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Original Article

Mitoxantrone hydrochloride injection for tracing helps to decrease parathyroid gland resection and increase lymph node yield in thyroid cancer surgery: a randomized clinical trial

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Abstract: The identification and preservation of parathyroid glands (PGs) during thyroid surgery can be challenging. Many techniques have been developed to help surgeons find PGs. We have developed a novel mitoxantrone hydrochloride injection that can be used for lymphatic targeting. After local application during surgery, mitoxantrone hydrochloride injection for tracing (MHI) helps surgeons better identify and preserve PGs and helps pathologists find more lymph nodes. We conducted an open-label, multicenter, randomized clinical trial (CTR20171137) in six centers in China from 08/2017 to 12/2018. Patients with thyroid carcinoma were randomized to the MHI group or the control group. All patients received total thyroidectomy and bilateral central compartment lymph node dissection. The primary outcomes were the PG resection rate and lymph node staining rate. The full analysis set (FAS) included 461 patients, of which 228 were assigned to the MHI group, and 233 were assigned to the control group. The PG resection rates of the MHI group and the control group were 6.6% (15/228) and 26.6% (62/233), respectively, with a significant difference ($P < 0.001$). No PGs were stained blue with MHI. The central lymph nodes were stained blue with MHI, and the staining rate was 90.5%±12.0%. More lymph nodes were detected in the MHI group than in the control group (13.0±7.3 vs. 10.1±6.4 nodes/patient, $P < 0.001$). No adverse events related to MHI were observed. MHI is a safe and effective tracer that may help to preserve PGs and identify more central lymph nodes in patients with thyroid cancer.

Keywords: Thyroidectomy, parathyroid glands, mitoxantrone hydrochloride injection for tracing, central lymph node dissection, neck dissection

Introduction

Since the 1980s, thyroid cancer incidence rates have rapidly increased, yet comparatively stable or even declining mortality rates have been observed in much of the world [1]. Thyroid cancer is responsible for 586,000 cases worldwide [2], ranking 9th in incidence in 2020. The incidence rate in women is 3-fold higher than that in men.

Thyroid cancer is a common malignant tumor characterized by a good prognosis but a high prevalence of cervical lymph node metastasis [3]. Thyroidectomy and lymph node dissection play important roles in treating thyroid cancer [4]. The location, size, and the number of lymph node metastases are associated with risk stratification and postoperative treatments [5]. The lymph node yield from the surgical specimen and the metastatic lymph node ratio (LNR) are predictors of disease recurrence [5, 6]. Therefore, precise and thorough lymph node dissection is crucial for better management of thyroid cancer.

The preservation of the parathyroid glands (PGs) is a major concern in thyroid surgery. PG injury and unintended resection may lead to many surgical complications, including postoperative hypoparathyroidism and hypocalcemia. Since PGs are often embedded among the fat tissue and have a similar yellow color, they are often accidentally resected during surgery, especially when central compartment lymph node dissection is performed [7]. Reported rates of temporary and permanent hypoparathyroidism after surgery are 6.9%-49% and 0.4%-33%, respectively [7]. Postoperative hypoparathyroidism increases the use of medication and biochemical tests and prolongs the hospital stay, adding to the overall cost of thyroidectomy. Numerous methods have been developed to help surgeons preserve PGs, including the usage of a contrast dye, near-infrared autofluorescence mapping, and improved surgical techniques [8, 9].

The commonly used contrast dyes to help identify PGs include indocyanine green (ICG), methylene blue, and carbon nanoparticles [10-12], which are administered intravenously or locally around the tissue. Label-free near-infrared autofluorescence devices were recently approved by the Food & Drug Administration (FDA) to help surgeons find PGs [13]. However, surgery must be performed under dim light conditions when using ICG. The frequency of allergic urticaria reactions caused by ICG deserves special attention. ICG is contraindicated in patients who are allergic to iodine or who have renal insufficiency [14]. Notably, 5.2% of patients have been reported to develop neurotoxic reactions after receiving methylene blue, including confusion, restlessness, disorientation, and somnolence. The central region may accidentally be stained black following the injection of carbon nanoparticles, which may increase the difficulty of surgery. Additionally, carbon nanoparticle staining is irreversible, with the black stain permanently remaining at the surgical site. This may cause difficulties for reoperation in cancer recurrence, which is not rare for patients with differentiated thyroid cancer (DTC).

