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the application in plastic and reconstructive surgery

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Indocyanine Green Imaging

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CHAPTER 1

Introduction and outline of the thesis

INTRODUCTION

Plastic and reconstructive surgery has always been on the frontier of utilizing novel, innovative technologies to increase patient safety during operative procedures and improve postoperative outcomes.[1] One such innovative imaging technique is indocyanine green imaging, also called near-infrared (NIRF) imaging. The use of this imaging technique is based on the administration of a fluorescent contrast agent, indocyanine green (ICG). Indocyanine green imaging has the ability to provide detailed information on anatomical structures and to assess tissue perfusion.[2] It is therefore increasingly applied in both medical diagnostics and plastic and reconstructive surgery, as it might facilitate the surgeon in critical preoperative, intraoperative and postoperative decision-making to enhance surgical precision and improve safety and outcomes for patients.

History of indocyanine green imaging

The use of indocyanine green imaging is not entirely new, as the principle has been used for more than sixty years in clinical medicine. Indocyanine green as a dye, was originally developed by the Eastman Kodak Company for use in infrared photography. In 1957, it was first introduced in clinical medicine and initially used for the assessment of cardiac output and hepatic function.[3,4] Following the introduction of indocyanine green imaging as a method to evaluate choroidal circulation in 1976, the principle has been adapted to currently available imaging devices.[5]

However, technological limitations at that time prevented extensive development of indocyanine green imaging and widespread acceptance of the technique was further delayed until the early 2000s when the resolution of digital imaging improved. Since then, indocyanine green imaging is frequently used in various surgical fields, including ophthalmology to guide macular hole surgery,[6] neurosurgery to identify vessel abnormalities[7] and in general surgery for real-time visualization of the biliary system during cholecystectomy and pancreaticoduodenectomy,[8] to identify hepatic malignancies and hepatic segments,[9] to localize and preserve the parathyroid glands during thyroidectomy,[10] to assess gastrointestinal anastomoses,[11] to identify sentinel lymph nodes and to target neoplastic cells.[2] Over the past decade, indocyanine green imaging is gaining popularity in plastic and reconstructive surgery, predominantly as an adjunct in the assessment of flap perfusion in free or pedicled flap surgery.[12-16] Additionally, it is used to assess tissue viability in nipple-sparing or skin-sparing mastectomy, traumatic deglovement injuries and burns, to perform lymphatic mapping and to evaluate lymphaticovenous anastomosis (LVA).[12,17-19]

Technical aspects of indocyanine green imaging

As described, indocyanine green imaging uses ICG as a fluorescent contrast agent, which can be visualised in the human body using a dedicated imaging system. ICG is a water-soluble tricarbocyanine dye that is rapidly and extensively bound to blood plasma protein following intravenous administration.[13] In case of intradermal injection, the ICG is drained via lymphatic capillaries of the lymphatic system, which are located within the interstitial space.[20] Therefore, ICG is an ideal contrast agent to obtain tissue perfusion assessment as well as to perform lymphatic vessel mapping.

ICG absorbs light in the near-infrared spectral range between 750 and 810 nanometres (nm). Light at this wavelength is minimally absorbed by water or haemoglobin and is not scattered by tissues, which allows for excellent visualization of blood vessels within the deep dermal plexus and subcutaneous fat up to a maximum depth of 2 cm from the skin surface.[15] When excited, ICG emits fluorescence light of approximately 840 nm, that can be recorded using a specialised imaging system with an infrared filter.[21] This system allows the fluorescence to be recorded in real-time for the human eye, as a real-time photograph or video. Most imaging systems contain both an excitation light source and a detection device to capture emitted fluorescence from the exited fluorophores with a single device. See Figure 1.

Indocyanine green imaging is fast (i.e., a matter of minutes), safe, and easy to perform. ICG is excreted rapidly into the bile with a plasma half-life of 3 to 4 minutes.[22] Furthermore, since the ICG dose by weight is limited to a safe dose of 5.0 mg/kg[23,24] and the most frequently used intravenous dose by weight is 0.5 mg/kg in the current literature, multiple assessments can be performed during one surgical procedure. The clinical use of ICG has proved to be safe in humans, with very few reports of anaphylactic reactions (incidence of 1 in 42,000).[23,25] As ICG contains sodium iodide, it was previously suggested that ICG should be used with caution in patients who have a history of allergy to iodine because of the risk of anaphylaxis.[26] However, a recent statement from the manufacturer describes that a patient with a known allergy to iodine will not be at risk of an allergic reaction to ICG as sodium iodide is a very small molecule which cannot be identified by the body as an antigen.[27] Nonetheless, allergic reactions may still occur to other excipients of ICG.

Indocyanine green (ICG) is administered intravenously to obtain tissue perfusion assessment, predominantly via a peripheral infuse system in the dorsum of the hand or foot. During surgery, ICG is visualised using a handheld near-infrared fluorescence camera. For most of the studies in this thesis, the handheld Fluobeam 800 (Fluoptics, Grenoble, France) is used. The camera head contains an excitation light source, filter sets and a camera

sensitive to record near-infrared fluorescence. The videos or photographs are displayed in real-time on a mobile monitor cart to which the handheld camera is connected. During indocyanine green imaging, the operation lights are turned off. Of note: to perform lymphography, ICG is injected intradermally in the webspace of the hand or foot.

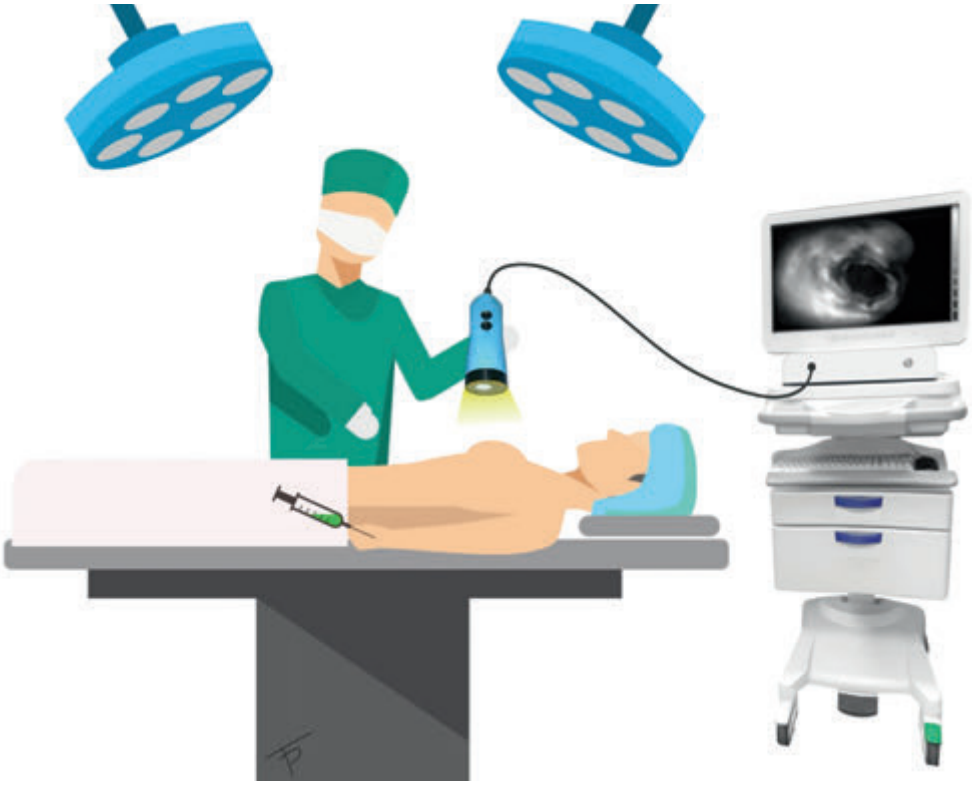

Figure 1. Schematic overview of indocyanine green angiography during surgery. The handheld Fluobeam 800 (Fluoptics©, Grenoble, France) is used. ICG is administered intravenously via a peripheral infuse system in the dorsum of the left hand. As illustrated, the operation room lights were turned off during ICG imaging.

