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# A Novel and Generic Workflow of Indocyanine Green Perfusion Assessment Integrating Standardization and Quantification Toward Clinical Implementation

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**Objective:** This study aims to generate a reproducible and generalizable Workflow model of ICG-angiography integrating Standardization and Quantification (WISQ) that can be applied uniformly within the surgical innovation realm independent of the user.

**Summary Background Data:** Tissue perfusion based on indocyanine green (ICG)-angiography is a rapidly growing application in surgical innovation. Interpretation of results has been subjective and error-prone due to the lack of a standardized and quantitative ICG-workflow and analytical methodology. There is a clinical need for a more generic, reproducible, and quantitative ICG perfusion model for objective assessment of tissue perfusion.

**Methods:** In this multicenter, proof-of-concept study, we present a generic and reproducible ICG-workflow integrating standardization and quantification for perfusion assessment. To evaluate our model's clinical feasibility and reproducibility, we assessed the viability of parathyroid glands after performing thyroidectomy. Biochemical hypoparathyroidism was used as the postoperative endpoint and its correlation with ICG quantification intraoperatively. Parathyroid gland is an ideal model as parathyroid function post-surgery is only affected by perfusion.

**Results:** We show that visual subjective interpretation of ICG-angiography by experienced surgeons on parathyroid perfusion cannot reliably predict organ function impairment postoperatively, emphasizing the importance of an ICG quantification model. WISQ was able to standardize and quantify ICG-angiography and provided a robust and reproducible perfusion curve analysis. A low ingress slope of the perfusion curve combined with a compromised egress slope was indicative for parathyroid organ dysfunction in 100% of the cases.

**Conclusion:** WISQ needs prospective validation in larger series and may eventually support clinical decision-making to predict and prevent postoperative organ function impairment in a large and varied surgical population.

**Keywords:** ICG angiography, quantification, standardization, perfusion, fluorescence

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Indocyanine green (ICG) has been used for many years in reconstructive surgery to determine compromise in anastomosis or evaluate tissue perfusion for flap reconstruction, for imaging of the lymphatic system, and to assess organ function and vascularization.[1,2] Despite its widespread use, there is a paucity of data illustrating a standard imaging procedure to quantify the fluorescence signal in a reproducible and reliable manner. This is in contrast to radiographic, magnetic resonance, or nuclear imaging modalities where standard diagnostic protocol is well established.[3]

Standardization and quantification methods for ICG-angiography purposes will lead to more objective and reproducible data, highlighting interoperator reliability. Moreover, it will overcome the main limitation, the (inter-)observer subjectivity in the visual assessment of the presence/absence of a fluorescence signal. Present literature indicates that visual assessment alone by the attending surgeon does not optimize the outcomes studied,[4–9] implying the need for a more objective, generic, reproducible, and verifiable semiquantitative ICG perfusion model.

This study aimed to generate a standardized quantification model for ICG-angiography within surgical innovation applications. To validate this model, we assessed the parathyroid glands during thyroidectomy by quantified ICG. Hypoparathyroidism was used as an endpoint, given that parathyroid function post-surgery is only affected by perfusion. Hypoparathyroidism can be measured objectively using parathyroid hormone (PTH) and serum calcium levels, as the criterion standard diagnostic test. Within this clinical application, we sought to generate an ICG-workflow model integrating standardization and quantification.

## METHODS

### Workflow of ICG-Angiography Integrating Standardization and Quantification

The first part of this study consisted of developing a Workflow model of ICG-angiography integrating Standardization and

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For this study, the Stryker SPY-Elite was used.

The authors report no conflicts of interest.

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Quantification, denominated as Workflow model of ICG-angiography integrating Standardization and Quantification (WISQ).

WISQ is depicted in Figure 1 and consists of 4 steps described in more detail in Supplemental Digital Content 1, http://links.lww.com/SLA/D181. In short, step 1 is: selecting suitable hardware. Step 2 is standardization of the imaging setup in the operating theater. Step 3 is image acquisition. Step 4 is post-processing and data interpretation. Perfusion graphs are produced by plotting time against the mean fluorescence intensity (MFI).

