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The role of autofluorescence and Indocyanine green (ICG) in Endocrine Surgery

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ABSTRACT

Parathyroid preservation during total thyroidectomy is crucial in preventing postoperative hypoparathyroidism. The use of intraoperative parathyroid hormone monitoring has been shown to reduce the incidence of hypoparathyroidism. However, this method has limitations, and alternative techniques such as autofluorescence and ICG angiography have emerged as potential options for parathyroid preservation. The use of ICG angiography and near-infrared autofluorescence has shown promise in improving parathyroid gland preservation and reducing the risk of postoperative hypocalcaemia and hypoparathyroidism. The combination of autofluorescence and ICG angiography has also been found to be useful in identifying parathyroid glands in challenging cases, such as reoperations for thyroid or parathyroid diseases. However, further studies are needed to establish its long-term safety and efficacy and to address the limitations of its implementation in clinical practice.

Key Words: *Parathyroid glands; hypoparathyroidism; autofluorescence; indocyanine green; thyroidectomy*

INTRODUCTION

Total Thyroidectomy (TT) is one of the most commonly performed operations in Endocrine Surgery. TT is a procedure which has evolved tremendously, zeroing out historically reported mortality and significantly reducing morbidity making it a safe although technically demanding surgical procedure associated with excellent postoperative and oncological outcomes, if performed by experienced surgeons. However, when complications do occur, they can become life threatening or have a major impact in the quality of life of the patients. The incidence of surgical pathology of the thyroid gland has led to the ability of endocrine surgeons to accumulate significant experience in surgical technique. In this context, endocrine surgeons must strive for even better outcomes in complications such as injury of recurrent laryngeal nerve and transient or permanent postoperative hypoparathyroidism, which still remains a challenge even for high volume endocrine surgeons [1,2].

The identification and vascular preservation of the parathyroid gland vasculature is a difficult, alas crucial step of TT. More specifically, this step is characterized by obvious difficulties such as identification of the parathyroid glands among the surrounding tissues and their highly fragile and submillimeter vessels which cannot be easily identified or preserved. In addition to this, variations in location (e.g. intrathyroidal parathyroid gland, intrathymic parathyroid gland, ectopic superior parathyroid gland), morphology and blood supply make the intraoperative identification of parathyroid glands demanding even with high expertise and experience. Unintentional injury of parathyroid glands and their vascularity or removal of parathyroid glands with surgical specimen during TT, may lead to transient or permanent hypoparathyroidism. Unfortunately, the result of permanent hypoparathyroidism, apart from the life-long dependance on calcium and Vitamin D supplementation, may severely impact the quality of life, the

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ability to work and also predispose to renal impairment and chronic kidney disease, which in turn increases the morbidity and mortality rates for these patients.

In recent decades, strategies for the assistance in identification and preservation of function of parathyroid glands during TT has been a field of intense research, in order to reduce the rate of postoperative hypocalcaemia (temporary and permanent). In the early 1970s, methylene blue was used for the first time as a means of identifying pathological and normal parathyroid glands, but its use did not show any benefit in relation to their simple intraoperative identification [3]. Additionally, the methylene blue technique had some potential complications at the site of its injection, such as neurotoxicity. In 2006, aminolevulinic acid, an agent that acts as a photosensitiser and accumulates in the parathyroid parenchyma was used, but without significant results [4]. Hyun et al described that some synthetic fluorescent agents may be useful for identifying parathyroid glands in animal models. Most of these agents and methods did not have significant results and were not applied in clinical practice [5].

Recently, some innovative techniques using near-infrared autofluorescence (NIR-AF) appear to be able to help overcome current limitations in locating and preserving parathyroid glands.

The use of Autofluorescence in Endocrine Surgery

Autofluorescence is the natural emission of light from intrinsic fluorophores. The term is used to distinguish light originating from artificially added fluorescent agents (exogenous contrast agent) such as indocyanine green (ICG). The application of this phenomenon to identify the parathyroid glands using near-infrared (NIR) wavelengths during thyroidectomy or parathyroidectomy is the most modern technique in the field of Endocrine Surgery.