This paper proposes yet another tracer to identify PGs. A novel lymph node-targeting delivery system has recently been developed [15]. In this system, mitoxantrone hydrochloride injection for tracing (MHI) has a high affinity and visibility for the lymphatic system. After local injection, mitoxantrone hydrochloride molecules spontaneously form spherical nanocrystals through noncovalent interactions under physiological conditions. The size of these spherical nanocrystals is approximately 100 nm. Since the diameter of the nanocrystals is larger than the capillary endothelial cell space (30-50 nm) and smaller than the capillary lymphatic endothelial cell space (500 nm), the nanocrystals selectively enter the lymphatic capillaries and accumulate in regional lymph nodes through lymphangion. Due to its dark blue color, lymph nodes are stained blue and selectively dissected by surgeons during surgery. Meanwhile, PGs can be identified and preserved because they are not stained.

In a previous phase I clinical trial conducted by the authors, MHI was shown to be a safe tracer. All PGs and lymph nodes were well visualized within 1-3 min in all 15 patients. The results supported the use of MHI in total thyroidectomy and bilateral central compartment lymph node dissection [16]. Here, we report this novel tracer's phase II/III clinical trial results. In this trial, we aimed to further confirm the phase I clinical trial results and verify the effectiveness and safety of MHI in a large sample of patients.

Materials and methods

Study design and participants

This trial was an open-label, multicenter, randomized clinical trial (CTR20171137) that enrolled patients scheduled to undergo total thyroidectomy and bilateral central compartment lymphadenectomy from August 2017 to December 2018. The study was conducted at six tertiary medical centers in five different cities in China. The study was approved by the ethics committee of each participating center. All participants signed informed consent forms prior to the initiation of any study procedures. The trial was conducted according to the tenets of the Declaration of Helsinki.

The inclusion criteria were patients 1) aged between 18 and 70 years; 2) clinically diagnosed with or highly suspected of having thyroid carcinoma; 3) planning a total thyroidectomy and bilateral central region lymphadenectomy; 4) with no contraindications identified before surgery, and 5) willing to sign the consent form. The patient exclusion criteria were 1) allergies to anthraquinone or related agents; 2) a history of severe allergic reactions to other agents; 3) a history of drug abuse within a year; 4) a history of thyroid surgery, radiofrequency ablation therapy, or radiation therapy in the neck region; 5) a hemoglobin level if < 90 g/L, a platelet count of < 75×10^9, an absolute neutrophil count of < 1.5×10^9, an alanine transaminase or aspartate transferase level > 3 times the upper limit of normal; 6) a creatinine level > 1.5 times the upper limit of normal; 7) having participated in another clinical trial within 3 months; 8) a history of a psychotic disorder; 9) pregnant or breast-feeding women or those planning to become pregnant within 6 months; 10) relatives of trial staff or 11) patients who were considered inappropriate to participate in the trial by the investigator.

Randomization

Blocked randomization was used in the study. Patients were assigned to the MHI group (undergoing total thyroidectomy and bilateral central compartment lymph node dissection with the help of MHI) or the control group (undergoing the same surgery but without any lymphatic tracers). Randomization was conducted on the morning of the operation day using a centralized computer system built with a random number table generated using SAS 9.4 (SAS Institute, Cary, NY, USA) and managed by a third-party statistician.

Surgery and follow-up

The baseline data were collected upon admission to the hospital. For the MHI group, MHI (mitoxantrone hydrochloride injection for tracing, provided by Shenyang Tianbang Pharmaceutical Co., Ltd., Shenyang, China) was administered at the beginning of the surgery. More specifically, after exposing the thyroid gland, 0.2-0.6 mL (according to the size of the thyroid gland) of MHI was injected directly into the thyroid parenchyma at two or three different sites in each lobe, depending on the size of the thyroid and the surgeon's experience (Supplemental Figure 1). After allowing approximately 5 min to let the MHI pass into the draining lymph nodes, the operation was performed as usual, including the removal of the thyroid gland and central neck dissection. Attempts were made to localize and preserve all four PGs *in situ*. All resected specimens were sent to the Pathology Department for evaluation, including counting the total number of resected lymph nodes, determining whether they were stained with MHI, and discovering whether PGs were resected.

The participants in the control group underwent the same procedures but without MHI injection. All patients were actively supplemented with calcium before and after surgery. Serum parathyroid hormone levels were measured with along vital signs and other safety tests before, immediately after (postoperative days 1-4), and 10-20 days after surgery.

Endpoints and assessments

The primary endpoints were the PG resection rate and the lymph node staining rate. The PG resection rate was defined as the total number of patients with resected PGs divided by the total number of participants ×100%. The lymph node staining rate was defined as the total number of stained lymph nodes divided by the total number of lymph nodes resected ×100%.

The secondary endpoints were the number of harvested cervical lymph nodes, metastatic lymph nodes, and lymph nodes with different sizes. Lymph nodes were divided into ≤ 2 mm, 2-5 mm (including 5 mm), 5-10 mm (including 10 mm), and > 10 mm groups, according to their sizes.

The adverse events were graded according to NCI CTCAE 4.0. The tumors were staged using the 8th edition of the American Joint Committee on Cancer (AJCC) tumor-node-metastasis (TNM) staging system (TNM-8).

