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Intraoperative Guidance in Parathyroid Surgery using Near-Infrared Fluorescence Imaging and Low-Dose Methylene Blue

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Abstract

Background—Identification of diseased and normal parathyroid glands during parathyroid surgery can be challenging. The aim of this study was to assess whether near-infrared (NIR) fluorescence imaging using administration of a low-dose Methylene Blue (MB) at the start of surgery could provide optical guidance during parathyroid surgery and assist in the detection of parathyroid adenomas.

Methods—Patients diagnosed with primary hyperparathyroidism planned for parathyroid resection were included. Patients received 0.5 mg/kg MB intravenously directly after start of anesthesia. During surgery, NIR fluorescence imaging was performed to identify parathyroid adenomas. Imaging results were compared to a previous published feasibility study in which 12 patients received MB after surgical identification of the adenoma.

Results—13 patients were included in the current study. In 10/12 patients with a histologically proven adenoma, the adenoma was fluorescent. Mean Signal to Background Ratio (SBR) was 3.1 ± 2.8 . Mean diameter of the resected lesions was 17 ± 9 mm (range 5 – 28 mm). Adenomas could be identified up to 145 minutes after administration, which was the longest timespan until resection. Interestingly, in 3 patients, a total of 6 normal parathyroid glands (median diameter 2.5 mm) with a SBR of 1.8 ± 0.4 were identified using NIR fluorescence imaging.

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CONFLICT OF INTEREST STATEMENT

Quirijn R.J.G. Tummers, M.D.: Nothing to declare

Abbey Schepers, M.D., Ph.D.: Nothing to declare

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John V. Frangioni, M.D., Ph.D.: FLARETM technology is owned by Beth Israel Deaconess Medical Center, a teaching hospital of Harvard Medical School. Dr. Frangioni has started three for-profit companies, Curadel, Curadel ResVet Imaging, and Curadel Surgical Innovations, which has optioned FLARETM technology for potential licensing from Beth Israel Deaconess Medical Center.

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Alexander L. Vahrmeijer, M.D., Ph.D.: Nothing to declare

Conclusion—Early administration of low dose MB provided guidance during parathyroid surgery by identifying both parathyroid adenomas and normal parathyroid glands. In patients where difficult identification of the parathyroid adenoma is expected, or when normal glands have to be identified, administration of MB could improve surgical outcome.

INTRODUCTION

Primary hyperparathyroidism is a common endocrine disorder caused by parathyroid adenomas or hypertrophic parathyroid glands (¹). Complete surgical resection of these lesions is the only curative treatment. For diagnosis and surgical planning, preoperative imaging can be performed with several imaging modalities. Available imaging modalities currently consist of ultrasound (US), radionuclide scanning, magnetic resonance imaging, and both conventional and “4 D” computed tomography (²). In daily practice, various combinations of these imaging options are used.

During surgery, adequate identification of parathyroid adenomas and parathyroid glands is of utmost importance for a curative treatment and to minimize the need for surgical exploration of the neck. However, few intraoperative imaging modalities are available. In 1971 Dudley et al. reported the use of intravenous administration of high dose Methylene Blue (MB) for the intraoperative identification of parathyroid adenomas (³). Since then, this technique has been repeated by many groups (^{4–6}). However, administration of high dose MB (7.5 mg/kg) is associated with serious adverse events such as toxic metabolic encephalopathy (⁷) and can lead to blue staining of the complete surgical field due to leakage when surgical procedures takes longer.

Besides the use of high dose MB, several intraoperative imaging modalities are described, all with their own disadvantages. Radio-guided identification using ^{99m}Tc-sestamibi and hand-held gamma probes lacks real-time visual guidance (^{5;8;9}). Moreover, nuclear isotopes are scarce in large areas of the world. Aminolevulinic acid (ALA) was successfully tested in clinical studies, but can cause phototoxic reactions for which patients have to be protected from direct light exposure for 48 hours (^{10–12}). Moreover, ALA is mainly fluorescent in the visual light spectrum, which leads to high absorption and scatter of visible light in living tissue.

