Carbon nanoparticles for identification of metastatic lymph nodes around the superior mesenteric artery in transverse colon cancer

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Research

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Abstract

Background Accurate identification of metastatic lymph nodes around the superior mesenteric artery (SMA), with or without metastasis, is vital for surgeons when dissecting lymph nodes in patients with transverse colon cancer. In the current study, we evaluated the prospect of using carbon nanoparticles in identification of lymph nodes around SMA.

Methods We recruited a total of 220 patients, with transverse colon cancer, and divided them into two groups. The first group (n=51) was carbon nanoparticle (CN) while the other (n=169) had no carbon nanoparticle (NCN) group. Intraoperative and post-operative data were compared between the groups.

Results We found that 117 patients were negative for D1 lymph nodes, while 3.4% were positive for D2 lymph nodes. Additionally, 169 patients were negative for D2 lymph nodes while 10.7% of patients were positive for D3 lymph nodes. Laparoscopy easily identified black-dyed lymph nodes in the CN group. Significantly higher numbers of positive station D3 lymph nodes (0.63 ± 2.43 vs 0.29 ± 1.03, p = 0.006), number of positive station 214 nodes (0.10 ± 0.13 vs 0.08 ± 0.17, p = 0.004), the number of positive lymph nodes (2.69 ± 5.38 vs 1.90 ± 4.22, p = 0.037), and the number of total lymph nodes (39.67 ± 19.33 vs 34.50 ± 18.99, p = 0.037) were found in the CN compared to NCN group. However, we found no significant differences between CN and NCN groups with regards to the number of positive station D1 and D2 lymph nodes.

Conclusions We successfully identified metastases 214 LNs around SMA in transverse colon cancer using carbon nanoparticles. These particles have potential to significantly increase the number of positive and negative lymph nodes.

Background

Successful surgery involves generating a proper dissection range of lymph nodes. American Society of Clinical Oncology (ASCO) and European Society for Medical Oncology (ESMO) guidelines on colon cancer do not recommend dissecting No. 214[1,2]. In fact, it is not clear whether is it necessary to dissect 214 lymph nodes in transverse colon cancer patients. It's about 3% of transverse colon cancer having 214 metastasis[3,4]. Several studies have attributed a high radical success in treatment of the disease to removal of lymph nodes around the root of the superior mesenteric artery (SMA), owing to more extensive lymph node clearance in the transverse colon[5,6]. Conversely, some studies have indicated that removing 214 lymph nodes in transverse colon cancer patients is not only time consuming, but also leads to more complications [7,8]. Currently, the use of 214 lymph node dissection in transverse colon cancer surgery remains unclear.

According to the Japanese Society for Cancer of Colon and Rectum (JSCCR)[9], station 214 lymph nodes are those that lie along the SMA, proximally at the lower border of the pancreas. Their metastasis rate is 3%[4]. Lymph node metastasis is a major prognostic factor for survival after transverse colon cancer

radical surgery, indicating the importance of pathologic examination of 12 or more nodes[10,11]. Therefore, it is necessary to harvest station 214 nodes to better understand long term survival, and generate more precise staging. However, 214 lymph nodes are to not easy to dissect. In this study, we sought to investigate a tracer that could detect 214 nodes so that decided whether dissection of 214 nodes is necessary.

Over the past decades, technological advancement has enabled a rapid development in the field of nanotechnology. Particularly, Carbon nanoparticles have been widely used as a lymph node tracer in different kinds of cancers[12–15]. Staining enables surgeons to find identify the cancers, with the method successfully used for breast, gastric, and thyroid cancers[16–18]. However, its suitability colon cancer remains a mystery. It has been confirmed that more accurate N staging and more precise oncologic prognosis assessment were achieved for patients with rectal cancer after neoadjuvant chemoradiotherapy, by increasing the number of lymph nodes counted using carbon nanoparticles[13]. Based on these previous studies, we surmised that carbon nanoparticles can identify lymph nodes of station 214 nodes around SMA in transverse colon cancer. Besides, as far as we know, this is the first study that investigated the use of carbon nanoparticles to detect 214 nodes.

