Fluorescence-guided cancer surgery using clinical available and innovative tumor-specific contrast agents

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Chapter 5

Intraoperative identification of normal pituitary gland and adenoma using near-infrared fluorescence imaging and low-dose indocyanine green

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ABSTRACT

Background: The intraoperative distinction between normal and abnormal pituitary tissue is crucial during pituitary adenoma surgery to obtain a complete tumor resection, while preserving endocrine function. Near-Infrared (NIR) fluorescence imaging is a technique to intraoperatively visualize tumors by using Indocyanine Green (ICG); a contrast agent allowing visualization of differences in tissue vascularization. Although NIR fluorescence imaging has been described in pituitary surgery it has, in contrast to other surgical areas, never become widely used.

Objective: To evaluate NIR fluorescence imaging in pituitary surgery both qualitatively and quantitatively, and to assess the additional value to resect adenoma tissue under NIR fluorescence guidance.

Methods: We included ten patients planned for transnasal transsphenoidal selective adenomectomy. Patients received multiple intravenous administrations of 5 mg ICG, up to a maximum of 15 mg per patient. Endoscopic NIR fluorescence imaging was performed at multiple points in time. The NIR fluorescent signal in both the adenoma and pituitary gland was obtained and the Fluorescence Contrast Ratio (FCR) was assessed.

Results: Four patients had Cushing's disease, one had Acromegaly and one had a Prolactinoma. Four patients had a non-functioning macro-adenoma. In nine out of 10 patients with a histologically proven pituitary adenoma, the normal pituitary gland showed stronger fluorescent signal than the adenoma. A FCR of normal pituitary gland to adenoma of 1.5 ± 0.2 was obtained. In two patients, adenoma resection actually took place under NIR fluorescence guidance, instead of under white light.

Conclusion: NIR fluorescence imaging can easily and safely be implemented in pituitary surgery. Timing of ICG administration is important for optimal results and warrants further study. It appears that injection of ICG can best be postponed until some part of the normal pituitary gland is identified. Subsequent repeated low-doses ICG administration improved the distinction between adenoma and gland.

INTRODUCTION

In transsphenoidal surgery it is important to be able to distinguish between normal pituitary gland and pituitary adenoma tissue for two reasons: 1) to obtain a complete tumor resection and 2) to preserve endocrine function. The vascular pattern between the normal pituitary gland and tumor tissue differs[1;2]. This difference can be shown with preoperative magnetic resonance imaging (MRI), making use of dynamic administration of gadolinium. Normal pituitary tissue is visualized by contrast enhancement[3]. However, during surgery, the identification of normal gland and adenoma is based on differences in tissue characteristics, such as colour and consistency. This difference can be difficult to detect.

Transsphenoidal surgery is an established and relatively safe procedure. More than 95% of the pituitary tumors are operated via this approach[4]. Unfortunately, remission rates of hormone secreting tumors are not perfect, while surgery induced pituitary deficiencies still occur[5]. Moreover, in patients with initial remission, recurrences occur due to endocrine activity of postoperative tumor remnants. It is therefore likely that the success rate of surgery can be improved by increasing visual differentiation between adenoma and the normal gland.

Near-infrared (NIR) fluorescence imaging is an innovative technique to visualize tumors, vital structures, and lymph nodes during surgery[6]. It is currently clinically used for several medical indications. These are intraoperative imaging of liver metastases, breast cancer and parathyroid adenomas[7-10]. One of the clinically available NIR fluorescent contrast agents is indocyanine green (ICG). ICG is an ideal agent for real-time visualisation of differences in tissue vascularisation. The excitation and emission profiles of ICG in the NIR light spectrum make it possible to visualize blood vessels covered by a few millimetres of tissue. At present, ICG-imaging is widely used in a variety of surgical procedures[6;11], including several cerebrovascular procedures in neurosurgical practice[12;13].

