Scientific Research Publishing

Journal of Cancer Therapy, 2019, 10, 134-145
http://www.scirp.org/journal/jct
ISSN Online: 2151-1942
ISSN Print: 2151-1934

Carbon Nanoparticles for Identifying Lymph Nodes during Surgery in Colorectal Cancer: A Meta-Analysis

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How to cite this paper: Liu, M., Shen, A., Li, W., Chen, L.H., Chen, X.F. and Sun, H. (2019) Carbon Nanoparticles for Identifying Lymph Nodes during Surgery in Colorectal Cancer: A Meta-Analysis. *Journal of Cancer Therapy*, **10**, 134-145.
https://doi.org/10.4236/jct.2019.102010

Received: January 6, 2019
Accepted: February 12, 2019
Published: February 15, 2019

Abstract

Aim: To investigative the efficacy of carbon nanoparticles (CNs) to identify the lymph nodes during radical surgery in colorectal cancer. Method: The MEDLINE, EMBASE and Cochrane Library databases were searched electronically to identify the studies that compared the use of CNs (CN group) with control group in patients undergoing colorectal cancer radical surgery (from January 2009 to November 2018). The primary outcome was the number of retrieved central lymph nodes. Results: This meta-analysis identified 2 randomized controlled trials and 5 non-randomized controlled trials. Compared with the control group, the CN group resulted in an average of 7.16 more lymph nodes removed per patient (WMD = 7.16, 95% CI = 3.76 to 10.57, $p < 0.01$), 7.26 minutes less required for retrieving lymph nodes (WMD = −7.26, 95% CI = −13.43 to −1.09, $p = 0.02$), and 15.1 ml less blood loss during operation (WMD = −15.11, 95% CI = −23.15 to −7.06, $p < 0.01$). Although there was no significant difference in the metastatic lymph nodes between the two groups (OR = 1.02, 95% CI = 0.79 to 1.31, $p = 0.87$), there was 1.45 times more metastatic lymph of the stained nodes in CN group than in the control group (OR = 1.45, 95% CI = 1.13 to 1.85, $p < 0.01$). In addition, lymph nodes less than 5 mm were detected significantly more in the CN group than in the control group (OR = 2.15, 95% CI = 1.77 to 2.63, $p < 0.01$). Conclusions: The technique of CNs labeled lymph node staining in curative colorectal carcinoma is easy and effective, which can improve the retrieved number of lymph nodes, especially for nodes < 5 mm. The black stained lymph node indicates higher risk of metastasis. Further high quality RCT is needed to verify these conclusions.

Keywords

Carbon Nanoparticles, Lymph Node, Colorectal Cancer

1. Introduction

Radical colorectal surgery is the main treatment for colorectal cancer [1]. The lymph node is the main way of colorectal cancer metastasis, so, it has a prognosis and therapeutic importance to stage lymph node metastasis accurately in patients with colorectal cancer [2]. In order to improve the accuracy of tumor staging, lymph node detection should be included as much as possible for pathological examination [3]. However, the traditional finger-pressure palpation method is time-consuming and easy to miss lymph nodes [4], especially the small lymph nodes and these lymph nodes also have the possibility of metastasis; small metastatic lymph node remnants are important causes of postoperative recurrence and death [5]. There are lots of studies reported; with the application of tracer, the retrieved rate of lymph nodes can be improved, especially the small ones [6] [7].

The carbon nanoparticles (CNs) can be injected as a suspension that can enter the lymphatic capillaries rather than the capillary vessels [8] [9]. After macrophage phagocytosis, CNs quickly gathers in the lymph nodes and dyes them black [10]. Because CNs are with high lymphatic system trends and specificity, their guidance value in the detection of the lymph node in a variety of solid tumor radical surgery has been confirmed [11]. However, no systematic review about the CNs value in radical colorectal surgery was reported.

Therefore, this meta-analysis was designed to evaluate the efficacy of CNs during radical colorectal surgery. This study included all comparative researches using CNs as a tracer for lymph node dissection or not during radical surgery for colorectal cancer. By summarizing initial recommendations on the injection site and total dose, and waiting time for the use of CNs in these studies, we can investigate the relationship between the number of nodes retrieved and the rate of node positivity, and evaluate the effect of submucosal CNs staining on improvements in lymph node detection and staging accuracy.

