Regional Lymph Nodes Distribution Pattern in Central Area of Right-sided Colon Cancer: In-Vivo Detection and the Update on the Clinical Exploration.

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Abstract

Background

Distribution of regional lymph nodes (LN) is decisive for the lymphadenectomy boundary in radical resection of a right-sided colon cancer (RCC). Currently, the data of LN in central area remains ambiguous and scarce. Herein we aim to provide a more detailed anatomical research on LN surrounding the superior mesenteric vessels for RCC and investigated the metastasis rate.

Methods

Carbon Nanoparticles (CNs) or Indocyanine Green (ICG) were used as dye and we laparoscopically observed the stained LN distribution pattern and analyzed the harvested LN combined with pathology report. Lastly, 137 RCC patients who received a “superior mesenteric artery (SMA)-oriented” hemicolectomy from September 2016 to September 2020 were included to calculate the probability of LN metastasis in our target area.

Results

20 patients diagnosed as RCC (mean age 55.55 years, 13 male) were included. 13 patients underwent CNs injection and 7 patients consented to the ICG, while 4 cases suffered from imaging failure. The unequal number of the regional LN located between SMV and SMA was detected in 17 cases (85%), posterior to SMV area in 6 cases (30%), and anterior to SMA in 11 cases (55%), respectively. The presence of LN posterior to SMV was associated with the crossing pattern of ileocolic artery ($\chi^2= 5.38, p= 0.020$). The probability of LN metastasis in the above areas (target areas) was 2.19% (3/137). No dyed LN occurred when the SMA sheath was exposed. What's more, the number of total harvested LN in patients with dye injection was significant more than dye-free RCC patients (22.44±13.78 vs 43.20±22.70, p<0.01).

Conclusion

Right-hemi colon-draining lymphatic vessels anteriorly/posteriorly traversed the SMV and arrived at the surface of SMA near the middle colonic artery (MCA) level, which highlights the potential need of CME to place the internal border anterior to SMA and the removal of mesenteric tissue in our target area on lymphatic resection.

Background

Since the concept of complete mesocolic excision (CME) was proposed in the last decade, it has been adopted to carry out more radical operations globally [1, 2]. Regional LN metastases are regarded as one of the most critical prognostic indicators for colorectal cancer (CRC), and the lymph node yield has been proved to be an independent risk factor in patients with CRC [3, 4]. Therefore, the complete removal of the mesentery wrapped by visceral peritoneum and mesenteric fascia that contain the regional draining lymph nodes of the tumor area, become the main component of CME [5].

The awareness of regional LN of RCC is undergoing continuous dynamics. Due to the complicated anatomical layers in the right colon, the extent of lymphadenectomy for RCC has not been defined clearly by NCCN or ESMO guidelines [6]. However, the central nodal metastasis rates (on the root of the artery) in RCC reported by different centers ranged from 0-5.8%[2, 7], which suggested the necessity for refining research involving LN distributed pattern in the central area.

Until now the internal border of the central area has remained fuzzy, and the data on regional LN around superior mesenteric vessels are scanty [8, 9]. Currently, most surgeons define SMV as the destination of RCC surgery, but this opinion is controversial. In 2013, Milan Spasojevic et al reported an anatomical postmortem study and established the presence of LN posterior to SMV, which was associated with the cross-modal relationship between an ileocolic artery (ICA) and SMV [10]. Recent postmortem research developed the understanding that the long right colonic lymphovascular bundles were across the SMV and described the midline of SMA as the watershed between the small bowel and right colon lymphatics [8]. This research had shown the potential necessity to define the internal border of RCC resection to the midline of SMA. However, the perioperative detection of lymph nodes from RCC patients has not been systematically revealed, due to the limitation of visual observation to distinguish LN from the adjacent connective tissue.
CNs and ICG were found to be reliable navigations in colonic surgery for lymph road mapping, guiding anatomical destination, precise positioning of the tumor, and reducing intraoperative and postoperative complications[11-14]. These dyes are available tools that keep LNs visible during surgery.

In this study, we aimed to investigate the regional LNs distribution pattern in the central area of the RCC patients and further discussed the subsequent need for the increased resection margins anterior to the SMA.