Litvack et al. first reported the use of ICG in endoscopic pituitary surgery in 2012[14]. In this feasibility study, up to 75 mg ICG was administered intravenously for each procedure. An endoscopic NIR fluorescence imaging system was used to capture fluorescent signal. The authors concluded that NIR fluorescence-guided endoscopy was a promising intraoperative modality towards visually distinguishing adenoma from normal gland tissue and thus possibly facilitating

complete tumor resection. Like MRI gadolinium enhancement, NIR fluorescence imaging showed illumination of the normal gland, and delay in illumination of the adenoma. However, unlike gadolinium use for MRI of pituitary tumors, NIR fluorescence imaging has never become widely accepted in clinical practice.

The aim of the current study was to assess the feasibility and potential value of NIR fluorescence imaging in endoscopic pituitary surgery. The detection of the normal gland, with the aid of low-dose ICG was studied and the detected fluorescent signal was scored both qualitatively and quantitatively. Moreover, the possibility to resect adenoma tissue under only NIR fluorescence guidance was evaluated.

METHODS

Patients

Patients included in the study were those diagnosed with a pituitary gland adenoma and having first been scheduled for their initial endoscopic transnasal transsphenoidal surgical resection between January 2013 and March 2014. Only one endoscope and light cable suitable for NIR fluorescence imaging were available, allowing a maximum of one patient per day to be included. All patients gave informed consent. Exclusion criteria were previous transsphenoidal surgery, pregnancy, lactation, renal impairment (eGFR<55) or an allergy to iodine or ICG.

Clinical trial

This clinical trial was performed at the Leiden University Medical Center, a tertiary referral center. The study was approved by the local Medical Ethics Committee and was performed in accordance with the ethical standards of the Helsinki Declaration of 1975.

Patients underwent a standard-of-care endoscopic transnasal transsphenoidal selective adenomectomy, conducted by a team of two neurosurgeons with a vast experience in pituitary surgery. During the procedure, 5 mg ICG was administered intravenously at different time points. The first ICG gift was after exposure of the sellar dura, but prior to opening. The NIR compatible endoscopic device (Image Hub I by Karl Storz GmbH & Co. KG Germany) was used to identify the fluorescent signal. After opening the dura,

the resection of the adenoma was performed making alternating use of visual and fluorescence guidance. Additional boluses of 5 mg ICG were administered after partial resection of the adenoma, in order to obtain new NIR fluorescence images. At the surgeons' judgment of complete resection, new NIR fluorescence imaging was performed to ensure identification of any residual adenomatous tissue. For analysis of fluorescence signal, images were obtained 45 seconds after ICG administration.

Intraoperative endoscopic near-infrared fluorescence imaging system

Intraoperative NIR fluorescence imaging of the pituitary gland was performed using a newly developed endoscopic high definition (HD) fluorescence imaging system by Karl Storz GmbH & Co. KG Germany, the Image Hub I. This system consists of a plasma light guide and a 0-degree angle 5.4-mm endoscope, applicable for white light (WL), autofluorescence and ICG-imaging. The system was used for intraoperative conventional imaging (WL mode) and real-time fluorescence imaging (760-nm light, ICG mode). It allows for easy switching between WL mode and ICG mode using a foot pedal. The overlay of conventional and fluorescent images is not yet possible but anatomical orientation could be maintained due to the easy switching between light modes. Images were recorded using a charge-coupled device camera.

Statistical and image analysis

SPSS statistical software package (Version 20.0, Chicago, IL) was used for statistical analysis. Fluorescent signal of pituitary adenoma and normal gland were reported both qualitatively and quantitatively. Snapshots were taken from the recorded operating videos, for further analysis. For qualitative analysis, fluorescent signal was classified as ++, +, +/- and -. Two observers – M.V. (one of the operating neurosurgeons) and Q.T. – scored the fluorescent signal independently. Where scoring discrepancies occurred, agreement was reached by reassessing the images. Quantitative fluorescent signal was measured using ImageJ version 1.49b (a public domain Java-based image processing program developed at the National Institute of Health). With ImageJ, regions of interest (RoI) were drawn circumventing the fluorescent signal of the adenoma and normal pituitary tissue. Fluorescence Contrast Ratios (FCRs) were calculated by dividing the fluorescent signal of both RoIs. FCRs were reported as mean. Patient age was reported as median and range.