One of the techniques that could overcome these issues is near-infrared (NIR) fluorescence imaging. NIR fluorescence imaging is an innovative technique to visualize tumors, vital structures and lymphatic channels and lymph nodes (¹³). Recently, our research group showed feasibility of intraoperative identification of parathyroid adenomas using NIR fluorescence imaging and low dose MB as fluorescent tracer (¹⁴). In this previous study, parathyroid adenomas were identified by surgical identification, whereafter 0.5 mg/kg MB (low-dose) was administered intravenously to assess feasibility. In 9 out of 12 patients, the exposed adenoma became fluorescent after MB administration within 5 – 10 minutes. For intraoperative guidance in parathyroid surgery, it would be of great added value if MB could be administered before surgical identification, resulting in faster identification of parathyroid adenomas.

Therefore, the aim of this study was to assess whether early MB administration provides guidance in parathyroid adenoma identification during surgery. Subsequently, the obtained results were compared with previous published data to compare early and late MB administration protocols.

MATERIALS AND METHODS

Clinical Study

This study was approved by the Medical Ethics Committee of the Leiden University Medical Center and was performed in accordance with the ethical standards of the Helsinki Declaration of 1975. All patients planning to undergo a resection of a parathyroid adenoma for primary hyperparathyroidism were eligible for participation in the study. Exclusion criteria were pregnancy or lactation, the use of serotonin reuptake inhibitors, serotonin, and noradrenalin reuptake inhibitors and/or tricyclic antidepressants, severe renal failure, G6PD-deficiency, or an allergy to MB. All patients gave informed consent and were anonymized. Patients received standard of care diagnostic work-up, which for our center included a preoperative cervical US and a preoperative ^{99m}Tc -sestamibi-SPECT, combined with a low-dose computed tomography (CT) scan for preoperative surgical planning.

Directly before or after start of anesthesia, 0.5 mg/kg MB was administered intravenously over 5 minutes. During surgery, after subplatysmal dissection, retraction and initial exploration, visual inspection combined with NIR fluorescence imaging was used for identification of parathyroid adenomas and normal parathyroid glands. After identification and resection of the adenoma, an intraoperative parathyroid hormone (PTH) assay was performed. Postoperative histopathological assessment of the resected specimen served as gold standard for parathyroid adenoma identification. No standard intraoperative frozen section analysis was performed.

Intraoperative NIR fluorescence imaging

For NIR fluorescence imaging, the Mini-FLARE imaging system was used (¹⁵). Briefly, the system consists of 2 wavelength isolated light sources: a “white” light source, generating 26,600 lx of 400 to 650 nm light, and a “near-infrared” light source, generating 1.08 mW/ cm^2 of ≈ 670 nm light. Color video and NIR fluorescence images are simultaneously acquired and displayed in real time using custom optics and software that separate the color video and NIR fluorescence images. A pseudo-colored (lime green) merged image of the color video and NIR fluorescence images is also displayed. The imaging head is attached to a flexible gooseneck arm, which permits positioning of the imaging head at extreme angles virtually anywhere over the surgical field. For intraoperative use, the imaging head and imaging system pole stand are wrapped in a sterile shield and drape (Medical Technique Inc., Tucson, AZ).

Comparison between early and late MB administration protocol

The obtained results were compared to the previous performed and published feasibility trial (NTR3403) (¹⁴). This trial was performed using the same imaging system and identical surgical workup. In both studies, patients received 0.5 mg/kg MB. The only difference was

that in the previous published study, MB was administered directly after surgical exposure of the suspected parathyroid adenoma. In the current study, MB was administered directly after start of anesthesia, before the surgical incision and assisted with parathyroid adenoma identification.

Statistical Analysis

For statistical analysis, SPSS statistical software package (Version 20.0, Chicago, IL) was used. Signal-to-background ratios (SBR) were calculated by dividing the fluorescent signal in the parathyroid adenoma by fluorescent signal of surrounding healthy tissue. Patient age and body mass index (BMI) were reported in mean and range and signal-to-background was reported in mean and standard deviation. To compare characteristics between different administration protocols, the independent-sample *t* test and chi-square test were used. *P* < 0.05 was considered significant.