Statistical analysis

The PG resection rate was estimated to be 17% and 30% in the MHI and control groups, respectively. The sample size was estimated to be at least 198 in each group at a two-tailed α level

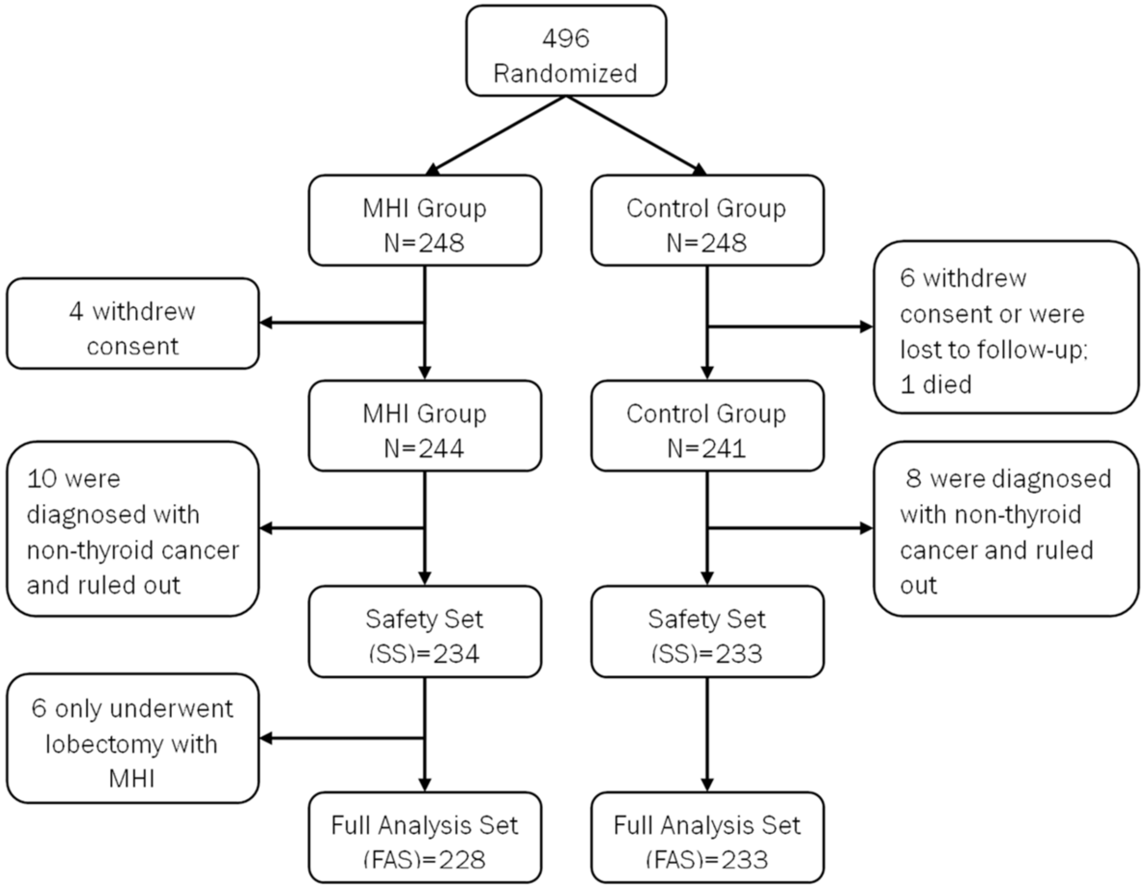

Figure 1. Flow chart. The study included 496 participants (248 in each group). In the MHI group, four patients withdrew informed consent and 10 patients did not receive the MHI injection. Another 6 patients have underwent lobectomy instead of total thyroidectomy. In the control group, six patients withdrew informed consent, and 1 patient died from posttracheotomy anastomotic fistula, organ dysfunction syndrome and septic shock. Eight patients were diagnosed with benign thyroid disease and did not receive total thyroidectomy.

of 0.05 and 90% power. The total withdrawal and loss to follow-up rate was set at 20%. Therefore, we planned to enroll 496 patients in the trial, with 248 patients in each group.

The endpoints were analyzed in the full analysis set (FAS). The FAS was a set of patients based on intention to treat (ITT), which refers to the datasets composed of all patients who participated in the trial, received treatment, and completed a baseline efficacy evaluation. The safety set (SS) included patients who completed at least one safety assessment. Continuous variables were tested for normal distributions using the Kolmogorov-Smirnov test; they are presented as the means ± standard deviations and were analyzed using Student's t test. As appropriate, the categorical data are presented as n (%) and were analyzed using the chi-square test or Fisher's exact test. All analyses were conducted using SAS 9.4 software (SAS Institute, Cary, NY, USA). A two-sided P value < 0.05 was considered statistically significant.