RESULTS

In the inclusion period 58 patients underwent a transnasal transsphenoid operation. Fourteen patients had a different pathology than adenoma. Six patients had been operated before. One patient was allergic to iodine. Ten patients refused participation in the study for a variety of reasons.

The light cable was temporarily broken leading to an inclusions stop for 4 weeks and subsequently exclusion of 7 patients. From the remaining 20 patients always the first patient of the two cases scheduled for surgery that day was included, leading to 10 included patients.

Median age was 50 years, ranging between 28 and 74. Six patients were female. Patients had either clinical features and imaging consistent with Non-Functioning macro-Adenoma (NFMA) (N=4), or were biochemically diagnosed with a functional adenoma (Cushing's disease (N=4), Acromegaly (N=1) and Prolactinoma (N=1)). Patient and tumor characteristics are shown in Table 1. All resected lesions were histologically proven to be a pituitary adenoma.

The first NIR fluorescence images were obtained before opening of the dura. Figure 1 shows an example of fluorescent images of the dura. A clear fluorescent signal in the blood vessels was visible, however no discrimination between normal gland and the adenoma was possible at this point. Enhanced vascularity due to dural invasion of the tumor was not present in our series.

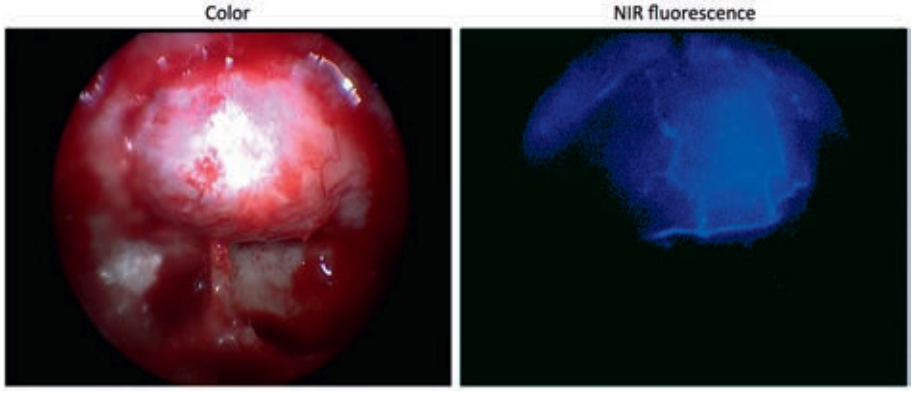

Figure 1. NIR fluorescence imaging of the dura

NIR fluorescence imaging of the unopened dura mater after administration of 5 mg ICG. Fluorescent vessels are visible on the dura. No identification of adenoma or normal pituitary gland is observed.

Table 1. Patient and tumor characteristics

Characteristic	Median	Range	
Age	50	28 - 74	
	N (n = 10)	**%**	
Gender			
M	4	40	
F	6	60	
	Preoperative		**Postoperative**
	N		**N**
Visual deficits			
No deficits	7		10
Minor deficits	2		0
Mild bitemporal deficits	1		0
Cranial nerve deficits			
None	8		8
Total N.III OS	1		0
Mild N.III OD	0		1*
Moderate N.III, slight N.VI OD	1		0
Minor N.VI OD	0		1*
Pituitary Function			
No deficits	6		8
Hypogonadism	3		1
Hypothyriodism	1		1
Hypersecretion			
None	4		10
ACTH	4		0
PRL	1		0
GH and PRL	1		0
Pathology			
Biochemical			
NFMA	4		4
Cushing's disease	4		4
Acromegaly	1		1
Prolactinoma	1		1
IHC			
GH+; PRL +	N/A		2
ACTH+	N/A		5
LH+	N/A		1
Null-cell	N/A		2

Abbreviations: ACTH, adrenocorticotropic hormone; DI, Diabetes Insipidus; GH, Growth hormone; IHC, immunohistochemistry; LH, Luteinizing hormone; N/A, not applicable; OD, oculus dextra; OS, oculus sinistra; PRL, prolactin.

