Autofluorescence of parathyroid glands during endocrine surgery with minimally invasive technique

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Abstract

Purpose Accidental injury to the parathyroid glands (PTGs) is common during thyroid and parathyroid surgery. To overcome the limitation of naked eye in identifying the PTGs, intraoperative autofluorescence imaging has been embraced by an increasing number of surgeons. The aim of our study was to describe the technique and assess its utility in clinical practice.

Methods Near-infrared (NIR) autofluorescence imaging was carried out during open parathyroid and thyroid surgery in 25 patients (NIR group), while other 26 patients underwent traditional PTG detection based on naked eye alone (NO-NIR group). Primary variables assessed for correlation between traditional approach and autofluorescence were number of PTGs identified and incidence of postoperative hypoparathyroidism (hypoPT).

Results 81.9% of PTGs were detected by means of fluorescence imaging and 74.5% with visual inspection alone, with an average of 2.72 PTGs visualized per patient using NIR imaging versus approximately 2.4 per patient using naked eye ($p = 0.38$). Considering only the more complex total thyroidectomies (TTs), the difference was almost statistically significant ($p = 0.06$). Although not statistically significant, the observed postoperative hypoPT rate was lower in the NIR group.

Conclusion Despite the limitations and technical aspects still to be investigated, fluorescence seems to reduce this complication rate by improving the intraoperative detection of the PTGs.

Keywords Parathyroid glands · Autofluorescence imaging · Near-infrared (NIR) autofluorescence imaging · Hypoparathyroidism · Intraoperative localization of parathyroid glands

Introduction

Hypoparathyroidism (hypoPT) is an endocrine disorder characterized by absent or inappropriately low concentrations of circulating parathyroid hormone (PTH), which leads to hypocalcemia, hyperphosphatemia and increased fractional excretion of calcium in the urine. The cut-off value used to define postoperative hypocalcemia differs, but most Authors agree on the biochemical diagnosis of hypocalcemia as a total serum calcium concentrations < 8 mg/dL or 2 mmol/L. Patients affected may be asymptomatic or show signs and symptoms such as paraesthesia and tingling up to cramps and muscle spasms (tetany), convulsions and alterations in the heart rhythm [1–5].

Parathyroid failure derives from an intraoperative damage to the parathyroid glands (PTGs) caused by a combination of factors such as mechanical or thermal trauma, gland devascularization, obstruction of venous outflow, inadvertent parathyroid excision, and parathyroid autotransplantation [3, 6, 7].

Most have transient hypoPT when parathyroid function recovers fully within 6 months, usually few weeks. HypoPT is considered permanent when it persists for more than 6 months after surgery with the need for replacement therapy and is generally due to an irreversible damage of all the PTGs [3–5].

Increased risk of postoperative hypoPT is mainly associated with the extent of surgery, neck dissection; redo surgery, reoperation for bleeding, large multinodular goiter

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(MNG), Graves' disease, coexistence of chronic lymphocytic thyroiditis (CLT), previous neck irradiation and radioactive iodine (RAI) ablation therapy [8–12].

The estimated prevalence of transient and permanent hypoPT according to a recent review and meta-analysis of Edafe et Al. [7] varies from 19 to 38% and from 0 and 3%, respectively, even if the real prevalence is probably underestimated [2, 4–6, 13–15].

Despite the knowledge of the anatomic landmarks and surgical techniques, postoperative parathyroid dysfunction is the most common complication after total thyroidectomy (TT) and a challenge for the endocrine surgeon [14–17].

To date, different techniques involving the use of dyes or fluorophores for the visualization of the PTGs have been studied, from the application of methylene blue to the aminolevulinic acid (ALA) as exogenous contrast agents [18–20]. Given the side effects (neurotoxicity for methylene blue, phototoxic effects in case of prolonged exposure to light for 5-ALA) and procedural complexity, none of these techniques has achieved the expected success and the clear identification of PTGs still remains a topic of debate and research [21–23].

In recent years, there has been growing interest in the use of the Indo Cyanine Green (ICG), which was first studied by Zaidi et al. in 2016 [24] and is currently the most promising fluorophore in the field of parathyroid fluorescence imaging.

When injected into a vein, ICG binds to plasma proteins, remaining intravascularly, and emits a fluorescence that can be visualized through a near-infrared (NIR) light source. Since the intensity of fluorescence is directly proportional to the blood supply of the structure to be evaluated, it leads to differentiate the PTGs, richly vascularized, from the surrounding tissues. In addition, ICG has a short half-life (after two minutes from the intravenous injection it reaches liver being eliminated through the bile without enterohepatic recirculation) allowing repeated administrations and a very low toxicity (it already acts at minimal doses (0.01 mg), and even doses 1000 times higher (5 mg/kg) are well tolerated). ICG angiography allows to visualize the vascular anatomy of the PTGs before dissection and to re-evaluate vascularization after dissection, which has been shown to correlate with the risk of postoperative hypoPT. Imaging with ICG has proved effective and very useful in the field of thyroid surgery [24–29]; however, all of these techniques mentioned so far always presuppose the use of exogenous contrast agents.

The breakthrough in intraoperative parathyroid imaging dates back to 2011, when a group of researchers from Nashville, Tennessee, with a study started in 2008, demonstrated that the PTGs have an intrinsic fluorescence, which allows them to be naturally distinguished from the surrounding tissues [30]. The PTGs, in fact, are able to emit spontaneously NIR light at peak wavelength of 820 nm upon excitation at 785 nm; then the radiation can be detected by a NIR camera or by probes with optical fibers connected to a NIR spectrometer. The PTGs may reach an intensity of fluorescence up to 11 times higher than that of the surrounding tissues; therefore, even a weak fluorescence signal in this range of wavelengths can provide high contrast.

However, the underlying molecular basis is still unclear. Currently, a calcium-sensing receptor (CaSR) is considered to be responsible for parathyroid autofluorescence, since it is present at highest concentrations in parathyroid chief cells, at lower concentrations in thyroid parafollicular cells, in the colon and in the kidneys, and absent in other cervical tissues [31].

McWade and his collaborators were the first to put into practice the discovery of autofluorescence in human thyroidectomy patients [32, 33]. They tested a modified NIR fluorescence imaging system (PPD camera, Karl Storz) on six patients operated for different pathologies (3 with primary hyperparathyroidism (pHPT), two with non-toxic MNG and one with papillary thyroid carcinoma): the NIR image was obtained by connecting a long-pass 808 nm filter in front of the camera, which blocked the 785 nm excitation light allowing the emitted NIR light to pass through. In all 6 patients, the PTGs were clearly visualized as they fluoresced 2.4 to 8.5 times the intensity of surrounding tissues, such as adipose tissue, muscle, thyroid, nerves and trachea. This study was followed by others that confirmed the potential role of autofluorescence in guiding the intraoperative identification of parathyroids, being safe and non-invasive for the patient [34–40].