Results

Characteristics of the participants

The study included 496 total participants (248 in each group). Twenty and 15 participants in the MHI and control groups did not complete the trial or were lost at follow-up, resulting in 228 participants in the MHI group and 233 in the control group (**Figure 1**). The baseline data are shown in **Table 1**. The baseline characteristics were comparable between the 2 groups, except for sex (33.3% vs. 24.5%, P=0.036).

Table 1. Baseline characteristics

Group	MHI (n=228)	Control (n=233)	*P* value
Age (years)	44.70±11.64	42.92±11.36	0.098
Sex			0.036
Male	76 (33.3%)	57 (24.5%)	
Female	152 (66.7%)	176 (75.5%)	
Body mass index (kg/m^2)	25.27±3.69	25.01±4.37	0.496
Ethnicity			0.488
Han Chinese	215 (94.3%)	216 (92.7%)	
Other Chinese	13 (5.7%)	17 (7.3%)	
Tumor pathology			0.568
Papillary thyroid carcinoma	214	218	
Benign goiter or adenoma	14	13	
Medullary thyroid carcinoma	0	1	
Metastatic clear cell carcinoma	0	1	
T stage			0.585
T1	185	189	
T2	22	22	
T3	0	3	
T4	1	2	
Tx	6	4	
Not applicable[a]	14	13	
N Stage			0.985
N0	63	62	
N1a	108	113	
N1b	43	45	
Not applicable[a]	14	13	
TNM stage			0.537
I	193	199	
II	21	19	
III	0	2	
Not applicable[a]	14	13	
Number of tumors			0.448
1	78	93	
≥ 2	136	127	
Not applicable[a]	14	13	

a: Fourteen and 13 patients in the MHI group and the control group, respectively, whose final diagnosis was not thyroid cancer, and they were not eligible for TNM staging and enumeration.

Primary endpoint

Seventeen PGs were resected in 15 participants from the MHI group, while 67 PGs were resected in 62 patients from the control group (**Table 2**). Based on the FAS analysis, the PG resection rates were 6.6% (15/228) in the MHI group and 26.6% (62/233) in the control group. A significant difference was observed between the two groups (P < 0.001). No PGs were stained with MHI. The lymph node staining rate with MHI was 90.5%±12.0%.

Secondary endpoints

A total of 66.2% (305/461) of patients had lymph node metastasis (N1a, N1b, or N2). More lymph nodes were detected in the MHI group than in the control group (13.0±7.3 vs. 10.1±6.4 nodes/patient, P < 0.001). MHI helped identify smaller lymph nodes (all P < 0.05 for lymph nodes ≤ 10 mm), but for lymph nodes > 10 mm, the detected numbers were not significantly different between the two groups (P > 0.05) (**Table 2**). The number of metastatic lymph nodes was not significantly different between the two groups (3.4±4.4 vs. 3.1±4.1 positive nodes, P=0.864).

Safety

The SS included 234 and 233 participants in the MHI and control groups, respectively. The percentages of adverse events (88.5% vs. 86.7%), serious adverse events (2.6% vs. 2.6%), and grade ≥ 3 adverse events (10.7% vs. 8.6%) were similar between the two groups (all P > 0.05) (**Table 3**). One participant in the MHI group discontinued treatment because of adverse events. All adverse events were unrelated to the MHI. Adverse events with a ≥ 10% incidence in the MHI group were oropharyngeal pain, productive cough, cough, hypoparathyroidism, hypocalcemia, nausea, abdominal discomfort and fever. The incidence of adverse events with a rate ≥ 10% was not significantly different between the two groups (**Table 4**).

Discussion

Accidental resection of PGs is common during thyroid surgery and lymph node dissection due

Table 2. Parathyroid gland outcomes

Group	MHI (n=228)	Control (n=233)	*P* value
PG resection rate	15 (6.6%)	62 (26.6%)	< 0.001
1 PG resected	13 (4.5%)	57 (24.5%)	< 0.001
2 PGs resected	2 (0.9%)	5 (2.1%)	0.450
≥ 3 PGs resected	0	0	
Total resected PGs	17	67	< 0.001
Hypoparathyroidism	80 (34.2%)	76 (32.6%)	0.719
Hypocalcemia	59 (25.1%)	54 (23.2%)	0.607

Table 3. Lymph node outcomes

Group	MHI (n=228)	Control (n=233)	*P* value
Lymph nodes detected	13.031±7.319	10.142±6.448	< 0.001
Metastatic lymph nodes	3.430±4.422	3.142±4.063	0.864
Size of lymph nodes detected			
Lymph nodes ≤ 2 mm	387	306	0.027
Lymph nodes ≤ 5 mm and > 2 mm	1416	1062	< 0.001
Lymph nodes > 5 mm and ≤ 10 mm	960	816	0.033
Lymph nodes > 10 mm	208	179	0.747