Factors of influence during indocyanine green imaging

Although recommendations on the use for indocyanine green imaging in plastic and reconstructive surgery have been advocated, there is still no consensus on imaging protocols, including the optimal dose of ICG.[16] Therefore, different medical groups around the world use their own experiences to determine the optimal ICG dosage.[21] However, there is evidence showing that ICG dosage influence fluorescence intensity and might therefore affect the result or the quality of indocyanine green imaging.[28]

In addition, there might be more factors that influence fluorescence intensity and potentially preclude an accurate assessment of indocyanine green imaging, including the timing of dye administration, the angle and distance of the camera head to the target of interest, ambient light during imaging and the use of vasopressors during surgery.[29-31] However, these factors are only briefly described in the current literature.

The role of indocyanine green imaging in flap surgery

Postoperative (partial) flap necrosis is one of the most feared complications following reconstructive flap surgery and represents a significant problem with an incidence of 4 to 19.4% depending on flap type.[32,33] The current golden standard for evaluating tissue perfusion relies on the surgeon's clinical evaluation of tissue colour, flap temperature, capillary refill, and edge bleeding of the flap.[15] However, the accuracy of this method highly depends on the surgeon's experience and is restricted by the visual performance limits of the human eye. Furthermore, evidence suggests that clinical evaluation alone is an unreliable predictor of insufficient tissue perfusion.[34]

In order to minimize the risk of postoperative tissue necrosis, surgeons need to be able to objectively evaluate tissue perfusion during surgery, as partial necrosis or total flap loss may be prevented by immediate intervention whenever perfusion appears to be insufficient. Such a method should be repeatable, reliable, user-friendly, accurate, inexpensive and applicable to all types of flaps.[35,36] Therefore, a broad range of different techniques, such as oxygen partial pressure, capillaroscopy, laser Doppler flow measurement, dynamic infrared thermography, and photoplethysmography have been evaluated clinically. However, none of these methods have yet been applied systematically due to high procedural costs, methodological complexity, low sensitivity, and high false positive as well as false negative rates.[15]

Since indocyanine green imaging was adopted in free flap surgery in 2002, it was found to be a simple, fast, reliable, and valuable perfusion assessment adjunct when compared to clinical evaluation with a reported sensitivity of 90.9% and an accuracy of 98.8%.[37,38] It offers the potential to provide objective and accurate intraoperative data to evaluate flap perfusion during pedicled and free flap surgery. Additionally, it can accurately guide debridement of non-vital tissue intraoperatively in order to minimize postoperative complications and reinterventions.[12] Furthermore, indocyanine green imaging can be used to assess skin viability of mastectomy flaps during breast reconstructive surgery, as well as to provide postoperative data to predict clinical outcome such as (partial) skin flap necrosis.[13]

The role of indocyanine green imaging in lymphedema surgery

Lymphedema is a chronic, debilitating condition, characterised by abnormal accumulation of subcutaneous protein-rich fluid due to failure of the lymphatic drainage.[39] The origin can be congenital or, more commonly, secondary to injuries. In developed countries, secondary lymphedema mainly occurs after cancer therapy and it is strongly associated with breast and prostate cancer.[40,41] Regardless of aetiology, patients with lymphedema experience a variety of symptoms including pain, skin tightness, recurrent periods of cellulitis, decreased range of motion, depression and anxiety. These symptoms can substantially affect patients' quality of life.[42,43]

Traditionally, lymphedema treatment consists of complex decongestive therapy, involving skin care, exercise, compression therapy, and manual lymphatic drainage.[42] However, the efficacy of this lifelong, time-consuming therapy largely depends on the patient's compliance.[44] Therefore, lymphaticovenous anastomosis (LVA) is proposed as one of the surgical options for lymphedema. LVA is a minimal invasive method that redirects excessive lymph fluid from the oedematous limb into the venous system, by anastomosis lymphatic vessels to subdermal venules.[45] Conventional imaging techniques to provide quantitative assessment of lymphatic function include direct contrast lymphography and lymphoscintigraphy, which are time-consuming and invasive.[13,15] Although LVA surgery was already proven to be a valuable procedure in 1977, it gained popularity after the introduction of indocyanine green imaging as imaging technique to enable direct visualization of the lymphatic system.[46]

Following intradermal injection into the webspace of the hand or foot, ICG enters the interstitial space. The lymphatic capillaries located within this area are highly permeable due to discontinuous button-like endothelial cell-to-cell junctions which allows for the entry of the ICG containing interstitial fluid.[20] Subsequently, dynamic images of the superficial lymphatic flow can be obtained to determine the stage of lymphedema and evaluate the functionality of lymphatic vessels.[47-50] Lymphoscintigraphy is the gold-standard examination for extremity lymphedema. However, indocyanine green lymphography is recommended to determine patient's suitability for LVA surgery in secondary lymphedema, since the diagnostic ability and its evaluation capability for lymphedema severity is similar to lymphoscintigraphy with less invasiveness and lower costs.[51] In addition, intraoperative indocyanine green lymphography can guide surgeons during LVA surgery by facilitating real-time decision making, leading to more reliable and improved outcomes following LVA.[45]

The future development of indocyanine green imaging

Although indocyanine green imaging has been used in various clinical applications routinely for sixty years, it is a relatively novel imaging technique in plastic and reconstructive surgery. As previously described, promising results were found in studies regarding the use of indocyanine green angiography and lymphography over the last decade, reflecting its potential to advance plastic and reconstructive surgery.[52]

However, the novel use of indocyanine green imaging in plastic and reconstructive surgery means that the imaging technique is still developing in many ways, and that there is still a lack of knowledge to facilitate surgeon confidence and education in its use.[1] For instance, it is currently unclear what specific imaging protocol needs to be followed to yield accurate, representative and reliable imaging. Consequently, more research is required in order to realize the full potential of this imaging technique and, eventually, to implement it in standard clinical care.

OUTLINE OF THE THESIS

This thesis is divided into two parts: preclinical evaluation and clinical application of indocyanine green imaging in plastic and reconstructive surgery. The aim of this thesis is to better understand the factors that influence indocyanine green imaging in order to optimize the use of indocyanine green imaging. Besides, some of the possible applications of indocyanine green imaging in plastic and reconstructive surgery will be evaluated in order to facilitate preoperative and intraoperative decision-making and minimize postoperative complications.

Part I: Preclinical evaluation of indocyanine green imaging
Part I describes systematic reviews and standardised experimental studies regarding factors that influence fluorescence intensity in indocyanine green imaging and the effect of indocyanine green imaging on preventing postoperative complications. In **Chapter 2**, currently used indocyanine green imaging protocols and factors of influence during indocyanine green imaging were evaluated in both a systematic review and ex vivo experiments. **Chapter 3** focusses on a porcine intestinal model, in which the potential effect of vasopressors on perfusion assessment using indocyanine green imaging was evaluated. **Chapter 4** describes a systematic review and meta-analysis summarizing protocols for mastectomy skin flap perfusion assessment and assessing the effect of indocyanine green imaging for preventing postoperative complications in breast reconstructive surgery.

Part II: Clinical application of indocyanine green imaging
Part I is the foundation for part II, in which the clinical application of indocyanine green imaging in plastic and reconstructive surgery in various surgical procedures are described. **Chapter 5** describes the use of indocyanine green imaging during autologous breast reconstruction and the impact of using the internal mammary artery on mastectomy skin flap perfusion in an observational study. In **Chapter 6** the use of indocyanine green imaging during abdominal wall reconstruction is evaluated in a pilot study. **Chapter 7** describes the role of indocyanine green lymphography to facilitate pre-operative decision making for patients undergoing lymphaticovenous anastomosis for extremity lymphedema.

Part III: Summary, discussion and impact
In **Chapter 8** all main results as presented in this thesis are summarised and discussed. In addition, future perspectives are given. **Chapter 9** contains a Dutch summary of this thesis, and **Chapter 10** describes the impact of the evidence collected in this thesis.