Given that several factors influence the interoperative signal intensity of ICG, it is understandable that perfusion cannot be determined by visual interpretation of the individual surgeon based on the absolute fluorescence intensity. As in other fields of diagnostic angiography, it should be determined by the dynamics of an inflow and outflow curve, characterized by the shape of the curve. WISQ is based on the concept that the optimal perfusion curve can be split into 2 phases, that is, the inflow and outflow phase (Fig. 1—step 4). We postulated that 4 different types of curves can be identified with the corresponding indication of inflow and outflow complications (Fig. 1—step 4, Supplemental Digital Content 1—Supplementary Figure 1, http://links.lww.com/SLA/D181). Perfusion patterns are analyzed based on descriptive perfusion graph characteristics and compared to the theoretical optimal perfusion curve. The 5 main quantification endpoints include the difference of inflow-to-outflow curves, described as the ingress slope [ingress slope = (MFImax−MFIstart peak)/(Tmax−Tstart), in arbitrary flow units (a.f.u.)/second], the time from MFIstart peak to reach the MFImaximum ($T_{in}$) (in seconds), the egress slope [egress slope = (MFImax-80%Fmax)/(Tmax-80%Tmax), in a.f.u./sec], MFI (in a.f.u.) and area under the perfusion curve (AUC) (Fig. 1−step 4).

## Parathyroid Perfusion for Perfusion Model Validation

The second part of this study consisted of assessing WISQ's clinical feasibility in parathyroid glands within thyroidectomy using the complication hypoparathyroidism as our endpoint. Hypoparathyroidism is defined biochemically as a low PTH (<14.1 pg/mL or <1.5 pmol/L) in the immediate postoperative setting and confirmed by low serum calcium levels with the need for calcium and calcitriol supplementation in the postoperative period.

As one well-vascularized parathyroid gland, the so-called least impacted gland (LIG) is sufficient for normocalcemia, we hypothesized that an adequate inflow-outflow perfusion curve of the LIG during thyroidectomy would predict postoperative normocalcemia without supplementation.[2,10]

The present study is a prospective, observational, multicenter, proof-of-concept study of 10 patients undergoing total thyroidectomy at two academic high-volume endocrine surgery units between August 2019 and February 2020. Exclusion criteria included allergy to ICG or iodinated contrast, pregnancy, lactating, or previous head