* Recovered completely after 6 months

After opening of the dura, the normal pituitary gland showed a more intense fluorescent signal than the adenoma in nine of the ten patients. In one patient intercavernous sinus venous bleeding prevented assessment of the fluorescent signal. Figure 2 shows images of the fluorescent signal in adenoma and normal gland during resection. In general, during resection, a bright fluorescent normal gland became visible after ICG administration.

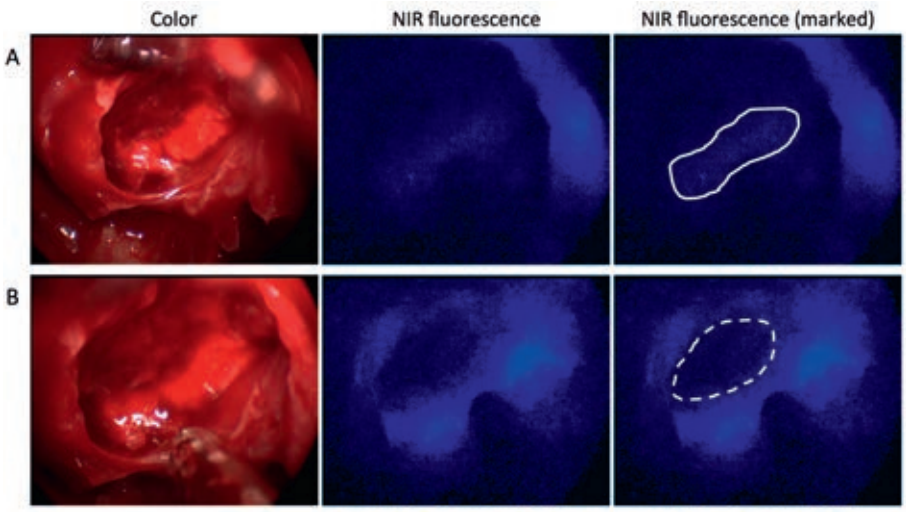

Figure 2. NIR fluorescence imaging of a pituitary adenoma

A. NIR fluorescence imaging of adenoma and normal gland after partial resection of the adenoma. ICG was administered just before opening of the dura, a second bolus of ICG was planned just after making this image. In the color image, a dark red colored remnant of the adenoma is visible. In the NIR fluorescence image, a slightly fluorescent normal gland is visible (circle). The adenoma remnant does not stain fluorescent. **B.** NIR fluorescence imaging of the same patient 60 seconds after the second ICG administration. A bright fluorescent normal pituitary gland is visible. The adenoma remnant remains still non-fluorescent (dashed circle).

Fluorescence intensities were measured 45 seconds after administration. Fluorescent signal with optimal contrast ratios were present for 1-2 minutes, where after the signal slowly decreased over time. The adenomas showed no, or only a weak fluorescent signal. After complete resection of the adenoma, a bright, consistent fluorescent pituitary gland was visible, and no non-fluorescent remnants of adenoma tissue were present anymore (Figure 3).

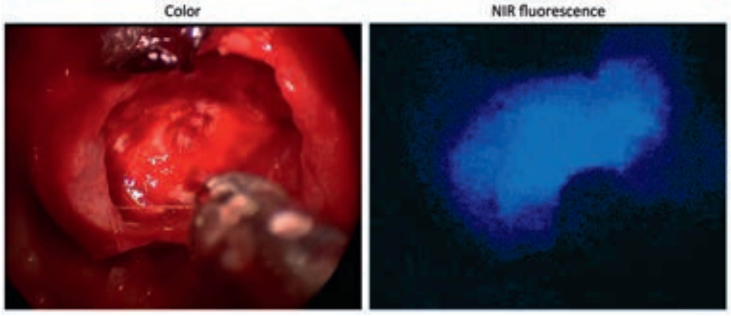

Figure 3. NIR fluorescence imaging after pituitary adenoma resection

NIR fluorescence imaging after resection of the adenoma. A complete fluorescent normal pituitary gland is visible. No non-fluorescent adenoma remnants can be identified.