Methods

Patients

Patients were excluded if they had CNs or ICG hypersensitivity, dye injection disagreement, or severe mental disease. 20 eligible patients at the Department of Colorectal Surgery in Guangdong Province Hospital of Traditional Chinese Medicine, with pathologically confirmed RCC, no history of abdominal surgery, were included in this research. Informed consent for research was obtained from patients and all the patients were scheduled for laparoscopic radical resection. The protocol for this study was approved by the ethical committee of Guangdong Provincial Hospital of Chinese Medicine. Besides, all ethical principles and applicable regulations to be followed in our research were certified. In addition, the clinical information of the 137 RCC patients from September 2016 to September 2020 who received the “SMA-orient” hemicolectomy was authorized to use.

Dyes

CNs or ICG were injected a day before the procedure. After bowel preparation, the patients were arranged for colonoscopy. Once we located and marked the lesion, the endoscopist used a 25-gauge needle to inject premixed CNs (50mg/1ml) into colonic submucosal space 1 cm away from the edge of the neoplasm. As for ICG, it was prepared by dissolving 25 mg powdered ICG into 10ml physiologic saline. The endoscopist used a 25-G sclerotherapy needle to inject ICG into the submucosal layer of the colon around the tumor. In the course of laparoscopic operation, the carbon-containing LNs were visible, and ICG was activated with a near-infrared LED at a wavelength of 760 nm as the light source.

Laparoscopic surgery

To our center, an abdominal enhanced CT scan would be acquired for a preoperative evaluation. Once R0 resection was realized, the “artery first” technique with beyond D3 lymph node dissection on the midline of the SMA that we firstly proposed in 2019 was given priority [15]. Step 1, the operator mobilized the tri-junction of the ileocecal area and exposed the duodenum and pancreatic head. Step 2, ileocolic artery/vein (ICA/ICV), right colonic artery/vein (RCA/RCV), and middle colonic artery/vein (MCA/MCV) were bared successively along the midline of SMA from caudal to cranial side and ligated these vessels at the root. Step 3, nearly 2/3 proximal gastrocolic ligament was separated and we laterally dissected the anterior lobe of the transverse mesocolon to free the hepatic flexure. The key time-points during the operation along with stained LNs were as shown in the Supplementary Figures 1-2.

Statistical analysis

Descriptive statistics were used. The Row Mean and Std. Deviation (SD) calculations on the clinical characteristics in this study were performed by Graphpad Prism (Version 8.0). Student t-test was conducted for single comparisons and \( p < 0.05 \) was considered significant.

Results

Patients’ background

A total of 20 patients (13 male, aged 55.55±14.42 years, BMI=21.75±2.452 kg/m\(^2\) [mean±SD]) accepted ICG or CNs injection. The levels of serum gastrointestinal tumor markers were preoperatively detected (CEA=48.38±185.40 μg/L, CA199=14.35±14.60 U/ml, AFP=3.49±2.07 ng/ml [mean ± SD]). The parameters including disease type, differentiation, tumor size, and the pathological stage were as shown in Table 1. No conversion to open surgery and severe postoperative complications occurred. One patient developed
liver metastasis (case 6) and consented to radiofrequency ablation. All the patients were discharged uneventfully and with no re-hospitalization within 30 days.

**LNs posterior to SMV**

As for LNs posterior to SMV, we could observe fluorescence aggregation or local carbon-containing in 3 cases, and it had been confirmed by pathological examination in 4 cases (Total 6 cases) (Figure 1 and Table 2). Under this circumstance, we bared SMV stereoscopically that helped avoid the incomplete dissection of potential positive LNs (Figure 1A-1C). Milan Spasojevic et al had reported that LNs anterior or posterior to SMV related to the crossing pattern of ICA and SMV [10]. In the above 6 cases, ICA crossed posterior to SMV in 5 of 6 cases while ICA crossed anteriorly to SMV in another ($\chi^2 = 5.38, p= 0.020$).

**LNs between SMV and SMA**

The clear presence of LNs between SMV and SMA was realized in 17/20 cases (Figure 2, and Supplementary Figure 3). However, no positive node in this area was detected. We noted that these nodes were presented within the area from ICA to MCA level, and did not affect the crossing pattern of the ICA/MCA to the SMV.