In the present study, we detected the PTGs also by using NIR autofluorescence during thyroid or parathyroid surgery and provided further evidence.

Primary endpoint was to evaluate whether autofluorescence can assist in intraoperative parathyroid identification and affect the incidence of postoperative hypoPT if compared with visual inspection alone.

Materials and methods

We considered all patients that underwent hemithyroidectomy (HT), TT and parathyroidectomy (PT) consecutively treated between May and October 2020, at the Division of General Surgery of Trieste, Italy.

The patients included in the studied where divided into two groupsas follows: one studied by visual inspection alone, while the other by visual inspection by NIR fluorescence.

All operations were performed through a cervicotomic incision, with a minimally invasive video-assisted technique with thyroid volume not exceeding 25 ml with nodules smaller than 35 mm. With increasing experience, minimally invasive thyroidectomy was proposed also in patients with

T1 papillary carcinoma without clinically apparent lymph node metastases (T1N0 according to the AJCC/TNM classification VIII edition of 2017) [41–44].

Mono- or bi-lateral central neck dissection (CND) and/or lateral compartment neck dissection (LND) was performed in addition to TT when there was certainty or suspicion of lymph node metastases intra- or pre-operatively, in accordance with the American Thyroid Association (ATA) guidelines [45]. In most cases, one or two drains were placed.

PTGs were identified intraoperatively by visual inspection (in number of four in case of TT, two if HT and generally one in case of PT) and, in some patients by means of NIR fluorescence, using the Spy-Phi camera (Stryker, inc) (Fig. 1).

After lateral mobilization of the thyroid and exposure of the recurrent laryngeal nerve, we visually searched for the classic site of the PTGs. After removing light interference in the room, the operative field was exposed to a NIR light emitted by the camera from a distance of about 5 cm and autofluorescence of parathyroids was measured in about 15 s. Absence of fluorescence was considered as negative result.

After dissection, we checked again the PTGs by fluorescence when a change in parathyroid color, suspicious for possible devascularization, was observed, in order to see if there were any differences from before dissection. In case of manifest damage to a PTG (evident color change, resection of the afferent pedicle or its accidental removal), the same was reimplanted in a pocket of the sternocleidomastoid muscle.

In patients undergoing NIR fluorescence, we considered total number of detected PTGs, fluorescence score for each parathyroid (0 = no fluorescence, 1 = weak or heterogeneous fluorescence, 2 = high fluorescence), and, in case of parathyroid autotransplantation, number of reimplanted PTGs and each fluorescence score before and after dissection.

Fig. 1 Images of Stryker's Spy-Phi column and camera

Postoperatively, all patients were monitored clinically and in laboratory. Normally, serum calcium levels were measured at 4 h and together with PTH 1 day after surgery [46, 47]; in case of hypocalcemia, it was dosed daily until discharge and the following blood chemistry checks were decided case by case.

In patients undergoing PT for pHPT, intraoperative serum PTH (IOPTH) was determined at baseline (pre-incision), at 5 and 10 min after gland removal, with a further dosage at 15 min if there has not been a sufficiently significant reduction within the first 10 min. Success was defined as a fall in IOPTH levels of > 50% at 10 min post-excision compared to baseline [48].

We collected demographic and clinical data from all patients, including age, sex, preoperative serum calcium, PTH and vitamin D values (normal ranges in our laboratory were: calcium 8.5–10.5 mg/dl; PTH: 11–73 pg/ml; Vitamin D3: 20–100 ng/ml), preoperative diagnostic investigations (US of the neck and possible fine-needle aspiration cytology, FNAC) and localization in case of pHPT (US, scintigraphy, CT, MRI, etc.).

The FNAC was performed only in patients with suspected thyroid nodules according to the ATA 2016 guidelines [45]. In patients with pHPT we never referred to FNAC because not recommended. In these patients, a cervical ultrasound with Technetium-99m-sestamibi scintigraphy were performed. In some case the hyperfunctioning parathyroid gland was visualized with a four-dimensional computed tomography (4DCT), with an MRI scan, or in some cases with a fluorine-18-choline PET/CT.

In addition, the assaying parathyroid hormone (PTH) in the washing liquid after FNAC (Parathyroid FNA with PTH washout) was never necessary in this series of patients with pHPT because in almost all cases the parathyroid gland with suspected adenoma was already visible and well localized with the preoperative imaging techniques.

Perioperative parameters evaluated wereas follows: type and time of operation, placement of drains, pathological staging according to TNM (classification AJCC/TNM VIII edition 2017), postoperative serum calcium and PTH levels, replacement therapy in case of hypocalcemia, postoperative complications.

For our aims, we considered only hypocalcemia after TT excluding from the study complications such as haemorrhage, recurrent paralysis, re-operations and surgical site infections. According to our laboratory ranges, postoperative hypoPT was defined as < 11 pg/mL (normal values 11–73 pg/mL) with serum calcium < 8.5 mg/dL (normal values 8.5–10.5 mg/dl). The replacement therapy was adjusted on the severity of hypocalcaemia and treatment response.

In this study, we reported the experience with the use of fluorescence in thyroid and parathyroid surgery at our Institution. In particular, we analyzed the technique focusing on false positives and negatives, the number of cases in which NIR fluorescence was helpful in identifying the PTGs and, in cases of TT, the incidence of postoperative hypoPT compared to traditional approach without fluorescence.

Statistical analysis

Data were collected in a database in Microsoft® Excel. Categorical variables were expressed as absolute and percentage frequencies and assessed using Fisher's exact test or chi-square test, when appropriate. Continuous variables were reported as mean or median, and evaluated by using Student's *t* test or Mann–Whitney *U* test, when appropriate. A *p* value of less than 0.05 was considered statistically significant. All statistical analyzes were performed using the GraphPad Software.

Results and discussion

Between May and October 2020, we analyzed 51 patients for a total of 165 PTGs. In 25 patients, classified as "NIR" group, after visual inspection NIR fluorescence was used to visualize the PTGs, while in the other 26 patients, classified as "NO-NIR" group, the PTGs were identified by visual inspection alone (Table 1). Overall, 123/165 PTGs (74.5%) were detected with naked eye.