Materials And Methods

Patients

This study was performed between January 2010 and June 2019 with the following inclusion criteria: patients were18 years or older, single transverse colon cancer, transverse colon biopsy pathologically confirmed cancer by endoscopy, and taking radical resection. Exclusion criteria included: those with obstruction, perforation, or cancer local excision by endoscopy. A total 220 patients were finally enrolled in the study cohort and divided into the carbon nanoparticle (CN), and no carbon nanoparticle (NCN) groups. Fifty-one (51) patients in the CN group were endoscopically injected with carbon nanoparticles into the submucosal layer at four points of primary tumor 1 day before surgery. The remaining 169 patients were put in the no carbon nanoparticle (NCN) group. Written informed consent was obtained from all patients prior to being included in the study.

Methods

We performed radical resection with D3 lymph node dissection through laparoscopy on all patients. Station D1 lymph nodes were defined as lymph nodes on the wall of the transverse colon, while Station D2 ones were defined as lymph nodes that lie along the artery (except SMA). Station D3 lymph nodes were those that lie on the root of the artery (except SMA), while Station 214 denoted lymph nodes that lie on the root of the SMA as well as the lower border of the pancreas[9]. Lymph nodes black-dyed were additionally dissected. Statistical analyses were performed using SPSS 22. We performed $\chi 2$ and t-tests to compare categorical and continuous variables respectively.

Results

Baseline and tumor characteristics in the two groups

A total of 220 (59 in the CN and 169 in the NCN groups) patients with transverse colon cancer were recruited in this study based on the aforementioned inclusion criteria. All patients underwent radical resection with D3 lymph node dissection. Similar baseline and tumor characteristics were found between the groups, except for pM and Grade (Table 1).

Table 1
Baseline characteristics of patients in CN and NCN groups

Characteristic	NCN group (n=169)	CN group (n=51)	P
Sex			0.827
Male	101 (59.8)	32 (62.7)	
Female	68 (39.4)	19 (37.3)	
Age, years			
< 65	119 (70.4)	35 (68.6)	0.944
≥ 65	50 (29.6)	16 (31.4)	
pT stage			0.373
T1	6 (3.6)	3 (5.9)	
T2	11 (6.5)	2 (3.9)	
T3	113 (66.9)	39 (76.5)	
T4	39 (23.1)	7 (13.7)	
pN stage			0.662
N0	81 (47.9)	26 (51.0)	
N1	59 (34.9)	14 (27.5)	
N2a	28 (16.6)	11 (21.6)	
N2b	1 (0.6)	0 (0.0)	
pM stage			0.032
M0	163 (89.3)	40 (100.0)	
M1	18 (10.7)	0 (0.0)	
pTNM stage			0.064
I	12 (7.1)	4 (10.0)	
II	63 (37.3)	22 (43.1)	
IIA	53 (31.4)	22 (43.1)	
IIB	8 (4.7)	0 (0.0)	
IIC	2 (1.2)	0 (0.0)	
III	76 (45.0)	24 (47.1)	
IIIA	2 (1.2)	0 (0.0)	

IIIB	60 (35.5)	16 (31.4)	
IIIC	14 (8.3)	8 (15.7)	
IV	18 (10.7)	0 (0.0)	
Grade			0.036
I	12 (7.1)	2 (3.9)	
II	135 (79.9)	40 (78.4)	
III	1 (0.6)	3 (5.9)	
Unknow	21 (12.4)	6 (11.8)	
LN total subgroup			0.607
< 12	15 (8.9)	2 (3.9)	
≥ 12	154 (91.1)	49 (96.1)	
Histology			0.180
Tubular or papillary adenocarcinoma	102 (60.4)	34 (66.7)	
adenocarcinoma	25 (14.8)	2 (3.9)	
Mucinous adenocarcinoma	37 (21.9)	12 (23.5)	
Signet ring cell carcinoma	3 (1.8)	2 (3.9)	
Unknow	2 (1.2)	1 (2.0)	
D1 metastasis			0.939
No	88 (52.1)	28 (54.9)	
Yes	78 (46.2)	23 (45.1)	
Unknow	3 (1.8)	0 (0.0)	
D2 metastasis			0.484
No	122 (72.2)	46 (90.2)	
Yes	15 (8.9)	3 (5.9)	
Unknow	32 (18.9)	2 (3.9)	
D3 metastasis			0.497
No	156 (92.3)	43 (84.3)	
Yes	17 (10.1)	8 (15.7)	
Unknow	12 (7.1)	0 (0.0)	

Site			0.084
Hepatic flexure	89 (52.7)	27 (52.9)	
Transverse colon	60 (35.5)	23 (45.1)	
Splenic flexure	20 (11.8)	1 (2.0)	

Intraoperative And Postoperative Images

Black-dyed lymph nodes were easily identified under laparoscopy in the CN group. Similarly, localization of the primary tumor and its surrounding lymph nodes were also easily identified in this group (Fig. 1A–F). Postoperative specimens were analyzed as indicated in Fig. 1A-F. Briefly, the specimen was opened after surgery and carbon nanoparticles observed around the hepatic flexure (Fig. 1A-B), transverse colon cancer (Fig. 1C-D), and spleen flexure (Fig. 1E-F). Dissection of station 214 LN is shown in Fig. 2. Subsequently, station D1, D2, D3, and 214 nodes were taken out and subjected to pathological analysis.

