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Intraoperative Parathyroid Gland Identification Using Autofluorescence: Pearls and Pitfalls

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Abstract

Due to their small size, variable location, variable aspect and tenuous vascularization, identifying and preserving Parathyroid Glands (PTG's) during thyroid surgery are challenging and have a steep learning curve. Temporary and permanent hypoparathyroidism are problematic in thyroid surgery particularly because this surgery is performed in most cases for benign disease or for cancers with an excellent prognosis. Surgery for hyperparathyroidism be it primary, secondary or tertiary, is challenging and locating ectopic disease can be daunting. For these reasons, intraoperative PTG autofluorescent imaging, a new non-invasive technique that can reliably aid in positively identifying PTG's, has been embraced by an increasing number of surgeons throughout the world and may lead to a revolution in thyroid, parathyroid and head and neck surgery. The aim of this report is to describe the technique and the currently published results and then to describe the false positives and false negatives and suggest ways to optimize the technique in clinical practice.

Keywords: Parathyroid; Autofluorescence; Imaging; Hyperparathyroidism; Thyroid surgery

Introduction

Due to their small size, variable location, variable aspect and tenuous vascularization, identifying and preserving Parathyroid Glands (PTG's) during thyroid surgery is challenging and has a steep learning curve. Temporary and permanent hypoparathyroidism are problematic in thyroid surgery particularly because this surgery is performed in most cases for benign disease or for cancers with an excellent prognosis. Surgery for hyperparathyroidism is it primary, secondary or tertiary, is challenging and locating ectopic disease can be daunting.

In the past, intraoperative intravenous injection of methylene blue dye was used by some to attempt to increase the color contrast between the thyroid gland, the paratracheal fat and the PTG's, but was not always reliable and not largely embraced by surgeons. Furthermore, methylene blue interferes with monitoring of blood oxygen levels during general anesthesia, and has fallen out of favor today due to a risk of serotonin toxicity with severe neurologic effects in patients treated with selective Serotonin Reuptake Inhibitors (SSRI's) but also in association with other less potent inhibitors or serotonin reuptake such as fentanyl and tramadol [1-6]. The product carries a specific warning issued by the American Food and Drug Administration [7].

For parathyroid adenomas and hyperplasia, radionuclide probe-guided surgery may aid in detecting and positively identifying the diseased glands, but for all practical purposes only in centers where collaboration with nuclear medicine teams is routine. This technique seems to be particularly helpful when preoperative localization with sestamibi scan is negative [8,9].

Other technologies such as infrared fluorescent methylene blue staining, 5-Aminolevulinic Acid (5-ALA) imaging, Indocyanine Green (ICG) angiography, optical coherence tomography, laser speckle contrast imaging, dynamic optical contrast imaging and Raman spectroscopy are currently being developed and tested for intraoperative PTG identification and/or angiography [10]. Few of these other techniques are currently adapted for routine use in the operating room, however, and the gold standard for identification of PTG's and for determining their viability is still the surgeon's subjective visual evaluation.

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For these reasons, intraoperative PTG autofluorescent imaging, a new non-invasive technique that can reliably aid in positively identifying PTG's, has been embraced by an increasing number of surgeons throughout the world and may lead to a revolution in thyroid, parathyroid and head and neck surgery. The aim of this report is to describe the technique and the currently published results and then to describe the false positives and false negatives and suggest ways to optimize the technique in clinical practice.

Technique and Results

The autofluorescent qualities of PTG's were discovered by Professor Mahadevan-Jansen and her team of scientists at Vanderbilt University in 2010 [11]. They found that when illuminated with a laser of a specific wavelength (785 nm), the parathyroid glands spontaneously give off light in the infrared spectrum with a fluorescence peak at 820 nm to 830 nm that can be detected using Near Infrared (NIR) spectroscopy or a NIR camera [12,13]. They showed that the intensity of the endogenous fluorescence signal given off by the PTG's is 2 to 10 times higher than that of the adjacent thyroid tissue, making it easy to distinguish the two structures visually or by using a specially developed fibered probe connected to NIR fluorescence spectrometer [13]. The measured intensity of the PTG is thus the relative intensity as compared to the thyroid, and not an absolute value.