In the NIR group, 68 out of 83 PTGs (81.9%) were visualized by means of fluorescence imaging (Table 2).

Table 1 Age, sex, treatment, intraoperative and postoperative findings of the two groups of patients

	NIR	NO-NIR	*p* value
Number of patients	25	26	
Median age (range)	52 (31–80)	55.5 (25–83)	ns
Gender (female/male)	17/8	19/7	ns
Type of surgery			
HT—benign nodule growing in size, TIR3B with no risk factors, pHPT	6	5	ns
HT + parathyroidectomy—HPT I and concomitant thyroid nodule growing in size	1	0	–
TT—MNG	7	4	0.5
TT—Graves-Basedow	3	2	ns
TT—plummer in MNG	1	0	–
TT—TIR3B in MNG or with risk factors	2	5	0.4
TT—thyroid carcinoma	3	6	0.4
With CND	1	4	0.35
Parathyroidectomy—pHPT	1	4	0.35
Median time HT, min (range)	72.5 (59–85)	110 (67.5–162.5)	0.2
Median time TT, min (range)	85 (67–101)	85 (76–104)	–
Median time parathyroidectomy, min (range)	52	59 (37–167)	–
Parathyroids detected (%)	68/83 (81.9%)	63/82 (76.8%)	ns
With naked eye (average)	60 (2.40)	63 (2.42)	
With NIR fluorescence (average)	68 (2.72)		
Parathyroids detected during TT (%)	55/68 (80.9%)	52/68 (76.4%)	ns
With naked eye (average)	51 (3.00)	52 (3.06)	
With NIR fluorescence (average)	55 (3.24)		
Parathyroidsreimplanted (%)	2/25 (8%)	1/26 (3.8%)	ns
HypoPT post-TT			
Transient (%)	3 (12%)	5 (19.2%)	ns
TT—Graves-Basedow	2	0	
TT with CND	0	2	
TT—MNG/TIR 3B in MNG	1	3	
Parathyroids detected (average)	10/12 (3.3)	13/20 (2.6)	ns
Permanent	0	0	–

HT hemythyroidectomy, *TT* total thyroidectomy, *pHPT* primitive hyperparathyroidism, *MNG* multinodular goiter, *CND* central compartment lymphadenectomy, *ns* not significant

Table 2 Data of "NIR" group about fluorescence

Parathyroids detected with NIR fluorescence (%)	68/83 (81.9%)
Fluorescence score	
0—absence of fluorescence: false negatives (%)	15 (18%)
Pathologic parathyroids	1/15 (6.7%)
Superior parathyroids	9/15 (60%)
Inferior parathyroids	5/15 (33.3%)
1—weak or heterogeneous fluorescence (%)	30 (36.1%)
2—high fluorescence (%)	38 (45.7%)
False positives	5
Brown fat	3
Thyroid tissue/colloid nodules	2
Metastatic lymph nodes	0
Parathyroidsreimplanted	2/83 (2.4%)
Fluorescence pre-dissection (score)	1, 2
Fluorescence post-dissection (score)	1, 2

Fluorescence allowed a clear distinction of the PTGs from the surrounding structures in most cases, in which the signal intensity was maximum (score 2; Figs. 2) and, in some patients, weak or heterogeneous (score 1; Fig. 3).

Only in few patients no fluorescence was detected (score 0). Particularly, in 2 cases all four PTGs did not show any fluorescence, in 3 patients only the superior parathyroids, while in one case the gland without fluorescence was later found to be pathologic (oxyphilic neoplasm with areas of carcinomatous transformation) (Fig. 4).

In 5 cases, other structures, especially brown fat and thyroid colloid nodules, expressed some fluorescence which, however, was always lower than that of the PTGs (Fig. 5).

Overall, fluorescence imaging was able to detect the PTGs in 15 patients (out of 17) undergoing TT: in 11 all four PTGs were visualized, in 3 patients only three and in one patient only two PTGs (Tables 1, 2, 3). In patients undergoing HT, except for one inferior parathyroid not visualized,

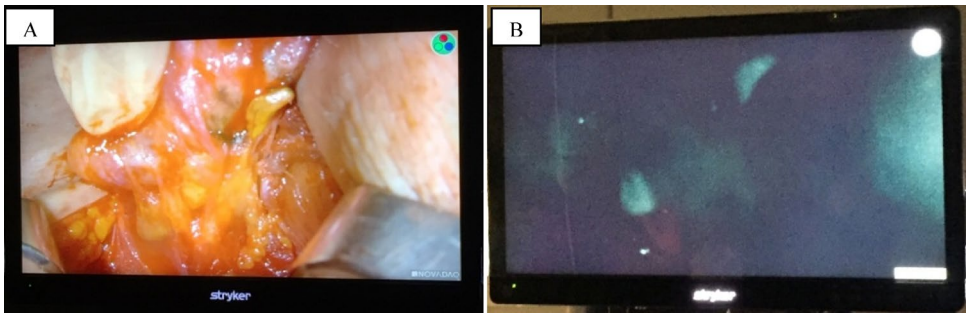

Fig. 2 Images of two ipsilateral parathyroids during thyroidectomy. **A** White light image after thyroid lobe lateralization. **B** Localization of the glands by NIR autofluorescence imaging (strong signal intensity)

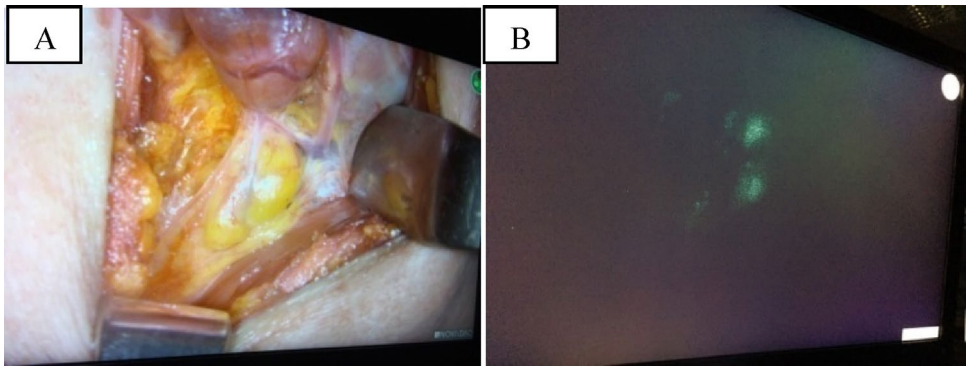

Fig. 3 Images of the left inferior parathyroid during total thyroidectomy. **A** The gland under white light imaging. **B** Photo of the gland exhibiting a heterogeneous pattern of fluorescence in the NIR mode

