of special devices that had limited availability in the hospital. Subsequently, Japanese multicenter trial demonstrated that the proportion of false negatives was too high in using ICG to show SLNs in gastric cancer [23]. In 2010, Quadros et al. [24] have performed lymphoscintigraphy using technetium-99m-phytate and patent blue to detect blue and/or radioactive retroperitoneal and/or lateral pelvic nodes (RLPN) which were examined histopathologically and immunohistochemically with a step-sectioning technique in ninety-seven rectal adenocarcinoma patients, which significantly improved the identification of RLPN metastases for selective indication of retroperitoneal and lateral pelvic lymphadenectomy. However, these methods were not widely used in clinical practice because they were time-consuming, labor-intensive, and toxic to both doctors and patients. In 2014, Kir et al. [25] have used ex vivo intra-arterial methylene blue injection (MBI) in the operation theater to improve the detection of lymph node metastases in colorectal cancer, and the total number of lymph nodes harvested; the number of cases with lymph node metastasis has also improved. However, ex vivo intra-arterial MBI was not helpful for real-time decision-making for D2 or D3 lymph node dissection.

Carbon nanoparticle suspension, which has been approved by Chinese Food and Drug Administration in clinic since 2007, is used to trace sentinel lymph nodes in breast cancers, thyroid cancers, and gastrointestinal cancer. The carbon nanoparticle suspension comprises nanosized carbon particles (average diameter, 150 nm), which ensure

that these particles enter into lymphatic vessel capillaries (diameter of 150–500 nm), but not blood vessel capillaries (diameter of 20–50 nm). Therefore, carbon nanoparticle suspension is a good DLNs tracer. Not only that, the method we used in the present study is simple and easy: one milliliter carbon nanoparticle suspension was endoscopically injected into the submucosal layer at four points around the site of the primary tumor 1 day before surgery. Once again, compared with the previously mentioned approach, our method that is based on using carbon nanoparticles to show decision-making lymph nodes around IMA appeared to be easier and less time consuming. Moreover, it was not toxic to both doctors and patients. In this study, we performed D3 lymph node dissection for all of participants, and found that intra-operative frozen results of DLNs black-dyed by carbon nanoparticles were consistent with post-operative pathological results of paraffin section of station 253 nodes. When intra-operative frozen pathological examination showed no tumor metastasis in DLNs, the following pathological results of paraffin section also showed no tumor metastasis in station 253 nodes after surgery. Our next step is to perform a multicenter clinical trial, which is to illustrate that standard D3 lymph node dissection is theoretically unnecessary for RC patients when intra-operative DLNs show no metastasis. If our next study was proven oncologically feasible in larger series, the ideal surgical approach for dissecting D2 or D3 lymph nodes in RC patients will be more accurate. In other words, for those RC patients without intra-operative DLNs metastasis, D2 lymph node dissection will be suitable as a "tailored surgery."

The limitation of this study was a case–control study. Therefore, a prospective randomized controlled trial is necessary and 68 patients must be included, which allowed detection of a 40% difference in decision-making lymph node dissection between carbon nanoparticle group and control group, with a power of 90%, using the Chi-square test and two-tailed alpha of 0.05. Currently, this prospective randomized controlled trial is ongoing in our hospital.

In conclusion, it is feasible to use carbon nanoparticles to show DLNs in station 253 nodes around IMA in RC. Carbon nanoparticle suspension is safe for submucosal injection. It is not necessary to perform D3 lymph node dissection when intra-operative DLNs show no metastasis in RC.

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Compliance with ethical standards

Disclosures Drs. Kai Li, Dexin Chen, Weisheng Chen, Zhangyuanzhu Liu, Wei Jiang, Xiumin Liu, Ziming Cui, Zhiyao Wei, Zhiming Li, and Jun Yan have no conflicts of interest or financial ties to disclose.

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