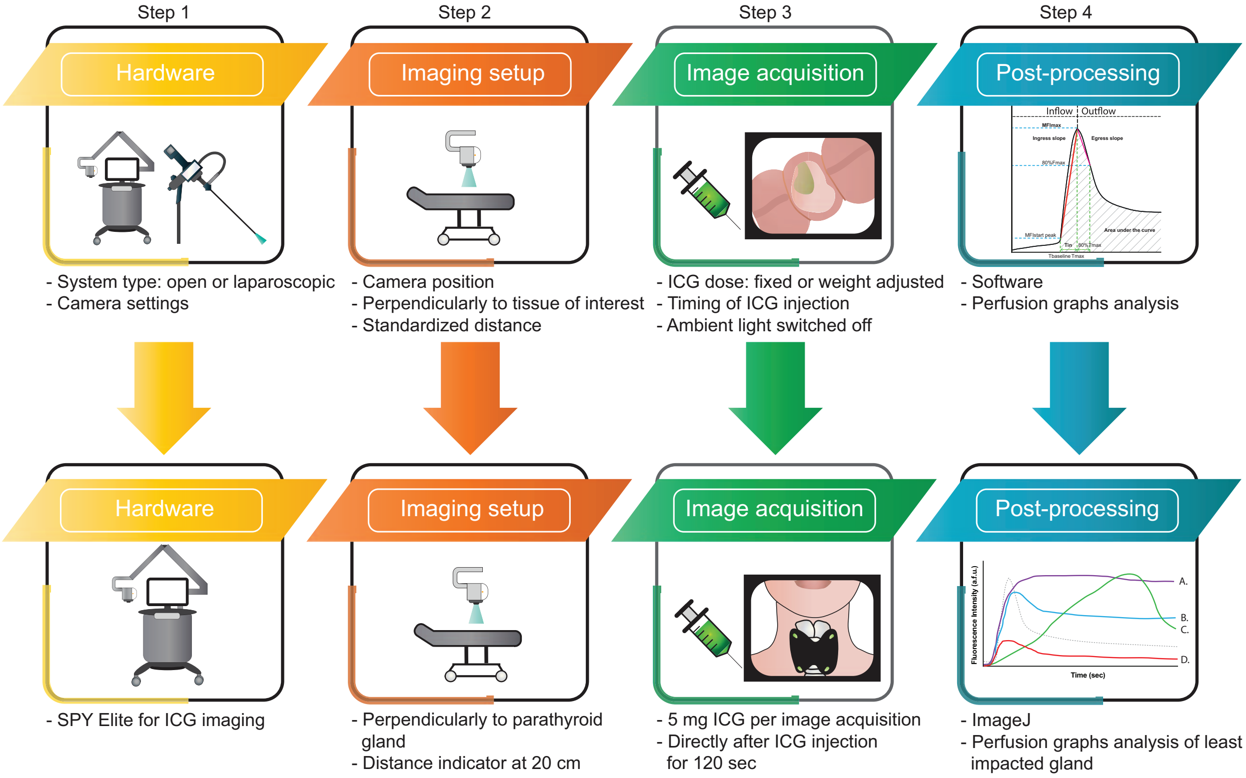

**FIGURE 1.** The 4-steps of the WISQ model. WISQ is graphically represented in the top row. The bottom row represents the respective devices and settings used in this exemplary study. Step 1 is the selection of the appropriate hardware; open or laparoscopic. The user controls basic camera settings. Step 2 concerns the standardization of the camera position, distance and positioning respective to the tissue of interest in the operation theater. Step 3 consists of image acquisition aspects such as ICG dose, time and injection rate and ambient lighting. The final step is data processing and data analysis based on the optimal perfusion curve. For more detail on perfusion curves, refer to Supplementary Figure 1, http://links.lw.com/SLA/D181. WISQ, workflow of ICG-angiography integrating standardization and quantification.

and neck surgery. The study was registered at ClinicalTrials.gov (NCT03969108) and approved by both centers' research ethics board [ID 19-5447 (UHN) and METc2019/379 (UMCG)]. For further details on the surgical procedure, refer to Supplementary Content 1, http://links.lww.com/SLA/D181.

### *Data Acquisition*

ICG was administered twice in all patients (after the respective hemithyroid was removed). A bolus of 5 mg of ICG (2.5 mg/mL) was injected intravenously by the anesthesiologist, followed by a flush of 10 mL NaCl. Data acquisition was conducted following WISQ and was translated to thyroid surgery (Fig. 1). For intraoperative imaging, the SPY Elite (Stryker, Toronto, Ontario, Canada) fluorescence camera system was used. The camera was placed perpendicularly above the tissue of interest with a standardized distance from the surgical field to the camera head (20 cm) using a laser distance indicator. For further details on post-processing and statistical analysis, refer to Supplementary Content 1, http://links.lww.com/SLA/D181.

### Visual Interpretation Versus Postoperative Outcome

To study whether a visual assessment of ICG-angiography videos alone without quantification can predict organ function impairment, 4 experienced endocrine surgeons from four academic centers reviewed the ICG-angiography perfusion videos of all parathyroid glands blinded for results of postoperative outcome (Supplementary Content 1, http://links.lww.com/SLA/D181). This visual interpretation of the LIG was compared to the postoperative outcome.

