RESEARCH ARTICLE

The role of carbon nanoparticle in lymph node detection and parathyroid gland protection during thyroidectomy for non-anaplastic thyroid carcinoma- a meta-analysis

Shaowei Xu[1], Zhifeng Li[2], Manbin Xu[1], Hanwei Peng[1] *

1 Department of Head and Neck Surgery, Cancer Hospital, Shantou University Medical College, Shantou, China, **2** Department of Oncology, Cancer Hospital, Shantou University Medical College, Shantou, China

* penghanwei@126.com

Abstract

Objective

To assess the efficiency of the carbon nanoparticles (CNs) in lymph node identification and parathyroid gland (PG) protection during thyroidectomy for non-anaplastic thyroid carcinoma (N-ATC).

Methods

A systematic literature search for relevant literatures published up to December 2018 in PubMed, EMBASE, Web of Science and Cochrane Library was performed. Both English and Chinese literatures were retrieved and analyzed. Randomized controlled trials or non-randomized controlled trials comparing the use of CNs with the use of methylene blue or a blank control in patients undergoing thyroidectomy for N-ATC were enrolled in this study. The primary outcomes included the number of lymph nodes harvested, the rate of lymph nodes involved, and the rates of accidental parathyroidectomy, hypoparathyroidism, and hypocalcemia. Weighted mean differences (WMDs), odds ratios (ORs) and risk differences (RDs) were calculated for the dichotomous outcome variables. Between study heterogeneity was tested using the Q tests and the I^2 statistics. All analyses were performed using Review Manager (version 5.3.5).

Results

25 studies comprising 3266 patients were included in this analysis. The total number of lymph nodes harvested in the CNs groups was significantly higher than that in the control groups (WMD, 2.36; 95% CI, 1.40 to 3.32; P <0.01). Administrating CNs was associated with a lower incidence of accidental PG removal (OR = 0.28, 95% CI = 0.21 to 0.37, P<0.01) and lower rates of both postoperative transient hypoparathyroidism (OR = 0.46, 95% CI = 0.33 to 0.64, P <0.01) and transient hypocalcemia (OR = 0.46, 95% CI = 0.33 to 0.65, P <0.01). No significant difference was found concerning lymph node metastatic rates between CNs group and control group. Subgroup analysis indicated that the application of CNs in reoperation thyroidectomy reduced both the rate of transient hypoparathyroidism

Competing interests: The authors have declared that no competing interests exist.

(OR = 0.21, 95% CI = 0.06 to 0.75, P = 0.02) and the possibility of accidental PGs removal (OR = 0.21, 95% CI = 0.07 to 0.62, P = 0.004, P<0.05).

Conclusions

The application of CNs in thyroidectomy for N-ATC results in higher number of lymph node harvested and better PG protection during both initial and reoperation thyroidectomy.

Introduction

Thyroid cancer is one of the most common types of cancer in the world, the incidence of which increased dramatically in the recent two decades [1]. More than 95 percent of the thyroid carcinomas were non-anaplastic thyroid carcinoma (N-ATC), including papillary thyroid carcinoma, follicular thyroid carcinoma, and medullary thyroid carcinoma [1]. Total thyroidectomy combined with level II-VI neck dissection for clinically N1b cases or central compartmental dissection (CND) for cN0 cases is widely advocated as one of the standard treatment protocols for N-ATC, whereas lobectomy is only accepted for T1 cases without high risk factors [2–4]. However, it's a challenge for surgeons to perform a total thyroidectomy with or without CND due to the potential risk of postoperative transient hypocalcemia/hypoparathyroidism (incidence rate 20%-60%) or permanent ones (incidence rate 2%-7%) based on the previous reports and even higher for those who undergo a reoperation [5, 6].

Carbon nanoparticles (CNs) have been successfully attempted for lymph node dissection in breast carcinoma, gastric carcinoma, and a few other malignancies [7, 8]. CNs have a mean diameter of 150 nm larger than the size of the capillary endothelial cell gap (20–50 nm), and thus cannot enter the blood vessels. However, they can penetrate the lymphatic capillary endothelial cell gap (120–500 nm) and may be phagocytized by macrophages. Thus, these CNs specifically accumulate in the lymph nodes, staining them black and easily been identified with naked eyes [9, 10]. However, the parathyroid glands (PG) remains unstained due to their possible lack of lymphatics. This so called "negative development" not only increases the number of lymph nodes harvested during compartmental dissection, but also facilitates the surgeons' distinguishing of the PGs from the lymph nodes and decreases the possibility of unintentional removal of PGs [11–13]. The pattern of CNs metabolism is the hypothetic basis that CNs can be used as a tracer to detect the sentinel nodes.

In the past decade, CNs had been successfully attempted as a negative developer to protect parathyroid gland (PG) during initial thyroidectomy [14]. Although a couple of meta-analyses evaluating the value of CNs in initial thyroidectomy had been published, the data need to be updated [15, 16]. Furthermore, there are still doubts about the efficiency of CNs in reoperation thyroidectomy due to the hypothesis that the lymphatic capillaries may be destroyed during initial surgery [17, 18]. Therefore, we performed a meta-analysis with more comprehensive and updated studies to summarize the role of CNs in lymph node detection and PG protection during both initial and reoperation thyroidectomy.

Materials and methods

Search strategy

Two authors conducted independently a search for relevant literatures up to December 2018 in PubMed, EMBASE, Web of Science, and Cochrane Library. The following medical search headings were used: ("thyroid gland"[MeSH Terms] OR ("thyroid"[All Fields] AND

"gland"[All Fields]) OR "thyroid gland"[All Fields] OR "thyroid"[All Fields] OR "thyroid (usp)"[MeSH Terms] OR ("thyroid"[All Fields] AND "(usp)"[All Fields]) OR "thyroid (usp)"[All Fields]) and ("carbon"[MeSH Terms] OR "carbon"[All Fields]). We manually searched the references of eligible studies and ClinicalTrials.gov to ensure identification of relevant published and unpublished studies. Chance-corrected agreement (i.e. kappa) in the screening stages and the eventual final set of included studies was calculated.

Inclusion criteria

Studies included in the meta-analysis need to fulfill the following criteria: (1) human N-ATC confirmed by pathology; (2) patients underwent thyroidectomy/lobectomy and/or neck dissection; (3) studies designed to compare the use of CNs with the use of methylene blue (MB) or with blank control; (4) studies on human beings; (5) full text available in English or Chinese.

Exclusion criteria

Studies were excluded if they (1) had a sample size less than 15; (2) included pregnancy or adolescent (aged<16); (3) included patients with benign and malignant thyroid diseases, and complete data of the malignancies were unavailable. Exclusion criteria (2) was set to reduce the publication bias because most of the studies reported only the cases in adult or adolescents over 16 years old. When two or more studies were reported by the same authors and/or institution, either the most recent study or the higher quality study was included in the analysis to excluded the possible duplicate cases.

Data extraction

Two reviewers (SW Xu and ZF Li) independently performed the first-stage screening of titles and abstracts based on the inclusion criteria. In the second-stage screening, the two reviewers retrieved and reviewed the possible relevant articles in full text to confirm the included articles, and then they recorded the following data independently: first author, publication year, sample size, description of study population (age, sex), study design (RCT or NRCT), surgical procedure (CNs injection dose, points and waiting time), lymph nodes details (number of harvested and involved LN), parathyroid protection outcome. Outcome of parathyroid protection was evaluated based on the following parameters: number of the PGs identified and/or incidentally removed and patient numbers with transient or permanent postoperative hypoparathyroidism/hypocalcemia. Both hypoparathyroidism and hypocalcemia were accepted as parameters to judge parathyroid function impair, and the threshold values in each report were adopted. When hypoparathyroidism or hypocalcemia persisted for 6 months or more, it was defined as permanent, otherwise it was defined as transient. Any discrepancies were resolved by discussion or referred to the corresponding author (HW Peng).