Post-operative Findings

Post-operative data across the study groups is summarized in Table 2. A significantly higher number of positive station D3 lymph nodes (0.63 ± 2.43 vs 0.29 ± 1.03, p = 0.006), number of positive station 214 nodes (0.10 ± 0.13 vs 0.08 ± 0.17, p = 0.004), the number of positive lymph nodes (2.69 ± 5.38 vs 1.90 ± 4.22, p = 0.037), and the number of total lymph nodes (39.67 ± 19.33 vs 34.50 ± 18.99, p = 0.037) were found in the CN relative to the NCN group. In addition, we found no significant differences between the groups with regards to the number of positive station D1 lymph nodes (1.84 ± 3.55 vs 1.43 ± 2.81, p = 0.093) and the number of positive D2 lymph nodes (0.13 ± 0.52 vs 0.22 ± 0.68, p = 0.259). Moreover, a summary of results on skip metastases is outlined in Table 3. Briefly, the risk of skip metastases in D2, when D1 was negative, was 3.4%, while that of D3 when D2 was negative was 10.7%. Furthermore, operation time, blood loss, post-operative hospital length of stay, post-operative complications for CN group are summarized in Table 4. Post-operative complications included anastomotic leakage, anastomotic hemorrhage, pneumonia, and incisional infection.

Table 2

Number of lymph nodes in the CN and NCN groups Post-operation

	CN group(n=51)	NCN group(n=169)	P
mean no. of total lymph nodes	39.67±19.33	34.50±18.99	0.037
mean no. of positive lymph nodes	2.69±5.38	1.89±4.22	0.044
mean no. of positive station D1 lymph nodes	1.84±3.55	1.43±2.81	0.093
mean no. of positive station D2 lymph nodes	0.13±0.52	0.22±0.68	0.259
mean no. of positive station D3 lymph nodes	0.63±2.43	0.29±1.03	0.006
mean no. of positive station 214 lymph nodes	0.10±0.13	0.08±0.17	<0.001

Table 3

Skip metastases in transverse colon cancer

	T1	T2	T3	T4	Total
D1(-) and D2(+) (n=117)	0.0%	0.0%	1.7%	1.7%	3.4%
D2(-) and D3(+) (n=169)	0.0%	0.6%	5.9%	4.1%	10.7%

Table 4
Post-operative complications in the CN group

Variable	CN group (n=51)
Gastrointestinal decompression (%)	27.5%
Using somatostatin (%)	35.3%
Operative time (min)	238.1±14.7
Blood loss (ml)	46.4±10
Post-operative hospital length of stay (days)	11.5±3
Post-operative complications (%)	23.5%
Septic shock (%)	2.0%
Intra-abdominal hemorrhage (%)	2.0%
Intra-abdominal infection (%)	3.9%
Incisional infection (%)	3.9%
Pancreatitis (%)	2.0%
Pneumonia (%)	11.8%
Urinary infection (%)	3.9%

Discussion

Previous studies have reported a positive lymph nodes rate of station 214 in transverse colon cancer to be 3%[4]. In fact, it remains unclear whether dissecting station 214 lymph node in transverse colon cancer patients is beneficial for surgeons. Based on the poor survival rates reported in patients with station 214 lymph node metastasis[4], identification of metastases station 214 lymph node could potentially help improve the situation. In the present study, we defined station 214 lymph nodes around SMA with carbon nanoparticles in the transverse colon with the aim of improving their identification for future treatment therapies. Currently, most of the surgeons use their experience to perform 214 lymph node dissection due to lack of specific markers for tracing lymph nodes. Consequently, this leads to unnecessary lymph node dissections.