## RESULTS

### Study Population

In total, 11 patients were enrolled in this proof-of-concept study. However, 1 patient was excluded after performing ICG-angiography due to non-adherence to WISQ. Patient characteristics are summarized in Supplementary Content 2, http://links.lww.com/SLA/D181. Three patients (30%) were diagnosed with hypoparathyroidism and started on calcium and calcitriol supplementation.

### Visual Interpretation of Expert Surgeons Versus Postoperative Outcome

Three of 4 surgeons misjudged the visual interpretation of ICG-angiography in one of the patients with hypoparathyroidism (Supplementary Content 2, http://links.lww.com/SLA/D181, Supplementary Figure 2, http://links.lww.com/SLA/D181). Furthermore, in 5 of 7 patients without hypoparathyroidism at least 1 surgeon would perform unnecessary autotransplantation. Overall, the surgeon's accuracy judging the viability of a parathyroid gland was 60%.

### ICG Quantification

### *Perfusion Curves*

Representative ICG-angiography images and perfusion graph analyses are depicted in Figure 2. Perfusion curves of all parathyroid glands for each patient with more detailed descriptions can be found in Supplementary Content 2, http://links.lww.com/SLA/D181. In 6 of 7 patients without postoperative hypoparathyroidism, the LIG showed a perfusion curve similar or near similar to the proposed optimal perfusion curve (Supplementary Figure 3, panel A, B, E, F, I and J, http://links.lww.com/SLA/D181). The last patient without hypoparathyroidism showed a sharp inflow peak, however, during the outflow phase the intensity did not decline, suggesting an outflow problem (Supplementary Figure 3, panel H, http://links.lww.com/SLA/D181). In this patient, only 3 parathyroid glands were identified intraoperatively, and the patient likely had a fully functioning parathyroid gland within the thymus not found by the surgeon.

In contrast, the LIG of the 3 patients with postoperative hypoparathyroidism showed multiple different types of perfusion problems (Supplementary Figure 3, panel C, D and G, http://links.lww.com/SLA/D181). These problems included minimal increase of fluorescence intensity over time, a low ingress slope and an outflow phase consisting of only a slightly decreasing fluorescence intensity.

### *Quantification Parameters*

In 86% of the patients without hypoparathyroidism, the ingress slope of the LIG was $\geq$5.0 a.f.u./sec, whereas in all patients with hypoparathyroidism, this was $\leq$4.1 a.f.u./sec (Fig. 2, Supplemental Digital Content 2, http://links.lww.com/SLA/D181—Supplementary Figure 4, panel A, http://links.lww.com/SLA/D181). $T_{in}$ of the LIG was in 71% of the patients without hypoparathyroidism $\leq$12.0 seconds and in all patients with hypoparathyroidism $\geq$14.1 seconds (Supplementary Figure 4, panel B, http://links.lww.com/SLA/D181).

In 71% of the patients without hypoparathyroidism, the egress slope of the LIG was $\leq -1.8$ a.f.u./sec seconds. In all patients with postoperative hypoparathyroidism, the egress slope was $\geq -1.7$ a.f.u (Fig. 2, Supplementary Figure 4, panel C, http://links.lww.com/SLA/D181).

The peak MFI of 86% of the patients without hypoparathyroidism was $\geq$64.8 a.f.u. versus $\leq$56.1 a.f.u. in 67% of the patients with hypoparathyroidism (Supplementary Figure 4, panel D, http://links.lww.com/SLA/D181).