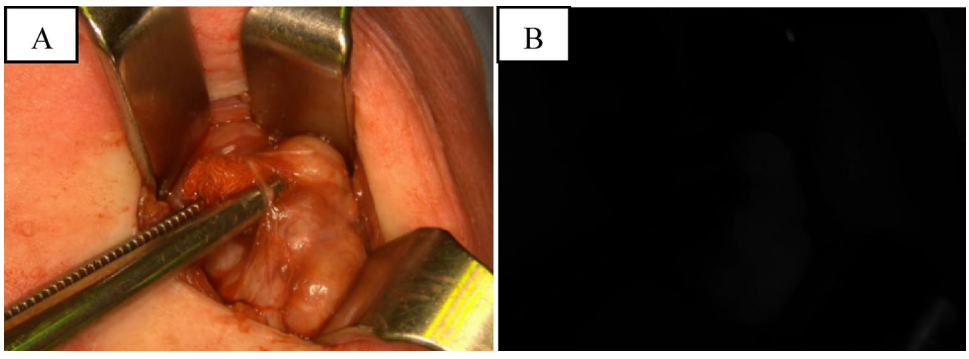

Fig. 4 Images of a pathologic left inferior parathyroid before resection. **A** White light image of the gland. **B** Absence of fluorescence in the NIR mode

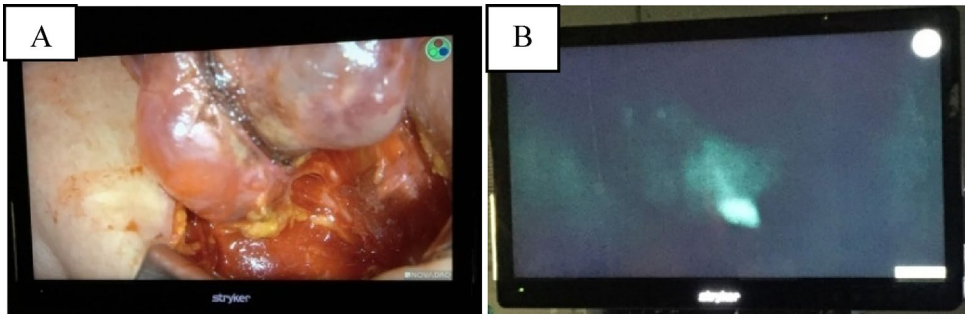

Fig. 5 Images of a parathyroid and a nearby thyroid colloid nodule. **A** White light image. **B** Autofluorescence of both parathyroid and nodule expose to NIR light

Table 3 Autofluorescence and hypoPT after total thyroidectomy in the "NIR" group

Parathyroid fluorescence	Number of patients	Postoperative hypoPT
Absence of fluorescence	2	0
Weak/heterogeneous/high fluorescence	15	3
4 parathyroids detected	11	2
3 parathyroids detected	3	1
2 parathyroids detected	1	0
1 parathyroid detected	0	0

all ipsilateral PTGs were correctly identified. In one of these patients, a concomitant PT for pHPT from ipsilateral adenoma was performed, with detection of both the adenoma and the other non-pathological ipsilateral gland. The other case of PT observed in this group, as described above, did not express any fluorescence, probably due to the carcinomatous transformation of the gland.

In the cohort of TTs, we classified a subgroup of 17 patients as more complex by pathology (Basedow, submerged goiter, associated chronic thyroiditis) and type of intervention (TT with CND) (Table 4). Fluorescence was used in 8 cases leading to detect a greater number of PTGs than by visual identification alone in all cases, except for one with mediastinal goiter in which both the inferior PTGs were located downwards and inaccessible to the camera.

Overall, the sensitivity of fluorescence in parathyroid identification was 81.9%, with an average of 2.72 PTGs visualized per patient using NIR imaging versus approximately 2.4 per patient using the naked eye alone ($p = 0.38$). In those undergoing TT, an average of 3.24 PTGs per patient were recognized by means of fluorescence versus approximately 3 per patient with the naked eye alone ($p = 0.55$). Stratifying the patients undergoing TT according to its complexity, the difference was almost statistically significant: 3.38 PTGs per patient detected by fluorescence imaging versus 2.75 PTGs per patient by visual inspection alone ($p = 0.06$).

The procedure took 3–5 min to set up the camera and 2–3 min to visualize the PTGs.

In 6 patients undergoing TT, visual inspection after dissection revealed a change in parathyroid color suspected for devascularization; in these cases, fluorescence remained unchanged compared to pre-dissection. In 2 patients the parathyroid was reimplanted in a pocket of the sternocleidomastoid muscle. None developed a postoperative hypoPT.

In the NO-NIR group, the PTG was reimplanted in only one patient, in whom, however, a postoperative hypoPT did not occur.

There was no difference between parathyroid visualization in terms of vascularization: as mentioned above, some

Table 4 Data of complex total thyroidectomies in the two groups

	NIR	NO-NIR	*p* value
Number of patients	8	9	
Type of surgery and pathology			
TT—Graves-Basedow	4	2	
TT—MNG/TIR3B in MNG and CLT	3	3	
TT—carcinoma with CND	1	4	
Median time TT, min (range)	89 (55–160)	85 (60–155)	ns
Parathyroids detected (%)	27/32 (84.4%)	25/36 (69.4%)	ns
With naked eye (average)	22 (2.75)	25 (2.76)	
With NIR fluorescence (average)	27 (3.38)		
Postoperative hypoPT	2 (25%)	4 (44.4%)	ns

TT total thyroidectomy, *CLT* chronic lymphocytic thyroiditis, *CND* central compartment lymphadenectomy, *ns* not significant

PTGs, despite a strong suspect of devascularization, mantained the same fluorescence than before dissection; on the other hand, the fluorescence intensity did not differ between hyperfunctioning parathyroid adenomas with increased vascularization documented on preoperative Doppler US and normal PTGs. We reported one case of hyperfunctioning parathyroid adenoma (Fig. 6) in which the fluorescence intensity did not change even after excision.

