Intraoperative rapid aspiration cytological method for parathyroid glands identification and protection

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Abstract. To explore new methods for intraoperative identification of parathyroid glands, 86 thyroid cancer patients, admitted to Xijing hospital from July 2017 to July 2018, were included. During lymph node dissection, parathyroid glands were firstly judged by clinician eyeballing, based on his clinical experience. Then, cytological detection was used for rapid identification via Diff-quick staining. PTH monitoring was performed by PTH detection kit. Finally, frozen pathology was examined and regarded as the golden standard. In this study, 172 suspicious parathyroid glands were observed. According to frozen pathology outcome, the accuracy, sensitivity and specificity of clinician eyeballing were calculated as 63.3%, 100%, and 13.9%. Kappa test showed poor consistency (kappa = 0.156), AUC area was 0.569 ± 0.045, 95%CI = (0.480–0.658), p = 0.123. For cytological and PTH detection, the accuracy, sensitivity and specificity were 91.7% vs. 92.3%, 93.6% vs. 93.8% and 89.0% vs. 90.3%. Kappa value was 0.829 vs. 0.842, indicating good consistency. AUC area was 0.908 ± 0.027 vs. 0.918 ± 0.025, 95%CI = (0.856–0.960) vs. (0.869–0.966), p < 0.001, indicating higher diagnostic value. Besides, compared with frozen pathology, cytological detection was easily and rapid. The time-taking between frozen pathology and cytological detection or PTH detection were 39.0 ± 6.59 min vs. 5.02 ± 0.78 min and 39.0 ± 6.59 min vs. 6.1 ± 1.23 min, p < 0.001. In conclusion, intra-operative cytological detection maybe potential for in-situ preservation of parathyroid glands.

Key words: Parathyroid glands, Intra-operative identification, Cytological detection, Thyroid cancer

THYROID CANCER is currently regarded as the most common endocrine cancer, new incidence of which has risen dramatically from 60,220 in 2013 to 62,980 in 2014 [1, 2]. The routine preventative central lymph node dissection has been regarded as a regular adjunct to thyroidectomy by most experts, collectively referred to as standard radical surgery [3]. However, the accidental removal or iatrogenic injury of the parathyroid glands, due to the difficult identification between parathyroid glands and lymph nodes, is always occurred during thyroidectomy, which finally results in hypocalcemia, the most common complication following total thyroidectomy [4, 5]. Based on a systematic meta-analysis, the median incidence of transient and permanent hypocalcaemia was 27% (19%–38%) and 1% (0–3%), respectively [6]. Hence, protecting parathyroid glands in situ is pretty crucial to thyroid cancer patients.

Recently, advances in surgical technique have not only highlighted the success of the operations but also focused on how these diseases affect patient-reported quality of life before and after surgery [7]. It is pretty significant to develop a strategy, effectively preventing the appearance of hypocalcemia [8]. Previously, people have recognized the important of parathyroid glands identification and blood supply protection during surgery. Although autotransplantation of parathyroid glands can be adopted for recovery, it might not completely restore the normal function [9, 10]. Currently, a variety of techniques has been used in China for parathyroid glands identification. Sentinel lymph node biopsy using methylene blue has been regarded as a safe and technically feasible procedure in thyroidectomy surgery [11, 12]. Nano-carbon suspension can proved the complete dissection of lymph node and prevent parathyroid damage [13]. In addition, NIR (near-infrared) fluorescence spectroscopy can detect the parathyroid regardless of tissue pathology based on the signal caused by calcium-sensing receptors present in the parathyroid intra-operatively [14]. Similarly, OCT (Optical Coherence Tomography) is also a highly sensi-
tive technique in distinguishing parathyroid tissue, thyroid tissue, lymph nodes and adipose tissue [15]. Furthermore, ultrasound scalpel, parathyroid hormone assay, together with magnetic resonance imaging are all improved the likelihood of the parathyroid glands preservation during thyroid surgery [16, 17]. Nevertheless, either from the low sensitivity, high false-negative rate or from the interference from fat tissue, there is still no satisfactory method occurred clinically in parathyroid glands recognition.