1.1. Identification of Trials

MEDLINE, EMBASE, PubMed, the Web of Science, and the Cochrane Library databases were systematically searched for all comparative studies from January 2009 to November 2018, using the keywords carbon nanoparticles, lymph node, colorectal cancer. The reference lists of relevant articles were manually searched to identify other relevant trials. Only trials published in English or Chinese were included. Studies in animals and review articles were excluded. Studies identified through the search were independently screened by two authors (Miao Liu and Ai Shen) for inclusion. Any disagreements were arbitrated by a third author

(Hao Sun).

Inclusion Criteria

Studies met the following criteria characteristics can included in this study. Included articles must compare the use of CNs with at least 1 control group which did not use a tracer during colorectal cancer under radical surgery. Only randomized controlled study (RCT) and non-randomized comparative study (NRCT) were included.

Subjects

Patients underwent a radical surgery for colorectal cancer with a diagnosis confirmed by pathology results.

Interventions

Patients in the experimental group were injected with CNs before surgery, and patients in the control group did not receive an injection of tracer.

Observation Indexes

The primary outcome was the number of retrieved central lymph nodes per patient. Other outcomes included the staining rates of lymph nodes, the rates of metastatic nodes in all retrieved central lymph nodes, operation time, time for dissection lymph nodes, blood loss, total number of lymph nodes and small ones (<5 mm). At least 1 index was described separately for each study group.

Exclusion Criteria

Non-independent clinical controlled trials, studies with a patient number < 15, or studies with incomplete data were excluded.

1.2. Quality Assessment

The quality of RCT studies was assessed according to the Jadad scoring system. The scale consists of 3 items: randomization (0 - 2 points), blinding (0 - 2 points), and descriptions of the withdrawals and dropouts (0 or 1 point). The total possible score was 5 points. Trials with a score of 0 to 2 were considered to be of low quality, and those with a score of 3 to 5 were considered to be of high quality (**Table 2**). For NRCTs, the Newcastle-Ottawa Scale was used (**Table 3**).

1.3. Statistical Analysis

All analysis was performed with Rev Man 5.2. Based on the results of heterogeneity tests and study designs, a random effects model was used for all analyses. Weighted mean differences (WMDs) were calculated for the continuous outcome variables, and odd ratios (ORs) and risk differences (RDs) were calculated for the dichotomous outcome variables. In addition, 95% confidence intervals (CIs) were calculated for the WMDs, ORs, and RDs. Differences were considered to be statically significant at $p < 0.05$. A sensitivity analysis was performed to exclusively examine the pooled results of these studies. If result of the comparison had changed, the result was presented.

2. Results

2.1. Study Selection and Description

A total of 156 references were initially identified. After reading titles and abstracts, 35 references were eliminated for non-colorectal cancers' study. Additional 28 references were excluded for non-clinical studies, duplicate publication and non-controlled trials (Figure 1). After carefully evaluating, there were 7 studies with high quality met the inclusion criteria [12]-[18]. Thus, 7 studies, including 921 patients, were included in this study (Table 1). Of the 921 patients, 531 (57.7%) patients in CN group, 390 (42.3%) patients in control group.

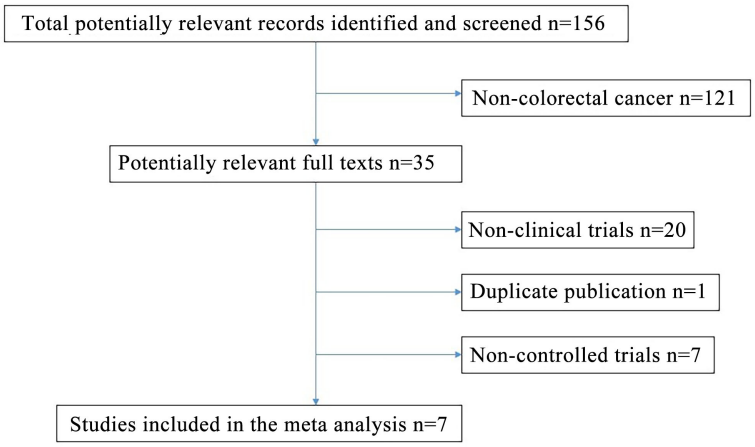

Figure 1. Flowchart showing the screening procedure used in the study.

Table 1. Characteristics of the 7 articles included in the meta-analysis.