## DISCUSSION

ICG-angiography is currently being applied in many areas of medicine to benefit patient outcomes with contradictory results concerning the visual assessment, indicating that visual assessment does not suffice.[4–9] We developed an objective, generic, reproducible, and semiquantitative ICG perfusion model (WISQ). We showed that the WISQ model was able to standardize and quantify ICG-angiography in the surgical theater. The analysis of perfusion curves is a suitable method to interpret ICG-angiography data. We noted that a low ingress slope ($\leq$4.1 a.f.u./sec) of the perfusion curve, when combined with a compromised egress slope ($\geq -1.7$ a.f.u./sec), resulted in parathyroid organ function impairment in 100% of the cases. WISQ can be applied uniformly within the surgical innovation realm since it can be used as guidance to maintain standardization and quantification for other tissues of interest. In the future, WISQ may support in conducting operator-independent cohort studies, possibly preventing postoperative complications.

Even in this small sample size, the ingress slope showed a trend to significance. The outflow parameter seemed less likely to predict viability than the ingress slope, but when we combined the 2 parameters, a predictive model for organ function emerged. Since this study was not intended to establish cutoff values applied across ICG applications, all quantification parameters should be prospectively investigated in large sample sizes for each ICG use and tissue of interest. Several attempts have been made to semiquantify the fluorescence signal in colorectal, esophageal, and plastic surgery.[11–13] Unfortunately, these studies are heterogeneous both in methodology and quantitative parameters. They lack a standardized imaging setup, including constant imaging conditions and a set distance and angle from the camera to the surgical field. Our model describes a possible solution to these issues, since we have used these efforts as a steppingstone to develop an ICG-workflow model integrating earlier attempts such as inflow parameters with additional inflow and outflow analysis strategies and a standardized imaging protocol.

We also showed that visual interpretation of ICG-angiography cannot reliably predict organ function impairment. Since both visual