Transient postoperative hypoPT occurred in 3 patients (12.5%) of the NIR group and in 5 patients (19.2%) of the NO-NIR group; in 50% of cases it developed after TT for Basedow (2 patients in the NIR group) or with concomitant CND (2 patients in the NO-NIR group), known independent risk factors for postoperative hypoPT (Table 1). Although not statistically significant, the observed postoperative hypoPT rate was lower in the NIR group, where at least 3 PTGs were detected by fluorescence if compared to an average of 2 PTGs by naked eye alone. On the contrary, postoperative hypoPT did not occur in the 2 patients where the camera did not allow detection of any PTG.

Stratifying the patients by surgical complexity, postoperative hypoPT, as expected, was higher in the group of patients undergoing TT for Basedow or substernal goiter, TT with associated CND and in patients with chronic thyroiditis. However, comparing both groups of patients (NIR and NO-NIR) undergoing complex thyroidectomy, postoperative hypoPT rate was still lower in that where NIR fluorescence was used and allowed to identify, in almost all cases (7 patients out of 8), more PTGs than with the naked eye alone.

In clinical practice, most surgeons still rely primarily on visual inspection to identify PTGs during thyroidectomy. However, interpretation of visual data does not always allow for correct discrimination especially in case of non-pathological PTGs, which are small and similar in appearance to the surrounding structures.

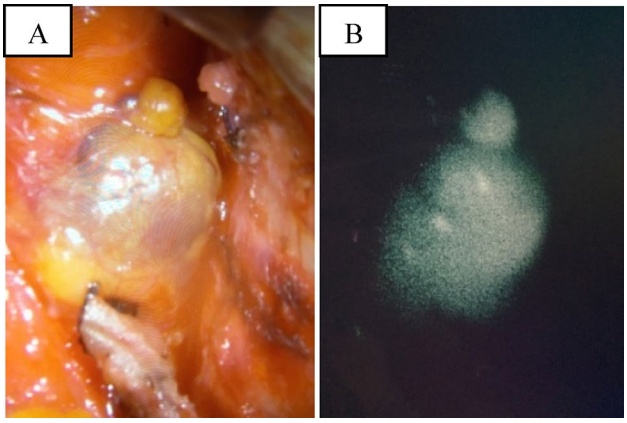

Fig. 6 Images of a parathyroid adenoma during parathyroidectomy. **A** White light image of the adenoma in situ. **B** High-intensity autofluorescence of the adenoma after resection

US, CT and MRI are used only in a few cases, usually for preoperative diagnosis of pathological PTGs. The extemporaneous intraoperative examination of the gland could be decisive, but it requires the partial removal of the parathyroid itself compromising its vascularization and function [49]; fine-needle aspiration (FNA) with PTH measurement, on the other hand, takes time and has low sensitivity [50].

Although the FNAC is a first-line investigation in the evaluation of neck nodules, it is not indicated in the parathyroid lesions.

FNA, generally considered a useful preoperative diagnostic tool for tumors, is not recommended for PA-suspected tumors owing to the chances of serious complications, such as massive hematoma, parathyromatosis, and misdiagnosis as malignancy during pathological diagnosis [50, 51]. Ultrasound-guided fine-needle aspiration can be performed to localize a parathyroid lesion and distinguish parathyroid tissue from surrounding structures. However, there are several innate risks of this procedure that can cause difficulties in diagnosis and management.

It is difficult to distinguish parathyroid lesions from thyroid lesions using FNAC because of their proximity and their similar cytomorphological features. FNA can cause misdiagnosis of cytomorphological findings between parathyroid and thyroid lesions.

The FNA can cause misdiagnosis of cytomorphological findings because of significant overlap in cytological features of parathyroid and thyroid lesions. Oncocytic parathyroid adenoma can be confused with Hürthle cell thyroid neoplasm, especially in the absence of clinical information [52]."

Autofluorescence, compared to these methods, offers the advantage of not being invasive and requiring only a few minutes for application.

In this study, we demonstrated that NIR fluorescence can accurately identify PTGs with a sensitivity of 81.5%, which was found to be slightly lower than other studies reporting rates above 90% [34, 53–56].

However, our detection system was developed to visualize tissue perfusion by ICG angiography and not to record the significantly weaker signal spontaneously expressed by the PTGs upon excitation. Furthermore, the camera is not as small as the operative field and the surface to be explored is often difficult to access, especially when a minimally invasive approach is preferred. For all these reasons, our system should be further improved by applying more suitable light source and camera.

To date, only the following two systems have been approved by the FDA for clinical use: the probe-based PTeye® system (AiBiomed, Santa Barbara, CA) and Fluorobeam 800® (Fluoptics, Grenoble, France). However, there is still no specific system to be applied for parathyroid

visualization that can superimpose the NIR image over the color video image, such as some ICG angiography systems. Despite the promising results, the technique still has some limitations and technical aspects that need to be further investigated and solved.

The first limit concerns false negatives and false positives [57]. Not all PTGs have the same fluorescence and this can derive either from a correct use of the technique or from a certain heterogeneity in the signal intensity within the same gland.

As already discussed, signal detection requires a very sensitive NIR camera as well as exclusion of interference as extraneous "flashes" from the room. The white light from patient monitors or a window can cause artifacts affecting the quality of image (Fig. 7) and generate false negatives, especially if the intensity signal is very low. To optimize the image, all external lights must, therefore, be excluded, starting from the lights of the operative room and those deriving from any other source (screens, windows,…).

Fluorescence intensity and image quality may also depend on other factors, such as the angle of the camera and the distance between the camera and the operative field, as demonstrated by McWade in a recent study, in which different measurements were obtained by modifying tha angle of the camera [32].

As concerns the heterogeneity of fluorescence in the parathyroid itself, Ladurner et al. observed that some normal glands were not fluorescent enough to be identify with the NIR camera, leading to a sensitivity of 90.2% [36]. Furthermore, pathologic glands seem to have an even more heterogeneous fluorescence intensity than normal ones, as reported in the study by DiMarco et al. [58], in which only 257/284 abnormal glands, either adenomas or multiglandular hyperplasia, were visualized with autofluorescence, with a false negative rate of 9.5% and a sensitivity of 90.5%. They also observed that the higher the preoperative calcium, the lower the fluorescence intensity ($p < 0.01$), strengthening the hypothesis that the CaSR may be the responsible fluorophore. The heterogeneity of signals recorded within the same gland has also been documented by other Authors [59]. In our study, we found one similar case of pathological parathyroid, confirmed as such at the definitive histological examination, which did not show any fluorescence upon repeated examination with the NIR camera, probably due to the presence of different fluorescent areas within the same gland, with a majority not fluorescing. However, efforts must be made to clearly identify the specific fluorophore involved in the mechanism of parathyroid fluorescence and its possible role in the function and regulation of these glands.