Study	Tumor Type	Number	Study Design	Injection Site	Dose ml	Waiting Time	Laparoscopic	Indices
Cai *et al.* [13]	Colorectal	60	RCT	4-quadrant region around the mass	1	10 min	No	①④⑨
Zhang *et al.* [17]	Rectal	87	NRCT	Around the submucosa of the rectum	5	30 min	In 96% cases	①④
Wang *et al.* [18]	Rectal	152	NRCT	3 points around the submucosal layer of mass	0.5	1 day	in 97% cases	①②⑧
Yang *et al.* [12]	Colorectal	65	RCT	4 - 6 points around the mass	0.15 - 0.25	10 min	Yes	①②③④
Zhang *et al.* [16]	Colorectal	53	NRCT	4 points around the submucosal layer of mass	0.15 - 0.25	1 - 3 day	Yes	①②③④⑧
Wang *et al.* [15]	Colorectal	54	NRCT	4 points around the submucosal layer of mass	1	10 min	Yes	①⑤⑦⑧
Wang *et al.* [14]	Colorectal	470	NRCT	4 - 6 points around the mass	1	30 min	No	①②⑤⑦

Observation indices: ① the number of retrieved lymph nodes in the CN and control groups, ② the total metastatic rate of the retrieved lymph nodes in the CN and control groups, ③ the metastatic rate of stained/non-stained lymph nodes in the CN and control groups, ④ the number of tiny lymph nodes (<5 mm) in the CN and control groups, ⑤ the surgical time in the CN and control groups, ⑥ the lymph node dissection time between the CN and control groups, ⑦ the surgical blood loss in the CN and control groups, ⑧ the number of patients who had dissected lymph nodes > 12 in the CN and control groups, and ⑨ the number of lymph nodes in the CN and MB groups. CN: carbon nanoparticles; MB: methylene blue.

Table 2. Details of quality assessment of RCTs.

Study	Randomization	Concealment of Allocation	Blinding	Loss to Follow-Up, %	Quality Assessment
Cai *et al.* [13]	No detailed description	Only mentioned randomized	Unclear	0	2
Yang *et al.* [12]	Divided randomly into label group	Sealed envelopes	No blinding	0	3

Table 3. Details of the quality assessment of nonrandomized controlled trial.

Study	Study Design	Selection	Comparability	Exposure	Quality Assessment
Zhang *et al.* [17]	Prospective controlled study	4	2	2	8
Wang *et al.* [18]	Prospective controlled study	4	2	2	8
Zhang *et al.* [16]	Prospective controlled study	3	2	2	7
Wang *et al.* [15]	Retrospective study	3	2	2	6
Wang *et al.* [14]	Prospective controlled study	4	2	2	8

Study characteristics and quality assessment. Two of the 5 studies were RCTs, involving 125 patients [12] [13]. Three of the 5 studies were NRCTs, involving 796 patients. All patients had confirmed colorectal cancer, based on postoperative pathologic examinations. The characteristics of the 5 included studies are presented in **Table 1**. Most investigators preferred multipoint injections (3 to 6 points) around the tumor, with a total CNs dose of 0.15 to 5 ml and a 10 minutes to 3 days wait before surgery.

Intervention Effects

Number and metastatic rate of retrieved lymph nodes in the CN group and control group:

Compared with the control group, the use of CNs resulted in an average of 7.16 more lymph nodes removed per patient (WMD = 7.16, 95% CI = 3.76 to 10.57, $p < 0.01$, **Figure 2**), with high heterogeneity (I^2 = 95%, $p < 0.01$). Meta-analysis demonstrated that the total metastatic rate of the retrieved lymph node was not significantly different between the CN and control group (OR = 1.09, 95% CI = 0.84 to 1.41, $p = 0.53$, **Figure 3**). But of the metastatic rate, the stained lymph nodes of CN group was 1.45 times more than the control group which didn't use the tracer technique (OR = 1.45, 95% CI = 1.13 to 1.85, $p < 0.01$, **Figure 4**). Two studies have reported these data. Similarly, according to these two studies above, there was no significant differences between the

non-stained lymph nodes in the CN group and the found lymph nodes in the control group (OR = 1.31, 95% CI = 0.64 to 2.65, $p = 0.46$, Figure 5). Only two researches have statistically reported detection rates for the tiny lymph nodes which diameter < 5 mm in both groups and found that the number rate of the tiny lymph node was significant higher in the CN group than the control group (OR = 2.32, 95% CI = 1.69 to 3.18, $p < 0.01$, Figure 6). The comparison of patients with detected LNs numbers excess than 12 was reported in 3 studies, and the pooled results showed that the CN group was significantly superior to the control group (OR = 13.03, 95% CI = 2.79 to 60.96, $p < 0.01$, Figure 7).