Table 2 shows the imaging characteristics of the detected lesions. In five patients, no fluorescent signal was observed in the adenoma. In three patients, only weak fluorescence (qualitatively rated as +/-) was observed and in the case of one patient, fluorescent signal was rated as + (#9). The observed FCRs were confirmed by quantitative analysis, wherein a mean FCR of normal pituitary gland to adenoma of 1.5 ± 0.2 was obtained. In addition, two patients suffering from a microadenoma (#6 and #7) exhibited an FCR of 1.3 and 1.4 respectively. In one patient (#2), no discrimination in NIR fluorescent signal between normal gland and adenoma could be observed due to suboptimal timing of the imaging. In this patient, NIR fluorescence imaging was performed according to protocol just before opening of the dura. During resection of the adenoma, venous bleeding occurred that stopped after resection of the adenoma. After the second ICG administration and imaging, no remnant tissue was observed. Therefore, no NIR fluorescent images of the adenoma could be obtained.

Table 2. Imaging characteristics

Patient ID	Identification of normal gland and stalk on MRI			NIR Fluorescence Imaging				
	Preoperative	Post-operative	WH-class. grade	Dose ICG received (mg)	Location of adenoma	Fluorescent signal pituitary gland	Fluorescent signal adenoma	FCR
1	Cranial on top of adenoma	NP	IV	10	R	++	-	1.4
2	Stalk R; clear normal gland R	Normal	IIBE left	10	L	*No distinction possible*		
3	Stalk L; suggestion of normal gland	Remnant left side	IIIa	10	R	++	+/-	1.7
4	Stalk R; no normal gland	Normal	IIC	10	Mid - L	+	-	1.8
5	Stalk R; clear normal gland R	Normal	IIAE left	10	L	+	-	1.3
6	Stalk mid; clear normal gland	Normal	I	10	R	+	+/-	1.3
7	Stalk mid; clear normal gland	Normal	I	10	R	+	+/-	1.4
8	Stalk R; clear normal gland R	Normal	IIAE left	10	L	+	-	1.4
9	Stalk L; suggestion of normal gland	Remnant left side	IIB	5	R	++	+	1.5
10	Stalk L; suggestion of normal gland	Remnant left side	IIBE right	15	R	++	-	1.8

Abbreviations: FCR, Fluorescence Contrast Ratio; L, left; mid; midline; N/A, not applicable; NP, not performed; R, right; WH-class, Wilson-Hardy classification

The total individual dose of ICG varied, depending on the ease of surgical resection of the adenoma. The initial ICG gift was prior to tumor removal, the second gift at the end of the procedure, to control for completeness of resection. Most patients thus received a total dose of 10 mg ICG. In one patient, NIR fluorescence imaging showed tumor remnants, which were subsequently removed. A third ICG dose was administered to again check for remnants. The longest time-interval between ICG administration and obtaining images in fluorescence mode was 62 minutes (#12). At this point, weak fluorescence signal was still present, but did not show any contrast ratio. According to study protocol, this led to additional administration of ICG.

Tumor resection was typically done with conventional white light. In two patients (#1 and #3) we performed the tumor resection using predominantly the blue light of NIR fluorescence imaging (up to 9:15 minutes after ICG administration). Although visibility under these lighting conditions is clearly much poorer than with conventional white light, tumor resection seemed facilitated. It was possible to remove the pituitary adenoma in a standard way with curretes and suction. Tumor resection was deemed complete when the gland showed an even bright enhancement under NIR fluorescence imaging. In one of these two patients, a Cerebro Spinal Fluid (CSF) leakage occurred at the opening of the dura and was not related to the resection of the tumour under NIR fluorescence imaging.

In this limited number of patients, we did not find a relation between tumour type and ICG signal. No adverse reactions associated with the use of ICG or the NIR fluorescence imaging system, were observed. Complications associated with the surgical procedure included: 1) leakage of intraoperative CSF in one case (#3); and 2) intraoperative venous bleeding in another case (#9).