Numerous methods for lymph node detection have been proposed [19–25]. For instance, Carbon nanoparticle suspension has been used to trace sentinel lymph nodes in breast, gastric, and thyroid cancers. In this study, we performed D3 lymph node dissection on enrolled patients and compared data across 2 groups with (CN) and without (NCN) carbon nanoparticles. Our results revealed a significantly higher number of positive station D3 lymph nodes, positive station 214 nodes, positive lymph nodes, and total lymph nodes in the group with nanoparticles compared to that without. Additionally, we found no significant differences between the groups with regards to the number of positive station D1 and D2

lymph nodes. This may be attributed to multiple options for surgery in transverse colon cancer. When cancer in the transverse colon is near the hepatic flexure, it can take the transverse colectomy or extended right hemicolectomy. When cancer in the transverse colon is near spleen flexure, it can take the transverse colectomy or extended left hemicolectomy. We found a similar scope of D1 and D2 lymph node dissection regardless of the kind of surgery taken. However, D3 and 214 lymph nodes were different, using carbon nanoparticles. This is important as they aided to identity lymph node trace and could help to select a surgery method for determining whether to dissect 214 lymph nodes. Although carbon nanoparticles do not increase the positive rate of lymph node detection, it significantly increases the number of detected lymph nodes (both positive and negative), especially for 214 lymph nodes.

In conclusion, Carbon nanoparticles successfully aided the identification of 214 LNs around SMA in transverse colon cancer. These nanoparticles led to a significant increase in the number of positive and negative lymph nodes.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors reviewed and approved the manuscript.

Availability of data and materials

Not applicable.

Conflict of interest
The authors have no conflict of interest to declare.
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Author's Contributions
YuXin Xu processed the data and carried out computational simulations.
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Figures