2.2. Operation Situation

There's a small amount of studies reported the time of surgery, with respect to the general surgical time, the CN group is slightly less than the control group (WMD = −7.54, 95% CI = −13.81 to −1.28, $p = 0.02$, Figure 8). Meanwhile, the CN group has shorter lymph node dissection times (WMD = −7.30, 95% CI = −8.51 to −6.09, $p < 0.01$, Figure 9) and less surgical blood loss (WMD = −15.11, 95% CI = −23.15 to −7.06, $p < 0.01$, Figure 10).

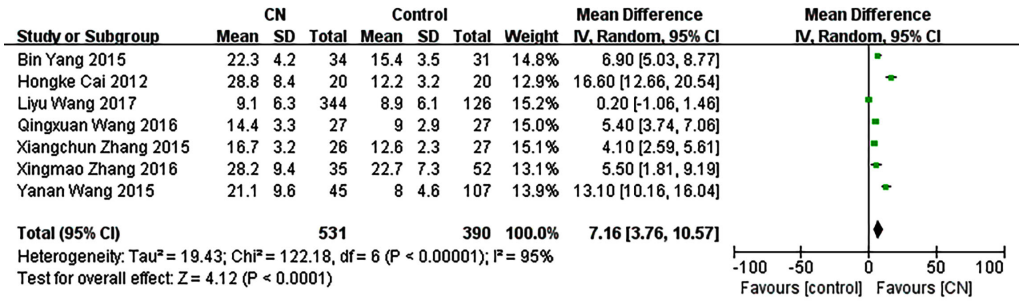

Figure 2. Total lymph nodes in the CN and control groups.

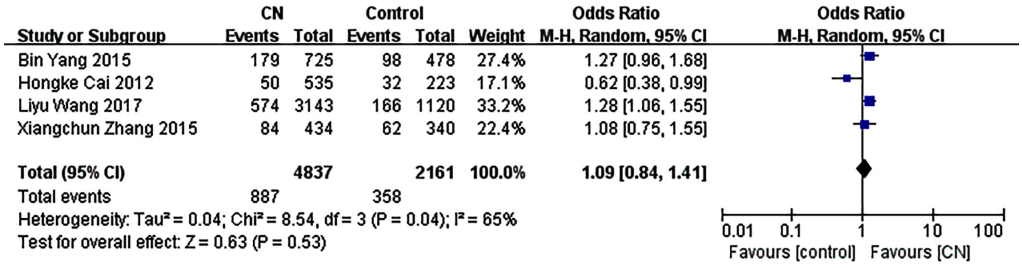

Figure 3. Total metastatic lymph nodes in the CN and control groups.

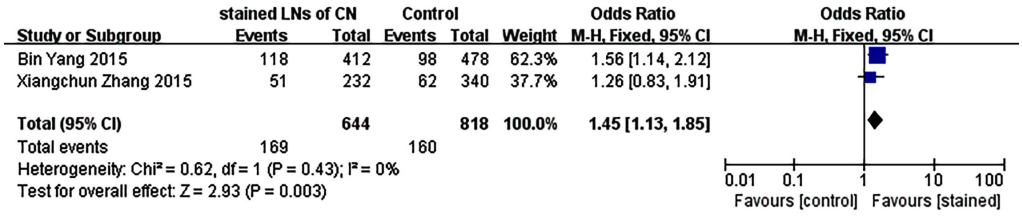

Figure 4. Metastatic lymph nodes in stained lymph nodes of CN and control group.

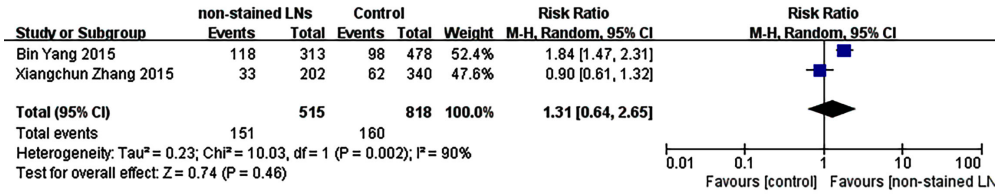

Figure 5. Metastatic lymph nodes in non-stained lymph nodes of CN and control group.

