

Peer Review File

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Reviewer A

Comment 1: It would be helpful in this paper to include in the Introduction some of the background to the use of nanoparticles. Is there a precedent for the technique you describe? What is the time-course of nanoparticles travelling to the lymph nodes? Does this take longer for lymph nodes further away?

Reply 1: The use of carbon nanoparticles (CNs) as tracer in malignant tumors has been well described in gastric cancer, breast cancer, as well as in thyroid cancer. CNs would lead to a more extensive central lymph node dissection, while improve preservation of the parathyroid glands in lobectomy and total thyroidectomy. It usually takes about five minutes as the thyroid gland is stained black, followed by the lymph nodes. It takes longer for CN to travel to the lateral lymph nodes. However, the specific time was not well documented.

Changes in the text: We added some description of CN in the introduction section, paragraph 3: “Carbon nanoparticle suspension is composed of nanosized polymeric carbon granules that would be absorbed by macrophages accumulating in lymph nodes. It is widely used for LN tracing in multiple cancers. In thyroid cancer, the use of carbon nanoparticles leads to a more extensive central lymph node dissection, while improves preservation of the parathyroid glands in total thyroidectomy”.

Comment 2: In the Introduction (line 96) you cite the probability of lymph node metastasis as 90%. This seems very high and I am not sure other sources would agree. In this sentence (lines 95-96) you refer to “cervical LN metastasis” and lateral neck metastasis” – are these not the same?

Reply 2: Under certain circumstances, the probability of LN metastasis could be as high as 90%. However, in a common scenario, the probability of LN metastasis is 50%-60% as reported by several studies. We agree with the reviewer that this sentence should be revised. “Cervical LN metastasis” refers to both central LN metastasis and lateral LN metastasis.

Changes in the text: In introduction, paragraph 1, we revised the last sentence as “Preventive central and lateral neck dissection showed that even for microcarcinoma with cN0, the probability of cervical LN metastasis was as high as 50-80% and the probability of lateral neck metastasis was 30%-60%”. One additional citation was added to this sentence.

Comment 3: Methods: line 123. You should stipulate that patients gave informed consent. It is implied in the previous sentence but it is better to state this clearly.

Reply 3: Yes, all patients gave informed consent.

Changes in the text: In Materials, paragraph one, we added “All patients gave informed consent.” after the last sentence.

Comment 4: There are some areas where some rewording would help the reader. In lines 169-171 where the analysis is case-based rather than node-based, this was not immediately apparent, and I had to re-read the paragraph several times to get the point. Some rewording would help.

Reply 4: Thank you for your suggestion. We did some rewording in the text to make it easier to understand.

Changes in the text: We revised the first sentence of Result- paragraph 3, “Then we focus the analysis on LNs in compartment IIA, III, and IV, and performed the analysis on per patient basis instead of per node basis.”

Comment 5: Though you quote the sensitivity of other tests (e.g. in line 210), you do not give any figures for specificity and sensitivity for your technique, or values for positive or negative predictive value. I suspect they may not be that high but that should not deter you as this paper is basically a starting point, with potential in future studies to improve these further. It does provide a means of comparison with other techniques.

Reply 5: We did not specify the specificity and sensitivity for the technique since the data was not mature now. Future comparisons with other techniques would be helpful for surgeons to choose the proper tracer for lateral LN mapping.

Comment 6: You have avoided referring to any of the dyed lymph nodes as “sentinel nodes”. Is there a particular reason for this, or would you not see these as sentinel nodes?

Reply 6: Yes, we have avoided referring the dyed LN as sentinel nodes for several reasons. Sentinel LN usually refers to the first stop of LN metastasis in cancer. For PTC, the sentinel LN is mostly central LN, not lateral LN. Furthermore, sentinel LN biopsy is used for occult LN metastasis. However, in our study, we included patients with indeterminate LN, which is different from previous studies. Thus, using the term “sentinel LN” might be misleading in the current study.

Comment 7: The final section on limitations (lines 234-239) requires some expansion. Comparison with established sentinel node techniques would be appropriate. Whether sensitivity and specificity might be improved by using your technique alongside a second technique might be worth a mention.

Reply 7: Thank you for your suggestion. However, we mentioned this limitation in the last sentence that “Lastly, we did not compare carbon nanoparticle with other tracers used for LN mapping. Therefore, it remained unknown which tracer or the combination of tracers is the best choice for mapping LNs in PTC.”. Again, we did not compare established sentinel LN techniques since the study populations were different. Previous studies mainly focus on occult metastasis while the current one focus on indeterminate lateral LN.

