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ORIGINAL ARTICLE

Intraoperative verification of parathyroid glands in primary and secondary hyperparathyroidism using near-infrared autofluorescence (IOPA)

Henning W. Wolf[1,2] · **Bernhard Grumbeck**[3] · **Norbert Runkel**[4,5]

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Abstract
Intraoperative verification of parathyroid glands relies on visual identification by the surgeon and, with some time delay, on serum parathormon measurements and frozen section. Fluorescence imaging, however, is an instant on-table method for direct visualization of parathyroid tissue which is known to exhibit increased autofluorescence intensity when exposed to near-infrared light. In this retrospective observational study, we evaluate the clinical use of this method in a series of patients with primary and secondary hyperparathyroidism. A total of 66 adenomatous and hyperplastic parathyroid glands were examined with intraoperative autofluorescence in 39 patients with primary and secondary hyperparathyroidism using a near-infrared system (KARL STORZ GmbH & Co. KG). The specimens were verified by conventional histology. Fifty-seven of 66 histologically proven adenomatous/hyperplastic glands exhibited autofluorescence. The sensitivity of near-infrared autofluorescence was 0.9 in pHPT and 0.83 in sHPT, respectively. The positive predictive value was 0.93 in pHPT and 1.0 in sHPT, respectively. Near-infrared autofluorescence guidance presents an innovative instant surgical imaging tool with sensitivity in detecting adenomatous and hyperplastic parathyroid glands comparable to current intraoperative methods. Due to its elegant and tracer-free design combined with low follow-up costs, this method can be useful for routine use.

Keywords Parathyroid surgery · Hyperparathyroidism · Endocrine surgery · Near-infrared autofluorescence

Introduction

Intraoperative detection and assessment of parathyroid glands rely on the surgeon's visual judgment. Confirmation by fresh frozen histology requires considerable time for transport and examination of the tissue specimens. Intraoperative parathyroid hormone monitoring (IOPTH) can verify whether or not all autonomously functioning parathyroid tissue has been removed with a high degree of accuracy in operations for primary hyperparathyroidism (pHPT) [1]. In renal secondary HPT (sHPT), however, declines of PTH > 50% from preexisting levels might not occur for half an hour or more after operation due to a slower renal elimination of PTH. Regardless of the scope of application, IOPTH is associated with a prolonged operation time or a standstill in the operating room.

In 2011, Paras et al. published a novel intraoperative instantaneous identification technique based on the phenomenon of autofluorescence of parathyroid glands when exposed to near-infrared light [2]. It could be shown that parathyroid autofluorescence is up to 8.5-fold higher than

✉ Henning W. Wolf
henning.wolf@ksa.ch

Bernhard Grumbeck
bernhard.grumbeck@klinikum-straubing.de

Norbert Runkel
norbert.runkel@sana.de

1 Department of General Surgery, Schwarzwald-Baar Klinikum Villingen-Schwenningen, Klinikstrasse 11, Villingen-Schwenningen 78052, Germany

2 Kantonsspital Aarau AG, Tellstrasse 25, 5001 Aarau, Switzerland

3 Department of Surgery, Klinikum Sankt Elisabeth, St.-Elisabeth-Strasse 23, 94315 Straubing, Germany

4 Schwarzwald-Baar Klinikum Villingen-Schwenningen, Klinikstrasse 11, Villingen-Schwenningen 78052, Germany

5 Sana-Klinikum Offenbach, Starkenburgring 66, 63069 Offenbach, Germany

surrounding tissue including thyroid. This method allows immediate and repetitive assessment with only minimal delay of the surgical progress. Few studies have been published since using this technique in small patient series or in ex vivo use only [2, 3, 11, 12].

We herein present our experience with in vivo intraoperative parathyroid assessment (IOPA) using a near-infrared (NIR) light system during standard parathyroidectomy for pHPT and sHPT.

Patients and methods

Patients

IOPA was applied in 39 patients with pHPT and sHPT. They were consecutive cases from between February 2012 and September 2015; however, some surgical cases were not examined due to organizational deficits and were thus not included in our analysis. Preoperative localization studies included surgeon-based ultrasound in all cases and sestamibi scans in pHPT patients with unclear ultrasound. The results of IOPA were documented prospectively and analyzed retrospectively. Approval of the local ethics committee was given for the analysis of autofluorescence data, but not for the biopsy of non-flourescencing tissue. Therefore, no specificity could be calculated.