Another factor affecting the sensitivity of the technique is the presence of ectopic PTGs which may be located intrathyroid (1–2%) or in the thymic residue (2–5%); NIR light can penetrate only a few millimeters into the tissues, so the technique will not work in these cases [60, 61]. Given the limit of NIR light penetration, the surface of the PTGs must be well-exposed [38] thus requiring more dissections than normal, with a potentially higher risk of gland devascularization.

Another limitation may concern the identification of the inferior PTGs, in their typical location between the branches of the inferior thyroid artery. To preserve their vascularization, an attempt is generally made to limit dissection in this site, often necessary to obviate the limit of light penetration. In our study, 60% of false negatives concerned the upper PTGs with a noticeable decrease in the sensitivity of the technique.

In addition to false negatives, false positives can also occur, usually associated with brown fat, colloid nodules, cystic lesions and lymph node metastasis of papillary thyroid carcinoma emitting a “false” fluorescence that can mimic that of the PTGs [57]. Brown fat is the main cause of false positives, as it has a yellow color too and may cover the underlying PTG hindering detection by the camera. Brown fat fluorescence is probably due to a high level of porphyrin, a natural fluorophore present in this tissue. However, it has been seen that the fluorescence peak of porphyrin is reached around 400 nm, unlike PTGs at 785 nm, so it would be enough to use different wavelengths to distinguish the two structures. Colloid nodules and cystic lesions also can emit high-intensity fluorescence, which can lead to the false impression of a subcapsular or intrathyroid PTG. Careful dissection can clarify the situation, although it requires longer operative time. Finally, lymph node metastasis of papillary thyroid carcinoma can mimic PTGs upon NIR excitation; also in this case, visual inspection, together with

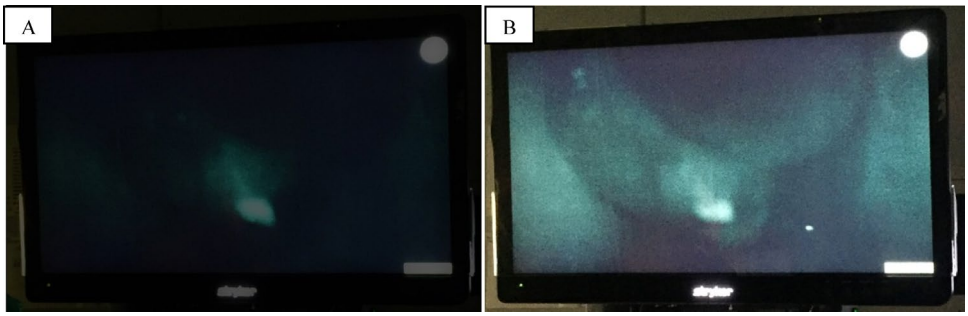

Fig. 7 Images of the same right inferior parathyroid acquired with lights off (**A**) and with interference from the lights inside the room (**B**)

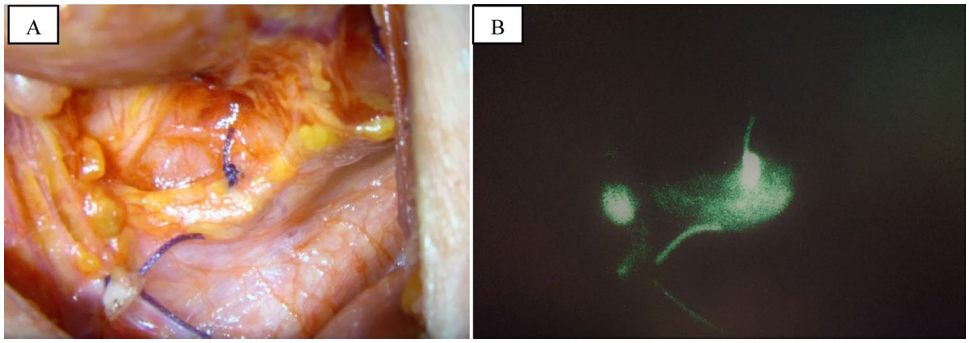

Fig. 8 Images of two ipsilateral parathyroids and of Vicryl® sutures during total thyroidectomy. **A** White light image. **B** Autofluorescence of both structures exposed with the NIR illuminator image

an extemporaneous intraoperative examination if available, will be able to discriminate between metastasis and parathyroid. Blue objects, especially Vicryl® sutures, tend to be highly fluorescent using this technique (Fig. 8) but, again, visual identification of the structure can allow easy distinction.

Unlike ICG imaging, autofluorescence was found to be independent of vascularity. It has been seen, in fact, that the intensity-based fluorescence signal does not vary between vascularized and devascularized PTGs nor between pathologically hypervascularized and normal ones, confirming once again that autofluorescence depends upon a fluorophore indipendent of vascularization. Therefore, on the one hand, autofluorescence does not enable to diagnose a possible gland devascularization, known risk factor for hypoPT; on the other hand, it allows to localize the PTGs at any time of surgery. This is important especially during CND, when the constant recognition of the PTGs is essential to avoid accidental injuries. Autofluorescence, in fact, has proved useful for identifying the lower PTGs, often difficult to be identified in the adipose tissue or between the lymph nodes during CND, especially in the context of lymphadenopathy.

The most important outcome we finally wanted to investigate concerns the incidence of postoperative hypoPT. From our experience, autofluorescence has not been shown to predict postoperative hypocalcemia on the intensity-based fluorescence signal. On the contrary, it allowed us to detect and preserve a greater number of PTGs than visual observation alone, especially in case of complex thyroidectomies, with a lower rate of postoperative hypoPT, although not statistically significant.

Therefore, we can hope that, with a larger sample, these data will be further confirmed, even reaching statistical significance.

Despite the promising results, further technical improvements are necessary to reduce false positives and especially false negatives, in our study attributable to the upper PTGs for more than half of cases. The technique would achieve greater sensitivity and specificity potentially eliminating many of these false positives and negatives, by optimizing image acquisition (light off, physician training, small and sensitive "dedicated" room, and standardized methodology) and objectively quantifying the fluorescence signal.

Conclusions

Autofluorescence has proved effective in identifying intraoperatively the PTGs even if with a slightly lower sensitivity than other studies in literature, mainly due to the limitations of the camera used. Recognition/isolation of the upper PTGs still remains an open problem, responsible for the majority of the false negatives observed, since they are more difficult to expose avoiding the gland devascularization.