Table 4. Safety profile

	MHI (n=234)	Control (n=233)	*P* value
Adverse events	207 (88.5%)	202 (86.7%)	0.563
Serious adverse events	6 (2.6%)	6 (2.6%)	0.994
Treatment-related adverse events	0	0	-
Treatment-related serious adverse events	0	0	-
Discontinuation related to adverse events	1 (0.4%)	0	0.318
Adverse events with an incidence ≥ 10%			
Oropharyngeal pain	80 (34.2%)	76 (32.6%)	0.719
Productive cough	32 (13.7%)	21 (9.0%)	0.112
Cough	34 (14.5%)	17 (7.3%)	0.012
Nausea	36 (15.4%)	30 (12.9%)	0.436
Abdominal discomfort	35 (15.0%)	31 (13.3%)	0.608
Fever	24 (10.3%)	18 (7.7%)	0.339

to their unpredictable location, shape and number [7]. Chang Myeon Song analyzed the number of PGs preserved during thyroidectomy in 454 patients [17]. Incidental parathyroidectomy occurred in 19.8% (one PG in 17.6%, two in 1.5%, and three in 0.7%). The most widely reported and applied techniques are autofluorescence [18] and ICG imaging [19]. However, they all require additional fluorescence imaging systems that are unavailable at most hospitals. Most current optical methods require an intravenous injection of an agent, which might be associated with safety issues because of the systemic distribution of the agents. This trial provides another method to localize and preserve PGs without using fluorescent devices. In this trial, we observed a similar incidence of accidental PG resection in the control group. Furthermore, the use of tracer in the MHI group significantly reduced the rate of accidental PG resection (6.6% vs. 26.6%). Upon MHI injection, PGs will be distinguishable from the lymph nodes in the surgical field within 1-5 min, allowing surgeons to preserve the PGs and their blood supply by applying vasculature-preserving techniques [1]. The trial confirmed that MHI helps surgeons identify and retain PGs. However, we did not detect differences in postoperative hypocalcemia and hypoparathyroidism, which might attributed to the fact that only one functioning PG is sometimes sufficient to produce hormones and maintain the calcium level. Three PGs still remained even when one was erroneously resected. In addition, active calcium supplementation before and after surgery in all patients exerted a certain effect on the incidence of hypocalcemia.

The lymph node status is strongly associated with cancer-specific mortality in population-based studies [5, 6, 20]. Increasing the total number of lymph nodes identified during surgery will influence the staging of cancer and subsequent treatments. Carbon nanoparticles are another tracer and contrast agent used to facilitate nodal dissection and PG preservation during thyroidectomy [21]. In a recent study, carbon nanoparticles were shown to play a role in increasing the total lymph node yield and PG protection rate [12]. The lymph node staining rate was 69.4%, which was lower than the staining rate achieved using MHI in this study (90.5%±12.0%). This study showed a significant difference in the numbers of lymph nodes detected between the two groups. Compared with the control group, the MHI group had a significantly increased number of lymph nodes removed by surgeons, especially small nodes (≤ 10 mm). In previous studies, the total lymph node yield was proposed to be associated with disease-free survival, and higher lymph node yield was associated with lower recurrence rates [22, 23]. Therefore, a higher lymph node yield helps with decision-making regarding adjuvant treatments.

The ratio between metastatic lymph nodes and the total resected lymph nodes (lymph node ratio, LNR) is an important prognostic factor in patients with differentiated thyroid carcinoma [23, 24]. A recent study involving 2384 patients indicated that the LNR is an independent factor for nodal and any-site recurrence [5]. An LNR > 0.3 is considered a risk factor for recurrence [5, 25, 26]. No significant difference in the number of lymph node metastases resected was observed between the two groups in this trial. This finding is consistent with the results from previous studies using other staining agents [27]. However, the number of patients with an LNR > 0.3 in the MHI group was significantly lower than that in the control group (35.5% vs. 45.5%, P=0.008). Future studies should investigate whether MHI provides a predictive value for survival outcomes.

Locally injected MHI did not induce more postoperative complications because the amount of drug used was relatively small. In addition, a phase I trial by the authors' group showed that systemic MHI was not detected after the surgery [16]. In this study, surgical time and fluency were not affected significantly by the injection procedure. The incidence of adverse events in this trial seems to be high. However, adverse events included all common symptoms after a surgical procedure, such as pain, nausea, cough and any blood test abnormalities. Thus, any discomfort or abnormality would be considered an adverse event, resulting in a rate exceeding 80%. The incidence of adverse events in the MHI and control groups was similar. Most of these adverse events quickly resolved, and according to the researcher's analysis, no adverse reactions were related to MHI in this trial.