Parathyroid surgery

All operations were performed by seven different surgeons at the Department of General Surgery in Villingen-Schwenningen in the black forest region of Germany, which is known for its endemic iodine deficit. A minimally invasive resection (30 mm incision) was applicable in 12 of 29 patients with pHPT after localization ultrasound was unambiguous among two different examiners. In ten patients with pHPT, excision of the parathyroid adenoma was combined with total or unilateral thyroidectomy for nodular goiter via Kocher´s incision. Bilateral neck exploration (BNE) was performed because of unclear preoperative localization in five patients and suspicion of a double adenoma in two patients, respectively (Table 1). BNE, total parathyroidectomy and cervical thymectomy were the procedures of choice in all ten patients with renal sHPT. One of ten patients with sHPT required a revision surgery for persistence of elevated PTH serum levels. Histology had shown that only three parathyroid glands had been removed at initial surgery. The fourth gland was successfully removed at the second operation with normalized PTH levels thereafter.

Table 1 Patient characteristics and operative procedures in primary and secondary hyperparathyroidism

Variables	Number (%)
Primary hyperparathyroidism	
Gender	
Male	9 (31)
Female	20 (69)
Age	
Mean ± SD	60 ± 13.8
Median	61
Range	25–85
Procedure	
Adenoma excision	19 (66)
Adenoma + thyroid resection	10 (34)
Incision	
Kocher's incision	17 (59)
Minimal invasive (< 3 cm)	12 (41)
Number of adenomas resected	
1	28 (97)
2	1 (3)
> 2	0
Reoperation needed	
No	27 (93)
Yes	2 (7)
Parathyroid adenoma weight (g)	
Mean ± SD	3.38 ± 5.46
Median	1.5
Range	0.07–25
Secondary hyperparathyroidism	
Gender	
Male	7 (70)
Female	3 (30)
Age	
Mean ± SD	57 ± 16.5
Median	64
Range	28–76
Procedure	
Parathyroidectomy	9 (90)
Parathyroidectomy + thyroid resection	1 (10)
Number of glands resected	
1	1 (10)
2	0
3	1 (10)
4	8 (80)
Parathyroid gland weight (g)	
Mean ± SD	0.56 ± 0.33
Median	0.63
Range	0.12–2

Technology

Near-infrared autofluorescence was excited using an NIR light source (D-Light P system, Karl Storz GmbH & Co KG, Tuttlingen, Germany) without the need of an exogenous tracer. Light was delivered to the tissue via a 10 mm Hopkins 0° ICG telescope. A high-resolution three-chip camera head and camera control unit (Tricam SL II, Karl Storz GmbH & Co KG, Tuttlingen, Germany) were then used to detect and evaluate the autofluorescence response. All documentation was carried out by a computer-based diagnostic software (AIDA, Karl Storz GmbH, Tuttlingen, Germany).

Intraoperative measurement

The first step: adenomatous or hyperplastic parathyroid glands were visually identified by the surgeon. Second step: the illumination of the surgical site with near-infrared light was performed after complete darkening of the operating room. The working distance between the distal end of the optic and the tissue was typically 2–3 cm. The surgeon and the surgical assistant classified the autofluorescence intensity of the visually identified glands as "non-autofluorescent" or "autofluorescent". A polyglactin suture (Ethicon Vicryl®) served as a reference for the examiner because it demonstrates an autofluorescence signal similar to parathyroid tissue. After photographic documentation, all autofluorescent glands were removed for histology. If IOPA was negative in a suspected lesion, search was continued by the surgeon. Finally, all tissue that were judged pathological by the surgeon was excised even when autofluorescence was absent. Conventional histology examination was performed of all resected specimens and served as reference. IOPTH was used in all patients with pHPT to determine if hyperfunctioning glands had been removed. Data analysis was performed using descriptive statistics.

Results

Primary hyperparathyroidism

The median preoperative serum parathyroid hormone level was 132 pg/ml (85–4042 pg/ml) in 29 patients with pHPT. Postoperative normalization occurred 27 times (93% of all patients) with normal ($n=16$) or subnormal values of PTH ($n=11$). One case demonstrated an inadequate postoperative decline of 9% despite a previously excised and histologically proven parathyroid adenoma. A second adenoma was then found and removed during a revision operation with subsequent biochemical normalization. In another patient with a large adenoma that had burst during surgery, postoperative PTH levels remained pathological despite a drop in parathyroid hormone levels by 91%. An uncomplicated level VI dissection for "seeding" of parathyroid cells resulted in a biochemical cure (Table 2). The cumulative PTH normalization rate was 100% for pHPT.