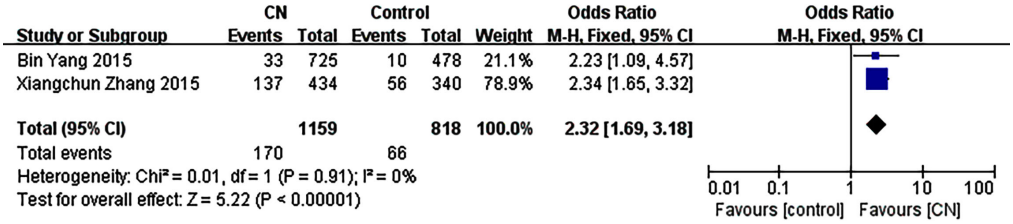

Figure 6. Tiny lymph node in CN and control group.

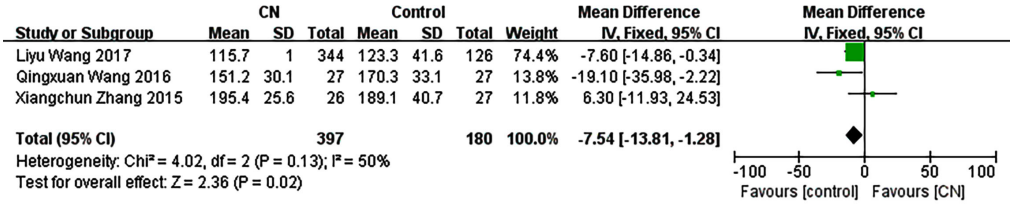

Figure 7. The number of patients of detected LNs > 12 in CN and control group.

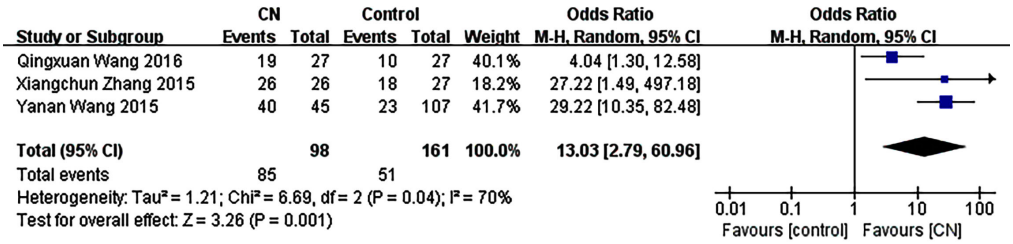

Figure 8. The time of surgery in CN and control group.

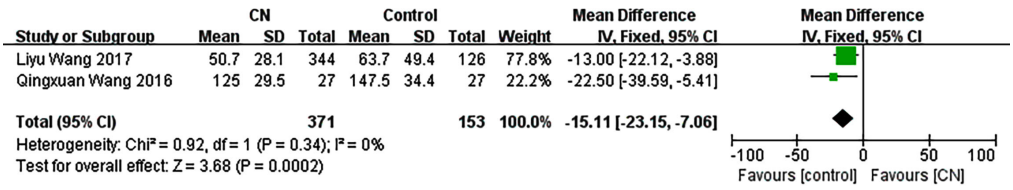

Figure 9. The time of lymph node dissection times in CN and control group.

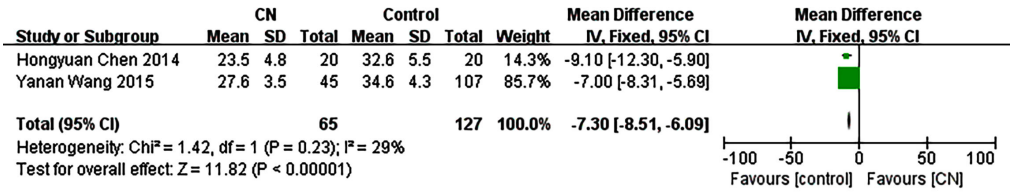

Figure 10. The surgical blood loss in CN and control group.