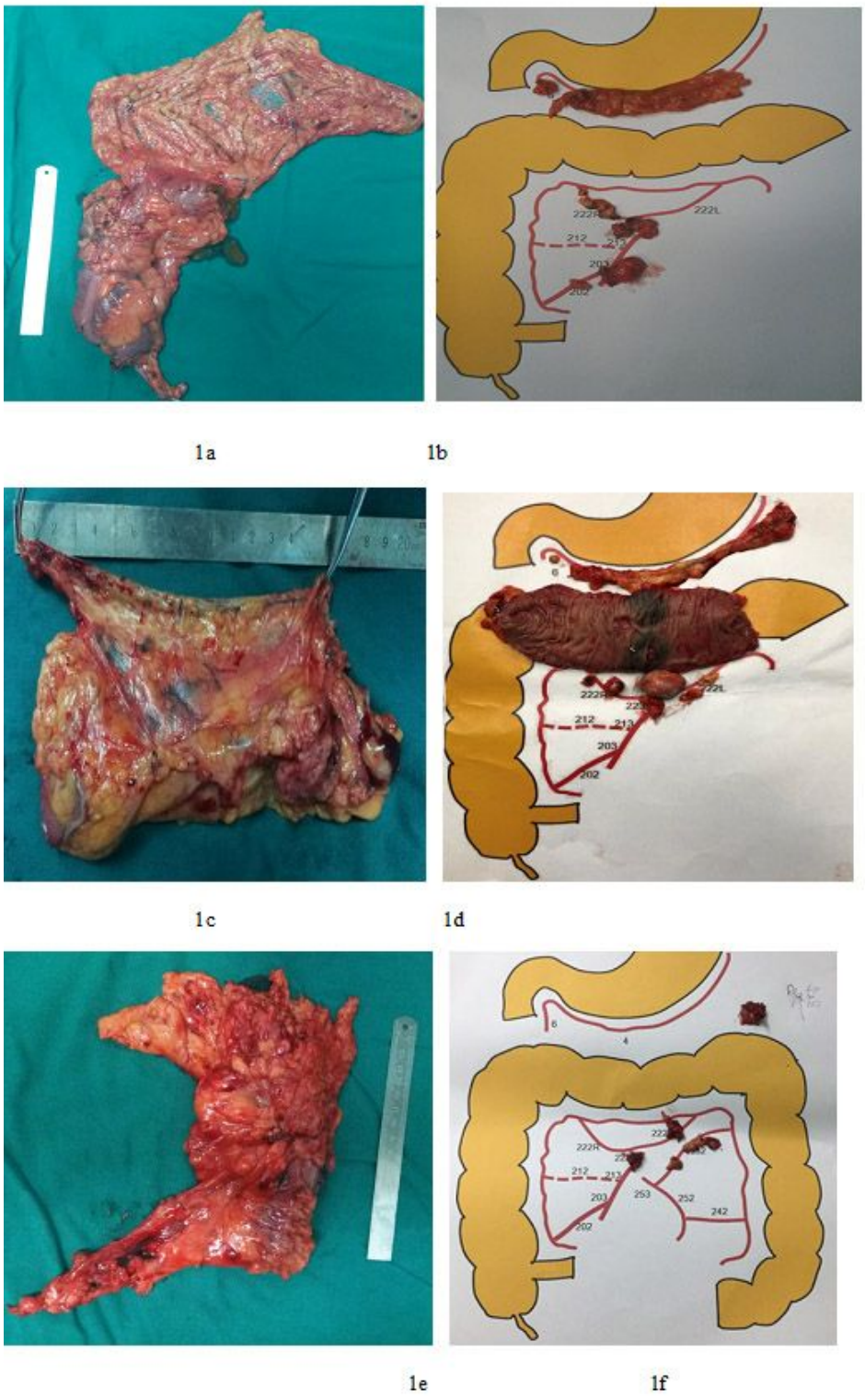

Figure 1

Localization of the primary tumor and its surrounding lymph nodes in postoperative specimens from the CN group

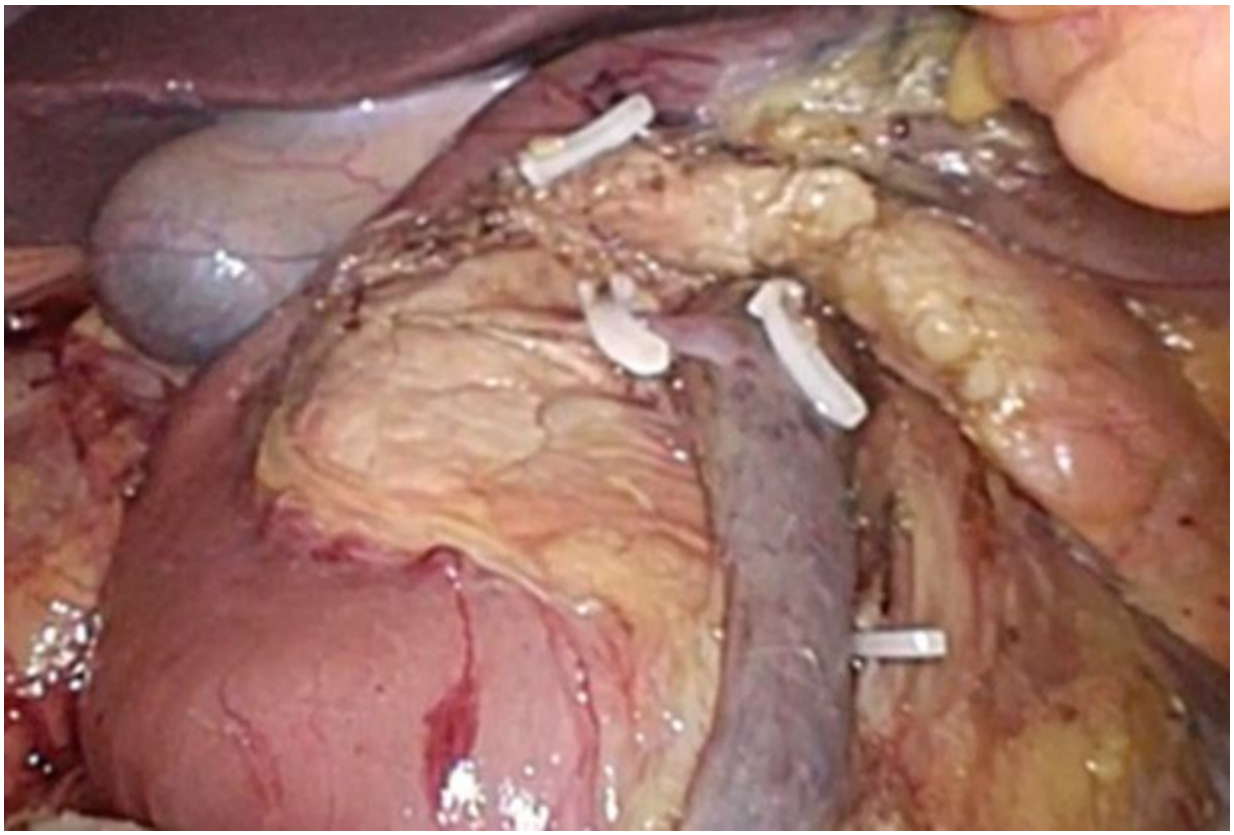

Figure 2

Dissection of station 214 LN

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