DTC is well known for its high prevalence of lymph node metastasis in the early stage of the disease [28]. As a chemotherapeutic agent, MHI was also shown to inhibit lymph node metastasis in animal studies [15]. Therefore, MHI has the potential to alter the recurrence rate by killing micrometastases after local injection. Long-term follow-up studies and more controlled trials are needed to evaluate this hypothesis.

The extent of thyroid surgery and prophylactic central neck dissection are controversial. All participants in this trial underwent total thyroidectomy and bilateral central lymph node dissection, potentially due to specific clinical practice challenges in China. The ATA guidelines [3] recommend total thyroidectomy for tumors greater than 4 cm, lobectomy or total thyroidectomy for tumors between 1-4 cm, and lobectomy or active surveillance for tumors < 1 cm. Prophylactic central lymph node dissection is not recommended for patients with cN0 tumors. This guideline provides important guidance for clinical practice. However, a gap exists between the ATA and Chinese guidelines [29]. In China, fine needle aspiration biopsy (FNAB) is not yet popular, and most patients only receive an ultrasound before surgery, resulting in a lack of a pathological diagnosis prior to surgery. Thus, a few participants in this trial actually had to have benign goiter or adenoma. Moreover, many patients presented with anxiety about tumor recurrence and requested "total and complete resection". A multicenter, prospective study [30] conducted in nine tertiary hospitals in China showed that 83.1% of patients with moderate- to high-risk differentiated thyroid cancer underwent total/near-total resection, and 99.0% of patients received lymph node dissection. Therefore, this trial enrolled some patients with cN0 thyroid cancer.

Another drawback of this study is that the functionality of the PG was not measured as in the

ICG trials. More tests and experiments are needed further to evaluate the fluorescence properties of MHI. This trial did not detect significant changes in postoperative hypocalcemia and hypoparathyroidism rates. However, with the help of MHI, surgeons may have a better chance of protecting the blood supply of the PGs and thus minimize calcium supplementation during the perioperative period.

Conclusions

MHI is a novel agent developed for surgical use. Its nanocrystalline nature leads to its accumulation in the lymph nodes and lymphatic ducts. When injected into the thyroid gland, it stains the lymph nodes a dark blue, thus helping the surgeons and pathologists localize PGs and find more lymph nodes. This trial has shown that MHI is a safe, feasible, and effective agent for use in thyroid cancer surgery to identify lymph nodes and significantly reduce the rate of unwanted PG resection. However, more studies are needed to determine the benefits of this novel tracer.

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All participants signed informed consent forms prior to initiating any study procedures.

Disclosure of conflict of interest

None.

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References

[1] Lortet-Tieulent J, Franceschi S, Dal Maso L and Vaccarella S. Thyroid cancer "epidemic" also occurs in low- and middle-income countries. Int J Cancer 2019; 144: 2082-2087.

[2] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021; 71: 209-249.

[3] Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawka AM, Schlumberger M, Schuff KG, Sherman SI, Sosa JA, Steward DL, Tuttle RM and Wartofsky L. 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 2016; 26: 1-133.

[4] Zhao W, You L, Hou X, Chen S, Ren X, Chen G and Zhao Y. The effect of prophylactic central neck dissection on locoregional recurrence in papillary thyroid cancer after total thyroidectomy: a systematic review and meta-analysis: pCND for the locoregional recurrence of papillary thyroid cancer. Ann Surg Oncol 2017; 24: 2189-2198.

[5] Nam SH, Roh JL, Gong G, Cho KJ, Choi SH, Nam SY and Kim SY. Nodal factors predictive of recurrence after thyroidectomy and neck dissection for papillary thyroid carcinoma. Thyroid 2018; 28: 88-95.

[6] Robinson TJ, Thomas S, Dinan MA, Roman S, Sosa JA and Hyslop T. How many lymph nodes are enough? Assessing the adequacy of lymph node yield for papillary thyroid cancer. J Clin Oncol 2016; 34: 3434-3439.

[7] Kakava K, Tournis S, Papadakis G, Karelas I, Stampouloglou P, Kassi E, Triantafillopoulos I, Villiotou V and Karatzas T. Postsurgical hypoparathyroidism: a systematic review. In Vivo 2016; 30: 171-179.

[8] Abbaci M, De Leeuw F, Breuskin I, Casiraghi O, Lakhdar AB, Ghanem W, Laplace-Builhé C and Hartl D. Parathyroid gland management using optical technologies during thyroidectomy or parathyroidectomy: a systematic review. Oral Oncol 2018; 87: 186-196.