3. Discussion

Carbon nanosuspensions have highly lymphoid system tropism with an average diameter of 100 nm [19]. When injected into local tissue, it can be quickly consumed by macrophages. Due to the gap of capillary endothelial cells is 20 - 50 nm, and the gap of capillary lymphatic endothelial cell is 120 - 150 nm, and the basal membrane is not growth completely, so the CNs were not enter into the blood vessels but quickly enter into the lymphatic vessels, and stranded in the lymph nodes, dyed the lymph node to black, fulfilled the vital staining of tumor areas, achieved the tracer role [20].

At present, the advantages of laparoscopic radical resection for colon cancer have been widely recognized and this micro-invasive method was written into the NCCN guidelines for colon cancer treatment. This method has become the standard surgical procedure for colon cancer. In addition, laparoscopic surgery for rectal cancer has also been widely carried out [21].

Laparoscopic surgery for colorectal cancer has a magnification effect of 2 - 3 times, with clear anatomical space and structure, which is easy to identification and conducive to nerve protection and lymph node dissection, as well as complete mesenteric resection (CME) and total mesenteric resection (TME) [22]. However, the current standard of laparoscopic lymph node dissection of TME and CME in colorectal cancer surgery are difficult to achieve generally due to the level differed in different hospitals and doctors [23]. The most important metastatic route of colorectal cancer is through the lymph nodes pathway, and positive lymph node residue is an important cause of recurrence and death [24]. In particularly, about 8% - 12% colorectal cancer may have lymph node metastasis. Therefore, how to ensure the dissection of D3 lymph node and the realization of TME and CME are important clinical challenges at present, especially in Laparoscopic colorectal cancer surgery [25]. In this study, it was found that carbon nanoparticle tracer technology can help solve these problems. It has been report that that CNs in the operation is helpful for detect the sentinel lymph node, because the dyeing time is short, and the lymph node staining of each station is insufficient, so, injecting CNs into tumor area 1 - 3 days before operation may help the lymph node at each stage fully stained, and these lymph nodes can be used to clean the lymph nodes at all sites and avoid missing cancerous tissue [26]. For the staining lymph nodes other than the third station, the lymph nodes need not be routinely cleaned, but can be excised and sent to rapid frozen pathological examination [27]. In colorectal surgery, lymph nodes in the corresponding area should be cleaned if there is metastasis, and nerve damage should be avoided if there is no metastasis. Therefore, for beginners of laparoscopic colorectal surgery with inadequate management, the standard D3 lymph node cleaning is hard to reach. However, using CNs lymph tracer technology can guide intraoperative lymph node cleaning, ensure the D3 radical surgery done, and help handle with the black dye lymph nodes beyond D3 stage if needed [28]. Therefore, the use of carbon nanoparticles can reduce surgery time and reduce

bleeding.

CNs can improve the detection rate of lymph nodes, guide pathological staging and postoperative adjuvant therapy. From the 2010 Edition of NCCN guidelines for the diagnosis and treatment of colorectal cancer, sufficient number of lymph nodes no less than 12 should be included in the postoperative pathological examination to ensure the accuracy and reliability of the pathological result evaluation and guide the postoperative adjuvant treatment [3]. However, most of the lymph nodes detected by pathologists are retrieved by hand without a tracer, and their sensitivity is very low. Many of the smaller lymph nodes are hidden in the mesenterium and fat, these lymph nodes cannot be easily touched, so it is difficult to guarantee the number of detected lymph nodes. Some studies have found that 45.4% metastatic lymph nodes are small lymph nodes with a diameter less than 5 mm, which is easy to be omitted, leading to low pathological staging and ultimately affecting the assessment of treatment and prognosis [29]. Lymph nodes stained black with CNs are easy to identify and can help pathologists find these tiny metastatic nodes more quickly and more often. This systematic analysis shows that the CN stained can effectively improve the detected number of lymph nodes, especially the small lymph nodes, and improve the accuracy of pathological staging.

This study has some limitations. First, the available articles were limited to English or Chinese publications. No publications written in other languages were included. The quality assessment scores of the 2 included randomized controlled trials were relatively low with the lack of blinding; the other 5 studies were nonrandomized controlled trial. Thus, further research is needed to verify the conclusions in the present study.

4. Conclusion

Carbon nanoparticles are helpful in curative colorectal surgery. CNs can improve the retrieved number of lymph nodes, especially for nodes < 5 mm. The black stained lymph node indicates higher risk of metastasis. A large scale high quality randomized controlled study is needed to verify these conclusions.

Funding Information

This study was supported by the project from Chongqing Science and Technology Bureau (cstc2018jscx-msybX0156).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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