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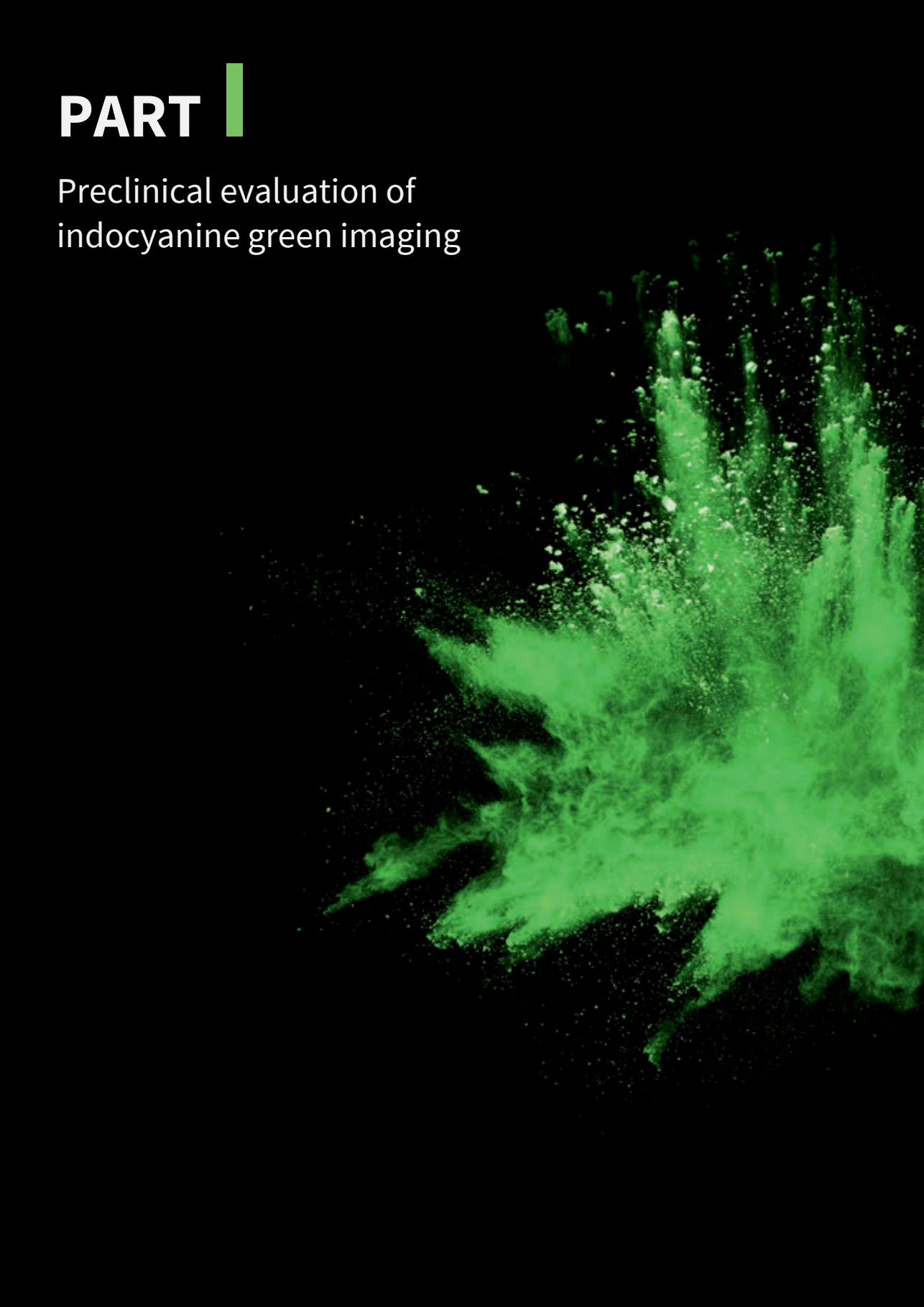
PART I

Preclinical evaluation of indocyanine green imaging



CHAPTER 2

Optimizing indocyanine green angiography in reconstructive flap surgery: a systematic review and ex vivo experiments

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ABSTRACT

Background

Indocyanine green angiography (ICGA) offers the potential to provide objective data for evaluating tissue perfusion of flaps and reduce the incidence of postoperative necrosis. Consensus on ICGA protocols and information on factors that have an influence on fluorescence intensity is lacking. The aim of this article is to provide a comprehensive insight of in vivo and ex vivo evaluation of factors influencing the fluorescence intensity when using ICGA during reconstructive flap surgery.

Methods

A systematic literature search was conducted to provide a comprehensive overview of currently used ICGA protocols in reconstructive flap surgery. Additionally, ex vivo experiments were performed to further investigate the practical influence of potentially relevant factors.

Results

Factors that are considered important in ICGA protocols, as well as factors that might influence fluorescence intensity are scarcely reported. The ex vivo experiments demonstrated that fluorescence intensity was significantly related to dose, working distance, angle, penetration depth, and ambient light.

Conclusion

This study identified factors that significantly influence the fluorescence intensity of ICGA. Applying a weight-adjusted ICG dose seems preferable over a fixed dose, recommended working distances are advocated, and the imaging head during ICGA should be positioned in an angle of 60° to 90° without significantly influencing the fluorescence intensity. All of these factors should be considered and reported when using ICGA for tissue perfusion assessment during reconstructive flap surgery.

INTRODUCTION

Postoperative (partial) flap necrosis is one of the most feared complications in reconstructive flap surgery for both the patient and the surgeon. With an incidence of 4 to 16% depending on flap type, it represents a significant problem.[1-3] Flap necrosis can lead to slower recovery, infection, repeat surgery, delayed adjuvant therapy, and increased health care costs. Patients may even encounter psychological distress with a decline in their quality of life.[1,4] In order to minimize the risk of necrosis, surgeons need to be able to objectively evaluate tissue perfusion during surgery, as partial or even total flap loss may be prevented by immediate intervention whenever perfusion appears to be insufficient. Likewise, consequences of insufficient flap edge circulation, including postoperative wound dehiscence and fat necrosis, could be prevented during surgery.[5]

The current gold standard for evaluating tissue perfusion relies on the surgeon's clinical judgement, that is, the subjective evaluation of tissue colour, flap temperature, capillary refill, and assessment of dermal edge bleeding. Although this method is accurate in 84 to 96% of cases (depending on flap type), the accuracy depends highly on the surgeon's experience and expertise, whereas (by definition) it is also restricted by the visual performance limits of the human eye. Evidence suggests that clinical judgement alone is an unreliable predictor of insufficient tissue perfusion.[6] Therefore, various medical imaging modalities are being developed to obtain real-time assessment of tissue perfusion in an objective and reproducible manner. One such innovative technique is near-infrared fluorescence (NIRF) imaging using indocyanine green (ICG), also known as indocyanine green angiography (ICGA).[5]

Since Flower and Hochheimer developed an imaging technique to evaluate choroidal circulation routinely in 1976,[7] the principle has been adapted to currently available imaging devices. ICGA uses ICG, a water-soluble tricarbocyanine dye, as a contrast agent. Following intravenous administration, ICG is rapidly and extensively bound to plasma proteins, making it an ideal contrast agent to evaluate tissue perfusion.[8] When exposed to near-infrared excitation in the wavelength range of 750 to 810 nm, ICG reemits light (fluorescence) with a wavelength of approximately 840 nm. A dedicated digital video camera, which filters out the excitation light, allows the fluorescence of ICG to be recorded in real time.[9]

ICGA offers the potential to provide objective data to support intraoperative decision-making regarding flap design and is a useful adjunct for evaluating tissue perfusion of flaps. Reported sensitivity and the accuracy of ICGA are 90.9% and 98.8%, respectively.[10] Clinical

use of ICG has proved to be safe in humans. The incidence of adverse events is about 1 in 42 000 patients.[11] Furthermore, ICG has a plasma half-life of approximately 3 to 5 minutes, which allows multiple injections throughout a procedure, limited up to a safe maximum dose of 5 mg/kg.[8]

ICGA is currently explored for multiple applications in surgery.[12] In plastic and reconstructive surgery, it is used predominantly to assess tissue perfusion in (free) flap surgery.[5,9,13-18] Although recommendations for use of ICGA have been previously reported,[18] there is still no consensus about the technical use of ICGA during reconstructive surgery, including timing of evaluation and optimal intravenous dose of ICG.[5] This is important because ICG dosage influences fluorescence intensity, thereby influencing the adequacy of perfusion assessment.[19] On top of that, there are other factors that can have an impact on fluorescence intensity within the collected images, including the distance and angle between the camera and region of interest, and ambient light during perfusion evaluation. These are either not considered to be important or only briefly described in current literature. Since ICGA is rapidly being introduced in clinical practice worldwide, it is important that surgeons are aware of factors that potentially play a role with regard to the feasibility of this imaging modality. Therefore, this study aims to provide a comprehensive insight in potential factors influencing the fluorescence intensity when using ICGA by performing a systematic review, regarding all studies reporting on ICGA to assess tissue perfusion during reconstructive flap surgery, and ex vivo experiments.