RESULTS

Patient characteristics

Thirteen consecutive patients with primary hyperparathyroidism planned for surgical resection of a parathyroid adenoma were included in this study. Patient and surgical characteristics are shown in Table 1. Mean age was 53 years (range, 21 – 75) and 5 patients were male. Mean BMI was 26.6 ± 3.6 , mean preoperative calcium level was 2.8 ± 0.1 mmol/L and mean preoperative PTH level was 11.9 ± 7.2 pmol/L. All patients were diagnosed with primary hyperparathyroidism and in one patient this was associated with multiple endocrine neoplasia (MEN) 1 syndrome. One patient was treated for recurrent disease, after an initial parathyroid adenoma resection. The mean diameter of the resected lesions was 17 ± 9 mm (range 5 – 28 mm).

Preoperative Imaging

Table 2 shows the preoperative imaging characteristics. In 12 out of 13 patients, a ^{99m}Tc -sestamibi-SPECT/CT was performed. In 8 of 13 patients, a suspected parathyroid adenoma could be localized, whereas in 4 patient no evidence for localization of the adenoma was found. In 12 of 13 patients preoperative US was performed. In 5 patients a lesion suspect for parathyroid adenoma was identified. In 1 patient no preoperative US was performed because she was referred from a foreign clinical for surgery and ^{99m}Tc -sestamibi-SPECT/CT showed clear localization of a parathyroid adenoma.

Intraoperative NIR Fluorescence Imaging

In 9 out of 13 patients, intraoperative NIR fluorescence imaging clearly identified a parathyroid adenoma or hyperplastic parathyroid gland. Figure 1 shows a representative example of the intraoperative fluorescent signal in a parathyroid adenoma. The mean SBR of the identified NIR fluorescent adenomas or hyperplastic parathyroid glands was 3.1 ± 2.8 .

In 1 patient, the intraoperatively identified parathyroid adenoma appeared non-fluorescent *in vivo*, but this was caused by a hemorrhage on the ventral side of the adenoma. *Ex vivo*, the deepest located part of this adenoma also showed clear fluorescence. In 12 patients,

histological evaluation confirmed that the resected tissue was parathyroid adenoma (N = 8) or hyperplastic parathyroid gland (N = 4). In 2 patients, the resected parathyroid adenoma did not fluoresce. In summary, in 10 out of 12 patients with a proven parathyroid adenoma or hyperplastic parathyroid gland, the lesion was fluorescent.

In 1 patient (#9), no adenoma was found during exploration of the neck. A thymectomy was performed and 6 non-fluorescent lymph nodes were removed. During histological assessment of the resected tissue, no parathyroid adenomas were identified as well, although her intraoperative PTH assay showed a decrease from 25.0 to 1.4 pmol/L.

Interestingly, in 3 patients, a total of 6 normal parathyroid glands were identified using NIR fluorescence imaging (median size 2.5 mm, range 1–4). Figure 2A and 2B show an example of 2 small (1 and 2 mm) normal parathyroid glands located in the left and right thymus. These were not identified without NIR fluorescence imaging. Both glands were biopsied for frozen section analysis and were found histologically to be normal parathyroid glands. Subsequently, the non-biopsied part of both glands were re-transplanted in the sternocleidomastoid muscle. Mean SBR of the normal parathyroid glands was 1.8 ± 0.4 . The smallest normal parathyroid gland identified by NIR fluorescence imaging was 1 mm.

Mean time interval between administration of MB and detection of the adenoma was 77 ± 34 minutes. Parathyroid adenomas could be identified up to 145 minutes after administration, which was the longest timespan until resection.

No adverse effects regarding the use of MB or NIRF fluorescence imaging were seen.