**LNs anterior to SMA**

Hardly any data indicated that regional lymphatic drainage of the right-sided colon could across the SMA, while this phenomenon was observed in 11/20 cases (55%) (Figure 3, and Supplementary Figure 3) and no metastatic node was detected from this area. However, the level of gastrointestinal tumor markers and tumor parameters lacked enough discriminative power to predict the appearance of the regional LNs anterior to SMA.

It is noteworthy that all these nodes were anterior to the SMA plane, none of them was detected in the opposite direction. The dyed LNs appeared more frequently near the MCA level.

**Intrathecal Lymph Tube of SMA**

In our previous research on the vascular sheath of SMA, we had confirmed that nerve fiber was the main component [16]. Meanwhile, a detailed lymphangiology put forward the concepts of the lymphovascular bundle to generalize the collecting lymph vessels attach to blood vessels [17]. However, when we exposed the vascular sheath, ICG aggregation or black-stained could not be observed (Figure 4). By comprehensive consideration, we currently favor the theory that intrathecal lymph tubes of SMA most probably deliver lymph from intrathecal tissues, like the nerve and vascular wall, instead of the mesentery.

**The Metastasis Rate of LNs in Target Area**

From September 2016 to September 2020, total 137 patients diagnosed as RCC were treated surgically with "artery first" laparoscopic right hemicolectomy, the demographic and clinical baseline characteristics were as shown in Supplementary Table 1. There were 2 cases of lymph node metastasis to the area between SMV and SMA, and 1 case posterior to SMV, with pathological findings. Therefore, as a rough estimation, the probability of lymph node metastasis in the target area was 2.19% (3/137). Furthermore, to raise the awareness of metastatic potential of LNs in our target area, we would like to present a case of isolated lymph node metastasis and recurrence after 6 years of right hemicolectomy. A 51-year-old male was diagnosed as RCC (moderately differentiated adenocarcinoma, pT3N0M0) in 2014, and received SMV-oriented radical resection. When we reviewed the abdominal CT scan, an abnormally enlarged lymph node located between SMV and SMA was clearly observed but had not been removed during the surgery (Figure 6A). Unfortunately, in the reexamination via abdominal enhanced CT scan in May 2020, the size of the node was increased to 23mm × 27mm (Figure 6B-6D). According to preoperative imaging we could found that SMA was surrounded by tumor, so we preformed radical tumor excision and SMA resection (Figure 6E). Postoperative pathology prompted that the primary disease responsible for this lymph node metastasis was colon cancer.

**Evaluation of Dyes**

The application of CNs and ICG allowed the operator to distinguish tumor-draining LNs from peripheral adipose tissue or normal nodes (Figure 5A-5B). The carbon-content was sustainably visualized while ICG needed to be activated by 760nm as the light
source. However, there were still some limitations in real-time LNs navigation. The fluorescence intensity was too weak in case 1 (Figure 5D) whereas too strong in another (Case 7) (Figure 5E), and the CNs failed to enter the regional lymphatic system in the other 2 cases (Figure 5F). This made it impossible to play an indicative role during laparoscopic surgery. However, taking the above 137 RCC patients into account, the number total harvested LNs in 20 patients with dye injection was significantly more than 117 dye-free RCC patients (22.44±13.78 vs 43.20±22.70, p<0.01, Figure 5G).

Discussion

The extent of regional LNs for RCC has undergone a long developing process. In the early 20th century, Jamison et al firstly demonstrated that lymphatics of the colon ran with blood-vessels, and proposed metastases to central LNs surrounding superior mesenteric vessels [18]. However, the LNs drainage area had not been precisely defined for many years [19]. Decades later, the traditional tumor drainage area that followed the order of pericolic, intermediate, and main lymphoid groups, was finally accepted [20, 21]. However, when it comes to the distribution pattern of LNs in the D3 area, the descriptions remained vague for a long time. In 2013, an anatomical study began to explore the nodes anterior or posterior to the superior mesenteric vessels and found out a significant correlation to the crossing manners between ICA and SMV [10]. Another post-mortems study described the number, size, and density of LNs in the ascending mesocolon through serial histological sectioning [3]. Later research declaimed that long lymph vessels delivered lymph from the right-side colon were able to traverse SMV to the midline of SMA [8]. To our knowledge, there is not data involving LNs around the central area in vivo, especially for LNs on the medial side of SMA.