Further studies using infrared cameras have confirmed these findings in vivo, but also ex-vivo, and even after heating to 95°C, after cyropreservation and after formalin fixation of PTG's in the pathology lab [14,15]. *In-vivo* clinical studies during thyroid and parathyroid surgery have demonstrated the feasibility of using different laser + infrared camera devices in routine clinical practice [15-20].

There are practical differences between the probe-based-fluorescence spectroscopy and camera-based techniques. Both techniques, using only light, with no intravenous injection, are completely non-invasive, although the probe for NIR spectroscopy lightly touches the tissues [14]. The NIR camera has the advantage of providing spatial information and mapping by providing a black and white or color image of the operating field allowing spatial localization as well as positive identification. Many of these cameras can also be used for other types of NIR imaging for other indications, particularly for intraoperative angiography using intravenous indocyanine green. But the NIR camera is bulkier than probe-based NIR spectroscopy and seems to be more sensitive to other light sources. The probe-based NIR spectroscopy may have a higher specificity with fewer false positives as spectroscopy is highly sensitive to the biochemical composition of the tissues. Most clinical studies currently published have used a NIR camera system as opposed to the probe-based NIR spectroscopy with its limited spatial resolution. A prospective randomized trial would more objectively provide information on the sensitivity and specificity of these two methods.

Falco et al. [18,19] were among the first to report that a greater number of PTG's were detected by using NIR camera imaging than by visual identification alone during thyroid and parathyroid surgery. In 74 patients, an average of 2.5 PTG's were visualized per patient using the naked eye versus 3.7 per patient using NIR imaging ($p<0.0001$), and all four glands were seen in 86% of patients using the NIR camera versus in only 12% of patients without it. Then, in a case-control study using historic controls, Benmiloud et al. [21] demonstrated that using NIR imaging for PTG preservation during thyroidectomy was

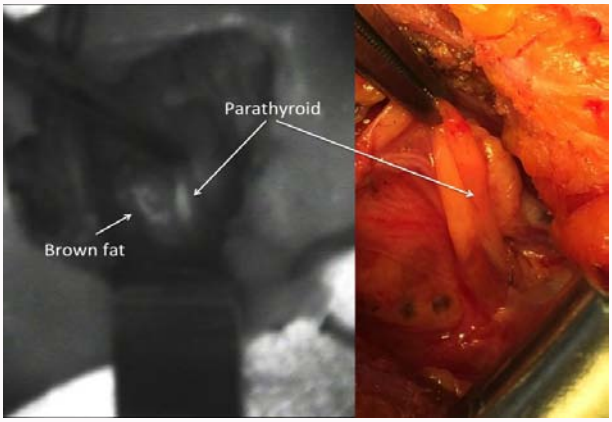

Figure 1: Parathyroid autofluorescence next to fluorescent brown fat (Fluobeam® Fluoptics, Grenoble, France).

associated with an increased mean number of identified parathyroids, a reduced rate of parathyroid autotransplantation, and a significantly lower rate of immediate postoperative hypocalcemia (5.2% *vs.* 20.9%), although no difference was observed in the rate of inadvertent PTG resection. Parathyroids were identified with the NIR camera before they were visualized by the surgeon in 68% of patients in this study.

Kahramangil et al. [22] reported the first multicenter study in 3 centers that included 210 patients undergoing total thyroidectomy, lobectomy or parathyroidectomy. With the detection of 584/594 PTG's by autofluorescence for a sensitivity of 97% to 99% in all three centers. In 46% of the glands (272/594), a PTG not seen with the naked eye was identified with the NIR camera, without further dissection. On a per-patient analysis, an additional PTG was visualized using the NIR camera versus visual identification alone in 77% of cases. Most recently, in a randomized controlled trial including 170 patients, Dip et al. [23] again confirmed the increase in the number of PTG's identified using a NIR camera as compared to classic visual identification during total thyroidectomy-2.6 glands identified per patient *vs.* 3.5 in the NIR camera group-with a corresponding decrease in temporary postoperative hypocalcemia ($p=0.005$).