Correlation ^{99m}Tc -sestamibi-SPECT/CT scan and NIR fluorescence imaging

In all patients where a suspected adenoma was identified on ^{99m}Tc -sestamibi-SPECT/CT, the resected parathyroid tissue was fluorescent (8 out of 8). Moreover, when analyzing all patients with an adenoma (N=12) that received a ^{99m}Tc -sestamibi-SPECT/CT (N=11), concordance between ^{99m}Tc -sestamibi-SPECT/CT status and NIR fluorescence status of the resected lesion was observed in 10 out of 11 patients. In 1 patient where the ^{99m}Tc -sestamibi-SPECT/CT was negative, the adenoma appeared to be fluorescent, and in 1 patient where the ^{99m}Tc -sestamibi-SPECT/CT was negative, 2 normal glands were identified using NIR fluorescence imaging.

Comparison between early and late MB administration protocol

There were no significant differences in patient characteristics between of the current study and the previous reported study regarding age, gender, BMI, preoperative calcium, preoperative PTH and diameter (Table 3). The most important difference, although not significant, was found in SBR between different administration protocols. Mean SBR was 3.1 in the early administration group versus 6.1 in the group that received MB after surgical exposure of the adenoma ($P=0.08$; mean difference 2.95; $-0.40 - 6.29$ 95% CI).

Combining the two groups showed that in 16/25 patients the preoperative ^{99m}Tc -sestamibi-SPECT/CT scan indicated an adenoma. In 15 out of these 16 patients, the detected adenoma was NIR fluorescent, leading to a positive predictive value of 94%. The only patient who

had a ^{99m}Tc -sestamibi-SPECT/CT positive lesion, that appeared non-fluorescent, was a patient who suffered from parathyroid carcinoma.

DISCUSSION

Despite the fact that surgical resection of parathyroid adenomas is a commonly performed and overall successful surgical procedure, intraoperative detection of an adenoma can be challenging. Moreover, the anatomical site where parathyroid adenomas are located contains many vital structures like arteries, veins, and nerves. When preoperative localization fails, and extended surgical neck exploration is needed, the risk of iatrogenic damage to these structures and associated complications increase (¹⁶). Therefore, intraoperative optical guidance in parathyroid surgery using exogenous contrast agents could assist in a faster and more accurate identification of parathyroid adenomas, without causing iatrogenic injury to surrounding vital structures.

Previously our research group showed feasibility of intraoperative identification of parathyroid adenomas using NIR fluorescence imaging and low-dose MB in humans (¹⁴). In that clinical trial, MB was administered after surgical exposure of the adenoma. The current study confirmed the results of the previous described study, showing the possibility to intraoperatively identify parathyroid adenomas using MB and its correlation with preoperative a ^{99m}Tc -sestamibi-SPECT/CT scan. Combining the two groups showed that 16 out of these 25 patients had a ^{99m}Tc -sestamibi-SPECT/CT scan indicating an adenoma. In 15 out of these 16 patients, the detected adenoma was NIR fluorescent, leading to a positive predictive value of 94% for a NIR fluorescent adenoma after a positive ^{99m}Tc -sestamibi-SPECT/CT scan. One could state that all patients with a positive ^{99m}Tc -sestamibi-SPECT/CT scan should receive MB for the improved identification of parathyroid adenomas.

In the current study, 2 parathyroid adenomas did not fluoresce. The histopathological analysis of these 2 adenomas were comparable with the NIR fluorescent positive adenomas, so the reason for non-fluorescence remains unclear.

Interestingly, we identified normal parathyroid glands, as small as 1 mm, during surgical exploration of the neck. These glands were not detected by the surgeon by visual inspection or palpation. Normal parathyroid glands can be difficult to distinguish during surgery because of their small size, their variability in location, and similar appearance to lymph nodes. Detection of these normal glands can be of great added value during subtotal parathyroid glands resection in the case of MEN1 syndrome, where 3.5 glands are removed and a portion of the most normal appearing gland is left in site or is auto-transplanted (¹⁷). Also when normal glands are suppressed by hyperfunctional parathyroid glands, as in patient #9 of the current study, imaging can be useful. These glands were very small, and located in the thymus on both sides, and would not have been auto-transplanted if not detected in the thymectomy specimens by NIR fluorescence imaging. Improved identification of both parathyroid adenoma and normal glands can limit the extent of neck exploration. Especially small normal glands are at risk of iatrogenic injury when not identified in time during neck exploration. The identification of normal glands using NIR