The tireless efforts to meet the demands toward the understanding of regional LNs can pave the way for precisely defining the extent of lymphadenectomy for RCC. Since Dr. Hohenberger proposed the technique on CME in colon cancer surgery, surgeons and guidelines have increasingly adopted a radical approach to satisfy the requirement [5, 22]. According to some points, dissection close to SMA increasing risk for damage to blood vessels and splanchnic nerves, and a Japanese investigation showed that lymph flowed towards central vessels hardly arrived at the left side of the anterior surface of the SMV [23]. Thus many surgeons insist that SMV serve as a landmark is concisely and safely, or ligated up feeding arteries 1 cm away from their origin [24, 25]. However, it cannot be overlooked that the central nodal metastases rate in colon cancer was considered nearly 3% [21, 26-28]. Some researchers demonstrated that patients diagnosed as stage II colon cancer, with or without LNs metastases could benet from enlarged lymphadenectomy [29, 30], which might be a possible hint that the above strategies could not completely consistent with CME, and may not have been optimal.

The anatomic view holds that lymphatic watershed between the right-side colon and small bowel is often located in the midline of SMA, and the position of watershed most probably anterior to SMA [8, 17]. It means that lymphatic vessels collecting from the right colon were capable of traversing SMV then approaching SMA. Therefore Japanese guidelines emphasized that D3 dissection required to remove the highest draining nodes, which might contain potential metastases [22]. Starting from 2016, our center has begun to develop an ‘artery-first’ approach to extend the internal boundary line of D3 cleaning from SMV to the right side wall of SMA [31]. With the deepening of understanding of the lymphatically draining rule, we have further performed resection along the midline of SMA in the caudal to cranial direction, which proved to be safe and feasible with the advantage of increasing lymph node yield. Performing the “artery-first” plus “caudal-to-cranial” technique could easy to expose the proper plane and reduce the manipulation of the tumor-bearing area so that the surgeon could better identify the retroperitoneal structures and more adopted the “no-touch” isolation principle [15, 31].

In this study, LNs distributed posterior to SMV, between SMV to SMA were observed and on the left side of SMA, during the laparoscopic radical colectomy for RCC. An important find in this article is the presence of regional LNs of RCC on the left side of SMA, especially under close to a physiological condition, which has not yet been reported. The above observation corresponds with the regional LNs distribution reported in the anatomical pieces of literature [8, 10]. In combination, lymphatic vessels collected right-hemi colon anteriorly/posteriorly across SMV passing through arteriovenous compartments, pooling at the MCA level, then traversing the surface of SMA and finally arriving at the left side of the artery.

However, the serum level of gastrointestinal tumor markers (CEA, CA199, and AFP) and tumor parameters (volume, differentiation, and pathological type) might weakly predict the appearance of regional LNs in the above area, or the clear trend would be seen if more cases included in the future. By analyzing the clinical data of 137 RCC patients who accepted “SMA-oriented” right
hemicolectomy in our center for nearly a year, the probability of LNs metastasis in our target area was 2.19%. Taking together, these observations explained the potential necessity for expanding the lymphadenectomy scope to some extent.

Except for keeping the mesenteric envelope more intact, locate the internal boundary to SMA can increase the lymph nodes yield in the surgical specimen. It’s currently believed that higher lymph nodes yield suggest a contribution of survival improvement regardless of the stage or LNs metastases [3, 32]. The growing number of LNs harvested resulting in an increased likelihood of identifying LNs harboring metastases, which is known as the "Will Rogers phenomenon". The World Congress of Gastroenterology recommended that at least 12 LNs should be pathologically assessed from surgical specimens to prevent under staging of colorectal cancer [33]. Although more emphasis should be placed on lymph nodes yield, fatty replacement is one of the major obstacles. This difficulty lied in the recognition of LNs in adipose tissue, and it had been reported that fatty infiltration was occurred in about 30% of LNs detected from specimen [4, 34]. Another obstacle is that more than 90% of LNs in mesocolons were smaller than 5mm and almost 60% were less than 2mm in maximum length [4]. Furthermore, RCC has been considered to have higher lymph nodes yield because of the longer colon needed to be respected, a greater proportion of tumors with microsatellite instability, and better antitumor immune response [35-38]. Thus some pathologists are encouraged to identify more than 12 LNs through other technology [39].