In this study, we developed a method for intraoperative parathyroid identification with satisfying accuracy, sensitivity and specificity, which may improve the in-situ protection of parathyroid glands. The protocol has been registered in clinicaltrials.gov (NCT03268785).

**Methods**

**Design**

This was a diagnostic study, comparing the efficacy between rapid aspiration cytological detection, PTH monitoring and eyeballing by two professors who have more than 15 years clinical experience. Accuracy, sensitivity and specificity were primary endpoint. The secondary endpoint was time-taking. Rapid cytological detection and frozen pathological examination were proceeded sequentially in each patient. During lymph node dissection process, parathyroid glands were firstly judged by clinician eyeballing, based on his clinical experience. Once suspicious parathyroid glands (unascertainable as parathyroid glands or seems like lymph nodes) were observed, its blood supply should firstly be protected. A puncture by matched 22 G needle of 5 mL sterile syringe was applied at 45 degree angle. The needle was initially thrust into the gland for 0.2 mm, and then advanced 0.1–0.2 mm deeper, while gently withdrawing the plunger of the syringe and maintaining negative pressure (Fig. 1). Parathyroid gland cells adsorbed in the needle was used for cytological detection via Diff-quick staining. At the same time, PTH monitoring was carried out by PTH detection kit. Finally, frozen pathological examination was routinely taken and regarded as the golden standard, by two pathologists with more than 10 years working experience. This protocol has been approved by the ethics committee of Xijing hospital (No. KY20162049-1) and registered on ClinicalTrials.gov (NCT. 03268785). All patients were asked to provide written informed consent before enrollment, in accordance with the Good Clinical Practice guidelines and the Declaration of Helsinki.

![Fig. 1](image.png) Design of intraoperative parathyroid identification. Regarding frozen pathology as golden standard, we evaluated the accuracy, sensitivity and specificity of Method 1 and Method 2 for intraoperative parathyroid glands identification.
Participants
The eligible patients diagnosed with thyroid carcinoma, from July 2017 to July 2018, were enrolled in this study. Inclusive criteria: (1) age ranges from 18 to 75 years old; (2) pre-operative detection, ultrasonic examination, as well as CT test demonstrated available indication of surgery; (3) patients diagnosed with thyroid cancer by fine needle puncture. Exclusive criteria: (1) patients diagnosed with more than one active malignant tumor, or other previously diagnosed parathyroid disorder that interfere the serum calcium concentration; (2) patients unsuitable for lymph node dissection.

Diff-quik staining
The needle puncture was repeated for 2–3 times from different orientation guaranteed the sample volume. Following the rapid emptying of syringe, the sample was smeared as a thin and uniform cytological smears. Once 5–20 seconds fixation finished, cell smears could be took out for cytological detection. A rapid Diff-quik staining (BASO Diagnostics Inc. Zhuhai) was used, according to the instruction, for intra-operative identification following complete fix in stationary liquid for 15 seconds within 2–4 seconds. Based on the outcomes achieved under high power microscope, the suspicious parathyroid could be identified by pathological experts.

PTH monitoring
When uncertain parathyroid glands were found during lymph node dissection, 5 mL sterile syringe was applied to puncture the nodes and absorb at least 90 uL liquid. According to the instruction of PTH detection kit (Bioda Diagnosis Co., Ltd), the liquid was dropped on specified test paper and cultured for 12 min. PTH detector was then used to assess PTH level, which ranged from 10 pg/mL to 1,000 pg/mL (Fig. 1). PTH dose more than 130 pg/mL of PTH was regarded as the standard to justify the node as parathyroid gland. Frozen pathology result was the golden standard.