Quality assessment

Concerning the quality of study design, the RCT was assessed according to the Jadad Scoring system, which 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. The Newcastle-Ottawa Scale was used for NRCT.

Statistical analysis

All analyses in the current meta-analysis were performed using RevMan 5.3.5 (free software downloaded from http://www.cochrane.org). The results are presented as weighted mean

differences (WMDs) and Odds ratios (ORs) with a 95% confidence interval (CI). A P value< 0.05 was considered statistically significant, except where otherwise specified. Moreover, study heterogeneities were quantified using the Q-test and the I^2 statistic. When $P>$ 0.1and $I^2<$ 50%, a fixed-effect model was used; otherwise, a random-effects model was applied. Possible publication bias was tested by Begg's funnel plot.

Results

Study selection and description

Fig 1 details the study flowchart of the initial search and the subsequent selection of relevant articles. The initial search retuned 4488 studies. In the first-stage screening, 4202 irrelevant references and 230 duplicates were excluded. The remaining 56 studies with full text were further evaluated in the second-stage screening, 31 studies of which were excluded due to the reasons listed in the diagram. Finally, 25 studies fulfilled the inclusion criteria for the meta-analysis [9–13, 18–37]. Begg's test for 25 studies present in Fig 2 and the Kappa Coefficient was 0.68 (95% CI:0.66–0.70). Table 1 summarizes all the individual studies and their characteristics. Of these 25 studies, 13 were RCT, 12 were NRCT. A total of 3266 patients were included in this meta-analysis, of which 1496 were included in the CN group, 1770 in the Control group (1496 in blank control and 181 in MB control). 3248 patients had PTC; 12 had FTC, and 6 had MTC. The two groups had no significant difference in terms of age (MD, -0.43; 95% CI, -1.35–1.26, P = 0.95) and sex (OR, 1.05; 95% CI, 0.87–1.25, P = 0.63). There were 3096 initial surgeries and 170 reoperations in this analysis.

The quality assessment details for the RCTs and NRCTs are presented in Tables 1 and 2 in S1 File.

Surgical procedure

The CNs were provided by Chongqing LUMMY Pharmaceutical Co., Ltd. In most of the studies, the CNs were injected underneath the fibrous thyroid capsules at two or three points around the tumor, 0.1–0.2ml for each point, with 5-10mins wait before thyroidectomy (Table 3 in S1 File).

Lymph node removal

Analysis of the number of harvested lymph nodes were possible in 21 studies. A random-effects model was applied in this analysis ($P<$0.01, I^2 = 97%). WMD analysis showed that the total number of harvested lymph nodes in the CN group was significantly higher than that in the control groups (WMD, 2.36; 95% CI, 1.40 to 3.32; $P<$0.01, Fig 3). The rate of LN black-stained varied between 73.3% and 95.3%. Eighteen out of the 21 studies provided adequate information for the analysis of LN metastatic rate (= number of tumors involved LN/ number of harvested LNs). A random-effects model was applied to assess heterogeneity ($P<$0.01, I^2 = 96%). No difference was found between the CNs group and the Control group regarding LN metastatic rate (OR = 1.07, 95% CI = 0.75 to 1.51, P = 0.71, Fig 4).

Parathyroid gland protection

A fixed-effects model was used to analyze the data (P = 0.89, I^2 = 0%). After analyzing the accidental removal of the PGs using 23 eligible studies, we found that the possibility of accidental PGs removal was decreased by 30% in CNs group compared with control group (OR = 0.28, 95% CI = 0.21 to 0.37, $P<$0.01, Fig 5).
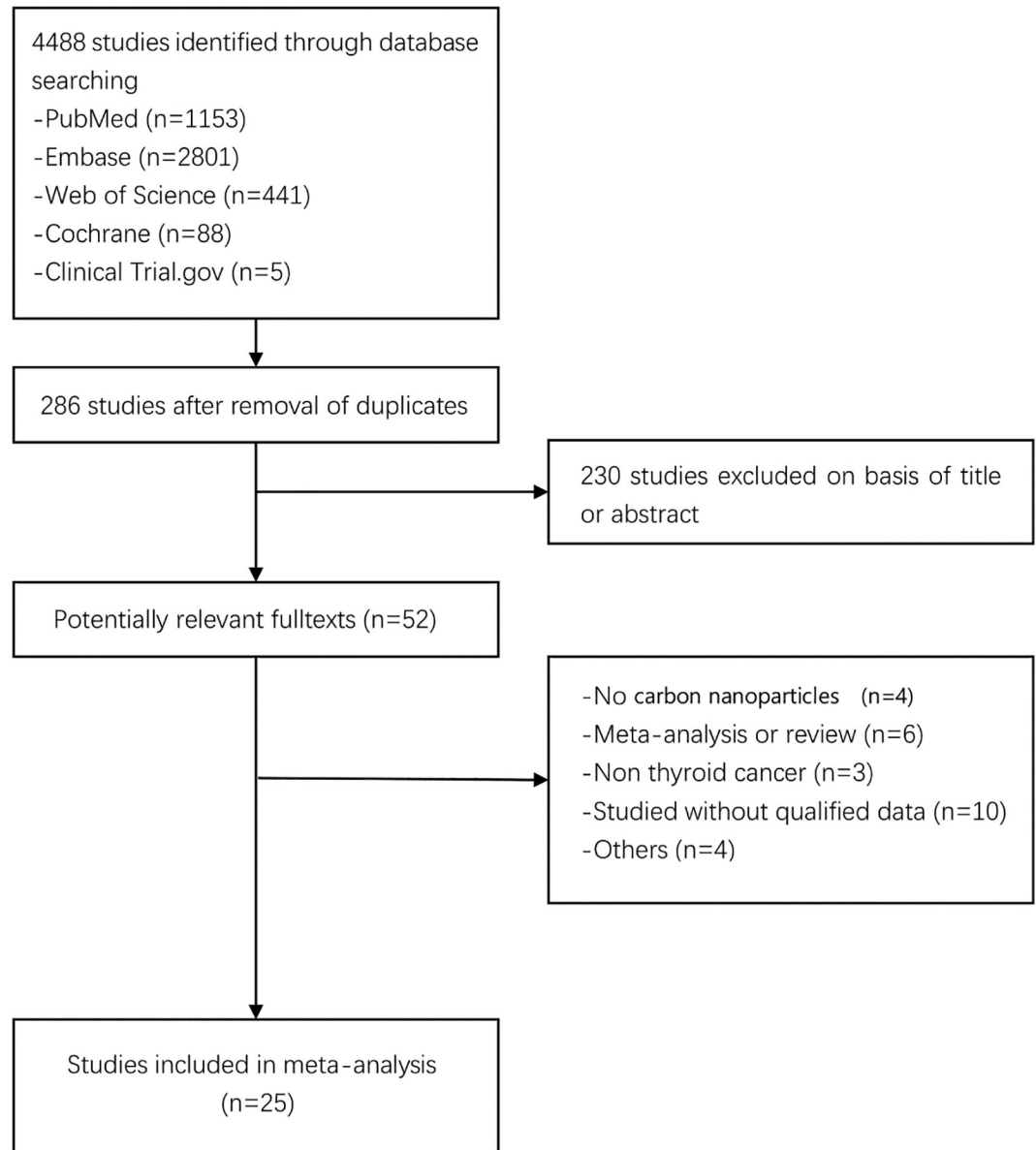
Fig 1. Study flow diagram.