Several pieces of research had suggested that intraoperative application of dyes could depict the extent of mesenterectomy more successfully and safely, and allowed higher adequate lymph nodes harvest [40-42]. In 1975, Ponsky et al firstly proposed preoperative colonoscopic tattooing for surgeons to localize tumors at operation [43]. Later, widely using dyes including ICG, CNs, methylene blue, and indigo carmine, it had been accepted that an important advantage of dyes was real-time visualization of regional LNs without radiation expose and might heighten the sensitivity of LNs metastasis detection [42, 44, 45]. The proper molecular weight and hydrodynamic diameter of ICG and CNs render them the promising lymphatic contrast tools to when they enter the regional lymphatic system by peritumoral injection [46, 47]. In our research, the mean number of LNs harvested from resected species was 43.20±22.70 (mean±SD), greatly exceed the lymph nodes yield benchmark of 12 [10]. Furthermore, the application of ICG and CNs could efficiently identify LNs in the D3 area, thus might help to precisely positioning the internal boundary of right hemicolectomy. However, there were still 4 imaging failure case, which was possible that the dosage of dye was too low, the blockage of lymph vessels, or the error layer injection. This suggested a high operational skills requirement of endoscopists during colonoscopic dye injection.

Further than increasing lymph nodes yield, however, the complications after bared the SMA including gastrointestinal dysfunction, severe post-operative diarrhea, and lymphatic leakage, should also be taken into account [2]. In the early days of exploration, we often open the vascular sheath of SMA for intrathecal cleaning, and we found the incidence of lymphatic leakage and diarrhea was increased after surgery. With the deepening of research, we had confirmed that a tiny fascial that contained autonomic nerve fibers was surrounding the SMA. A postmortem study had reported the long lymph vessels within the lymphovascular bundle were crossing the SMV then cranial and caudal along the SMA [8]. In our study, neither ICG aggregation nor CNs-stained occur when we exposed the SMA sheath. Therefore, we prefer the viewpoint that the lymph vessels which attach to the sheath of SMA were delivering lymph from intrathecal tissues, like the nerve and vascular wall, instead of from mesentery. Taken together, it was reasonable to protect the autonomic nerve fibers by preserving the sheath of SMA, and consistent with the principle of standardized D3 cleaning.

However, this study had several limitations. First of all, our data was based on small sample size, hence the potential for a type II error was ineluctable, and the observation of 20 cases was not enough to come to the final conclusion. Therefore, a more consistent and comprehensive interpretation of LNs distributed in central areas should be defined in further exploration involving a larger cohort. Second, due to the heterogeneity between the application of ICG and CNs, the intraoperative LNs development might be greatly affected by different dyes, so a more refined grouping is necessary and the results of classifications might change when the consistency has been improved. Third, this study was retrospective, in the early stage of the LNs pathologic examination, the lymph node stations were simply separated into N1 (pericolic), N2 (intermediate), and N3 (central) stations. Thus the anatomic location between LNs and SMA/SMV in the D3 area did not be a specific description in the pathological report, and the report could not reflect the intraoperative observation well. Finally, because of the small sample size, no metastasis node was identified in the target anatomic area of 20 patients, the metastasis rate needs to be substantiated in a larger sample size.
Conclusion

In summary, our research described the regional LNs distribution pattern in the central area of the right-sided colon cancer. Lymphatic vessels collected right-hemi colon anteriorly/posteriorly across SMV, passing through arteriovenous compartments, pooling anterior to SMA at the MCA level (Figure 7). This phenomenon illustrated the potential necessity for expanding the scope of the tumor-draining area of RCC. Meanwhile, we recommended preserving the vascular sheath of SMA when SMA-oriented procedure was performed. This study was supporting a more radical anatomic destination in the lymphadenectomy for RCC and may form a basis for a real-time navigation laparoscopic surgery. Acknowledgements

Abbreviations

Regional lymph nodes (LNs);
Right-sided colon cancer (RCC);
Colorectal cancer (CRC);
Carbon Nanoparticles (CNs);
Indocyanine Green (ICG);
Superior mesenteric vein (SMV);
Superior mesenteric artery (SMA);
Ileocolic artery/vein (ICA/ICV);
Right colonic artery/vein (RCA/RCV);
Middle colonic artery/vein (MCA/MCV).