MATERIALS AND METHODS

This report is composed of 2 parts. First, a systematic search of the literature was conducted to provide a comprehensive overview of currently used ICGA protocols in reconstructive flap surgery, focusing on ICG dosage, timing of both application and assessment, working distance, and other possible influencing factors as discussed above. In the second part, ex vivo experiments were performed to further investigate the practical influence of potentially relevant factors.

Systematic Review

A systematic literature search was conducted in July 2018 in the following databases: National Library of Medicine (PubMed) database, EMBASE database (via OvidSP), and Cochrane Library CENTRAL, using the methodology described in the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement.[20] The following terms were used (including synonyms and closely related words) as index terms or free-text words: “flap surgery”, “indocyanine green”, “angiography”, “perfusion”, and “imaging”.

The search syntax applied to each database, and the PRISMA 2009 checklist are attached as Supplementary Material. First, all titles and abstracts derived from the search were screened and independently reviewed for eligibility by 2 researchers (TP and RS). There were no restrictions on language in this review. Letters and comments on articles, conference abstracts, case reports including less than 10 flaps, studies conducting research other than on human subjects, reviews, and meta-analyses were excluded. Studies were considered eligible if they

1. Reported on ICGA in free flap, pedicled flap, or mastectomy skin flap surgery;
2. Reported on ICGA to assess tissue perfusion;
3. Described an ICGA protocol.

In case of uncertainty, full-text reports were screened to determine eligibility. Any differences in the resulting derived articles were discussed by the 2 aforementioned researchers. If no consensus was reached, a third author (SQ) decided after discussion. Other sources including the reference lists of included articles and recent review articles were screened for relevant articles not identified by the online databases based on previously described criteria. A data extraction sheet was developed containing items on the type of study included, the operated flap type, the applied imaging system, the dose of ICG, the working distance (eg, distance from imaging head to tissue), the timing of evaluation, the timing from administration of ICG to evaluation, the method to evaluate tissue perfusion, and the decision that was taken to excise tissue in the study. The data extraction sheet was completed for all eligible studies by 2 independent researchers (TP and RS).

Ex Vivo Experiments

The methods for the ex vivo experiments have been previously reported by 2 of the authors (JB and FW), using a laparoscopic NIRF imaging system.[19] In this study, a handheld NIRF camera (Fluobeam, Fluoptics, Grenoble, France) was applied, provided with integrated near-infrared light source with excitation between 750 and 800 nm and maximum fluorescein emission detection between 780 nm and 850 nm. In the author's institution, this system is used in daily practice for perioperative tissue perfusion evaluation as well as for mapping of lymphatic collecting vessels in the outpatient clinic.[21,22]

Experiments were performed using ICG diluted within 40 mg/mL albumin in a 0.9% NaCl dilution. This was done accordingly as ICG is considered to bind to albumin in vivo, which modifies its optical properties; 40 mg/mL was chosen as a stable point within the normal reference range for serum albumin, which is 35 to 55 g/L. In all experiments, a total of 18

different concentrations of ICG were used, ranging between 0.01 and 0.0001 mg/mL, representing 50 to 0.5 mg of total dose of ICG administered intravenously in a female patient, weighing 77.0 kg, with a blood volume of 5000 mL, estimated using MedCalc300 (Medscape, 2018). A bodyweight of 77.0 kg was considered average after obtaining chart data on 25 consecutive patients, who had undergone a deep inferior epigastric artery flap in the authors' institution.[2]

Next to the ICG dilutions, wells plates and beeswax plates were used for this experiment. From each dilution, 9 times 3 mL of the ICG-containing mixture was placed on a wells plate in order to completely fill the wells with fluid, to minimize fluid-to-beeswax plate air layer. The influence of distance was measured fixating the imaging head at 12 distances varying from 50 to 5 cm from the surface of the dye. This was then repeated for all distances with, respectively, 1 and 2 beeswax plates of exactly 1 mm thickness, stacked to the wells plate. Beeswax plates (Stockmar, Kaltenkichen, Germany) were chosen because it approaches the scattering behavior and translucent light penetration of human tissue.[23]

The experiments were performed in darkness (windows covered) with only one computer screen left on. The aforementioned experiment with 1 beeswax plate was repeated with uncovered windows to measure the influence of ambient light. The influence was measured at a distance of 15 cm for all ICG dilutions. The fluorescence intensity of the middle cup was measured at incident angles of 90°, 75°, 60°, and 45° between the imaging head and middle wells surface plane. The penetration depth was evaluated with the use of beeswax plates progressively stacked one by one to increase thickness until it was not possible anymore to distinguish the dilution-filled wells from its surroundings. In addition, the influence of beeswax plates themselves on fluorescence intensity was analysed. The setup of the ex vivo experiment is illustrated in Figure 1. In all experiments, fluorescence intensity was measured on a greyscale from 0 to 255 using ImageJ software (Version 1.51, ImageJ, National Institutes of Health, Bethesda, MD). Zero is black and 255 is white on this scale. Values in between make up different shades of grey.

Statistical Analysis

The association between dose and fluorescence intensity for different experimental conditions were first visualised using scatter plots with lines fitted using either linear spline regression, in case of an observed ceiling effect, or locally weighted scatterplot smoothing (LOESS), in case of an observed curvilinear association.

To quantify the associations between covariates of the experiment and fluorescence intensity, regression coefficients were estimated using multivariable, or adjusted, linear

regression analysis. No univariate analyses were performed as the estimates would be too dependent on the setting of other covariates of the experiment. In case a ceiling effect was present, observations for which the intensity was >254 on the 0 to 255 scale were omitted to prevent biased estimates. Curvilinear associations were estimated using polynomial regression (i.e., the linear regression model was extended with quadratic and cubic terms for continuous variables and tested for significance). All analyses were performed using R version 3.3.3 (R Foundation for Statistical Computing, Vienna, Austria) and the rms package version 5.1-0.

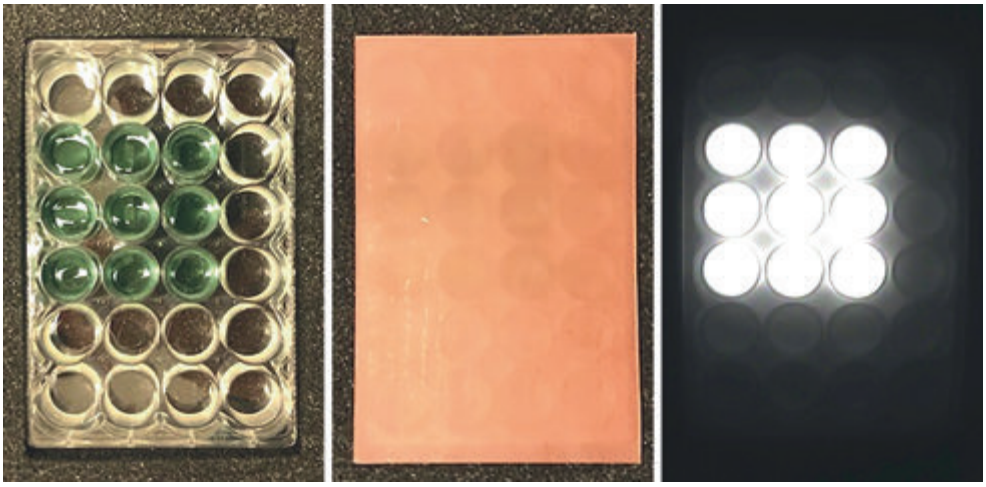

Figure 1. Setup of the ex vivo experiment.