fluorescence imaging and MB was described by Antika et al. ⁽¹⁸⁾ in rabbits. They used escalating dosages from 0.025 mg/kg to 3 mg/kg (human equivalent dose 0.008 mg/kg to 0.97 mg/kg calculated by using allometric dose translation based on body surface area ⁽¹⁹⁾) for identification of thyroid and parathyroid glands. However, to the best of our knowledge, this is the first clinical trial to demonstrate the intraoperative identification of normal glands using NIR fluorescence imaging and MB.

When early administration of MB is compared to late administration (after visual identification of the adenoma), a mean SBR of the adenoma of 3.1 in the early administration group versus 6.1 in the late administration group is seen. Although this difference was not significant ($P = 0.08$), it shows that MB follows the kinetics of a perfusion tracer, and don't show clear (active) accumulation in parathyroid adenomas. Nevertheless, a mean SBR of 3.1 is suitable for intraoperative identification of parathyroid adenoma's and normal glands as clearly shown in the current study.

Developing probes that specifically accumulate in parathyroid glands could enhance SBR and the time window of which optimal SBRs are achieved. A recent published study of our research group showed the high-sensitivity identification of parathyroid and thyroid glands in several animal models using novel tissue-specific NIR fluorophores ⁽²⁰⁾. However, probes such as these are not clinically available yet, and development of these probes is expensive and time consuming. Therefore, it is important to exploit clinically available contrast agents, such as MB ⁽²¹⁾.

Recently, several studies reported the use of autofluorescence characteristics of parathyroid tissue for discrimination between different tissue types using spectroscopy ^(22;23). They found autofluorescent properties in parathyroid and thyroid glands with a peak fluorescence at 822 nm when excited with 785 nm light. Using autofluorescence would make the development of exogenous contrast agents superfluous. However, no 2-D optical guidance from spectroscopy is yet possible. And, although the spectroscopy results were validated in a larger patient cohort using a customized endoscopic imaging system, ⁽²⁴⁾ this autofluorescent signal has not been seen in previous studies **by** our research group using our imaging system in humans or different animal species ^(14;20).

NIR fluorescence imaging is a growing field of interest worldwide, more imaging systems have been introduced for both open and endoscopic imaging ^(25;26). Prices of NIR fluorescence imaging systems are starting from \$40.000. NIR fluorescence imaging in parathyroid surgery has the potential to speed up the surgical procedure by assisting in the identification of parathyroid adenomas and normal parathyroid gland. In the current study, time interval between administration and detection of adenomas are reported, however they could not be compared to patients not undergoing NIR fluorescence-assisted surgery. Therefore, randomized controlled trials in which patients are receiving NIR fluorescence imaging or not are needed.

To conclude, NIR fluorescence imaging using MB can provide guidance during parathyroid surgery by identifying both parathyroid adenomas and normal parathyroid glands, for at least 145 minutes after MB administration. In patients where difficult identification of the

parathyroid adenoma is expected, or when normal glands have to be identified, administration of MB could potentially improve surgical outcome.

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Figure 1. Intraoperative NIR fluorescence-guided resection of a parathyroid adenoma located in the neck

During minimally-invasive parathyroid surgery, a 19 mm parathyroid adenoma is identified using NIR fluorescence imaging (arrow).

Figure 2. Intraoperative NIR fluorescence imaging of normal parathyroid glands

A. A 2 mm normal parathyroid gland (arrow) was identified in the right thymus using NIR fluorescence imaging after thymectomy. **B.** A 1 mm normal parathyroid gland (arrow) was identified in the left thymus using NIR fluorescence imaging after thymectomy. Both glands in A and B could not be identified using visual inspection.