The overall postoperative transient hypoparathyroidism data were available in 16 studies. A random-effects model was used, $P<0.04$, $I^2 = 42\%$), and subgroup analysis of transient hypocalcemia was possible 15 studies (A random-effects model was applied, $P<0.08$, $I^2 = 36\%$). Data analysis showed that the application of CN decreased the rate of both postoperative transient hypoparathyroidism and transient hypocalcemia equally by 46% (OR = 0.46, 95% CI = 0.33 to 0.64, $P<0.01$, Table 2, Fig 1 in S2 File; OR = 0.46, 95% CI = 0.33 to 0.65, $P<0.01$, Table 2, Fig 2 in S2 File). A random-effects model was applied to our data ($P = 0.44$, $I^2 = 0\%$), and 7 studies had a followed-up time of at least 6 months, thus the data of permanent hypocalcemia were analyzed. No significant difference concerning permanent hypocalcemia was found between the CNs group and control group (OR = 0.55, 95% CI = 0.09 to 3.43, $P = 0.52$, Table 2, Fig 3 in S2 File).
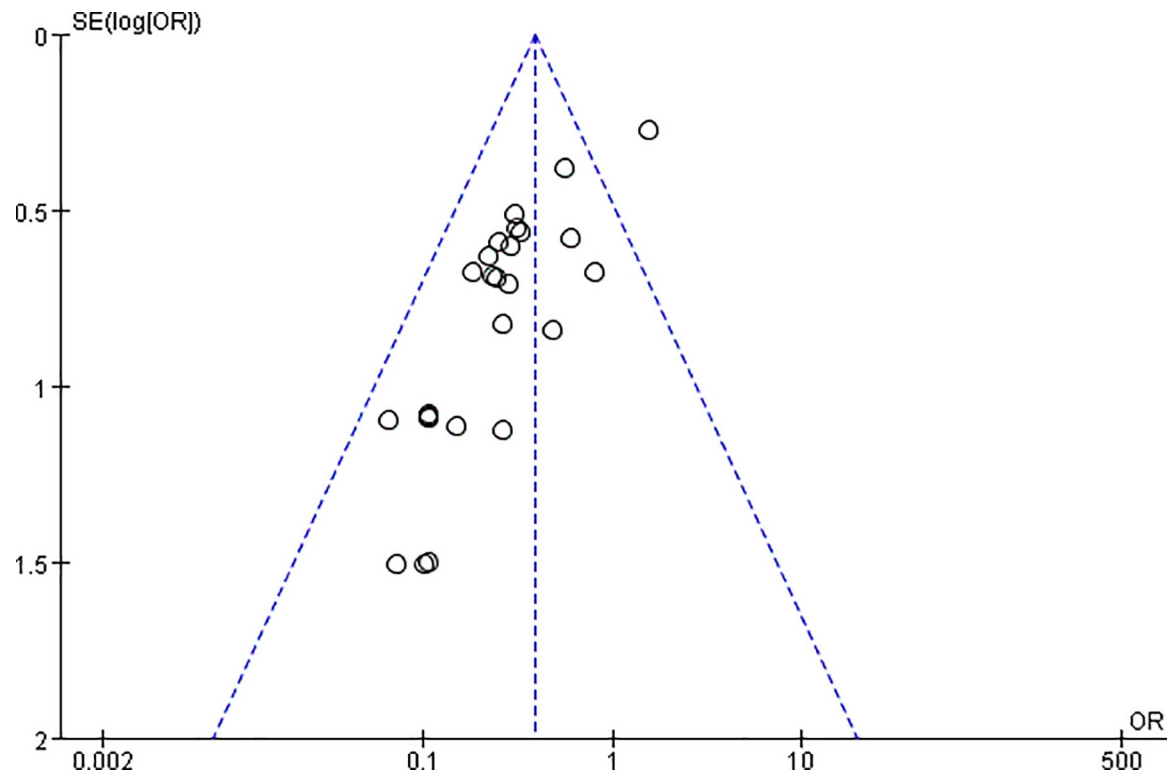
Fig 2. The funnel plots of the publication bias calculated from the Begg's test.

CNs application during reoperation

Two out the 25 included studies focused on reoperation thyroidectomy. Subgroup analysis of the data using a random-effects model demonstrated that the use of CNs significantly reduced the postoperative transient hypoparathyroidism rate by 21% (OR = 0.21, 95% CI = 0.06 to 0.75, P = 0.02, Table 2, Fig 4 in S2 File), and the possibility of accidental PGs removal was decreased by 21% (OR = 0.21, 95% CI = 0.07 to 0.62 P = 0.004, Table 2, Fig 5 in S2 File).

Discussion

Protection of the PGs and the recurrent laryngeal nerves are important techniques related to thyroidectomy, particularly total thyroidectomy combined with CND or reoperation thyroidectomy. Anatomically, the location of PGs, particularly the lower two, are variable, which results in a high risk of being accidentally removed during surgery [38]. Removal of all PGs inevitably leads to transient or permanent hypoparathyroidism and hypocalcemia, which has an adverse impact on the quality of life of the patients. Exposure and identification of the PGs intraoperatively facilitates preservation of the PG function. In addition, PTC has a trend of involving youngers and the 5-year survival rate is reported up to 98% [1]. As a result, omission of micro-metastases of the central compartmental lymph nodes may lead to reoperation, which may subsequently increase the incidence of complications and mortality. Therefore, effective techniques are urgently needed to improve the thoroughness of CND while preserving the PGs, particularly for reoperation.

CNs, approved by China Food and Drug Administration, have been efficiently attempted to assist lymph node dissection for gastric cancer, breast cancer and other malignancies [7, 8]. In the recent years, CNs are used as a novel and safe surgical technique to trace the regional