HE Staining
HE (Hematoxylin and Eosin) staining was used for frozen pathological verification. Samples were isolated and fixed with 4% paraformaldehyde for 12 h, embedded in paraffin and cut into 3-μm serial sections. Corresponding sections were stained with hematoxylin (BASO Diagnostics Inc. Zhuhai) for 10 min at room temperature. Then, sections were washed with running water. Subsequently, sections were washed with Scott promote blue liquid for 1 min, 1% hydrochloric acid alcohol differentiation liquid for 20 s, and Scott promote blue liquid for 1 min. Then, sections were stained with eosin (BASO Diagnostics Inc. Zhuhai) for 30 s. Sections were washed with running water and sealed for observation. Finally, sections were observed by Image-Pro Plus 5.0 software (Media Cybernetics, Inc., Bethesda, MD, USA).

Endpoint evaluation
Based on golden standard, the sensitivity and specificity of intra-operative parathyroid glands identification were calculated as TP/(TP + FN) and TN/(FP + TN) respectively. TP (true positive): parathyroid glands were correctly identified. TN (true negative): lymph nodes and adipose tissue were correctly identified as non-parathyroid tissue. FP (false positive): lymph nodes and adipose tissue were identified as parathyroid tissue. FN (false negative): parathyroid glands were not identified as such [15].

Statistical analysis
Statistical analysis was performed using the SPSS 22.0 statistical software package (SPSS Inc., Chicago, IL). Consistency analysis was evaluated by Kappa test, kappa < 0.4 demonstrated lower consistency, 0.4 < kappa < 0.7 indicated common consistency, kappa > 0.75 was regarded as good consistency with the golden standard. Besides, ROC curve was drew, AUC area was divided into 3 degree, including lower diagnostic value (0.5–0.7), medium value (0.7–0.9) and high diagnostic value (>0.9).

Results
Baseline
Eighty-six eligible patients diagnosed with thyroid carcinoma, from July 2017 to July 2018, were enrolled in this study. The baseline characteristics were listed in Table 1.

Accuracy, sensitivity and specificity of clinician eyeballing
During the operation, 172 suspicious parathyroid glands were observed by the clinician among 86 participants. There are 3 nodes failed for frozen pathological examination due to insufficient sample and excluded. For remaining 169 suspicious parathyroid glands, 107 nodes were recognized correctly via clinician’s judgement (97 true positive, 10 true negative). False positive occurred in 62 glands. Hence, the accuracy of clinician eyeballing was 107/169 = 63.3%, sensitivity of clinician eyeballing was 97/97 + 0 = 100%, and specificity of clinician eyeballing was 10/10 + 62 = 13.9%, respectively. Kappa test showed poor consistency between eyeballing and frozen pathology, kappa value = 0.156. ROC curve was shown in Fig. 2, AUC area was 0.569 ± 0.045, 95%CI = (0.480–0.658), p = 0.123, indicating lower diagnostic value (Table 2).
Table 1  Baseline characteristics

| Index | Data (N = 86) | Index | Data (N = 86) |
|-------|---------------|-------|---------------|
| Age   |               | N     |               |
| Average | 40.6 ± 11.64 | N0    | 16 (18.6%)    |
| Medium | 36 (22–61)   | N1    | 65 (75.6%)    |
| Sex    |               | N2    | 5 (5.8%)      |
| Female | 65 (75.6%)    | Pathology |        |
| Male   | 21 (24.4%)    | PTC   | 46 (53.5%)    |
| Tumor size |       | PTMC  | 40 (46.5%)    |
| >1 cm  | 46 (53.5%)    | Surgery |       |
| ≤1 cm  | 40 (46.5%)    | A     | 44 (51.2%)    |
| Lesion |               | B     | 42 (48.8%)    |
| Unifocal | 62 (72.1%)  |       |               |
| Multifocal | 24 (27.9%) |       |               |

Notes: PTC, papillary thyroid carcinoma; PTMC, papillary thyroid microcarcinoma; A, total thyroidectomy + lymph node dissection; B, unilateral lobectomy + lymph node dissection; N, lymph nodes; N0, no metastatic lymph nodes; N1, regional lymph node metastasis; N2, metastases to Level VI (pretracheal, paratracheal, and prelaryngeal/Delphian lymph nodes) or metastases to unilateral, bilateral, or contralateral cervical (Levels I, II, III, IV, or V) or retropharyngeal or superior mediastinal lymph nodes (Level VII).