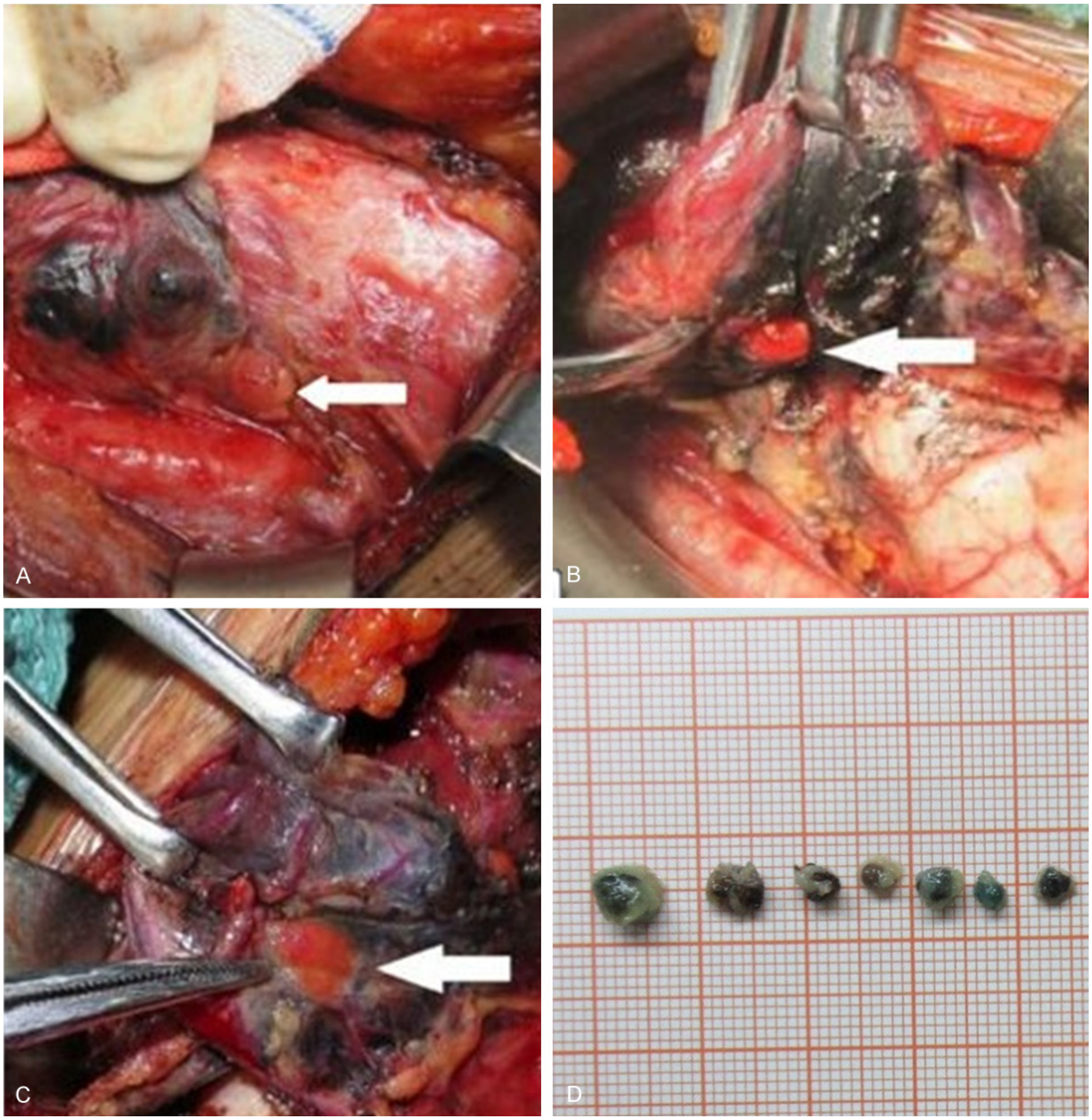
[9] Park I, Rhu J, Woo JW, Choi JH, Kim JS and Kim JH. Preserving parathyroid gland vasculature to reduce post-thyroidectomy hypocalcemia. World J Surg 2016; 40: 1382-1389.

[10] Liu F, Zhu Y, Qian Y, Zhang J, Zhang Y and Zhang Y. Recognition of sentinel lymph nodes in patients with papillary thyroid cancer by nano-carbon and methylene blue. Pak J Med Sci 2017; 33: 1485-1489.

[11] Hillary SL, Guillermet S, Brown NJ and Balasubramanian SP. Use of methylene blue and near-infrared fluorescence in thyroid and parathyroid surgery. Langenbecks Arch Surg 2018; 403: 111-118.

[12] Min L, Lang BHH, Chen W, Ai Q, Jiang J and Huang ZH. Utility of activated carbon nanoparticle (CNP) during total thyroidectomy for clinically nodal positive papillary thyroid carcinoma (PTC). World J Surg 2020; 44: 356-362.