Comment 8: Minor items around the English language. I would suggest you use the term “non-palpable” rather than unpalpable or impalpable. “Occlusion of” (meaning blockage, lines 106 & 119) – I suspect you mean “proximity to”.

Reply 8: Thank you for your suggestion. We have revised the language as indicated.

Changes in the text: Throughout the text, we have changed “unpalpable” to “non-palpable”. We also changed “occlusion of” to “proximity to”.

Reviewer B

Comment 1: In which respect does the method described by the author differ from that published

by the Department of Head and Neck Surgery, The First Hospital of Jiaying? (J Nanosci Nanotechnol. 2021 Nov 1;21(11):5408-5413. doi: 10.1166/jnn.2021.19472). This publication should be quoted and the differences should be discussed. In this paper the authors also conclude that the metastasis rate of the dyed lymph nodes in areas III and IV was significantly higher than that of non-dyed lymph nodes ($P < 0.05$) in 73 patients. To the same conclusion came also Biomed Res Int. 2021; 2021: 6693585. doi: 10.1155/2021/6693585 (quotation 23). Since these two studies have similar conclusions, the author should explain, how different their method is and underline the novelty of their study compared to those.

Reply 1: The discussion of Chen et al.'s work could be found in discussion, paragraph 4. Both these above studies used similar surgical technique to map the lateral LN as in our study, and they also identified that the metastasis rate of the dyed lymph nodes in areas III and IV was significantly higher than that of non-dyed lymph nodes. However, Chen et al.'s work discussed about clinically node-negative PTC, and only 10 sides/patients received neck dissection of compartment II-IV due to the positive result of frozen section, and their study population was different from the current study. Qian et al.'s work was a single center study with a lower rate of lateral LN metastasis. Unlike their studies, we included patients with indeterminate lateral LN and calculated the relative risks of metastasis in dyed LNs compared with undyed LNs in each neck compartment. We also conducted subgroup analysis and demonstrated that the distribution of dyed LNs was independent of clinical features, suggesting lateral LN mapping could be used in patients regardless of sex, age, multifocality, microscopic ETE and tumor location. We revised the paragraph as indicated by the reviewer.

Changes in the text: We added a paragraph of discussion in discussion, paragraph 2: "In previous studies, similar surgical technique has been described in lateral LN mapping in PTC. However, Chen et al. used endoscopy to explore the lateral neck for LN biopsy, and Qian et al. used open surgeries. Consistent with the current study, they both identified compartment III-IV exhibited higher metastasis rate in dyed LN. Nevertheless, unlike their studies, we conducted the first multi-center prospective study of carbon nanoparticle guided lateral neck mapping and calculated the relative risks of metastasis in dyed LNs compared with undyed LNs in each neck compartment. We also performed subgroup analysis and demonstrated that the distribution of dyed LNs was independent of clinical features, suggesting lateral LN mapping could be used

in patients regardless of sex, age, multifocality, microscopic ETE and tumor location.”

Comment 2: The median of LNs was 6 per case (range: 1-33). “ : was any patient with 1 lymph node resected included in this study ? I thought all patients received radical modified neck dissection – see 132-3.

Reply 2: Yes, all patients received radical modified neck dissection in the study. The median of dyed LNs was 6 per case, which is not all LNs, but only those dyed with carbon nanoparticles. The minimum number of dyed LN was 1 in this study, but the number of resected LN was not.

Comment 3: Conclusion: I am not sure I would call carbon nano particle a new method. I would rather write that this is the first prospective multicenter study using this method for mapping lateral lymph nodes in PTCs (there are several studies on CN particles in central lymph nodes in PTC).

Reply 3: Yes, we agree that it was not appropriate to call it a new method. The conclusion has been revised accordingly.

Changes in the text: In the abstract- conclusion, we revised the text as: “It was the first prospective multicenter study to map the lateral neck LNs with carbon nanoparticles, which could help surgeons visualize the suspicious LNs during surgery.” In the conclusion, we revised the text as: “This was the first prospective multicenter study using carbon nanoparticles to map the draining lateral LNs in PTC intraoperatively.”

Comment 4: what is known and what is new: “The management of the lateral neck remained controversial in PTC”. “ – I’d rather write ,, carbon nano particles have been previously shown to be a method for visualizing metastatic lymph node metastasis”.

Reply 4: We agree that it is appropriate to mention carbon nanoparticles have been previously used to visualize metastatic lymph node, especially central LNs.