Statistical Analysis

The association between dose and fluorescence intensity for different experimental conditions were first visualised using scatter plots with lines fitted using either linear spline regression, in case of an observed ceiling effect, or locally weighted scatterplot smoothing (LOESS), in case of an observed curvilinear association.

To quantify the associations between covariates of the experiment and fluorescence intensity, regression coefficients were estimated using multivariable, or adjusted, linear regression analysis. No univariate analyses were performed as the estimates would be too dependent on the setting of other covariates of the experiment. In case a ceiling effect was present, observations for which the intensity was >254 on the 0 to 255 scale were omitted to prevent biased estimates. Curvilinear associations were estimated using polynomial regression (i.e., the linear regression model was extended with quadratic and cubic terms for continuous variables and tested for significance). All analyses were performed using R version 3.3.3 (R Foundation for Statistical Computing, Vienna, Austria) and the rms package version 5.1-0.

RESULTS

Systematic Review

Forty-nine articles,[24-72] including 1996 surgical flaps, were selected for the review. Study designs included prospective cohort studies (n=27),[31,34,35,38,39,41-43,47-50,56,58-60,62-71] retrospective cohort studies (n=16),[25-27,29,32,33,36,40,44,45,51,55,57,61,72] prospective pilot studies (n=3),[28,37,52] and retrospective case series (n=3).[46,53,54] No randomised controlled trials have been performed yet that concern ICGA in plastic and reconstructive flap surgery. A detailed overview of the study selection is presented in a PRISMA flow chart (see Figure 2). A summary of findings from the included articles is presented in Table 1.

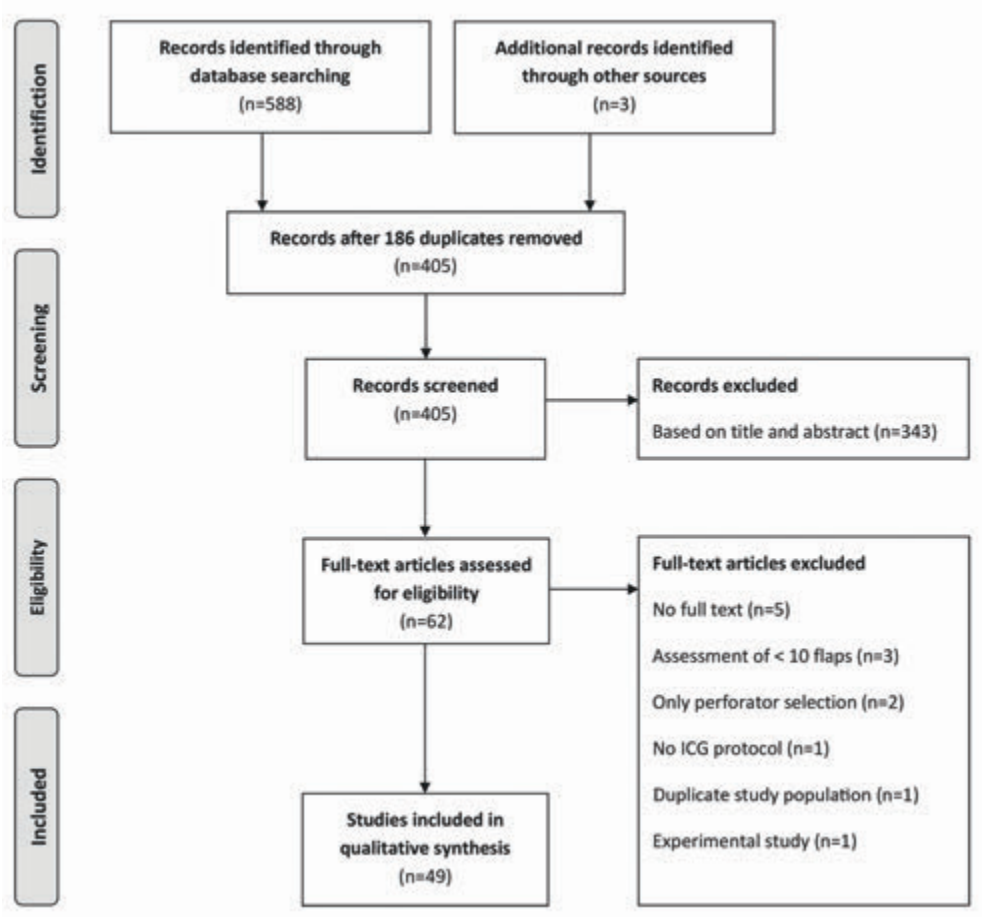

Figure 2. PRISMA flowchart showing selection of articles for review.

Table 1. Summary of Findings From the Component Articles.

Year	Author	P/R	Flap Perfusion	No.	System	Dose	Timing	Perfusion Assessment	Excision
Mastectomy skin flap and nipple-areolar complex perfusion									
2018	Yang et al[22]	P	Mastectomy skin	10	SPY	3 cc	1. After TE inset with 50% fill (skin closed temporarily) 2. Same with 100% 3. Same with 150%	Grayscale: ingress/egress rate analysis*	No
2018	Wang et al[27]	R	NAC	17	SPY	7.5 mg	1. With implant sizer	Grayscale: ingress/egress rate analysis	Yes
2018	Hammer-Hansen et al[24]	R	Mastectomy skin	66	SPY	NR	1. After mastectomy 2. With implant sizer 3. Dermis sutured	Relative perfusion: cutoff <33%	Yes
2018	de Vita and Buccheri[25]	R	Mastectomy skin	44	Quest spectrum	0.2 mg/kg	1. Before implant inset 2. After implant inset	NR: "areas of low fluorescence indicating limited flap perfusion"	No
2018	Venturi et al[26]	P	NAC and mastectomy skin	32	SPY	NR	1. Directly after mastectomy 2. After TE fill	Relative perfusion: threshold ≤5% of surrounding normal tissue perfusion	No
2017	Giorsi et al[23]	R	Mastectomy skin	100	PDE	5 mg	1. After TE inset, but before skin closure	Grayscale: "surgeon marked the nonenhanced areas"	Yes
2016	Rinker[28]	P	Mastectomy skin	20	SPY	25 mg	1. After mastectomy	NR: "nonperfusing areas were marked and excised"	Yes
2016	Mattison et al[41]	P	Mastectomy skin	55	SPY	NR	1. After mastectomy 2. After dissection 3. After TE or implant inset	Absolute flow value and relative perfusion	No
2016	Harless and Jacobson[42]	R	Mastectomy skin	213	SPY	NR	1. After mastectomy 2. After implant or TE inset	NR: "assessment of perfusion with LA-ICGA"	Yes
2016	Diep et al[22]	R	Mastectomy skin	61	SPY	NR	1. After TE inset	NR: "When ischemia was noted on ICGA the surgeon resected that ischemic tissue"	Yes
2015	Bertoni et al[72]	R	NAC	54	SPY	6.25-7.5 mg	1. Before skin incision	Analysis of arterial inflow pattern (in %)	No
2014	Munabi et al[47]	P	Mastectomy skin	62	SPY	10 mg	1. After completion of reconstruction	Absolute flow value	No
2014	Duggal et al[21]	R	Mastectomy skin	184	SPY	NR	NR	NR: "SPY perfusion analysis"	Yes
2013	Sood and Glat[25]	R	Mastectomy skin	39	SPY	3 cc	1. After mastectomy, prior to reconstruction	NR: "Areas of low fluorescence were noted"	Yes
2012	Phillips et al[28]	P	Mastectomy skin	51	SPY	17.5 mg	1. After TE inset and skin temporarily closed	Grayscale: "area of poor perfusion marked"	No
2012	Moyer and Losken[27]	R	Mastectomy skin	15	SPY	5 cc	1. Within 30 minutes after mastectomy 2. After completion and skin closed	Relative perfusion: "poorly perfused tissue resected when possible"	Yes
2010	Newman et al[40]	R	Mastectomy skin	20	SPY	NR	1. After mastectomy	Grayscale and relative perfusion assessment	No
Free flap perfusion									
2018	Fan et al[29]	P	Superthin ALT	40	SPY	0.1 mg/kg	1. Before ALT thinning 2. After ALT thinning	Relative perfusion: cutoff <30%	Yes
2018	La Padula et al[29]	R	ALT	13	PDE	NR	1. After flap isolation on perforator with selective perforator clamping	Grayscale: "resect black area, preserve gray area"	Yes
2016	Ludolph et al[28]	P	DIEP and ms-TRAM	35	SPY	10 mg	1. After flap harvest	Relative perfusion: cutoff <30%	Yes