DISCUSSION

In this study, successful identification of the normal pituitary gland and pituitary adenomas was possible using endoscopic NIR fluorescence imaging using low dose ICG. In all assessable patients with a histologically proven adenoma, a contrast ratio between adenoma and normal gland was observed. In the only one study on NIR fluorescence imaging in pituitary surgery of Litvack et al.[14] the administered dose of ICG (up to 75 mg) was relative high for NIR fluorescence

imaging. In comparison, in other studies an ICG dose between 2.5 mg, or 0.2 – 0.5 mg/kg was demonstrated successful for the assessment of tissue perfusion after intravenous administration[15-17]. For tumor imaging of colorectal or uveal melanoma liver metastases, intravenous administration of 10 mg ICG proved to be sufficient[7;18]. Furthermore it allows for repeated injections, so that NIR fluorescence imaging can be used during various phases of the tumor resection. The lower dose used in this study compared to the dose used by Litvack et al. appears sufficient and leads to satisfactory results within the timing windows of imaging we used.

The observed FCR between normal gland and tumor tissue was 1.5 ± 0.2. The dynamic character of the imaging facilitated detection of a clear gradient between the normal pituitary gland and the adenoma. Our obtained quantitative ratios cannot be compared to results obtained by others, because only semi-qualitative results are reported yet[14]. To the best of our knowledge, to-date, no quantitative analysis of NIR fluorescence imaging in pituitary surgery had been reported. In future studies, quantitative analysis of NIR fluorescence imaging in pituitary surgery should become a standard in order to further objectively assess the additional value of this technique. A contrast ratio above 2 is generally considered optimal for intraoperative differentiation between tissues. For example, in colorectal liver metastases[18], breast cancer[10] and parathyroid adenomas[9] imaging, FCRs of respectively 7.0, 2.4 and 6.1 are reported. The FCR in pituitary surgery is thus relatively low, although sufficient. In the future hopefully improved contrast agents and imaging systems could potentially further enhance the FCR in pituitary surgery as well.

Optimal timing of ICG administration is crucial to obtain optimal distinction between normal and abnormal tissue. In the current research protocol, the first ICG administration was performed before opening of the dura. The vascularisation of the dura was thereby visualised, but no additional value for identification of the gland or the adenoma was obtained. In our experience, this first gift of ICG was too early. It may help to assess changes in the vascularisation of the dura, suggesting invasive growth of the adenoma. However, we believe that the optimal timing of ICG administration is when the surgeon detects differences in tissue colour and consistency, suggesting that the normal pituitary gland may be identified. The FCR was sufficient to reliably differentiate adenoma from normal gland. Interestingly, adenoma resection only under NIR fluorescence guidance, e.g. without alternating between white and blue

light, was technically possible. Possibly tumor resection under continuous NIR fluorescence guidance, instead of only checking for tumor remnants at the end of surgery may be the best way to use NIR fluorescence guidance in pituitary surgery. From the current study, no optimal interval between ICG administration and peak fluorescence signal can be recommended. For all measurements, an interval of 45 seconds was taken to obtain and analyse fluorescent signal. In our experience, the fluorescent signal and contrast ratio stayed optimal for 1-2 minutes in all patients, and than slowly decreased over time. Timing of ICG administration warrants further systematic study.

For NIR fluorescence imaging during pituitary surgery a special endoscope is needed equipped with an excitation light source to excite fluorophores, and a detection device to capture emitted fluorescence from the exited fluorophores. The dimensions and functionality of this scope are otherwise identical with the regular HD Storz endoscope. ICG was readily available at the pharmacy. The technique of imaging was not complicated and was not associated with any sided effects. In one patient cavernous sinus bleeding prevented assessment of the fluorescence. As long as venous bleeding is well controlled, NIR fluorescence imaging can be used to remove adenoma tissue from the cavernous sinus. NIR fluorescence imaging during endoscopic pituitary surgery is easily implemented in current daily practice. As ICG can be visualized under specially equipped surgical microscopes, which are already clinically available, NIR fluorescence imaging is also possible in microscopic transsphenoidal procedures.