Declarations

Ethics approval and consent to participate

Informed consent for research was obtained from patients and all the patients were scheduled for laparoscopic radical resection. The protocol for this study was approved by the ethical committee of Guangdong Provincial Hospital of Chinese Medicine.

Consent for publication

The authors report no conflicts of interest, and manuscript is approved by all authors for publication.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions
Dechang Diao, Xiaochuang Feng and Hongming Li designed the study; Dechang Diao, Xiaochuang Feng, Yisen Ke contributed to the patient recruitment and collected the data. Weilin Liao and Jiahao Wang performed the statistical analysis. Dechang Diao, Jialiang Chen, and Jin Wan contributed to administrative, technical, or material support. Dechang Diao and Xiaochuang Feng wrote the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1: Baseline clinical characteristics of 20 patients with dye injection.
Table 2: Intraoperative details and lymph nodes harvested.

| Case | Gender | Age  | BMI   | Disease type  | Differentiation | Tumor size (cm) | Pathological stage | CEA (μg/L) | CA199 (U/ml) | AFP (ng/ml) |
|------|--------|------|-------|--------------|----------------|-----------------|-------------------|------------|--------------|-------------|
| 1    | Male   | 52   | 21.97 | Adenocarcinoma | Moderate       | 9.5×6.5         | T3N0M0            | 4.27       | 2.73         | 3.28        |
| 2    | Male   | 80   | 21.51 | Adenocarcinoma | Poor           | 14.0×8.5        | T4N2M0            | 0.97       | 3.41         | 1.03        |
| 3    | Male   | 61   | 18.45 | Adenocarcinoma | Moderate       | 7.0×5.0         | T3N0M0            | 17.17      | 9.91         | 3.58        |
| 4    | Male   | 53   | 20.20 | Adenocarcinoma | Moderate       | 3.8×3.5         | T3N0M0            | 2.67       | 20.92        | 3.24        |
| 5    | Female | 56   | 20.70 | Adenocarcinoma | Moderate       | 6.5×4.5         | T3N0M0            | 6.52       | 5.95         | 2.88        |
| 6    | Male   | 32   | 21.55 | Adenocarcinoma | Moderate       | 4.5×3.5         | T4N2M1            | 791.1      | 3.37         |             |
| 7    | Female | 67   | 22.19 | Adenocarcinoma | Moderate       | 6.5×6.0         | T4N0M1            | 10.8       | 22.60        | 1.78        |
| 8    | Male   | 53   | 21.43 | Adenocarcinoma | Moderate       | 6.5×4.2         | T3N0M0            | 3.63       | 19.24        | 3.33        |
| 9    | Female | 31   | 17.57 | Adenocarcinoma | Moderate       | 3.5×1.5         | T4N2M0            | 0.73       | 35.39        | 3.96        |
| 10   | Female | 39   | 24.89 | Adenocarcinoma | Poor           | 7.8×6.5         | T3N1M0            |            |              |             |
| 11   | Male   | 42   | 23.31 | Adenocarcinoma | Poor           | 4.5×3.0         | T4N2M0            | 12.35      | 34.63        | 1.49        |
| 12   | Male   | 56   | 20.37 | Adenocarcinoma | Moderate       | 3.0×2.0         | T4N1M0            | 0.36       | 2.62         | 3.44        |
| 13   | Female | 40   | 24.22 | Adenocarcinoma | Poor           | 3.5×3.0         | T3N0M0            | 1.43       | 50.23        | 1.35        |
| 14   | Male   | 51   | 25.16 | Adenocarcinoma | Moderate       | 5.5×4.0         | T3N0M0            | 3.15       | 0.60         | 4.54        |
| 15   | Male   | 59   | 21.59 | Adenocarcinoma | Moderate       |                 | T1N0M0            |            |              |             |
| 16   | Male   | 52   | 24.82 | Adenocarcinoma | Moderate       | 9.5×8.0         | T3N0M0            | 2.50       | 3.63         | 2.05        |
| 17   | Male   | 56   | 24.81 | Adenocarcinoma | Moderate       | 9.5×8.0         | T3N0M0            | 2.5        | 3.63         | 2.05        |
| 18   | Male   | 70   | 23.03 | Adenocarcinoma | Moderate       | 6.5×6.5         | T3N0M0            | 2.47       | 19.28        | 1.61        |