Accuracy, sensitivity and specificity of cytological detection

Finally, there are 167 suspicious glands available for cytological detection and frozen pathology examination (3 nodes failed for frozen pathological examination, 2 nodes failed for cytological measurement due to insufficient sample). After intraoperative Diff-quik detection, we identified 93 parathyroid glands, based on the pathological features of parathyroid cells (Fig. 3). The remaining 74 suspicious parathyroid glands were recognized as non-parathyroid glands. Judged by frozen pathology outcome, we demonstrated that 153 nodes were recognized correctly (88 true positive, 65 true negative). False positive and false negative occurred in 8 and 8 glands, respectively. Hence, the accuracy, sensitivity and specificity of intraoperative cytological detection were 91.7% (153/167), 93.6% (88/88 + 6) and 89.0% (65/65 + 8), respectively. Consistency analysis demonstrated that the kappa value was 0.829, showing good consistency with the golden standard. ROC curve was shown in Fig. 2, AUC area was 0.908 ± 0.027, 95%CI = (0.856–0.960), p < 0.001, indicating higher diagnostic value (Table 2).

Accuracy, sensitivity and specificity of PTH monitoring

Except for 3 nodes failed for frozen pathological

![ROC curve](image.png)

Fig. 2  ROC curves of eyeballing judgement (A), cytological detection (B) and PTH monitoring (C)

Table 2  Diagnostic and consistency analysis

| Item      | Accuracy (%) | Sensitivity (%) | Specificity (%) | Kappa value | AUC area    | 95%CI       | p value |
|-----------|--------------|----------------|-----------------|-------------|-------------|-------------|---------|
| Cytology  | 91.7         | 93.6           | 89.0            | 0.829       | 0.908 ± 0.027 | 0.856–0.960 | <0.001  |
| Eyeballing| 63.3         | 100            | 13.3            | 0.156       | 0.569 ± 0.045 | 0.480–0.658 | 0.123   |
| PTH       | 92.3         | 93.8           | 90.3            | 0.842       | 0.918 ± 0.025 | 0.869–0.966 | <0.001  |
examination due to insufficient sample, PTH level of the remaining 169 nodes were assessed. The level of PTH was ranged from <10 pg/mL to >1,000 pg/mL. Based on the golden standard, 156 suspicious nodes were identified exactly, 91 glands were identified as parathyroid gland (PTH > 130 pg/mL), 65 nodes were detected as non-parathyroid glands (PTH < 40 pg/mL). False positive and false negative occurred in 6 and 7 glands, respectively. Hence, the accuracy, sensitivity and specificity rate of PTH monitoring were 92.3%, 93.8% and 90.3%, respectively. Consistency analysis demonstrated that the kappa value was 0.842. ROC curve was shown in Fig. 2, AUC area was 0.918 95%CI = (0.869–0.966), \( p < 0.001 \), indicating higher diagnostic value (Table 2).

**Time-taking analysis**

Compared with frozen section, cytology procedure and PTH monitoring were rapid and easy to process. The mean time to perform cytology and PTH monitoring were 5.02 ± 0.78 minutes and 6.1 ± 1.23 minutes respectively, significant less than the mean time to perform frozen section (39.0 ± 6.59 min, \( p < 0.001 \)).

**Discussion**

Thyroid cancer is the commonest endocrine malignancy and total thyroidectomy combined with lymph node dissection is currently the main treatment strategy. However, postoperative hypocalcemia due to parathyroid misresection, significantly limit the life-quality of patients. As reported, the incidence of hypocalcemia was related to the number of preserved glands, two or more preserved parathyroid glands group possessed 19.7% lowered rate of hypocalcemia [18]. Therefore, much attention has been paid on the protection of parathyroid glands during thyroid cancer surgery. Here we introduced an intraoperative identification method for parathyroid glands protection.