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

Study	Study period	Group	Patients (N)	Age	M	F	Etiology
Bai, Y.C (2013)	Jun 2010 to Mar 2012	Experimental	48	46.3±9.2	9	39	48PTC
		Control	73	N	7	33	73PTC
Chaojie (2016)	Feb2011 to Feb 2014	Experimental	64	N	11	53	64PTC
		Control	52	N	9	43	52PTC
Chen, W (2014)	Jan2013 to Dec 2013	Experimental	36	38.23±10.67	5	31	36PTC
		Control	36	34.64±8.75	8	28	36PTC
Deng, W (2014)	July 2011 to Dec 2013	Experimental	18	N	4	14	18PTC
		Control	33	N	12	21	33PTC
Fu, H (2017)	Oct 2015 to May 2016	Experimental	75	45.41±10.2	21	54	75PTC
		Control	73	45.32±9.74	21	52	7PTC
Gao, B (2015)	Jan 2012 to Dec 2014	Experimental	27	49.4±2.5	2	25	27PTC
		Control	27	52.5±1.8	2	25	27PTC
Gao, Q (2014)	Jan2010 to Oct 2012	Experimental	50	42±1.28	12	38	50PTC
		Control	50	N	9	41	50PTC
Gu, J (2015)	Jun 2012 and Aug 2014	Experimental	50	46.98±9.027	10	40	47PTC+1FTC+2MTC
		Control	50	47.76±13.91	6	44	48PTC+1FTC+1MTC
Hao, R. T (2012)	Jan2008 to Dec 2009	Experimental	100	41	14	86	100PTC
		Control	100	44	11	89	100PTC
Liu, F (2017)	Apr 2016 and Feb 2017	Experimental	48	51.98 ± 3.1	N	N	48PTC
		Control	48	51.98 ± 3.1	N	N	48PTC
Liu, Y (2018)	Feb 2013 to May 2015	Experimental	45	46.17 ±10.20	17	28	45PTC
		Control	47	45.39±12.03	12	35	47PTC
Long, M (2017)	Jan2012 to May 2013	Experimental	42	44.5 ± 9.6	9	33	42PTC
		Control	46	43.8 ± 10.3	11	35	46PTC
Shen, H (2014)	Mar 2012 to Jul 2013	Experimental	45	N	N	N	42PTC+3FTC
		Control	64	N	N	N	57PTC+6FTC+1MTC
Shi, C (2016)	Jan2014 and Feb 2015	Experimental	52	45.2±5.8	6	46	52PTC
		Control	45	42 ±4.3	6	39	45PTC
Su, A. P (2016)	Apr 2013 to Mar 2015	Experimental	195	43.38 ± 11.65	57	138	55PTC
		Control	181	45.67 ± 13.50	55	126	181PTC
Tian, W (2014)	Apr 2012 to Oct 2013	Experimental	50	36.4±2.5	5	45	50PTC
		Control	50	44.5±5.8	11	39	46PTC+4FTC
Wang, B (2015)	Mar 2013 to Mar 2014	Experimental	28	30.25 ± 6.04	1	27	28PTC
		Control	27	29.44 ± 6.27	2	25	27PTC
Wang, B (2016)	Jan2013 to Jan2014	Experimental	90	44.36 ± 11.48	25	65	90PTC
		Control	141	44.09 ± 12.41	37	104	141PTC
Wang, X. L (2009)	NM	Experimental	18	44	10	8	17PTC+1MTC
		Control	18	40	7	11	16PTC+1FTC+1MTC
Xu, X. F (2017)	Sep 2013 to Aug 2014	Experimental	57	45.37±10.71	5	52	57PTC
		Control	57	42.68±14.43	4	53	57PTC
Xue, S (2018)	Jan2010 to Dec 2012	Experimental	106	44.88 ± 7.78	20	86	106PTC
		Control	300	44.35 ± 10.28	66	234	300PTC
Yu, W (2016).	Aug 2012 to Jun 2013	Experimental	41	41.6±17.1	8	33	41PTC
		Control	41	41.7±18.9	11	30	41PTC
Yu, WB (2016)	Jan2012 to Jun 2013	Experimental	70	44.5 ± 17.4	14	56	70PTC
		Control	70	45.5 ± 19.0	17	53	70PTC
Zhu H (2017)	Jan 2013 to Feb 2015	Experimental	60	60.4±7.18	24	36	60PTC

(Continued)

Table 1. (Continued)

Study	Study period	Group	Patients (N)	Age	M	F	Etiology
		Control	60	62.5±7.65	25	35	60PTC
Zhu, Y (2016)	Apr 2010 to Apr 2011	Experimental	81	46.75±12.09	14	67	81PTC
		Control	81	44.31± 10.37	16	65	81PTC

M/F = male/female; CN = carbon nanoparticles; PTC = papillary thyroid cancer; FTC = follicular thyroid cancer; MTC = medullary thyroid cancer

lymph nodes and localize the PG during thyroidectomy [23]. So far, no toxic side effects have been reported in humans. However, when the CNs were injected improperly and they spread out of the thyroid capsule, they may stain the surgical field, which makes surgical procedure more difficult [23]. In most of the included studies, the dose of CNs is 0.1 to 0.2 ml per point and 2–3 points around tumor within the thyroid capsule were recommended. After being properly injected, the CNs enter the lymphatic capillaries and stain lymph nodes in 10-15min, rather than PGs and the recurrent laryngeal nerve [10, 13, 18–20, 27, 32–37]. It is hypothesized that this technique facilitates intraoperative identification of LNs and improves thoroughness of LN dissection for thyroid carcinoma.

The current meta-analysis demonstrates that the total number of lymph node harvested in the CN group was approximately 2.36 more than that in the control groups. But the ratio of metastatic lymph nodes has no significant difference between the two groups. The results were similar to previous studies. This denotes that CNs play a key role in accurate identification of the lymph nodes, but they cannot improve the detection of metastatic lymph nodes. The
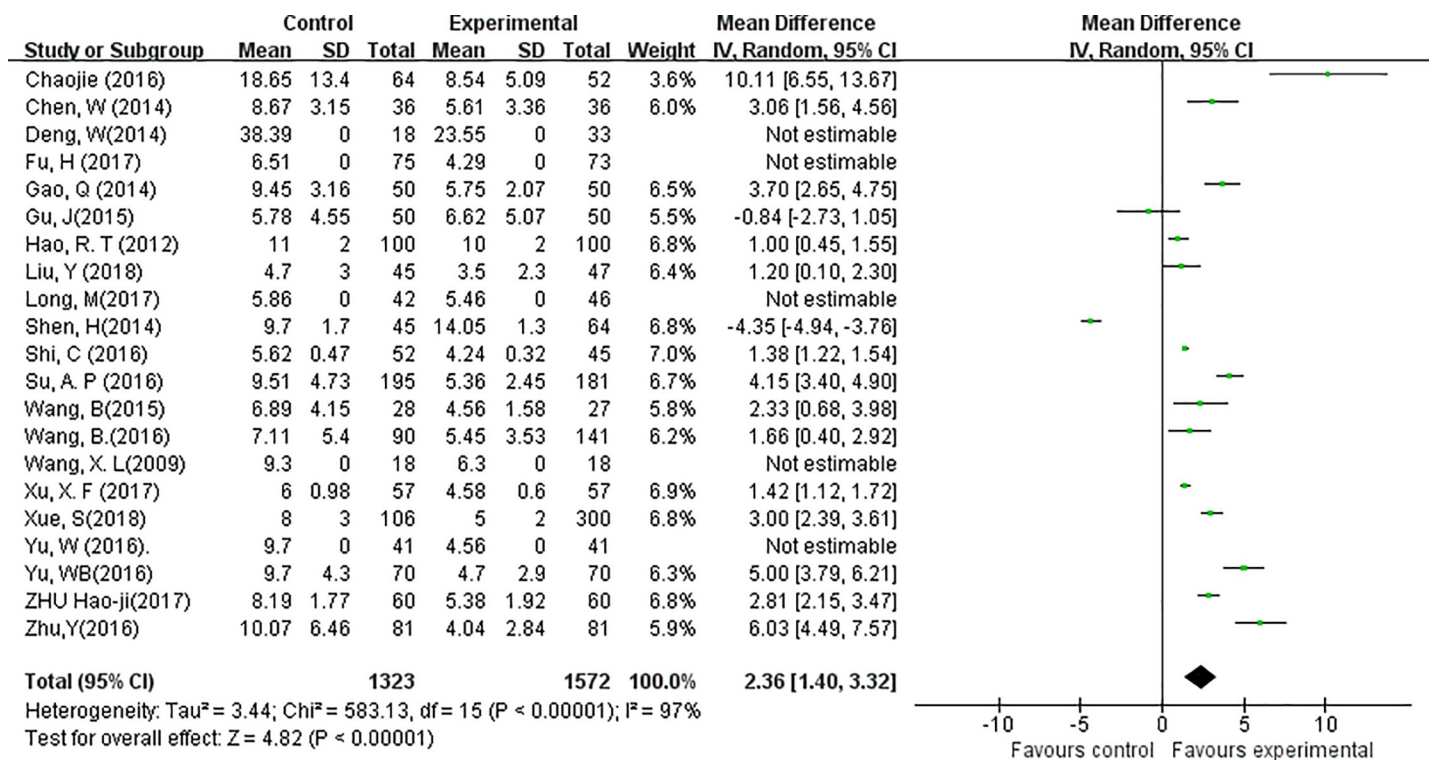