Although not able to predict a possible postoperative hypoPT, fluorescence seems to reduce this complication rate by improving the intraoperative detection of the PTGs, even if further studies and/or a broader case series are necessary.

Despite the limitations and technical aspects still to be investigated, NIR autofluorescence can, however, be considered a useful tool to assist the surgeon during surgery and increase his expertise, along the lines of recurrent laryngeal nerve neuromonitoring already widely used in clinical practice.

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Data availability All Authors can access the data supporting the conclusions of the study. Data collection was carried out on a database (Excel database). Patient's data remained anonymous.

Declarations

Conflict of interest All authors affirm that there is no conflict of interest about the publication of this manuscript.

Research involving human participants and/or animals The study was performed in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Written informed consent was obtained from all study patients.

References

1. Bollerslev J, Rejnmark L, Marcocci C et al (2015) European Society of Endocrinology Clinical Guideline: treatment of chronic hypoparathyroidism in adults. Eur J Endocrinol 173:G1–G20. https://doi.org/10.1530/EJE-15-0628
2. Lorente-Poch L, Sancho JJ, Muñoz-Nova JL et al (2015) Defining the syndromes of parathyroid failure after total thyroidectomy. Gland Surg 4:82–90
3. Mannstadt M, Bilezikian JP, Thakker RV et al (2017) Hypoparathyroidism. Nat Rev Dis Primers 3:17055. https://doi.org/10.1038/nrdp.2017.55
4. Sitges-Serra A, Ruiz S, Girvent M et al (2010) Outcome of protracted hypoparathyroidism after total thyroidectomy. Br J Surg 97:1687–1695
5. Stack BC Jr, Bimston DN, Bodenner DL et al (2015) American association of clinical endocrinologists and American college of endocrinology disease state clinical review: postoperative hypoparathyroidism—definitions and management. Endocr Pract 21(6):674–685
6. Jensen PVF, Jelstrup SM, Homøe P (2015) Long-term outcomes after total thyroidectomy. Dan Med J 62(11):A5156
7. Edafe O, Antakia R, Laskar N et al (2014) Systematic review and meta-analysis of predictors of post-thyroidectomy hypocalcaemia. Br J Surg 101:307–320
8. Shoback DM, Bilezikian JP, Costa AG et al (2016) Presentation of hypoparathyroidism: etiologies and clinical feautures. J Clin Endocrinol Metab 101:2300–2312
9. Kiernan CM, Schlegel C, Kavalukas S et al (2016) Does concomitant thyroidectomy increase risks of parathyroidectomy? J Surg Res 203:34–39
10. Thomusch O, Machens A, Sekulla C et al (2000) Multivariate analysis of risk factors for postoperative complications in benign goiter surgery: prospective multicenter study in Germany. World J Surg 24(11):1335–1341
11. Viola D, Materazzi G, Valerio L et al (2015) Prophylactic central compartment lymph node dissection in papillary thyroid carcinoma: clinical implications derived from the first prospective randomized controlled single institution study. J Clin Endocrinol Metab 100:1316–1324
12. Giordano D, Valcavi R, Thompson GB et al (2012) Complications of central neck dissection in patients with papillary thyroid carcinoma: results of a study on 1087 patients and review of the literature. Thyroid 22(9):911–917
13. Ritter K, Elfenbein D, Schneider DF et al (2015) Hypoparathyroidism after total thyroidectomy: incidence and resolution. J Surg Res 197:348–353
14. Sonne-Holm E, Holst Hahn C (2017) Prolonged duration of surgery predicts postoperative hypoparathyroidism among patients undergoing total thyroidectomy in a tertiary referral centre. Eur Thyroid J 6:255–262
15. Uhre Jorgensen C, Homoe P, Dahl M et al (2020) High incidence of chronic hypoparathyroidism secondary to total thyroidectomy. Dan Med J 67(5):A11190647
16. Bergenfelz A, Jansson S, Kristoffersson A et al (2008) Complications to thyroid surgery: results as reported in a database from a multicenter audit comprising 3,660 patients. Langenbecks Arch Surg 393(5):667–673
17. Bilezikian JP, Khan A, Potts JT et al (2011) Hypoparathyroidism in the adult: epidemiology, diagnosis, pathophysiology, target-organ involvement, treatment, and challenges for future research. J Bone Miner Res 26(10):2317–2337
18. Dudley NE (1971) Methylene blue for rapid identification of the parathyroids. Br Med J 3(5776):680–681
19. Van der Vorst JR, Schaafsma BE, Verbeek FPR et al (2014) Intraoperative near-infrared fluorescence imaging of parathyroid adenomas with use of low-dose methylene blue. Head Neck 36(6):853–858
20. Prosst RL, Weiss J, Hupp L et al (2010) Fluorescence-guided minimally invasive parathyroidectomy: clinical experience with a novel intraoperative detection technique for PTGs. World J Surg 34(9):2217–2222
21. Han N, Bumpous JM, Goldstein RE et al (2007) Intra-operative parathyroid identification using methylene blue in parathyroid surgery. Am Surg 73(8):820–823
22. Suzuki T, Numata T, Shibuya M (2011) Intraoperative photodynamic detection of normal PTGs using 5-aminolevulinic acid. Laryngoscope 121(7):1462–1466
23. Patel HP, Chadwick DR, Harrison BJ et al (2012) Systematic review of intravenous methylene blue in parathyroid surgery. Br J Surg 99(10):1345–1351
24. Zaidi N, Bucak E, Yazici P et al (2016) The feasibility of indocyanine green fluorescence imaging for identifying and assessing the perfusion of PTGs during total thyroidectomy". J Surg Oncol 113(7):775–778
25. Fortuny JV, Belfontali V, Sadowski SM et al (2016) PTG angiography with indocyanine green fluorescence to predict parathyroid function after thyroid surgery. Br J Surg 103(5):537–543
26. Lavazza M, Liu X, Wu C et al (2016) Indocyanine green-enhanced fluorescence for assessing parathyroid perfusion during thyroidectomy. Gland Surg 5(5):512–521
27. Jitpratoom P, Anuwong A (2017) The use of ICG enhanced fluorescence for the evaluation of PTG preservation. Gland Surg 6(5):579–586
28. Fortuny JV, Sadowski SM, Belfontali V et al (2018) Randomized clinical trial of intraoperative PTG angiography with indocyanine green fluorescence predicting parathyroid function after thyroid surgery. Br J Surg 105(4):350–357
29. Fanaropoulou NM, Chorti A, Markakis M et al (2019) The use of Indocyanine green in endocrine surgery of the neck: a systematic review. Medicine (Baltimore) 98:e14765. https://doi.org/10.1097/MD.0000000000014765
30. Paras C, Keller M, Mahadevan-Jansen A et al (2011) Near-infrared autofluorescence for the detection of PTGs. J Biomed Opt 16(6):067012. https://doi.org/10.1117/1.3583571
31. Brown EM, MacLeod RJ (2001) Extracellular calcium sensing and extracellular calcium signaling. Physiol Rev 81:239–297
32. McWade MA, Paras C, White LM et al (2013) A novel optical approach to intraoperative detection of PTGs. Surgery 154(6):1371–1377
33. McWade MA, Paras C, White LM et al (2014) Label-free intraoperative parathyroid localization with near-infrared autofluorescence imaging. J Clin Endocrinol Metab 99(12):4574–4580