In 2011, Paras and colleagues described the property of parathyroid gland autofluorescence in the near-infrared light spectrum and their ability to emit light at a peak wavelength of 820 nm when illuminated with light at 785 nm [6]. They showed that parathyroid glands acquire the strongest autofluorescence intensity at 820 nm, in contrast to the thyroid gland which has a lower intensity. Analysing the above data and results, they concluded that parathyroid glands can be distinguished from the surrounding tissues (adipose tissue, lymph nodes, muscles, trachea, thyroid gland, nerves) using this technology [6].

While several possible fluorophores have been proposed that may be responsible for the autofluorescence of the parathyroid glands, to date, none of them have been proven. Porphyrins produce the most persistent fluorescence in biological tissue, but have a peak emission of 600–700 nm, i.e. significantly lower than that of parathyroid glands [6]. Melanin is known to be the major fluorophore in the skin, but is not found in the parathyroid or thyroid gland [7,8]. The calcium-sensing receptor (CaSR) has been suggested as the most likely fluorophore in the parathyroid glands because of its presence and its high concentration in them, however, this has not been proven [6].

The use of near-infrared autofluorescence (NIR -AF) during Total Thyroidectomy

In 2014, McWade et. Al. reported the identification of parathyroid gland autofluorescence in patients during TT, by using the modified NIR -AF imaging system, for the first time [9]. The NIR -AF image was obtained by attaching an 808 nm long-pass filter in front of the camera, which blocks the laser light at 785 nm, while allowing the emitted NIR light to pass through. According to this study, intraoperative imaging of the parathyroid glands was applied to six patients (three patients diagnosed with primary hyperparathyroidism, two with nontoxic multinodular goiter, and one with papillary thyroid carcinoma) with an average time of imaging of six minutes. The NIR-AF technique was successful in 100% of the parathyroid glands regardless of the indication of surgery, normal parathyroids in patients undergoing thyroidectomy had a statistically significantly higher signal than affected/hypercellular parathyroids in patients undergoing parathyroidectomy [9].

The majority of NIR -AF devices designed and used in Endocrine Surgery have similar technical characteristics and are developed in a similar manner. The team of Palazzo and DiMarco from the United Kingdom described their experience of using the Fluobeam800 (Fluoptics, Grenoble, France) in 365 patients (96 parathyroid and 269 thyroid) [10,11]. More specifically, the camera is connected to the Fluobeam processor which is connected to a laptop that hosts the Fluosoft software and displays the image on its screen. The camera head is placed in a sterile case for use in the surgical field. After preparation and mobilisation of one lobe of the thyroid gland has been performed in the usual manner, the surgeon uses the NIR -AF camera head in its sterile case approximately 20 cm above the "target" area of the surgical field they want to be depicted. Then, the operating lights and all background lights should be turned off. The generated images are displayed on the screen, so the surgeon must correlate the surgical findings with what is displayed on the screen. The importance of this technology during thyroidectomy is to confirm and/

or identify the parathyroid glands which are preserved in the thyroid bed after TT and to confirm a possible subcapsular parathyroid gland which is at high risk of inadvertent removal with the surgical specimen, and prevent this removal making it suitable for parathyroid autotransplantation [10,11]. In the study conducted by DiMarco et al., a total of 257 (90.5%) parathyroid glands were identified using autofluorescence, while only three glands were not identified at all in autofluorescence. One hundred seventy-three parathyroids showed high signal using autofluorescence, 61 indicated medium and 23 low signal. A statistically significant negative correlation was found between blood calcium levels and signal intensity using autofluorescence ($p<0.01$).