Earlier studies tried several methods for identification of parathyroid glands. In 2012, Patel HP applied 1% methylene blue for abnormal parathyroid glands identification and showed desirable sensitivity and specificity [19]. In 2016, McWade MA established the clinical utility of autofluorescence spectroscopy for parathyroid detection [20]. In 2017, Wang L reported a meta-analysis, reviewing and supporting the application of carbon nanoparticles in lymph node dissection and parathyroid protection [21]. In 2017, Kim SW applied near-infrared autofluorescence imaging (NIR) for parathyroid gland detection and showing excellent accuracy rate [22]. In 2018, Ladurner R et al. identified parathyroid glands during thyroidectomy by displaying their near-infrared autofluorescence [23]. In this study, we developed intraoperative cytological detection method, which might improve the operative success rates and the in-situ preservation of parathyroid glands. Meanwhile, the method maybe of greater benefit for the patients undergoing re-operation, because the tissues and nodes of them have been damaged to some extend in the previous surgery, which increase the difficulty of intraoperative recognition.

According to the previous reports, PTH monitoring provided an opportunity for parathyroid retention. The sensitivity, specificity, and diagnostic efficiency of PTH-5 (detect PTH 5 minutes after surgery) were approximately 80%, 100%, and 91%, while that of PTH-60 (detect PTH 60 minutes after surgery) presented as 93%, 82%, and 87% [24]. Herein, we reported the accuracy, sensitivity and specificity rate of 92.3%, 93.8% and 90.3%, for intraoperative PTH monitoring with high diagnostic value. Compared with intraoperative cytological detection, tissue samples were needless for PTH monitoring, which produced less damage to parathyroid glands. Besides, cytological outcomes depend on the subjective judgement of pathologists, different with the objective achievement of PTH level via PTH detection kit. In spite of the above advantages of PTH detection, its accuracy rate was 0.6% lower than cytological judgement, which still needs further verification in the coming trial.
Traditionally, identification of parathyroid glands may be challenging and time consuming. But herein, the identification of the parathyroid was facilitated by performing rapid distinction. The rapid detection could shorten the operation time, which is of great economic benefits [25]. However, this method may have some limitations. Firstly, the method has high command of clinical experience and professional skill for clinician. So, we tried our best to decrease the megascopic and pathological deviation by sufficient training and communication. Besides, all the clinician and pathology technician were all senior specialists, which guaranteed the pathological detection. Secondly, insufficient sample may affect the smear detection and re-puncture can be an effective remedy. Before the study start, we have practiced the puncture skills, ensuring the successfulness of puncture. Thirdly, the number of patients enrolled is relatively small, we need to enroll more cases and conduct prospective study in the future to achieving much more convincing and representative results.

In summary, intra-operative rapid cytological detection may be potential for in-situ preservation of parathyroid glands. The outcome will be more convincing once a larger clinical trial was completed.

Key points
- Significant findings of the study
  We explored a new method for intraoperative parathyroid identification.
- What this study adds
  Cytological was sensitive and rapid for parathyroid retention in situ.

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Conflict of Interest Disclosure
We declared no conflicts of interests.

ClinicalTrials.gov Registration
NCT03268785 (Intra-operative Rapid Identification of Lymph Node and Parathyroid).