Changes in the text: We added in the introduction, paragraph 3, “Carbon nanoparticle suspension is composed of nanosized polymeric carbon granules that would be absorbed by macrophages accumulating in lymph nodes. It is widely used for LN tracing in multiple cancers. In thyroid cancer, the use of carbon nanoparticles leads to a more extensive central lymph node

dissection, while improves preservation of the parathyroid glands in total thyroidectomy”. We also added in the discussion, paragraph 2 “In previous studies, similar surgical technique has been described in lateral LN mapping in PTC [11,12]. However, Chen et al. used endoscopy to explore the lateral neck for LN biopsy, and Qian et al. used open surgeries. Consistent with the current study, they both identified compartment III-IV exhibited higher metastasis rate in dyed LN. Nevertheless, unlike their studies, we conducted the first multi-center prospective study of carbon nanoparticle-guided lateral neck mapping and calculated the relative risks of metastasis in dyed LNs compared with undyed LNs in each neck compartment. We also performed subgroup analysis and demonstrated that the distribution of dyed LNs was independent of clinical features, suggesting lateral LN mapping could be used in patients regardless of sex, age, multifocality, microscopic ETE and tumor location.”

Comment 5:110-112: instead of ,, In the current study, we aimed to develop a new method to map the draining LNs in the lateral neck by injecting tracer during operation and explore its potential to guide lateral neck dissection. “In the current study, we aimed to find out if carbon nanoparticles could also be used to map the draining LNs in the lateral neck by injecting tracer during operation and explore its potential to guide lateral neck dissection.”

Reply 5: Thank you for your suggestion. The above sentence was revised accordingly.

Changes in the text: In introduction, the last paragraph, we revised the last sentence “In the current study, we aimed to find out if carbon nanoparticles could also be used to map the draining LNs in the lateral neck by injecting tracer during operation and explore its potential to guide lateral neck dissection.”

Comment 6: I believe the role of radioiodine treatment for cleaning micrometastasis should be discussed. Even if some patients have micrometastasis in laterl non-enlarged lymph node, they might be treated by radioiodine treatment.

Reply 6: It is generally believed that micrometastasis in PTC was not a significant risk factor for inferior survival, since it would not progress to clinically relevant even if untreated for years. Previous studies suggested that even though small-volume microscopic LN metastases appear to be present in up to 80% of patients diagnosed with papillary microcarcinoma, locoregional

recurrence rates in treated patients range from 2%–6% regardless of the extent of LN dissection and whether radioactive iodine (RAI) was given as adjuvant therapy after surgical resection. Another reason we did not discuss micrometastasis in the current study because we aim to discuss indeterminate lateral LN instead of occult lateral LN metastasis. We believe that these were different topics and the discussion of micrometastasis was beyond the scope of the current study.

Comment 7: 223 is „random lymph node biopsy “really performed by the authors ? With which results?

Reply 7: We believe that it might be inappropriate to say “random” LN biopsy. What we really wanted to express was unguided LN biopsy, compared with visualize LN dissection guided by carbon nanoparticles.

Changes in the text: We revised “random LN biopsy” to “unguided LN biopsy” both in the abstract and in the discussion.

Comment 8: Is there any possibility to use carbon nanoparticles if thyroid tissue has already been removed? To me this is another limitation of this method. Lateral lymph node dissection is not rarely performed months/years after thyroidectomy in clinical routine.

Reply 8: We believe it is not possible to use the carbon nanoparticles if the thyroid tissue has already been removed since the tracer has to be injected into the thyroid lobe. It is indeed another limitation of this method.

Changes in the text: In discussion, the last paragraph, we added “Then, since the tracer had to inject into the thyroid tissue, the application of this method was confined to patients without prior thyroidectomy.”

Comment 9: 241-44: I would rephrase the conclusion in “Carbon nanoparticles can be used for identifying PTC micrometastasis in lateral lymph nodes at the time of thyroidectomy”

Reply 9: As mentioned above, the primary aim of the current study was to map the draining lateral LNs and help surgeons visualize suspicious lateral LNs. These LNs were indeterminate LNs which was not proven metastatic by FNA but suspicious on ultrasound and/or CT.

Micrometastasis, on the other hand, referred to a pathological definition that metastasis was less than 0.2cm. Micrometastasis could not be recognized before surgery and was another topic of interest. We believe that these were different topics and the discussion of micrometastasis was beyond the scope of the current study.

Changes in the text: We revised the conclusion as “This was the first prospective multicenter study using carbon nanoparticles to map the draining lateral LNs in PTC intraoperatively. This surgical procedure could help surgeons visualize suspicious lateral LNs and serve as an evaluation method in the decision making of neck dissection.”