Table 1. (continued)

Year	Author	P/R	Flap Perfusion	No.	System	Dose	Timing	Perfusion Assessment	Excision
2016	Hitier et al[37]	P	Fibular, DIEP and ALT	20	Fluobeam	0.025 mg/kg	1. After anastomosis	Grayscale: "Fluorescence calculated as mean of collected gray levels in a ROI, corrected by the exposure time in milliseconds."	No
2016	Akita et al[25]	P	DIEP, SCIP, ALT and TD	60	PDE	0.25 mg/kg	1. After flap harvest	Grayscale: "No-stained area (not enhanced in 60s) was not used for reconstruction."	Yes
2015	Valerio et al[44]	R	Osseous	16	SPY	7.5 mg	1. After flap harvest 2. After anastomosis	Absolute flow value: cutoff value of 6.0	Yes
2015	Bigdeli et al[42]	P	ALT, TFL, and DIEP	10	Vision-sense	0.5 mg/kg	1. After anastomosis	Relative perfusion: cutoff <33%	Yes
2015	Beckler et al[45]	R	Free fibula	25	SPY	25 mg	1. At least 30 minutes after tourniquet release, before flap pedicle division	Relative perfusion: cutoff ≤33%	Yes
2014	Iida et al[30]	P	SCIP	12	PDE	5 mg	1. Preoperatively to mark perforator 2. After flap raised on pedicle	NR: "PDE was performed to confirm perfusion."	NR
2014	Pestana et al[31]	P	SIEA, DIEP	24	SPY	10 mg	NR	Absolute flow value	Yes
2014	Nagata et al[46]	P	ALT, RF, and fibula	30	PDE	25 mg	1. After anastomosis	Grayscale: "Perfusion considered maintained when pinprick blood was fluorescent."	No
2013	Green et al[32]	R	LD, gracilis, vastus lateralis, ALT, fibula	55	SPY	7.5 mg	1. After flap harvest 2. After anastomosis	Absolute flow values: cutoff value of 6.0	Yes
2010	Komorowska-Timek and Gurtner[29]	P	Mastectomy skin, LD, DIEP, SIEA	24	SPY	10 mg	Alloplastic: 1. After mastectomy 2. After TE inset Autologous: 1. Before incision and harvest 2. After flap inset	Grayscale: "Areas where no perfusion was seen were marked and resected."	Yes
2009	Pestana et al[40]	P	Gracilis, ALT, RF, TRAM, SGAP, SIEA, DIEP, fibula, femoral corticoperiosteal	29	SPY	10 mg	1. After flap inset	Grayscale: "Areas of poorer perfusion by SPY were removed."	Yes
2009	Newman and Samson[43]	P	Mastectomy, DIEP, free TRAM	10	SPY	10 mg	1. After flap harvest 2. After anastomosis 3. After flap inset	Grayscale: "The area of questionable perfusion was debrided."	Yes
2008	Prantl et al[41]	P	Parascapular	10	IC-View	0.5 mg/kg	NR	Maximum fluorescence intensity and relative perfusion	No
2008	Holm et al[47]	P	SIEA	25	IC-View	0.5 mg/kg	1. With and without clamping deep epigastric system	Relative perfusion	No
2007	Holm et al[48]	P	SIEA	10	IC-View	0.5 mg/kg	1. After having raised the flap completely on a unilateral superficial system	Relative perfusion	NR
2006	Holm et al[49]	P	DIEP	15	IC-View	0.5 mg/kg	1. Immediately after pedicle dissection	Relative perfusion	No
2004	Mothes et al[39]	P	Various free flaps (NR)	11	IC-View	0.5 mg/kg	1. After flap harvest 2. After anastomosis	Relative perfusion	Yes

Table I. (continued)

Year	Author	P/R	Flap Perfusion	No.	System	Dose	Timing	Perfusion Assessment	Excision
Pedicled flaps									
2018	Alstrup et al[39]	R	LD, ms-LD, TRAM	77	SPY	7.5 mg	I. Perfusion	Relative perfusion: cutoff <33%	Yes
2016	Kuriyama et al[34]	P	Divided LD	11	HEMS	0.1 mg/kg	I. After clamping troublesome intercostal perforators (ICP)	NR	No
2016	Yano et al[36]	R	Pericranial	22	PDE	0.1 mg/kg	I. After flap harvest	Grayscale	No
2015	Surowitz and Most[40]	P	Paramedian forehead	10	SPY	NR	I. Before and after the initial flap transfer 2. Before pedicle division with clamp 3. Directly after pedicle division	Relative perfusion	No
2013	Piwkowski et al[37]	P	IMF	37	PDE	2.5 mg	I. 30-40 minutes after flap harvest 2. After suturing of flap	Grayscale: "Poorly fluorescing regarded as inadequately perfused."	Yes
2004	Yamaguchi et al[35]	P	Unipedicled TRAM flap	10	IC-View	0.5 mg/kg	I. Immediately after harvest of anastomosis	"Subjective measurement of fluorescence intensity."	Yes
Various free and pedicled flaps									
2015	Green et al[38]	R	Pedicled and free flaps (NR)	136	SPY	7.5 mg	NR	Absolute flow value cutoff 6.0 and relative perfusion cutoff ≤25%	Yes
2013	Wu et al[24]	R	Mastectomy skin, fasciocutaneous, myocutaneous	43	SPY	8.75-12.5 mg	I. Mastectomy skin 20 min after mastectomy, NR for other flaps	Perfusion percentage: cutoff ≤25%	Yes
2012	Losken et al[26]	P	DIEP, TRAM, and free ms-TRAM	77	SPY	5 mg	I. After flap harvest	Absolute flow values	No
2002	Holm et al[47]	P	TRAM, LD, RF, temporalis fascia, serratus anterior muscle, parascapular and serratus anterior	20	IC-View	0.5 mg/kg	I. After flap inset	Mean signal intensity and relative perfusion with cutoff 60%	No
2002	Holm et al[48]	P	Pedicled groin, sural island, vertical rectus abdominis, reversed forearm, forehead and random-pattern skin	15	IC-View	0.5 mg/kg	I. After suturing of the flap	Mean signal intensity and relative perfusion	No
1999	Still et al[33]	P	NR	21	NR	0.1 mg/kg	I. After anastomosis and temporary suturing	Grayscale	No

Abbreviations: TE, tissue expander; NAC, nipple-areolar complex; NR, not reported; PDE, Photodynamic Eye; ALT, anterolateral thigh; DIEP, deep inferior epigastric perforator; ms-TRAM, muscle-sparing transverse rectus abdominis musculocutaneous; SCIP, superficial circumflex iliac artery perforator; TD, thoracodorsal; TFL, tensor fascia lata; SIEA, superficial inferior epigastric artery; RF, radial forearm; SGAP, superior gluteal artery perforator; ms-LD, muscle-sparing latissimus dorsi; HEMS, Hyper Eye Medical System; IMF, intercostal muscle flap.

[a]Ingress rate: the rate of fluorescence intensity increase from baseline to peak intensity over time (eg, arterial inflow). Egress rate: the rate of intensity decrease from peak fluorescence intensity to the end of the study (eg, venous outflow).[27,31]

Flap perfusion: clinical applications.