Pathology confirmed a total of 30 parathyroid adenomas during the primary operation in 29 patients with pHPT. Twenty-six of 27 autofluorescent glands were first found and identified by the surgeon and then confirmed by autofluorescence (Fig. 1). One autofluorescent gland was first recognized by screening the exposed field with near-infrared light and then dissected and confirmed macroscopically by the surgeon. Two samples judged as parathyroid adenoma by the surgeon's eye and by autofluorescence proved to be brown adipose tissue in a 41-year-old woman and ectopic thyroid tissue in a 74-year-old woman. Fluorescence activity was homogenous throughout the adenomas in most cases and inhomogeneously patchy in six cases, respectively. Histology of these cases showed sclerotic or partially degenerated parathyroid tissue. The median weight of all removed adenomas was 1.5 g (0.07–25 g). A correlation between

Table 2 Laboratory results preoperative (preOP) and on postoperative day 1 (POD1) in patients with primary and secondary hyperparathyroidism

Variables	Value
Primary hyperparathyroidism	
Calcium preOP (mmol/L)	
Mean ± SD	2.86 ± 0.32
Median	2.82
Range	2.25–3.65
Calcium POD1 (mmol/L)	
Mean ± SD	2.31 ± 0.29
Median	2.24
Range	1.81–3.51
PTH preOP (pg/mL)	
Mean ± SD	368 ± 718
Median	132
Range	85–4042
PTH POD1 (pg/mL)	
Mean ± SD	44 ± 70
Median	26
Range	1–362
Secondary hyperparathyroidism	
PTH preOP (pg/mL)	
Mean ± SD	1251 ± 424
Median	1242
Range	681–1973
PTH POD1 (pg/mL)	
Mean ± SD	18 ± 29
Median	6
Range	0–91

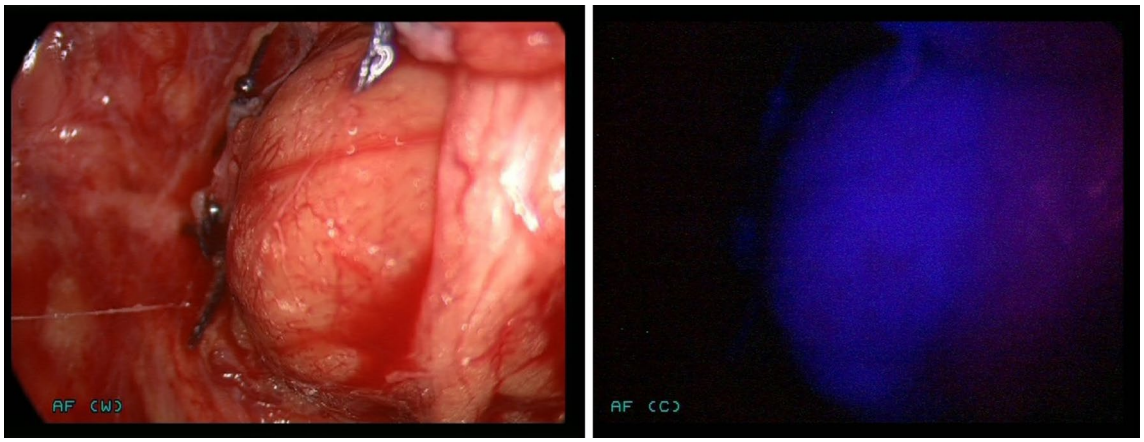

Fig. 1 Parathyroid adenoma in a patient with pHPT illuminated with white light (left) and after illumination with near-infrared light (right)

Table 3 Conformity of IOPA-positive tissue and histological confirmation of adenomatous/hyperplastic parathyroid tissue

Primary hyperparathyroidism	
True positive	27
False positive	2
False negative	3
Secondary hyperparathyroidism	
True positive	30
False positive	0
False negative	6

weight and fluorescence activity could not be established. The overall sensitivity of NIR autofluorescence in pHPT was 0.9. The positive predictive value of NIR autofluorescence was 0.93 (Table 3).

Secondary hyperparathyroidism

The median preoperative serum level of ten operated patients with renal sHPT was 1242 pg/mL (681–1973 pg/mL). Total parathyroidectomy without autotransplantation was performed in all cases. Early biochemical cure was archived by surgery in all patients: the PTH level dropped to normal (PTH 15–65 pg/mL, $n=3$) or subnormal (PTH < 15 pg/mL, $n=7$) values. A total of 36 hyperplastic/nodular glands were found in these ten patients, 4 in every patient with the exception of two patients, a 58-year-old man with 3 hyperplastic glands only and a 65-year-old man who underwent revision surgery for a single parathyroid gland left behind after prior subtotal excision of 3 glands. Postoperative PTH levels also dropped to normal values in these cases (Table 2).