Fig 3. Forest plots of the total number of lymph nodes harvested in groups. (Experimental = Carbon nanoparticle group, Control = Blank or methylene blue group, Total = total number of LN harvested, Mean = the average number of LN harvested in each patient, SD = Standard deviation).
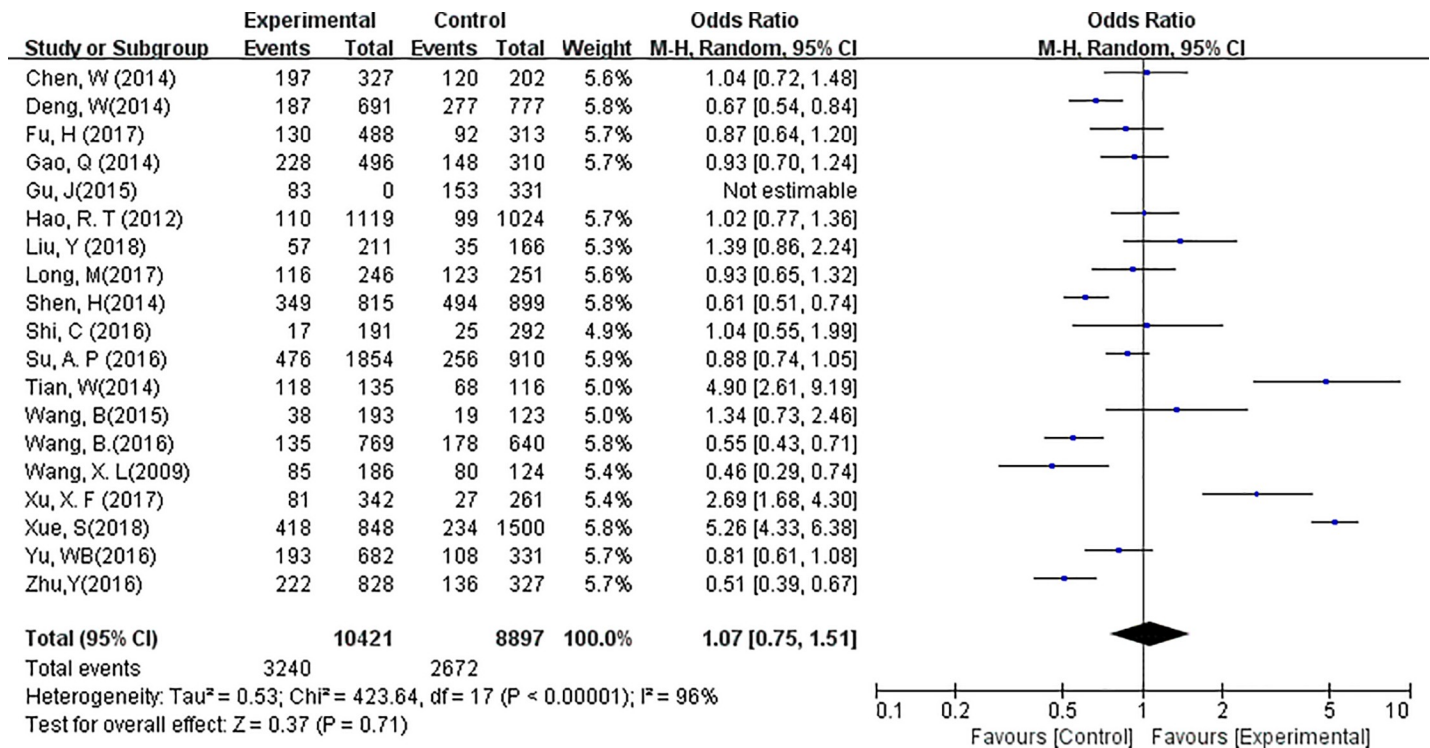
Study or Subgroup	Experimental Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Chen, W (2014)	197	327	120	202	5.6%	1.04 [0.72, 1.48]
Deng, W(2014)	187	691	277	777	5.8%	0.67 [0.54, 0.84]
Fu, H (2017)	130	488	92	313	5.7%	0.87 [0.64, 1.20]
Gao, Q (2014)	228	496	148	310	5.7%	0.93 [0.70, 1.24]
Gu, J(2015)	83	0	153	331		Not estimable
Hao, R. T (2012)	110	1119	99	1024	5.7%	1.02 [0.77, 1.36]
Liu, Y (2018)	57	211	35	166	5.3%	1.39 [0.86, 2.24]
Long, M(2017)	116	246	123	251	5.6%	0.93 [0.65, 1.32]
Shen, H(2014)	349	815	494	899	5.8%	0.61 [0.51, 0.74]
Shi, C (2016)	17	191	25	292	4.9%	1.04 [0.55, 1.99]
Su, A. P (2016)	476	1854	256	910	5.9%	0.88 [0.74, 1.05]
Tian, W(2014)	118	135	68	116	5.0%	4.90 [2.61, 9.19]
Wang, B(2015)	38	193	19	123	5.0%	1.34 [0.73, 2.46]
Wang, B.(2016)	135	769	178	640	5.8%	0.55 [0.43, 0.71]
Wang, X. L(2009)	85	186	80	124	5.4%	0.46 [0.29, 0.74]
Xu, X. F (2017)	81	342	27	261	5.4%	2.69 [1.68, 4.30]
Xue, S(2018)	418	848	234	1500	5.8%	5.26 [4.33, 6.38]
Yu, WB(2016)	193	682	108	331	5.7%	0.81 [0.61, 1.08]
Zhu,Y(2016)	222	828	136	327	5.7%	0.51 [0.39, 0.67]
Total (95% CI)		10421		8897	100.0%	1.07 [0.75, 1.51]
Total events	3240		2672			

Heterogeneity: Tau² = 0.53; Chi² = 423.64, df = 17 (P < 0.00001); I² = 96%
Test for overall effect: Z = 0.37 (P = 0.71)

Fig 4. Forest plots of the total metastatic rate of lymph nodes harvested in groups. (Experimental = Carbon nanoparticle group, Control = Blank or methylene blue group, Total = total number of LN, Events = the number of LN with metastasis).

possible explanation is that CNs facilitate identification of the tiny LNs which are black dyed by both surgeons and pathologists. However, CNs have no tumor trophism and they dye both the normal LNs and the metastatic LNs black without preference [26, 35].

Hypoparathyroidism is a common complication after thyroid surgery, especially after reoperation.

It is a disorder characterized by hypocalcemia, low or inappropriately normal intact parathyroid hormone (PTH) levels, and often hyperphosphatemia, which may be transient when it recovers within a few weeks or one month after thyroid surgery, or permanent when hypoparathyroidism persists for at least six months postoperatively. The incidence of transient hypoparathyroidism had been reported to be 20% to 60%, while that of permanent hypoparathyroidism was 0% to 7% [5, 6]. Our study revealed that application of CNs during thyroidectomy with or without CND reduces the incidence of accidental PG removal by approximately 30% and reduces the incidence of both postoperative transient hypoparathyroidism and transient hypocalcemia equally by 46%. However, there were still doubts about whether this technique leads to protection of long-term parathyroid function. In our subgroup analysis of permanent hypocalcemia using 7 studies who had a followed-up period of at least 6 months, no significant better PG function outcome was found in CNs group. We hypothesize that CNs facilitate identification of the PGs and lead to better protection of the glands' structures and blood supply. However, the benefit still cannot reach a statistic significance base on the data of these 7 studies yet.