Indocyanine green angiography has been reported for several types of flaps in plastic and reconstructive flap surgery in a clinical setting. The majority of included articles used ICGA to assess tissue perfusion of mastectomy skin flap (n=14) intraoperatively;[25,26,31-33,39-41,47,51,55,57,58,61] the remainder focused on the intraoperative assessment of nipple-areolar complex perfusion,[27,72] a combination of nipple-areolar complex and mastectomy skin flap perfusion,[24] anterolateral thigh flap,[28,29] deep inferior epigastric perforator flap,[38,56,66] transverse rectus abdominis myocutaneous flap,[38,56,70] superficial inferior epigastric artery flap,[64,65,73] superficial circumflex iliac artery flap,[50] free pericranial flap,[36] latissimus dorsi flap,[34] free parascapular flaps,[63] paramedian forehead flap,[43] intercostal muscle flap,[52] osseus free flaps,[44] free fibula flap,[45] and various pedicled and/or free flaps.[30,35,37,42,46,48,53,54,59,60,62,67,69,71] Two studies described preoperative perforator selection in addition to perfusion assessment.[50,73] See Table 1.

Imaging Systems.

Several ICGA systems have been described in the literature. In the majority of included studies (n=29),[24,26-28,30-32,38-41,43-47,51,53-62,72,73] the SPY imaging system (Novadaq Technologies, Inc, Toronto, Canada) was used for tissue perfusion assessment; the remainder used the IC-View System (Pulsion Medical Systems, Munich, Germany; n=8),[63-70] the Photo Dynamic Eye (Hamamatsu Photonics KK, Hamamatsu, Japan; n=7),[29,33,35,36,48,50,52] the Visionsense 3D high-definition near-infrared-guided indocyanine green video angiography system (ICG-NIR-VA, Orangeburg, NY; n=1),[42] Hyper Eye Medical System (Mizuho Medical Co, Ltd, Tokyo, Japan; n=1),[34] the Fluobeam device (Fluoptics, Grenoble, France; n=1),[37] and Quest spectrum TM (Quest Medical Imaging, Akron, OH; n=1).[25] One study did not state what kind of device was used.[71]

Dye and Dosing.

In almost every study (n=48), conventional ICG was used as fluorescent dye. In only one of the included articles, monopeak infracyanine green (Infracyanine, SERB Laboratory, Paris, France) was used.[37] The latter concerns an iodine-free preparation. The majority (n=37) of included articles reported a clear dosing regimen for ICGA, ranging from 2.5 to 25 mg or 0.025 to 0.5 mg/kg when a fixed dose (n=21)[27,30,33,38,39,44-48,50,52-54,56,58-60,62,72,73] or weight-adjusted dose (n=16)[25,28,34-37,42,63-69,71,74] was applied, respectively. Nine studies did not report any ICG dosing.[24,26,29,32,40,41,43,51,61] Three reports described a dose of 3 and 5 cc but did not report the concentration of ICG[55,57,75] (see Table 1). Ten milligrams and 0.5 mg/kg were the most frequently used fixed dose and weight-adjusted dose, respectively (see Figure 3). In addition, 9 articles report a flush of saline following ICG injection.[25,27,35,47,55,56,59,72,75]

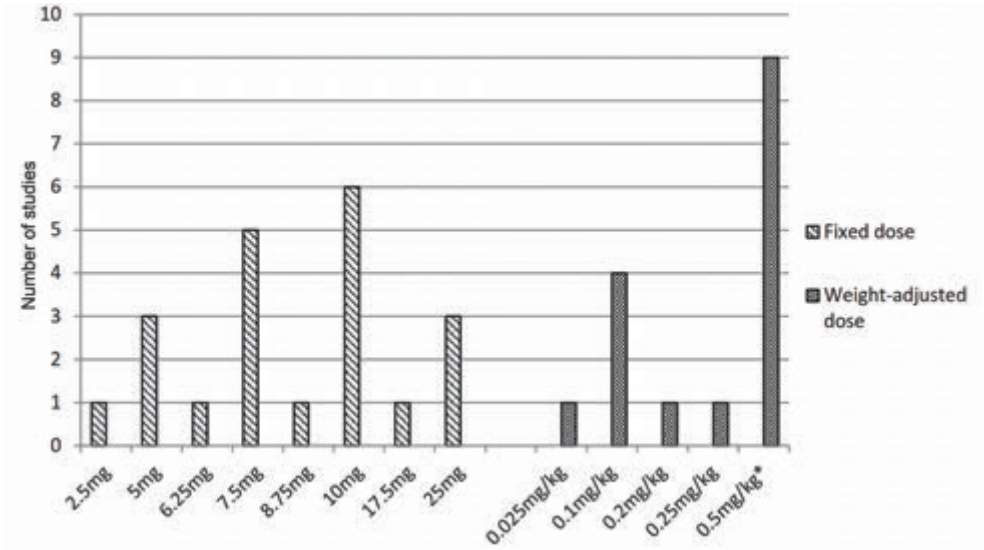

Figure 3. Frequencies of studies (N=37), reporting difference doses of indocyanine green in plastic and reconstructive flap surgery. Holm et al[64-68] reported 5 studies with 0.5 mg/kg dose.

Working Distance.

The working distance, defined as the distance between the imaging head of the ICGA system and the area of interest (i.e., skin of the flap), is reported in only 11 articles. Working distances of 20 cm,[25,37,39,57] 30 cm,[33,42,60] 30 to 100 cm,[63,69] and 20 to 40 cm[48] have been reported. Valerio et al. report a 2-dot laser-guided marker in the SPY system, which aids in identifying the optimal distance from soft tissue.[44]

Time to and Duration of Assessment.

The time to assessment, defined as the elapsed time from dye administration to perfusion assessment, is reported in 21 of the included studies. Most of the studies describe recording directly following ICG administration (n=11),[27,28,30,36,39,44,47,48,53,63,71] while other studies start recording 15 seconds (n=3)[29,49,59] or 60 seconds (n=2) after intravenous administration, respectively.[52,56] Five studies report to start recording when first fluorescence change is detected in the flap.[31,33,37,41,60] Total duration of ICGA from start of recording to the end is reported in 14 studies and is predominantly ranging between 60 and 120 seconds.[27,29,30,33,39,41,47,50,54,62] Assessments of up to 200[31,58] and 300 seconds[36,71] have been reported as well.

Intraoperative Timing of ICGA.

Timing, defined as the moment of perfusion assessment during operation, varies with the flap type to be assessed. In the included articles, no major difference exists in timing of assessment. For example, mastectomy skin flap perfusion is mainly assessed after

mastectomy, prior to and after inset of an implant.[26] Free flaps are predominantly assessed after flap harvest when the flap is raised on its pedicle and/or after transplantation of the flap to the recipient site.[44] See Table 1.

Resection of Tissue and Perfusion Assessment.

A total of 27 studies used ICGA imaging to guide resection of insufficiently perfused tissue (see Table 1).[26-30,32,33,35,38-40,42,44-46,51-55,57,59,60,62,69,70,73] The most applied methods to assess tissue perfusion were

1. Relative perfusion assessment (i.e., "percentage of perfusion");
2. Assessment of fluorescence intensity with greyscale imaging;
3. Absolute flow value assessment, based on greyscale imaging.

In 18 studies, quantitative software was used to calculate relative perfusion of the region of interest, compared with normal tissue quantified as a reference with 100% perfusion.[24,26,28,30,38,42,43,45,54,57,64-69] Perfusion assessment using absolute flow value is reported in 9 studies[41,44,46,47,53,56,61,63,73] and a combination of the aforementioned in 3 studies.[41,46,63] In 9 of these studies, cut-off values ranging from 25 to 60% to excise flap tissue have been reported for relative perfusion.[26,28,30,38,42,45,46,54,67] For absolute flow value assessment, in which a point value from 0 to 255 is based on a greyscale that corresponds to the signal intensity, higher values equate to superior perfusion. In the remaining 3 studies, a value of 6.0 was reported as the lower limit of acceptable perfusion[44,46,53] (see Figure 4).