False negatives

Not all PTG's fluoresce with the same intensity. In the initial studies using fluorescence spectroscopy, there were no false negatives-that is, no true PTG's that did not autofluorescent-but there was definite heterogeneity in their intensity, to the point that when calculating the normalized intensity ratio as compared to the thyroid gland, the optimal intensity cutoff for PTG identification was only 1.29 times that of the thyroid gland [14]. A further study by our team using a near infrared camera (Fluobeam® Fluoptics, Grenoble, France) found that most normal PTG's autofluorescent with an intensity around 1 to 3 times that of the thyroid gland, with a minority (<2%) fluorescing less and many (around 35%) fluorescing even more [15]. Even within the same patient, normal PTG's may have different intensities. Despite this heterogeneity, this study of PTG's *in-vivo* found a sensitivity of 98.1%, meaning that a true PTG will rarely not be detected with the NIR camera. Ladurner et al. [24], using an endoscopic NIR camera device also found that some normal glands were not autofluorescent enough to be detected; this false negative detection occurred in 4 glands (1 patient) out of 41 glands for a sensitivity of 90.2%.

Abnormal PTG's seem to be much more heterogeneous in their

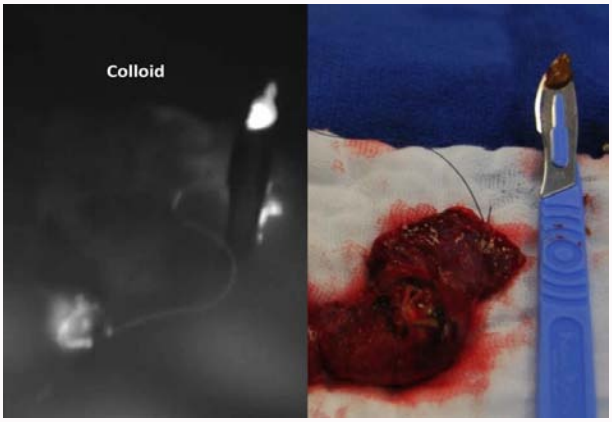

Figure 2: High intensity autofluorescnce of a colloid nodule and colloid on the end of the scalpel.

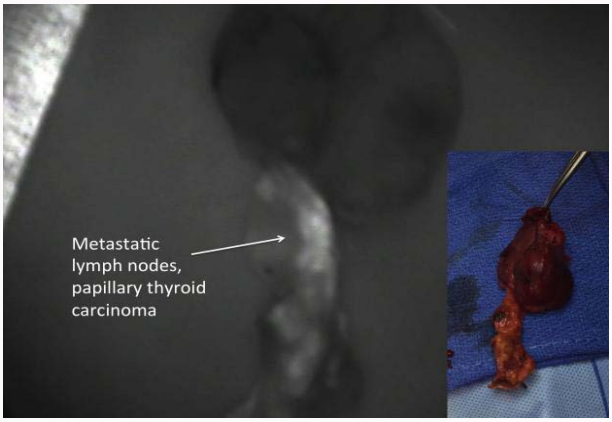

Figure 3: High intensity autofluorescence of lymph node metastases from papillary thyroid carcinoma.

autofluorescent intensities than normal PTG's. In the first systematic study of abnormal PTG's, either in the context of adenomas or multigland hyperplasia, DiMarco et al. [25] observed that only 257/284 PTG's were actually visualized with autofluorescence, for a 9.5% false negative rate and a sensitivity of 90.5%. They found that the higher the preoperative calcemia, the lower the fluorescence intensity ($p<0.01$). Furthermore, several teams have observed that these abnormal glands often have a heterogenous appearance within the same gland on NIR imaging, with brighter zones surrounded by less bright or non-fluorescent areas, making positive identification difficult [26].