Thirty of 36 parathyroid glands (83%) showed an autofluorescence signal after exposure to near-infrared light and were identified histologically as hyperplastic parathyroid tissue (Fig. 2). Six glands (17%) showed no autofluorescence. There were no false positive results (Table 3). One secondary hyperplastic gland presented a patchy fluorescence pattern; histology confirmed a partially sclerotic degenerated gland. The median weight of all secondary hyperplastic glands was 0.63 g (0.12–2 g). A relationship between weight and fluorescence activity could not be established. The sensitivity of NIR autofluorescence in sHPT was 0.83. The positive predictive value was 1.0.

The sensitivity of NIR autofluorescence for the entire population with HPT was 0.86, and the positive predictive value was 0.97.

Discussion

Intraoperative adjuncts for the localization of parathyroid glands are limited. Methylene blue carries a risk of toxic metabolic encephalopathy [4, 5], 5-aminolevulinic acid is limited by the need of complex photosensitization [6], and radioactive markers have a radiation exposure risk [4]. Recently, fluorescence imaging has gained attention for the identification and assessment of parathyroid glands using indocyanine green-9 (ICG) as a non-toxic dye that becomes fluorescent once excited with near-infrared light [7]. Zaidi et al. [8] demonstrated a more than 90% uptake of ICG in parathyroid glands, but its use to direct the course of parathyroid exploration was limited by concomitant ICG uptake by the thyroid gland. In their experience, ICG fluorescence-guided surgery was most useful in those patients with ectopic glands or in re-operative situations in which the thyroid had already been removed. Potential advantages of ICG fluoroscopic angiography are a reliable assessment of perfusion of remnant parathyroid tissue after subtotal parathyroidectomy [8, 9] and of parathyroid glands after thyroid resection to avoid postoperative hypoparathyroidism [7].

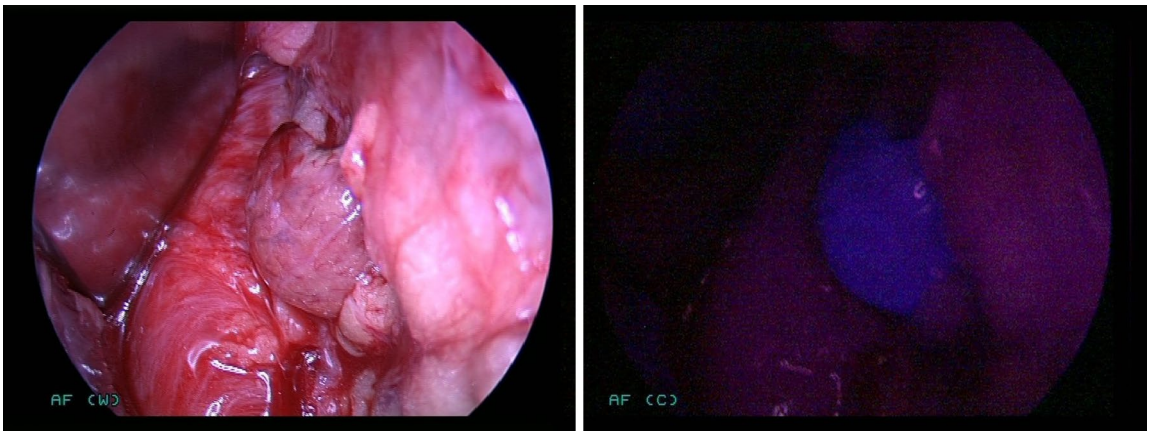

Fig. 2 Hyperplastic parathyroid gland in a patient with chronic renal failure illuminated with white light (left) and after illumination with near-infrared light (right)

The cutting edge of parathyroid real-time visualization is autofluorescence-guided surgery without the need of a tracer substance [3]. This technique is based on the observation that parathyroid tissue demonstrates higher fluorescence intensity compared to the thyroid gland and background tissue. This non-invasive technology offers an intraoperative, real-time, low-effort and unlimitedly repeatable method for the identification of each parathyroid gland. The first proof-of-concept study [2] and a follow-up study [10] by a group from Nashville, Tennessee, showed an excellent 100% detection rate of both, healthy and diseased parathyroid glands. The most recent study from Nashville included 137 patients (264 parathyroid glands) undergoing parathyroidectomy and/or thyroidectomy [11]. Near-infrared fluorescence correctly identified 256 of 264 (97%) glands. Excellent detection rates were also confirmed in Argentina in 28 patients including 10 patients with pHPT.