References
1. Siegel R, Naishadham D, Jemal A (2013) Cancer statistics, 2013. CA Cancer J Clin 63: 11–30.
2. DeSantis CE, Lin CC, Mariotto AB, Siegel RL, Stein KD, et al. (2014) Cancer treatment and survivorship statistics, 2014. CA Cancer J Clin 64: 252–271.
3. Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, et al. (2009) Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 19: 1167–1214.
4. Puzziello A, Rosato L, Innaro N, Orlando G, Avenia N, et al. (2014) Hypocalcemia following thyroid surgery: incidence and risk factors. A longitudinal multicenter study comprising 2,631 patients. Endocrine 47: 537–542.
5. Cmilansky P, Mrozova L (2014) Hypocalcemia—the most common complication after total thyroidectomy. Bratisl Lek Listy 115: 175–178.
6. Edafe O, Antakia R, Laskar N, Uttley L, Balasubramanian SP (2014) Systematic review and meta-analysis of predictors of post-thyroidectomy hypocalcaemia. Br J Surg 101: 307–320.
7. Adler JT, Sippel RS, Schaefer S, Chen H (2008) Preserving function and quality of life after thyroid and parathyroid surgery. Lancet Oncol 9: 1069–1075.
8. Prazenica P, O’Keeffe L, Holy R (2015) Dissection and identification of parathyroid glands during thyroidectomy: association with hypocalcaemia. Head Neck 37: 393–399.
9. Abd Elmaksoud AE, Farahat IG, Kamel MM (2015) Parathyroid gland autotransplantation after total thyroidectomy in surgical management of hypopharyngeal and laryngeal carcinomas: a case series. Ann Med Surg (Lond) 4: 85–88.
10. Kihara M, Yonemura H, Miyauchi A, Matsuoka K (2000) Recovery of parathyroid function after total thyroidectomy. Surg Today 30: 333–338.
11. Dudley NE (1971) Methylene blue for rapid identification of the parathyroids. Br Med J 3: 680–681.
12. Ji YB, Lee KJ, Park YS, Hong SM, Paik SS, et al. (2012) Clinical efficacy of sentinel lymph node biopsy using methylene blue dye in clinically node-negative papillary thyroid carcinoma. Ann Surg Oncol 19: 1868–1873.
13. Tian W, Jiang Y, Gao B, Zhang X, Zhang S, et al. (2014) Application of nano-carbon in lymph node dissection for thyroid cancer and protection of parathyroid glands. Med Sci Monit 20: 1925–1930.
14. McWade MA, Paras C, White LM, Phay JE, Mahadevan-Jansen A, et al. (2013) A novel optical approach to intra-operative detection of parathyroid glands. Surgery 154: 1371–1377; discussion 1377.
15. Ladurner R, Halfeldt KK, Al Arabi N, Stepp H, Mueller S, et al. (2013) Optical coherence tomography as a method to identify parathyroid glands. Lasers Surg Med 45: 654–659.
17. Pelizzo MR, Sorgato N, Isabella Merante Boschin I, Marzola MC, Colletti PM, et al. (2014) Does the ultrasound dissector improve parathyroid gland preservation during surgery? *Eur J Surg Oncol* 40: 865–868.

18. Kim YS (2012) Impact of preserving the parathyroid glands on hypocalcemia after total thyroidectomy with neck dissection. *J Korean Surg Soc* 83: 75–82.

19. Patel HP, Chadwick DR, Harrison BJ, Balasubramanian SP (2012) Systematic review of intravenous methylene blue in parathyroid surgery. *Br J Surg* 99: 1345–1351.

20. McWade MA, Sanders ME, Broome JT, Solorzano CC, Mahadevan-Jansen A (2016) Establishing the clinical utility of autofluorescence spectroscopy for parathyroid detection. *Surgery* 159: 193–202.

21. Wang L, Yang D, Lv JY, Yu D, Xin SJ (2017) Application of carbon nanoparticles in lymph node dissection and parathyroid protection during thyroid cancer surgeries: a systematic review and meta-analysis. *Onco Targets Ther* 10: 1247–1260.

22. Kim SW, Lee HS, Ahn YC, Park CW, Jeon SW, et al. (2018) Near-infrared autofluorescence image-guided parathyroid gland mapping in thyroidectomy. *J Am Coll Surg* 226: 165–172.

23. Ladurner R, Al Arabi N, Guendogar U, Hallfeldt K, Stepp H, et al. (2018) Near-infrared autofluorescence imaging to detect parathyroid glands in thyroid surgery. *Ann R Coll Surg Engl* 100: 33–36.

24. Freire AV, Ropelato MG, Ballerini MG, Acha O, Bergada I, et al. (2014) Predicting hypocalcemia after thyroidectomy in children. *Surgery* 156: 130–136.

25. Ambe PC, Bromling S, Knoefel WT, Rehders A (2014) Prolonged duration of surgery is not a risk factor for postoperative complications in patients undergoing total thyroidectomy: a single center experience in 305 patients. *Patient Saf Surg* 8: 45.