It is assumed that lymphatic tracer cannot accurately drains to the sentinel nodes, the first lymphatic station from the primary cancer in case of reoperation, because the local structures could possibly be destroyed in the initial surgery. Whether previous surgeries disturb the role
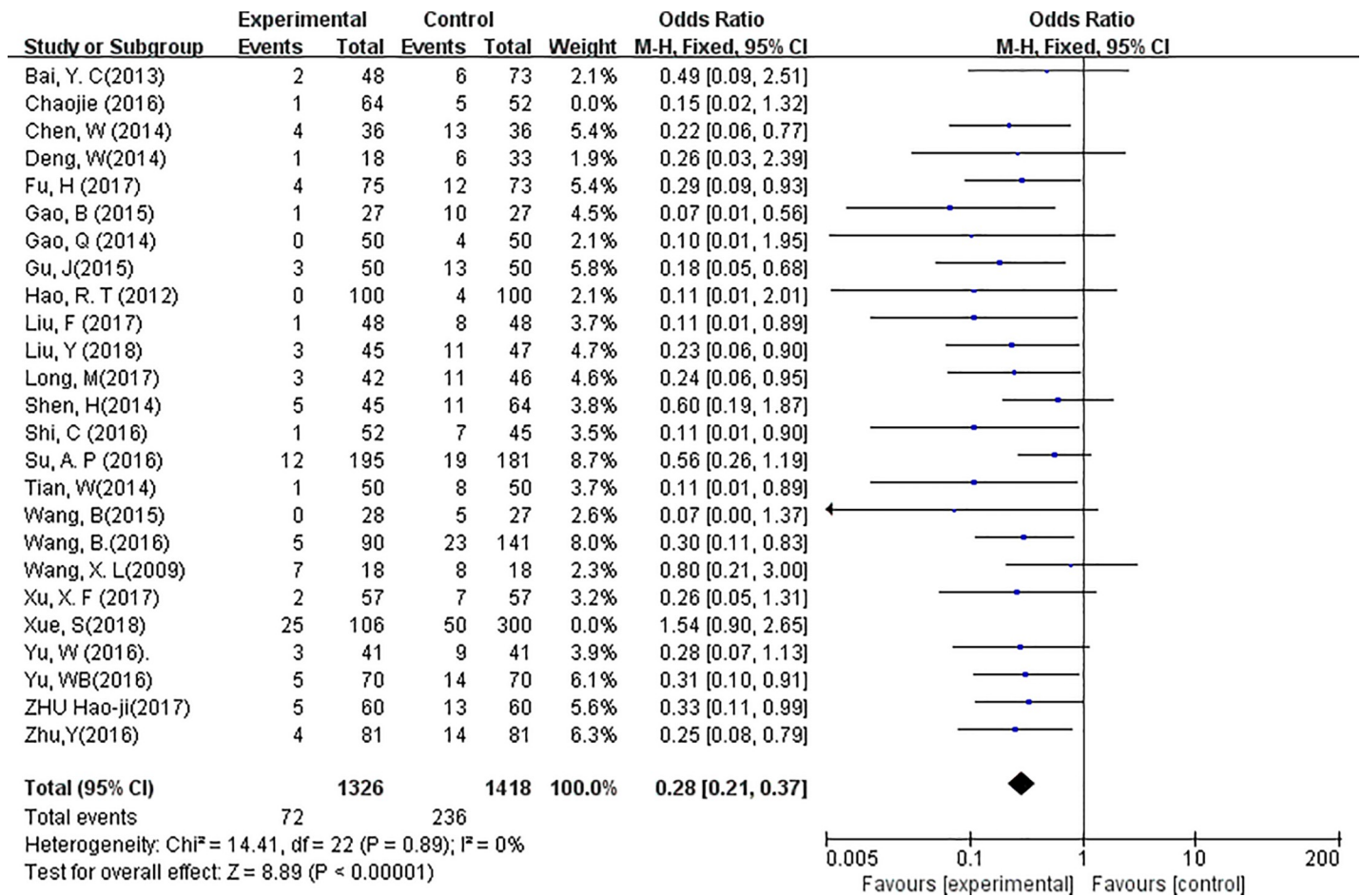
Study or Subgroup	Experimental Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Bai, Y. C(2013)	2	48	6	73	2.1%	0.49 [0.09, 2.51]
Chaojie (2016)	1	64	5	52	0.0%	0.15 [0.02, 1.32]
Chen, W (2014)	4	36	13	36	5.4%	0.22 [0.06, 0.77]
Deng, W(2014)	1	18	6	33	1.9%	0.26 [0.03, 2.39]
Fu, H (2017)	4	75	12	73	5.4%	0.29 [0.09, 0.93]
Gao, B (2015)	1	27	10	27	4.5%	0.07 [0.01, 0.56]
Gao, Q (2014)	0	50	4	50	2.1%	0.10 [0.01, 1.95]
Gu, J(2015)	3	50	13	50	5.8%	0.18 [0.05, 0.68]
Hao, R. T (2012)	0	100	4	100	2.1%	0.11 [0.01, 2.01]
Liu, F (2017)	1	48	8	48	3.7%	0.11 [0.01, 0.89]
Liu, Y (2018)	3	45	11	47	4.7%	0.23 [0.06, 0.90]
Long, M(2017)	3	42	11	46	4.6%	0.24 [0.06, 0.95]
Shen, H(2014)	5	45	11	64	3.8%	0.60 [0.19, 1.87]
Shi, C (2016)	1	52	7	45	3.5%	0.11 [0.01, 0.90]
Su, A. P (2016)	12	195	19	181	8.7%	0.56 [0.26, 1.19]
Tian, W(2014)	1	50	8	50	3.7%	0.11 [0.01, 0.89]
Wang, B(2015)	0	28	5	27	2.6%	0.07 [0.00, 1.37]
Wang, B.(2016)	5	90	23	141	8.0%	0.30 [0.11, 0.83]
Wang, X. L(2009)	7	18	8	18	2.3%	0.80 [0.21, 3.00]
Xu, X. F (2017)	2	57	7	57	3.2%	0.26 [0.05, 1.31]
Xue, S(2018)	25	106	50	300	0.0%	1.54 [0.90, 2.65]
Yu, W (2016).	3	41	9	41	3.9%	0.28 [0.07, 1.13]
Yu, WB(2016)	5	70	14	70	6.1%	0.31 [0.10, 0.91]
ZHU Hao-ji(2017)	5	60	13	60	5.6%	0.33 [0.11, 0.99]
Zhu,Y(2016)	4	81	14	81	6.3%	0.25 [0.08, 0.79]
Total (95% CI)		**1326**		**1418**	**100.0%**	**0.28 [0.21, 0.37]**
Total events	72		236			

Heterogeneity: Chi² = 14.41, df = 22 (P = 0.89); I² = 0%
Test for overall effect: Z = 8.89 (P < 0.00001)

Fig 5. Forest plots of accidental parathyroid removal rate in groups. (Experimental = Carbon nanoparticle group, Control = Blank or methylene blue group, Total = The number of patient, Events = the number of parathyroid glands removed accidentally).

https://doi.org/10.1371/journal.pone.0223627.g005

of CNs in black-staining the lymph nodes and negative developing the PGs during thyroidectomy remains unknown. To the best of our knowledge, no previous meta-analysis concerned the outcome of the application of CNs for reoperation thyroidectomy has been reported so far. Our systemic review retrieved only 2 studies who focused on CNs application for reoperation thyroidectomy. These reports demonstrated CNs technique not only have a benefit of PGs identification but also decrease the incidence of postoperative transient hypoparathyroidism

Table 2. Summary for subgroup analysis in the meta-analysis.