We tested the accuracy of NIR autofluorescence imaging in a homogenous group of patients with pHPT ($n=29$) and sHPT ($n=10$). Our detection rates of adenomatous/hyperplastic glands using NIR autofluorescence were 90% in pHPT (30 glands) and 83% in sHPT (27 glands), respectively. The corresponding figures from Nashville were 100% among 36 patients with pHPT and only 54% in 13 glands from 4 patients with sHPT [11].

Previous studies used laser light, whereas we used a xenon light source with a downstream infrared filter to induce light in the near-infrared range [3, 10]. The xenon light technique used here provided a safe working environment for the medical staff without specific safety precautions such as laser goggles. Transmission of the light signal was carried out with a 10 mm laparoscope, which simultaneously served as the detection unit via the connected camera. To achieve maximum autofluorescence and contrast to the surrounding tissue, the distance between the illuminated tissue and optics was reduced to less than 2 cm. This required the sterilization of optics and light cables. The mobile device design (integrated intraoperative imaging tower) enabled the easy application in different operating rooms.

Laser and near-infrared light differ in regard to the depth of tissue penetration [10]. Although NIR penetrates several millimeters, we experienced that glands covered with adipose tissue or blood significantly reduced the fluorescence signal. We learned that the tissue in question required surgical exposure prior to measurement. Therefore, NIR autofluorescence is an accurate method for in vivo confirmation of parathyroid glands, but its capability for intraoperative screening is low. NIR autofluorescence-assisted guidance may enhance the surgeon's expertise, but it will not substitute his experience. Furthermore, the new method has a potential training effect for both, advanced and novice endocrine surgeon.

The endogenous fluorophore that induces autofluorescence is still unknown. Paras et al. [2] assume that the calcium-sensing receptor (CsR) is the unknown fluorophore, as this receptor can be found in high concentration in both parathyroid and, in lower concentrations, in the C cells of the thyroid tissue [2, 10]. As part of the pathogenesis of parathyroid adenomas, a mutation-based clonal proliferation of parathyroid cells as well as an increased parathyroid hormone synthesis caused by a set-point shift is discussed. This may result in a decreased expression of CsR [12, 13]. Six parathyroid adenomas in patients with pHPT showed ex vivo a macroscopically inhomogeneous consistency with partly fibrotic, partly necrotic areas. These areas could not be excited to produce autofluorescence. Macroscopically homogeneous adenomas, however, demonstrated a strong fluorescence throughout the entire adenoma. One can speculate whether macroscopically homogeneous tissues have a higher expression of CSR, while this receptor is downregulated or

not expressed in fibrotic or necrotic tissue. A decline in CsR expression is also a characteristic phenotype of hyperplastic parathyroid cells in patients with sHPT [14]. One study even showed an inverse relationship between the expression of the CsR on the one hand and parathyroid gland weight and PTH secretion on the other hand [15].

This may be a possible explanation for the lower sensitivity of NIR autofluorescence imaging in sHPT. As a consequence, the entire gland should be exposed before testing. Our 10 mm optic system allowed us to visualize the entire or at least large areas of the gland surface on the screen. The cumulative autofluorescence in inhomogeneously emitting glands may be superior to collecting multiple spots with the 2 mm tip of the optical probe placed in direct contact with the surface of the tissue. This notion is supported by our higher detection rate with the 10 mm system (83%) than with the 2 mm system (54%) [11].

The autofluorescence intensity was not quantified in our study. Instead, we chose a simplified "yes or no" interpretation by the conducting surgeons based on the subjective comparison of the putative parathyroid with the surrounding autofluorescence. This resulted in a false negative result in an ectopic thyroid nodule, because thyroid tissue is known to exhibit weak autofluorescence [2]. The cause of another false positive test in brown adipose tissue in a female patient remains unclear.

Conclusion

Intraoperative identification of adenomatous and hyperplastic parathyroid glands with near-infrared light activated autofluorescence (IOPA) is easy, fast and reliable. This new method can be useful for instantaneous confirmation of glands by the surgeon rather than for the search per se. The technique is no substitute for surgical expertise. Further research must show whether autofluorescence may serve as parathyroid monitoring during thyroid resection to minimize the risk of postoperative hypoparathyroidism.

Compliance with ethical standards

Funding Wolf HW received financial support and technical equipment from Karl Storz GmbH, Tuttlingen, Germany.

Conflict of interest The authors Runkel N and Grumbeck B declare that thy have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Human and animal rights This article does not contain any studies with animals performed by any of the authors. As this study is designed as a retrospective study, formal consent is not required.

Informal consent As data were collected as part of a regular hospital stay and analyzed retrospectively, informed consent was not obtained. The study was approved by the local ethics committee and registered at the German trial register. Trial registration number: DRKS00009840.

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