Indocyanine green (ICG) angiography

ICG is an amphiphilic tricarbocyanine dye with a molecular weight of 751.4 Daltons, with a maximum absorption spectrum of 805 nm and re-emission at 835 nm. Immediately after its intravenous administration, it binds to plasma proteins and circulates only within the intravascular space [12]. This process allows ICG to act as a real-time intravascular contrast agent, with a half-life of 3-5 minutes, and elimination after 15-20 minutes from the time of absorption. ICG is finally excreted by the biliary system [13].

In 2015, Suh et al. first described the use of ICG and NIR-AF for the visualisation of parathyroid glands in dogs [2]. More specifically, they described the autofluorescence intensity response according to the administered dose of ICG and showed that the method could determine the optimal concentration of ICG for parathyroid imaging in experimental animals [2]. Angiography using ICG as fluorescent dye can be performed in patients undergoing thyroidectomy to visualise the vessels of parathyroid glands that have been previously identified by autofluorescence [12]. Several studies have described that patients who had at least one parathyroid gland with adequate perfusion maintained an adequate parathyroid function postoperatively, as well. ICG angiography allows a direct assessment of parathyroid gland feeding vessels, which are at high risk of injury during TT. In addition, it can play a crucial role in decision making about whether or not a parathyroid gland should be autotransplanted after TT [12]. Although the results of using ICG autofluorescence for the identification of normal parathyroid glands are promising, several limitations have been found, because parathyroid glands are in close relation to the thyroid gland, and thus their clear distinction is not always possible, as ICG is also absorbed by the thyroid gland.

Bibliographic data on the techniques of autofluorescence and the use of intraoperative ICG angiography during Total Thyroidectomy

In recent years, numerous studies from the majority of high-volume centers of Endocrine Surgery centers have been published, comparing and evaluating both the capability of each available device, as well as the contribution of these techniques-devices to the reduction of postoperative rates of short-term hypoparathyroidism after thyroidectomy.

Considering the meta-analysis conducted by Kim et al., the use of NIR-AF is a valuable intraoperative tool for identifying parathyroid glands during both thyroidectomy and parathyroidectomy [14]. Among a total of 17 studies that have been reviewed and analyzed, authors highlighted that NIR-AF can provide high identification rates of parathyroid glands, regardless of the type of device (probe based or image-based device). However, NIR-AF can produce false-negative and false-positive results, as the detection of parathyroid glands located in deeper layers and their dissection from the surrounding tissues are quite demanding. For this reason, the classic surgical approach for finding the parathyroid glands during thyroidectomy cannot be replaced by using NIR-AF and ICG [14].

With regards to the use of these promising techniques and the prevention of postoperative hypocalcemia after thyroidectomy, the meta-analysis conducted by Barbieri et. al., stated that patients who underwent TT with NIR-AF and ICG had significantly lower rates of short-term postoperative hypocalcaemia, compared to patients who underwent classical thyroidectomy without the use of further technologies and devices [15]. Regarding long-term hypocalcaemia and short-term hypoparathyroidism, there were insufficient data on whether autofluorescence and ICG reduce their incidence rates. What NIR-AF has proven to offer is the ability to review the surgical specimen in order to rule out a possible inadvertent parathyroidectomy and the ability to autotransplant an excised parathyroid gland. The possibility of autotransplantation is certainly an important aid to the surgeon and his constant concern of reducing the rate of postoperative hypoparathyroidism [15].

Finally, in 2020, the PARAFLUO randomised controlled trial was published, in which 245 patients were studied who were divided into two groups: patients who underwent classical thyroidectomy and patients who underwent thyroidectomy using NIR-AF. PARAFLUO study highlighted that the NIR-AF group had a higher rate of identification and preservation of parathyroid glands and a lower rate of postoperative hypocalcaemia [16].

In conclusion, although all published meta-analyses consist of heterogeneous studies with poorly stratified patients with respect to the indication for thyroidectomy, they certainly depict a potential benefit in preserving the parathyroid glands after their safer identification using autofluorescence and ICG angiography, which so far has not replaced the classic technique of thyroidectomy.

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