False positives

Brown fat is the main source of falsely fluorescent tissue that visually resembles PTG due to its brownish-yellow color and its location in the areas surrounding the inferior PTG in the central neck. Careful comparison between the operating field and the NIR image may help distinguish the two. Brown fat generally has an elongated structure and a more diffuse fluorescent signal, with blurry margins on NIR images whereas PTG images generally have a more well-defined margin (Figure 1). This distinction may be difficult, particularly in the presence of a moderately-fluorescent PTG. Careful dissection may show the absence of a vascular pedicle in the case of brown fat, whereas the PTG will generally have very fine vascular pedicle. When in doubt, removing the specimen (if autotransplantation is being considered) and placing it in water will show if it floats (fat) or if it sinks (PTG). Frozen section analysis of a small fragment is also warranted in cases of thyroid cancer, however, because lymph nodes sink too. The fluorescent signal in brown fat may be due to the high porphyrin level in this tissue, a natural fluorophore (Dr Mahdeva-Jensen, personal communication). If this is the case, the unique spectral properties of porphyrin, with several wavelengths that constitute peaks in the absorption spectrum-particularly around 400 nm, as compared to the PTG that absorbs maximally at 785 nm could be employed to distinguish these two tissues, by using different laser wavelengths. Hyperspectral imaging enables simultaneous imaging of in multiple wavelengths, and is being developed with fluorophores for deep infrared imaging [27]. For now, the currently existing probe-based NIR fluorescence spectroscopy devices may decrease the discrepancy between brown fat and PTG's, with their capability of averaging several intensity readings, as compared to currently available camera-based techniques, but this requires further investigation.

Colloid nodules and colloid as a structure often have a highly intense fluorescent signal in NIR imaging. This may lead to a false impression of a subcapsular or intrathyroidal PTG. Dissection generally can rectify the diagnosis, but takes extra time in the operating room (Figure 2).

Finally, and importantly, lymph node metastases from papillary thyroid carcinoma have been shown to autofluorescence using commercially available NIR cameras (personal observation and Jordy Vidal-Fortuny, personal communication). Cystic lesions seem to fluoresce with a higher intensity, and visual inspection may aid in discriminating metastases from PTG's. However, as stated above, when in doubt, frozen section analysis is warranted before reimplantation to rule out metastatic disease (Figure 3).

Tips and Tricks

PTG autofluorescence is a very low-intensity signal, as compared to indocyanine green fluorescence or other fluorphores used in medicine. Detection of this low signal requires a very sensitive NIR camera, so other structures and configurations can give way to false positive images or extraneous "flashes" when using NIR imaging for PTG identification.

The NIR camera is sensitive to white light from the operating room and even from an open window, and "specular reflectance" just the bouncing of the white light off of an object such as a retractor-may cause stray bright images to appear. To optimize the imaging, the operating room lights need to be turned off, any windows closed, and lights from any other source (screens) covered or turned off. After that, in general, comparing the operating field with the image on the NIR screen can help differentiate true autofluorescence from any extraneous light just reflecting on the operating field. Blue objects, particularly Vicryl® sutures tend to autofluorescence using this technique, but, again, visual identification of the structure tends to alleviate any discrepancies.

Light can penetrate only a few millimeters so that the surface of the PTG must be accessible to the light or not too hidden under opaque tissues. Deep-seated PTG's will not be visualized using NIR autofluorescence without exposing the surface of the gland to the laser light and to the camera [28]. This may require more dissecting of the PTG than is normally required for naked eye visualization, with possibly a higher risk of devascularizing the gland. In addition, for the 1% to 2% intrathyroidal PTG's and that 2% to 5% that may be

Table 1: Potential pitfalls in parathyroid gland autofluorescent imaging.

Potential False Positives	Potential False Negatives
Brown fat Thyroid tissue/colloid nodule Lymph node metastasis (cystic, from papillary carcinoma)	Deep PTG Blood or water in the field Camera too far away Too much stray light in the room Pathologic glands Some normal glands (<2%)

in the thymic remnant, this light-based technology will not function [29,30].

Despite this technical drawback, in their studies of the identification of PTG's during surgery, however, Kahramangil et al. [22] found more PTG's using a NIR camera than with the naked eye, without more dissection than was needed for a normal operation.