34. McWade MA, Sanders ME, Broome JT et al (2016) Establishing the clinical utility of autofluorescence spectroscopy for parathyroid detection. Surgery 159(1):193–203
35. Ladurner R, Sommerey S, Al Arabi N et al (2017) Intraoperative near-infrared autofluorescence imaging of PTGs. Surg Endosc 31(8):3140–3145
36. Ladurner R, Al Arabi N, Guendogar U et al (2018) Near-infrared autofluorescence imaging to detect PTGs in thyroid surgery. Ann R Coll Surg Engl 100(1):33–36
37. Ladurner R, Lerchenberger M, Al Arabi N et al (2019) Parathyroid autofluorescence-how does it affect parathyroid and thyroid surgery? A 5 year experience. Molecules 24(14):2560
38. Kim SW, Lee HS, Lee KD (2017) Intraoperative real-time localization of PTG with near infrared fluorescence imaging. Gland Surg 6(5):516–524
39. Falco J, Dip F, Quadri P et al (2017) Increased identification of PTGs using near infrared light during thyroid and parathyroid surgery. Surg Endosc 31(9):3737–3742
40. Kahramangil B, Dip F, Benmiloud F et al (2018) Detection of parathyroid autofluorescence using near-infrared imaging: a multicenter analysis of concordance between different surgeons. Ann Surg Oncol 25(4):957–962
41. Ruggieri M, Straniero A, Genderini M et al (2007) The eligibility of MIVA approach in thyroid surgery. Langenbecks Arch Surg 392(4):413–416
42. Ruggieri M, Straniero A, Genderini M et al (2007) The size criteria in minimally invasive video-assisted thyroidectomy. BMC Surg 25(7):2
43. Ruggieri M, Fumarola A, Straniero A et al (2008) The estimation of the thyroid volume before surgery—an important prerequisite for minimally invasive thyroidectomy. Langenbecks Arch Surg 393(5):721–724
44. Tuttle RM, Haugen B, Perrier ND (2017) Updated American joint committee on cancer/tumor-node-metastasis staging system for differentiated and anaplastic thyroid cancer (eighth edition): what changed and why? Thyroid 27:751–756
45. Haugen BR, Alexander EK, Bible KC et al (2016) 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 26:1–133
46. Julián MT, Balibrea JM, Granada ML et al (2013) Intact parathyroid hormone measurement at 24 hours after thyroid surgery as predictor of parathyroid function at long term. Am J Surg 206:783–789
47. Lombardi CP, Raffaelli M, Princi P et al (2006) Parathyroid hormone levels 4 hours after surgery do not accurately predict postthyroidectomy hypocalcemia. Surgery 140(6):1016–1023
48. Irvin GL, Dembrow VD, Prudhomme DL (1991) Operative monitoring of PTG hyperfunction. Am J Surg 162:299–302
49. Dobrinja C, Santandrea G, Giacca M, Stenner E, Ruscio M, de Manzini N (2017) Effectiveness of intraoperative parathyroid monitoring (ioPTH) in predicting a multiglandular or malignant parathyroid disease. Int J Surg 41(Suppl 1):S26–S33. https://doi.org/10.1016/j.ijsu.2017.02.063
50. Bancos I, Grant CS, Nadeem S et al (2012) Risks and benefits of parathyroid fine-needle aspiration with parathyroid hormone washout. Endocr Pract 18(4):441–449
51. Wei CH, Harari A (2012) Parathyroid carcinoma: update and guidelines for management. Curr Treat Options Oncol 13(1):11–23
52. Paker I, Yilmazer D, Yandakci K, Arikok AT, Alper M (2010) Intrathyroidal oncocytic parathyroid adenoma: a diagnostic pitfall on fine-needle aspiration. Diagn Cytopathol 38(11):833–836
53. De Leeuw F, Breuskin I, Abbaci M et al (2016) Intraoperative near-infrared imaging for ptg identification by auto-fluorescence: a feasibility study. World J Surg 40:2131–2138
54. Falco J, Dip F, Quadri P et al (2016) Cutting edge in thyroid surgery: autofluorescence of PTGs. J Am Coll Surg 223:374–380
55. Benmiloud F, Rebaude S, Varoquaux A et al (2018) Impact of autofluorescence-based identification of parathyroids during total thyroidectomy on postoperative hypocalcemia: a before and after controlled study. Surgery 163:23–30
56. Dip F, Falco J, Verna S et al (2019) Randomized controlled trial comparing white light with near-infrared autofluorescence for PTG identification during total thyroidectomy. J Am Coll Surg 228:744–751
57. Hartl D, Obongo R, Guerlain J et al (2019) Intraoperative PTG identification using autofluorescence: pearls and pitfalls. World J Surg Res 2:1166
58. DiMarco A, Chotalia R, Bloxham R et al (2019) Autofluorescence in parathyroidectomy: signal intensity correlates with serum calcium and parathyroid hormone but routine clinical use is not justified. World J Surg 43(6):1532–1537
59. Kose E, Kahramangil B, Aydin H et al (2019) Heterogeneous and low-intensity parathyroid autofluorescence: patterns suggesting hyperfunction at parathyroid exploration. Surgery 165(2):431–437
60. Pellitteri PK (2003) Directed parathyroid exploration: evolution and evaluation of this approach in a single-institution review of 346 patients. Laryngoscope 113(11):1857–1869
61. Bahar G, Feinmesser R, Joshua BZ et al (2006) Hyperfunctioning intrathyroid PTG: a potential cause of failure in parathyroidectomy. Surgery 139(6):821–826