Subgroup analysis	Studies	Experiment (n)	Control (n)	OR	I^2(%)	P
The number of LN harvested	21	1323	1572	(WMD)2.36	97	<0.01
The metastatic rate of LN	18	1208	1469	1.07	96	0.71
Accidental parathyroid gland removal	23	1326	1418	0.3	0	<0.01
Transient hypoparathyroidism.	16	1017	1275	0.46	42	<0.01
Transient hypocalcemia	15	881	1147	0.46	36	<0.01
Permanent hypocalcemia	7	590	840	0.50	0	0.45
Reoperation transient hypoparathyroidism	2	91	79	0.21	25	0.02
Reoperation accidental PGs removal	2	91	79	0.21	0	0.004

https://doi.org/10.1371/journal.pone.0223627.t002

by 20% [26, 35]. We believe although the disturbance of the drainage routes of the CNs leads to failure of sentinel node localization, it does not impact their drainage to the regional LNs, which facilitates identification of the black-stained LN and negative developing of the PGs.

The current meta-analysis has some limitations and the results should be interpreted with caution. First, all the studies including in this meta-analysis were conduct only in China and the subjects were all Chinese. Second, long-term PG function outcome is difficult to figure out because most of the studies had a follow-up period of less than 6 months. Third, average Jadad Score and Newcastle-Ottawa score were only 3 and 7.4 respectively, which indicate the study design was of moderate quality. Thus, further research is needed to verify the conclusions of the current study.

Conclusions

This is the meta-analysis so far, firstly focused on the application of CNs during both initial thyroidectomy and reoperation thyroidectomy for N-ATC. This meta-analysis demonstrates that the application of CN for thyroidectomy improve the lymph node detection and PG protection not only for initial surgery but also reoperation.

Supporting information

S1 Checklist. PRISMA 2009 checklist.
(DOC)

S1 File.
(DOCX)

S2 File.
(DOCX)

S3 File.
(DOCX)

Author Contributions

Conceptualization: Shaowei Xu, Zhifeng Li.

Data curation: Shaowei Xu, Manbin Xu.

Formal analysis: Shaowei Xu, Zhifeng Li, Manbin Xu, Hanwei Peng.

Investigation: Shaowei Xu, Zhifeng Li, Manbin Xu.

Methodology: Shaowei Xu, Manbin Xu.

Supervision: Hanwei Peng.

Writing – original draft: Shaowei Xu.

Writing – review & editing: Shaowei Xu, Hanwei Peng.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin. 2019; 69(1):7–34. https://doi.org/10.3322/caac.21551 PMID: 30620402

2. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and

Differentiated Thyroid Cancer. Thyroid: official journal of the American Thyroid Association. 2016; 26 (1):1–133.

3. Haugen BR. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: What is new and what has changed? Cancer. 2017; 123(3):372–81. https://doi.org/10.1002/cncr.30360 PMID: 27741354

4. Ya M. [Interpretation of the management guidelines for patients with thyroid nodules and differentiated thyroid cancer (2012 Chinese edition)]. Lin chuang er bi yan hou tou jing wai ke za zhi = Journal of clinical otorhinolaryngology, head, and neck surgery. 2013; 27(16):917–20. PMID: 24260876

5. Dedivitis RA, Aires FT, Cernea CR. Hypoparathyroidism after thyroidectomy: prevention, assessment and management. Curr Opin Otolaryngol Head Neck Surg. 2017; 25(2):142–6. https://doi.org/10.1097/MOO.0000000000000346 PMID: 28267706

6. Shoback DM, Bilezikian JP, Costa AG, Dempster D, Dralle H, Khan AA, et al. Presentation of Hypoparathyroidism: Etiologies and Clinical Features. J Clin Endocrinol Metab. 2016; 101(6):2300–12. https://doi.org/10.1210/jc.2015-3909 PMID: 26943721

7. Sawai K, Hagiwara A, Shimotsuma M, Sakakibara T, Imanishi T, Takemoto Y, et al. Rationale of lymph node dissection for breast cancer—from the viewpoint of analysis of axillary lymphatic flow using activated carbon particle CH40. Gan To Kagaku Ryoho. 1996; 23 Suppl 1:30–5. PMID: 8702308

8. Okamoto K, Sawai K, Minato H, Yada H, Shirasu M, Sakakura C, et al. Number and anatomical extent of lymph node metastases in gastric cancer: analysis using intra-lymph node injection of activated carbon particles (CH40). Jpn J Clin Oncol. 1999; 29(2):74–7. https://doi.org/10.1093/jjco/29.2.74 PMID: 10089947

9. Gu J, Wang J, Nie X, Wang W, Shang J. Potential role for carbon nanoparticles identification and preservation in situ of parathyroid glands during total thyroidectomy and central compartment node dissection. International journal of clinical and experimental medicine. 2015; 8(6):9640–8. PMID: 26309638

10. Zhu H-jCM-fZY-f. Protective effect of nano-carbon tracers on the parathyroid glands. Chinese journal of tissue engineering research. 2016; 20(30):4476–82.

11. Liu Y, Li L, Yu J, Fan YX, Lu XB. Carbon nanoparticle lymph node tracer improves the outcomes of surgical treatment in papillary thyroid cancer. Cancer biomarkers: section A of Disease markers. 2018; 23 (2):227–33. https://doi.org/10.3233/CBM-181386 PMID: 30198867

12. Wang B, Qiu NC, Zhang W, Shan CX, Jiang ZG, Liu S, et al. The role of carbon nanoparticles in identifying lymph nodes and preserving parathyroid in total endoscopic surgery of thyroid carcinoma. Surgical endoscopy. 2015; 29(10):2914–20. https://doi.org/10.1007/s00464-014-4020-x PMID: 25761552

13. Su AP, Wei T, Liu F, Gong YP, Li ZH, Zhu JQ. Use of carbon nanoparticles to improve the dissection of lymph nodes and the identification of parathyroid glands during thyroidectomy for papillary thyroid cancer. International journal of clinical and experimental medicine. 2016; 9(10):19529–36.

14. Li J, Li X, Wang Z. Negative developing of parathyroid using carbon nanoparticles during thyroid surgery. Gland surgery. 2013; 2(2):100–1. https://doi.org/10.3978/j.issn.2227-684X.2013.04.05 PMID: 25083465

15. Li Y, Jian WH, Guo ZM, Li QL, Lin SJ, Huang HY. A Meta-analysis of Carbon Nanoparticles for Identifying Lymph Nodes and Protecting Parathyroid Glands during Surgery. Otolaryngology—head and neck surgery: official journal of American Academy of Otolaryngology-Head and Neck Surgery. 2015; 152 (6):1007–16. https://doi.org/10.1177/0194599815580765 PMID: 25897006

16. Su AP, Wei T, Gong YP, Gong RX, Li ZH, Zhu JQ. Carbon nanoparticles improve lymph node dissection and parathyroid gland protection during thyroidectomy: a systematic review and meta-analysis. International journal of clinical and experimental medicine. 2018; 11(2):463–73.