[13] Solorzano CC, Thomas G, Baregamian N and Mahadevan-Jansen A. Detecting the near infrared autofluorescence of the human parathyroid: hype or opportunity? Ann Surg 2020; 272: 973-985.
[14] Zhang X, Li JG, Zhang SZ and Chen G. Comparison of indocyanine green and carbon nanoparticles in endoscopic techniques for central lymph nodes dissection in patients with papillary thyroid cancer. Surg Endosc 2020; 34: 5354-5359.
[15] Mao Y, Liu J, Shi T, Chen G and Wang S. A novel self-assembly nanocrystal as lymph node-targeting delivery system: higher activity of lymph node targeting and longer efficacy against lymphatic metastasis. AAPS PharmSciTech 2019; 20: 292.
[16] Chen S, Chen G, Wang S, Sun J and Zhao W. Mitoxantrone hydrochloride as a novel lymphatic tracer in thyroid surgery: a phase I clinical trial. Boston, USA: World Congress of Thyroid Cancer; 2017.
[17] Song CM, Jung JH, Ji YB, Min HJ, Ahn YH and Tae K. Relationship between hypoparathyroidism and the number of parathyroid glands preserved during thyroidectomy. World J Surg Oncol 2014; 12: 200.
[18] Paras C, Keller M, White L, Phay J and Mahadevan-Jansen A. Near-infrared autofluorescence for the detection of parathyroid glands. J Biomed Opt 2011; 16: 067012.
[19] Fortuny JV, Sadowski SM, Belfontali V, Guigard S, Poncet A, Ris F, Karenovics W and Triponez F. Randomized clinical trial of intraoperative parathyroid gland angiography with indocyanine green fluorescence predicting parathyroid function after thyroid surgery. Br J Surg 2018; 105: 350-357.
[20] Yang L, Shen W and Sakamoto N. Population-based study evaluating and predicting the probability of death resulting from thyroid cancer and other causes among patients with thyroid cancer. J Clin Oncol 2013; 31: 468-474.
[21] Li Y, Jian WH, Guo ZM, Li QL, Lin SJ and Huang HY. A meta-analysis of carbon nanoparticles for identifying lymph nodes and protecting parathyroid glands during surgery. Otolaryngol Head Neck Surg 2015; 152: 1007-1016.
[22] Heaton CM, Chang JL and Orloff LA. Prognostic implications of lymph node yield in central and lateral neck dissections for well-differentiated papillary thyroid carcinoma. Thyroid 2016; 26: 434-440.
[23] Noel JE and Orloff LA. Recognizing persistent disease in well-differentiated thyroid cancer and association with lymph node yield and ratio. Otolaryngol Head Neck Surg 2020; 162: 50-55.
[24] Vas Nunes JH, Clark JR, Gao K, Chua E, Campbell P, Niles N, Gargya A and Elliott MS. Prognostic implications of lymph node yield and lymph node ratio in papillary thyroid carcinoma. Thyroid 2013; 23: 811-816.
[25] Schneider DF, Mazeh H, Chen H and Sippel RS. Lymph node ratio predicts recurrence in papillary thyroid cancer. Oncologist 2013; 18: 157-162.
[26] Suzuki H, Koide Y, Hanai N, Nishikawa D, Beppu S, Mikami S and Hasegawa Y. Lymph node density in papillary thyroid carcinoma is a prognostic factor after adjusting for pathological stage. Oncotarget 2018; 9: 26670-26678.
[27] Wang L, Yang D, Lv JY, Yu D and Xin SJ. Application of carbon nanoparticles in lymph node dissection and parathyroid protection during thyroid cancer surgeries: a systematic review and meta-analysis. Onco Targets Ther 2017; 10: 1247-1260.
[28] Zhao W, Chen S, Hou X, Liao Q, Chen G and Zhao Y. Predictive factors of lateral lymph node metastasis in papillary thyroid microcarcinoma. Pathol Oncol Res 2019; 25: 1245-1251.
[29] National Health Commission of the People's Republic of China. National guidelines for diagnosis and treatment of thyroid cancer 2022 in China (English version). Chin J Cancer Res 2022; 34: 131-150.
[30] Ming J, Zhu JQ, Zhang H, Sun H, Wang J, Cheng RC, Xie L, Li XR, Tian W and Huang T. A multicenter, prospective study to observe the initial management of patients with differentiated thyroid cancer in China (DTCC study). BMC Endocr Disord 2021; 21: 208.



Supplemental Figure 1. Thyroid staining and parathyroid visualization. This figure shows thyroid staining and parathyroid gland visualization under low dose (0.2 mL, A), medium dose (0.4 mL, B) and high dose (0.6 mL, C) MHI. Stained lymph nodes were picked up by pathologists from specimen (D).