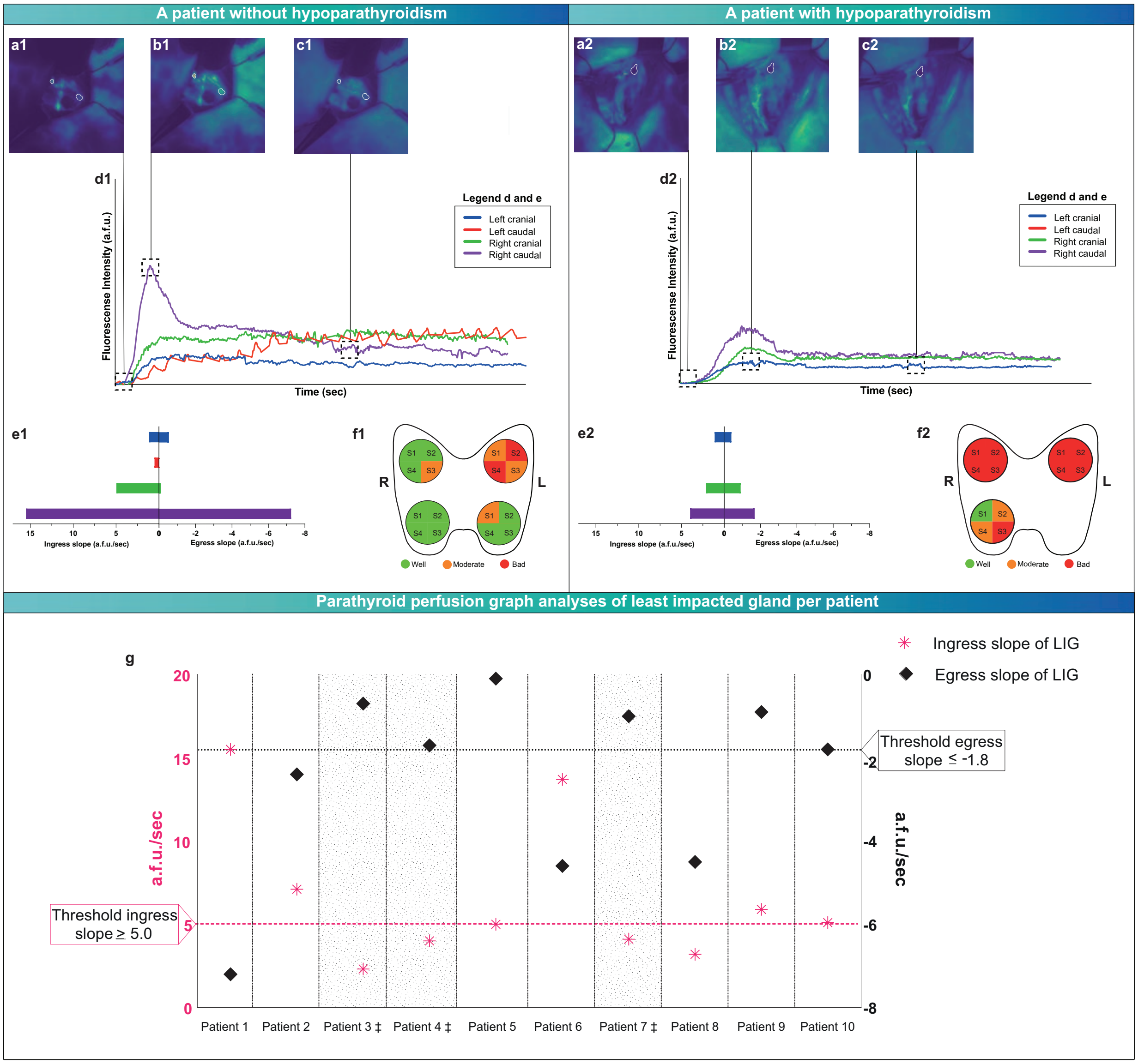

**FIGURE 2.** Representative ICG images and perfusion graph analyses according to WISQ. The images are presented for a patient without (1) and a patient with postoperative hypoparathyroidism (2). A–C, ICG-angiography images over time at the start (A), peak (B) and end (C) of the fluorescence signal. D, Perfusion curves of all intraoperatively identified parathyroid glands. E, Ingress slope and egress slope in a.f.u./sec of the perfusion graph for all identified parathyroid glands. F, Visual interpretation of four experienced endocrine surgeons of the identified parathyroid glands on ICG-angiography. G, Parathyroid perfusion graph analyses of least impacted gland per patient. *L,* left; *R,* right; S1, surgeon 1; *S2,* surgeon 2; *S3,* surgeon 3; *S4,* surgeon 4; *WISQ,* workflow of ICG-angiography integrating standardization and quantification; 🟥 = devascularized; 🟧 = moderately well vascularized; 🟩 = well vascularized; ▨ = and ‡ = diagnosed with postoperative hypoparathyroidism; *LIG,* least impacted gland.

interpretation and the maximum absolute fluorescence intensity could not predict organ function impairment, this emphasizes the importance of an ICG quantification model.

One of the limitations of WISQ is that the analysis was performed post-hoc by analyzing video recordings rather than real-time during the operation. We believe WISQ can easily be applied within the intraoperative setting as demonstrated in other studies showing nonstandardized use.[14] Future studies should focus on determining the optimum concentration, route and timing of ICG administration. Although our small cohort limits the utility of establishing cutoff values for perfusion parameters, a validation study using our model may achieve this for thyroid surgeons. This study showed the feasibility of WISQ in predicting organ function impairment in parathyroid glands and provided an estimated value for a future sample size calculation.

## CONCLUSION

This study presents a generic and reproducible ICG-workflow for perfusion assessment integrating standardization and quantification, denominated as WISQ. Visual interpretation of ICG-angiography could not predict organ function impairment, emphasizing the importance of an ICG quantification model. WISQ was able to standardize and quantify ICG-angiography in the surgical theater and provided robust and reproducible perfusion curve analysis. Using parathyroid gland perfusion to validate this model, we showed that a low ingress slope, combined with a compromised egress slope, was indicative for organ function impairment in 100% of the cases. Application of this model to other tissue perfusion platforms using ICG is the ultimate goal for generalizing and standardizing its use to support further clinical decision-making to predict and ultimately prevent postoperative organ function impairment.

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