17. Liu X, Chang S, Jiang X, Huang P, Yuan Z. Identifying Parathyroid Glands with Carbon Nanoparticle Suspension Does Not Help Protect Parathyroid Function in Thyroid Surgery. Surgical innovation. 2016; 23(4):381–9. https://doi.org/10.1177/1553350615624787 PMID: 26783268

18. Shen H, Wei B, Feng S, Zhou Q. [Efficiency of carbon nanoparticles in level VI lymphadenectomy for thyroid carcinoma and prevention of postoperative hypoparathyroidism]. Zhonghua er bi yan hou tou jing wai ke za zhi = Chinese journal of otorhinolaryngology head and neck surgery. 2014; 49(10):817–20. PMID: 25567436

19. Bai YC, Cheng RC, Hong WJ, Ma YH, Qian J, Zhang JM. [Thyroid lymphography:a new clinical approach for protecting parathyroid in surgery]. Zhonghua er bi yan hou tou jing wai ke za zhi = Chinese journal of otorhinolaryngology head and neck surgery. 2013; 48(9):721–5. PMID: 24330873

20. Xue S, Ren P, Wang P, Chen G. Short and Long-Term Potential Role of Carbon Nanoparticles in Total Thyroidectomy with Central Lymph Node Dissection. Scientific reports. 2018; 8(1):11936. https://doi.org/10.1038/s41598-018-30299-8 PMID: 30093623

21. Hao RT, Chen J, Zhao LH, Liu C, Wang OC, Huang GL, et al. Sentinel lymph node biopsy using carbon nanoparticles for Chinese patients with papillary thyroid microcarcinoma. Eur J Surg Oncol. 2012; 38 (8):718–24. https://doi.org/10.1016/j.ejso.2012.02.001 PMID: 22521260

22. Liu F, Zhu Y, Qian Y, Zhang J, Zhang Y, Zhang Y. Recognition of sentinel lymph nodes in patients with papillary thyroid cancer by nano-carbon and methylene blue. Pakistan journal of medical sciences. 2017; 33(6):1485–9. https://doi.org/10.12669/pjms.336.13940 PMID: 29492083

23. Yu W, Zhu L, Xu G, Song Y, Li G, Zhang N. Potential role of carbon nanoparticles in protection of para-thyroid glands in patients with papillary thyroid cancer. Medicine. 2016; 95(42):e5002. https://doi.org/10.1097/MD.0000000000005002 PMID: 27759629

24. Yu W, Cao X, Xu G, Song Y, Li G, Zheng H, et al. Potential role for carbon nanoparticles to guide central neck dissection in patients with papillary thyroid cancer. Surgery. 2016; 160(3):755–61. https://doi.org/10.1016/j.surg.2016.04.017 PMID: 27241116

25. Wang XL, Wu YH, Xu ZG, Ni S, Liu J. Parathyroid glands are differentiated from lymph node by acti-vated-carbon particles. Zhonghua er bi yan hou tou jing wai ke za zhi [Chinese journal of otorhinolaryn-gology head and neck surgery]. 2009; 44(2):136–40. PMID: 19558888

26. Chaojie Z, Shanshan L, Zhigong Z, Jie H, Shuwen X, Peizhi F, et al. Evaluation of the clinical value of carbon nanoparticles as lymph node tracer in differentiated thyroid carcinoma requiring reoperation. International journal of clinical oncology. 2016; 21(1):68–74. https://doi.org/10.1007/s10147-015-0855-y PMID: 26081253

27. Shi C, Tian B, Li S, Shi T, Qin H, Liu S. Enhanced identification and functional protective role of carbon nanoparticles on parathyroid in thyroid cancer surgery: A retrospective Chinese population study. Medi-cine. 2016; 95(46):e5148. https://doi.org/10.1097/MD.0000000000005148 PMID: 27861338

28. Gao Q, Zhao D. [Clinical application of carbon nanoparticles labeled lymph node in cervical lymph node dissection with papillary thyroid cancer staged preoperatively as N0]. Lin chuang er bi yan hou tou jing wai ke za zhi = Journal of clinical otorhinolaryngology, head, and neck surgery. 2014; 28(24):1938–40. PMID: 25895310

29. Deng W, Li H, Chen Y, Gao Y, Huang H, Lin S, et al. [Clinical application of carbon nanoparticles in sur-gery for papillary thyroid carcinoma in young patients]. Zhonghua er bi yan hou tou jing wai ke za zhi = Chinese journal of otorhinolaryngology head and neck surgery. 2014; 49(10):812–6. PMID: 25567435

30. Zhu Y, Chen X, Zhang H, Chen L, Zhou S, Wu K, et al. Carbon nanoparticle-guided central lymph node dissection in clinically node-negative patients with papillary thyroid carcinoma. Head & neck. 2016; 38 (6):840–5. https://doi.org/10.1002/hed.24060 PMID: 25832013

31. Long M, Luo D, Diao F, Huang M, Huang K, Peng X, et al. A Carbon Nanoparticle Lymphatic Tracer Pro-tected Parathyroid Glands During Radical Thyroidectomy for Papillary Thyroid Non-Microcarcinoma. Surgical innovation. 2017; 24(1):29–34. https://doi.org/10.1177/1553350616668088 PMID: 27634477

32. Tian W, Jiang Y, Gao B, Zhang X, Zhang S, Zhao J, et al. Application of nano-carbon in lymph node dis-section for thyroid cancer and protection of parathyroid glands. Medical science monitor: international medical journal of experimental and clinical research. 2014; 20:1925–30. https://doi.org/10.12659/MSM.890721 PMID: 25311844

33. Chen W, Lv Y, Xie R, Xu D, Yu J. Application of lymphatic mapping to recognize and protect parathyroid in thyroid carcinoma surgery by using carbon nanoparticles. Lin chuang er bi yan hou tou jing wai ke za zhi [Journal of clinical otorhinolaryngology, head, and neck surgery]. 2014; 28(24):1918–20, 24.

34. Xu XF, Gu J. The application of carbon nanoparticles in the lymph node biopsy of cN0 papillary thyroid carcinoma: a randomized controlled clinical trial. Asian journal of surgery / Asian Surgical Association. 2017; 40(5):345–9.

35. Gao B, Tian W, Jiang Y, Zhang S, Guo L, Zhao J, et al. Application of carbon nanoparticles for parathy-roid protection in reoperation of thyroid diseases. International journal of clinical and experimental medi-cine. 2015; 8(12):22254–61. PMID: 26885201

36. Wang B, Du ZP, Qiu NC, Liu ME, Liu S, Jiang DZ, et al. Application of carbon nanoparticles accelerates the rapid recovery of parathyroid function during thyroid carcinoma surgery with central lymph node dis-section: A retrospective cohort study. International journal of surgery (London, England). 2016; 36(Pt A):164–9. https://doi.org/10.1016/j.ijsu.2016.10.037 PMID: 27989915

37. Fu H, Zhang ZL, Tang ZN, Liu QL. [Application of carbon nanoparticle in neck lymph node dissection for papillary thyroid carcinoma]. Lin chuang er bi yan hou tou jing wai ke za zhi = Journal of clinical otorhino-laryngology, head, and neck surgery. 2017; 31(14):1089–92. https://doi.org/10.13201/j.issn.1001-1781.2017.14.009 PMID: 29798247

38. Sadowski SM, Fortuny JV, Triponez F. A reappraisal of vascular anatomy of the parathyroid gland based on fluorescence techniques. Gland surgery. 2017; 6:S30–S7.