The low signal intensity requires a sensitive camera with a long fluorescence signal integration time (around 1second) in order to improve the signal-to-noise ratio. Extraneous light can cause artefacts. The camera thus needs to be held very still and kept at the same distance from the tissue while imaging, to allow within patient and interpatient analysis. The intensity of the image is closely related to the camera angle and distance which requires a certain experience, and small, deep operating fields such as are used for minimally invasive thyroid and parathyroid surgery may be difficult to access with the laser light and camera. In our experience, there is a learning curve for the surgeon and for the operating room team that we would estimate at 10 to 15 cases, but this needs to be confirmed by objective studies. The bulk of some of the commercially available cameras complicate matters, but new systems are becoming available, and an endoscopic camera is already available [24]. Finally, to our knowledge, there is no system currently available specifically for PTG that can superimpose the NIR image on the color video image, as some systems can for indocyanine green NIR angiography, although some systems designed for indocyanine green angiography are capable of detecting PTG autofluorescence (the Quest Spectrum® camera, Quest Innovations, Middenmeer, The Netherlands, for example).

Future perspectives

The intensity of the autofluorescence of PTG's is dependent on the excitation wavelength and, as far as is currently known, only the 785 nm laser wavelength stimulates PTG fluorescence emission. Optimization of the fluorescence signal acquisition in the operating room (light off, physician training, and a standardized methodology) may lead to a higher sensitivity and specificity, and help "weed out" some of the causes of false positives. Objective quantification of the emitted light intensity would ideally provide a highly reliable and reproducible measurement and thus eliminate any variability related to thyroid tissue intensity, currently used as the standard with the probe-based techniques and which may cause false-negative readings with the NIR camera, as well. Even normal thyroid tissue may vary in fluorescent intensity from one individual to another, due to the presence of natural fluorophores such as flavins, lipofuscins and possibly even bilirubin [31,32]. A higher powered laser is unfortunately not an option for improving the penetration of the light because higher power would lead only to more diffusion, decreasing the signal-to-noise ratio, and may even be harmful by creating heat within the tissues. Combining spectroscopy and a camera may be a way to improve sensitivity and specificity. Identification of the natural fluorophore in PTG's may open a whole new realm of possibilities for the development of new fluorescent imaging technologies in the operating room.

In the clinical domain, the difference in intensity between abnormal and normal PTG's, with lower mean autofluorescent intensity and a heterogenous fluorescent pattern may aid in distinguishing normal parathyroids from abnormal glands during surgery for hyperparathyroidism [26]. This technology has been employed for identifying PTG's in laboratory animals and human donors for research. In extensive surgery for hypopharyngeal squamous cell carcinoma with thyroidectomy this technology is being evaluated to identify, preserve and reimplant parathyroid glands with the aim to reduce the rate of hypocalcemia in these already nutritionally-challenged patients (personal experience of the authors).

Finally, NIR autofluorescence may also prove to be a significant adjuvant, particularly for low-volume surgeons, to improve patient outcomes, and may be a tool to aid in teaching parathyroid and thyroid surgery, along the lines of recurrent laryngeal nerve neuromonitoring. New technology with superimposition of the PTG autofluorescent image on the operating field, for enhanced reality, is currently being tested [33,34]. The cost-effectiveness of this technology in terms of reduction in morbidity versus overall costs-investment, depreciation and operating room time remains to be evaluated, as well. Despite high-level evidence in favor of routine use of this technology or cost-effectiveness, two systems have already been approved by the FDA for clinical use: the probe-based PTeye® system (AiBiomed, Santa Barbara, CA) and the camera-based Fluobeam 800® (Fluoptics, Grenoble, France).

Conclusion

For now, in the absence of standardization, high-level evidence and recommendations, PTG autofluorescence remains a surgical adjunct and the surgeon's eye and intraoperative frozen section analysis remain the standard of care. More and more data suggest that using this technology can improve patient outcomes in thyroid surgery. Ongoing studies aim to increase the data and evidence base for NIR imaging of PTG's in diverse types of thyroid and parathyroid surgery, and to identify the mysterious structure or molecule that acts as the fluorophore in PTG's.

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