TABLE 1

Patient and surgical characteristics

Patient no.	Sex	Age	BMI	Disease	Previous treatment	Preoperative calcium, mmol/L	Preoperative PTH, pmol/L	Surgical procedure	Histology	Maximum diameter, mm
1	F	40	24.0	PH	No	2.70	7.60	PAR right	Parathyroid adenoma	20
2	M	34	31.8	PH	No	3.03	28.30	PAR left	Parathyroid adenoma	25
3	M	60	26.8	PH	No	2.80	13.30	PAR left, thymectomy right, hemithyroidectomy left	Parathyroid hyperplasia	6; 7
4	M	59	24.0	PH	PAR right	2.85	6.90	PAR right, thymectomy right	Parathyroid adenoma	14
5	M	21	27.8	PH by MEN1	No	2.89	11.80	PAR right, parathyroidectomy left and right, thymectomy	Parathyroid adenoma and normal PTG	23
6	F	67	25.1	PH	No	2.72	24.00	PAR left	Parathyroid adenoma	19
7	F	60	24.8	PH	No	2.89	9.30	PAR right	Parathyroid adenoma	20
8	F	75	21.5	PH	No	2.72	6.60	PAR left	Parathyroid adenoma	14
9	F	44	28.1	PH	No	2.88	15.70	Identification normal PTGs, LN resection, thymectomy left	Reactive lymph nodes	N/A
10	F	47	32.9	PH	No	2.62	12.60	PAR left	Parathyroid hyperplasia	6
11	M	62	30.7	PH	No	2.66	4.20	PAR left	Parathyroid adenoma	5
12	F	70	26.8	PH	No	2.78	9.80	PAR left and right, thymectomy	Parathyroid hyperplasia and normal PTGs	28; 20
13	F	52	22.8	PH	No	2.67	8.30	PAR left	Parathyroid hyperplasia	22

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Abbreviations: BMI, body-mass index; LN, lymph nodes; MEN1, multiple endocrine neoplasia 1 syndrome; N/A, not applicable; PAR, parathyroid adenoma resection; PH, primary hyperparathyroidism; PTG, parathyroid gland; PTH, parathyroid hormone.

TABLE 2

Identification characteristics of parathyroid adenomas.

Patient no.	Sestamibi-SPECT-CT	Preoperative ultrasound	NIRF detection	PTH decrease post-resection	Time between administration and NIRF detection (minutes)	SBR
1	+	+	+	+	106	1.5
2	-	-	-	+	N/A	N/A
3	+	-	+	+	102 and 132	5.7 and 2.3
4	+	-	+	+	113	2.3
5	+	+	+	+	115; 109 and 134	2.4 (adenoma); 1.4 and 2.0 (normal PTGs)
6	+	+	+	+	34	10.7
7	NP	+	-*	+	60	1.8 (ex vivo)
8	+	+	+	+	87	1.8
9	-	-	*** +	+	78 and 78	2.4 and 1.6 (normal PTGs)
10	-	-	-	+	N/A	N/A
11	-	-	+	+	54	1.7
12	+	NP	+	+	61 and 145; 135 and 136	3.8 and 1.7 (hyperplasia); 1.4 and 1.7 (normal PTGs)
13	+	-	+	+	41	2.2

Abbreviations: N/A, not applicable; NIRF, Near-infrared fluorescence; NP, not performed; PTG, parathyroid gland; PTH parathyroid hormone; SBR, Signal-to-Background ratio; SPECT, single photon emission computed tomography.

* No in vivo NIRF detection of parathyroid adenoma. Ex vivo, the deepest located part of adenoma appeared to be fluorescent.

** Identification of normal PTGs only using NIRF imaging.

Table 3

Comparison between early and late Methylene blue administration

Characteristic	Early MB administration (N=13)	Late MB administration (N=12)	P-value
Sex			0.67
Female	8	9	
Male	5	3	
	Mean	Mean	
Age	53	56	0.65
BMI	26.7	27.1	0.82
Preoperative calcium	2.79	2.68	0.42
Preoperative PTH	12.2	119.4	0.22
Diameter	17	16	0.80
SBR	3.11	6.10	0.08