Although transsphenoidal surgery is an established, relatively safe procedure, iatrogenic pituitary dysfunction is still a considerable drawback. A postoperative rate of hypopituitarism of 22% is reported[4]. Diabetes insipidus (DI) and pituitary insufficiency are commonly reported following transsphenoidal surgery. DI can be divided in temporary DI (incidence if 10 - 60%) and the uncommon permanent DI (0.5 – 15%)[4]. The cause of DI has been ascribed to stalk manipulation during surgery, and is more common after resection of microadenomas. Postoperative pituitary insufficiency is reported in 1-10% of patients; however outliers of 27% are documented[4]. In a recent structured review and meta-analysis of the available literature, Roelfsema et al. reported the onset of new, surgery-related pituitary insufficiency in 6.9 ± 1.8% of patients treated for prolactinoma, 6.7 +/- 1.6 for acromegaly, 12.5 +/- 4.6 % for NFA and 25.4 +/- 3.6 % for Cushing's disease[5].

Furthermore, surgical remission is not always achieved after resection of a functional adenoma and recurrence of a pituitary adenoma after apparent cure is also frequently reported[5]. Mean remission values and ranges of 68.8% (27-100) in prolactinoma, 47.3% (3-92) in NFA, 61.2% (37-88) in acromegaly, and 71.3% (41-98) in Cushing's disease, are reported. Overall remission rates of 53–64% for macroadenomas and 84-88% for microadenomas are reported[4].

Patients can suffer from recurrent disease, with the incidence peaking between 1 and 5 years after surgery. It is suggested that recurrence originates from small postoperative tumor remnants[5]. Consequently, there is a clear unmet need for improved peri-operative identification of pituitary adenomas, normal gland and surrounding structures, so as to improve surgical outcomes. A more aggressive attempt to attain complete adenoma resection will increase the number of patients achieving biochemical remission but will also increase the likelihood of postoperative complications such as DI and hypopituitarism. Thus, it is plausible to postulate that improved optical guidance during surgery could assist in resecting the adenoma in total while minimizing iatrogenic injury.

Only a limited number of imaging techniques during surgery, are described to improve completeness of tumor resection; for example intraoperative MRI[19] and intraoperative transcranial-transdural real-time ultrasonography[20]. Both techniques are reported as complementary imaging techniques and require the use of different, additional imaging devices that until now, interfere with the normal surgical procedure. Moreover, both techniques do not allow real-time merging of the surgical field and additional tumor imaging. NIR fluorescence imaging, as we describe here, has shown the ability to overcome these constraints.

We noticed several limitations to the described technique. Firstly, NIR fluorescence imaging identification was based on the principle that the normal gland stained after ICG administration, whereas tumor tissue stains to a lesser degree and with a time delay. For high-sensitive identification of adenomas and especially microadenomas, fluorescence signal in tumor tissue instead of healthy tissue would be of greater value. Should tumor tissue be stained, only fluorescent tissue has to be resected. The development of tumor-specific contrast agents targeting specific ligands could potentially facilitate this. Secondly, the imaging system used in the current study did not allow real-time overlay of color (white light) and NIR (blue light) images. Therefore, anatomical

orientation was difficult in some procedures and surgery under fluorescence guidance could only be performed when sufficient fluorescent signal was present. This would also allow a more detailed analysis of the optimal window between administration of ICG and peak fluorescence measurements, and gives a better insight in the decrease of fluorescent signal over time, as fluorescent signal can be measured all procedure long. In addition, to improve visual contrast, the fluorescence images displayed by the endoscopy system as blue on black could be converted to white on black. Further technical developments of endoscopic NIR fluorescence imaging systems are in progress and aim to improve the real-time intraoperative display of NIR fluorophores[17;21;22].

One limitation in the study was that only one endoscope and light cable suitable for NIR fluorescence imaging was available. Therefore only one patient could be included daily, potentially leading to selection bias. An aselect procedure was used to prevent this bias: always the first patient of the day was included, the order of cases was determined by chance.

CONCLUSION

NIR fluorescence imaging using intraoperative intravenous administration of low-dose ICG, can safely and easily be implemented in daily clinical practice. A useful differentiation between adenoma and pituitary gland was possible with NIR fluorescence.

Timing of the ICG administration warrants future study to optimize this technique. Better discrimination of tumor versus normal pituitary tissue, will likely facilitate improved surgical remission rates and reduced surgical morbidity after endoscopic transnasal transsphenoidal selective adenomectomy. With improved techniques and imaging protocols, we advocate that the potential additional value has to be evaluated prospectively in a randomized study design.

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