| 19   | Female | 81   | 17.1  | Adenocarcinoma | Moderate       | 4.5×4.0         | T4N1M0            | 4.64       | 1.14         | 5.72        |
| 20   | Female | 76   | 24.7  | Adenocarcinoma | Moderate       | 3.5×3.0         | T3N2M0            | 5.01       | 4.87         | 7.83        |
| Case | Dye | ICA | MCA | Total number of LN | LNs posterior to SMV | LNs between SMA/SMV | LNs anterior to SMA |
|------|-----|-----|-----|-------------------|---------------------|---------------------|---------------------|
| 1    | CNs | ant.| ant.| 54                | +                   | +                   | +                   |
| 2    | ICG | ant.| ant.| 16                | +                   | +                   | +                   |
| 3    | CNs | post.| ant.| 96                | +                   | +                   | +                   |
| 4    | CNs | ant.| ant.| 45                | +                   | +                   | +                   |
| 5    | ICG | post.|    | 46                | +                   | +                   | +                   |
| 6    | CNs | ant.| ant.| 49                | +                   | +                   | +                   |
| 7    | ICG | post.|    | 18                | +                   |                     |                     |
| 8    | CNs | ant.| ant.| 84                | +                   | +                   | +                   |
| 9    | ICG | post.| ant.| 53                | +                   | +                   | +                   |
| 10   | ICG | ant.| ant.| 31                | +                   | +                   | +                   |
| 11   | CNs | ant.| ant.| 47                | +                   |                     |                     |
| 12   | CNs | ant.| ant.| 78                | +                   | +                   | +                   |
| 13   | CNs | ant.| ant.| 17                | +                   |                     |                     |
| 14   | ICG | post.| ant.| 47                | +                   | +                   | +                   |
| 15   | CNs | ant.|    | 12                | +                   |                     | +                   |
| 16   | CNs | ant.| ant.| 41                | +                   |                     | +                   |
| 17   | CNs | post.| post.| 41              | +                   |                     | +                   |
| 18   | CNs | ant.| ant.| 38                | +                   |                     | +                   |
| 19   | CNS | ant.|    | 23                | +                   | +                   | +                   |
| 20   | IGC | ant.| ant.| 35                | +                   | +                   | +                   |

ant. = anterior; post. = posterior; + = Detected; Blank = Not detected.

**Figures**
Figure 1

The ICG imaging (A-D) or carbon-containing (E) LNs posterior to SMV. B-C: cleaning the LNs posterior to SMV.

Figure 2

The carbon-containing LNs between SMV/SMA.
Figure 3

The carbon-containing (A-B and D) or ICG imaging (C) LNs anterior to SMA.

Figure 4
ICG aggregation (A, B and D) or CNs-stained (C) LNs could not be observed in the vascular sheath of SMA.

Figure 5

Advantage and disadvantage of dyes application. A-B: It is easy to distinguish unequivocally regional LNs (white arrow) from adipose tissue or not regional LNs (blue arrow); C-F: ICG imaging (C-D) and INK staining failure (E-F); G: Box plots of total harvested lymph nodes in patients with dye-free or dye injection.
Figure 6

A case of lymph node metastasis in target area. A: An abnormally enlarged lymph node was observed between SMV and SMA on preprocedure CT image. B-D: The assessment of tumor progression in a follow up CT 6 years after surgery. E: Intraoperative photo during reoperation.
Figure 7

Schematic depiction of the regional LNs distributed pattern in our research.

Supplementary Files

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