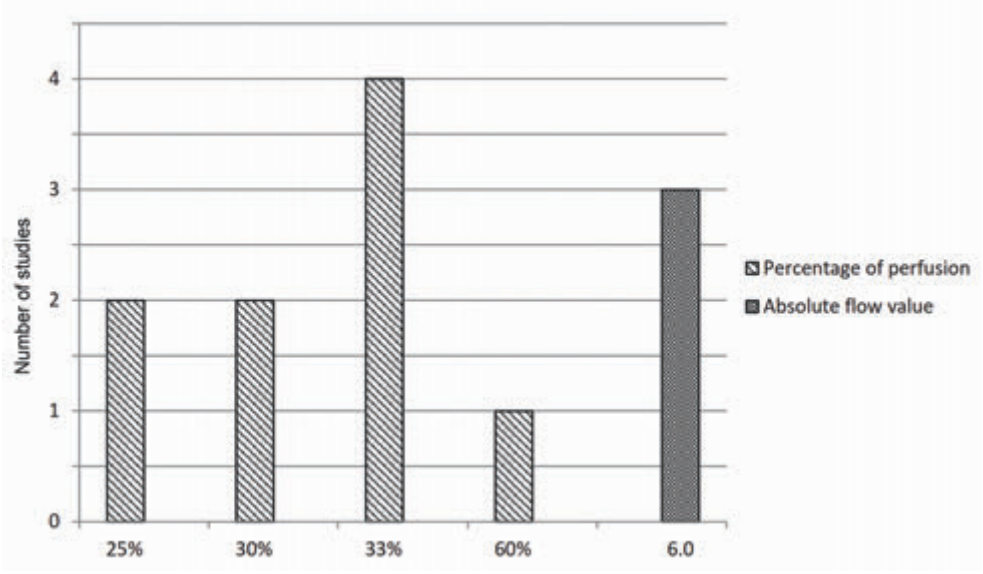

Figure 4. Frequencies of studies (N=12) reporting a cut-off value for tissue excision using indocyanine green angiography to measure tissue perfusion as percentage of perfusion (scale 0 to 100%) or as absolute flow value (scale 0 to 255 based on a greyscale).

For example, Green et al. report that areas of poor flap perfusion with absolute flow value under 6.0, as objectively assessed with SPY Q analysis software, were excised before definitive inset of the flap.[53] Perfusion assessment according to greyscale imaging was performed in 12 studies.[27,29,31,33,36,48,52,58-60,62,71] For example, Gorai et al. marked the nonenhanced areas according to the greyscale image on the monitor.[33]

Factors of Influence During ICGA.

Only 12 of the included studies report factors that might influence the assessment of tissue perfusion, including ambient light[31,35,39,42,47,70] the use of epinephrine containing injections,[26,31-33,47,54,58] the use of papaverine or other vasodilating agents,[66] systolic blood pressure,[30] stretch level of the mastectomy skin flap,[39] use of absorbent compress surrounding or underneath the flap in order to reduce artifacts,[28,42,52] use of the electric knife during assessment,[70] and the angle of the imaging head to the region of interest.[70]

Of these studies, only 4 explicitly report that all operating room lights were turned off during the recording to avoid interference of ambient light with the detection of fluorescence.[35,39,47,70] One study reported that ICGA was always performed under room light conditions.[42] None of these studies report the influence of ambient light on assessment.

Diep et al. found that more patients developed severe flap necrosis when they received tumescence-containing epinephrine during their mastectomy.[32] Due to the difficulty in interpreting ICGA, the authors discontinued the use of tumescent solution. Munabi et al. observed false-positive results in flap assessment due to the use of the tumescent technique, rendering ICGA less reliable to predict necrosis.[47] Hammer-Hansen et al. report that no local anaesthetics with adrenalin were used at any time during surgery in order to not impair visualization of the mastectomy flap perfusion when performing ICGA.[26] The other studies considering epinephrine as an influencing factor only describe that no epinephrine was injected into the surgical site.[33,54,58,75]

Alstrup et al. performed ICGA measurements with a mean systolic blood pressure above 100 mm Hg and during the assessment, a Doppler confirmed flow through the pedicle.[30] Yamaguchi et al. report that systolic and diastolic blood pressure is registered at the beginning of the analysis, without describing the purpose.[70]

Rinker placed laparotomy sponges in the breast pocket after completion of the mastectomy, prior to ICGA, to fill the dead space and to allow the skin flaps to lie flat without areas of redundancy, but also without stretch.[39] Two reports described that flaps were surrounded with clean surgical towels during ICGA assessment to avoid background signal

noise from the other vascular tissues.[28,52] Another group used an absorbent compress to fill the dead space underneath the flap in order to reduce artifacts.[42]

Yamaguchi et al. reported that the electric knife had to be switched off to prevent artifacts in ICGA imaging, a commonly seen interference by electromagnetic interference. The authors also suggest that ICGA recording should be performed before flap reshaping, since the video camera must be perpendicular to the flap surface.[70]

Ex Vivo Experiments

The results of the ex vivo experiments are depicted in Figures 5 and 6.

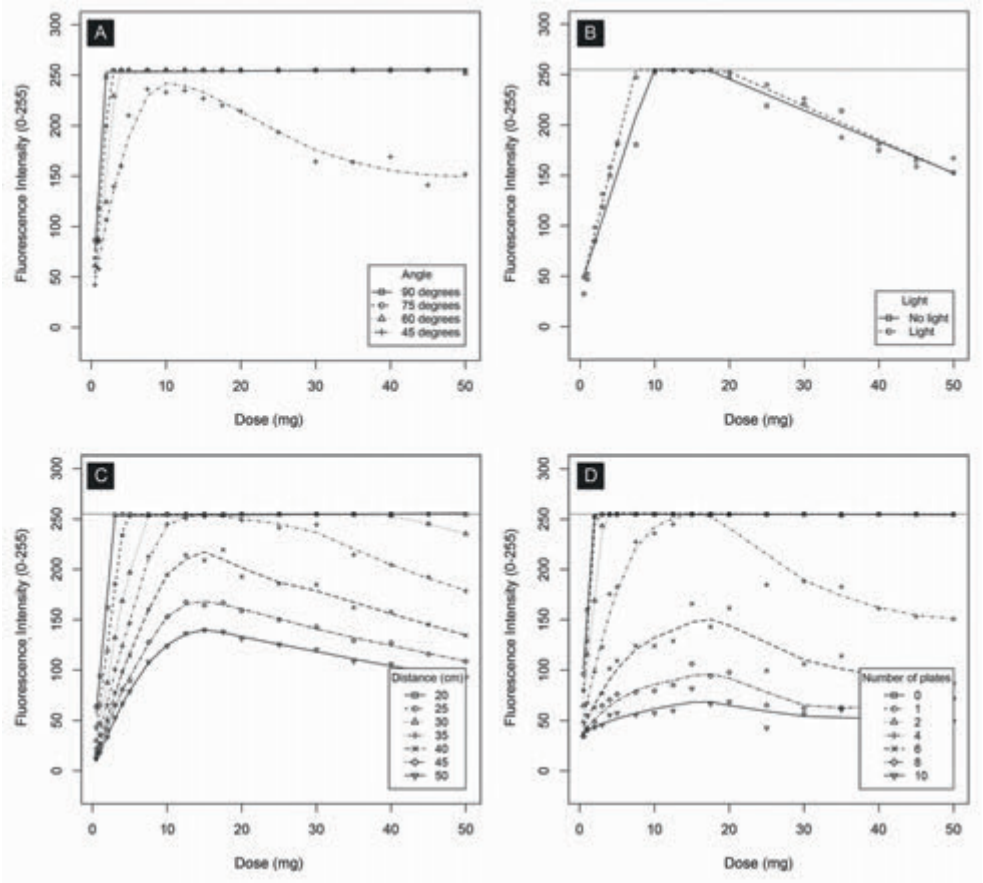

Figure 5. Results of ex vivo experiments. The graphs show fluorescence intensity for a range of doses. The different curves on each graph show differences between (A) various angles, (B) with and without ambient daylight at a distance of 30 cm, (C) various distances (in cm), (D) various penetration depths (in number of beeswax plates). Fluorescence intensity is measured on a greyscale from 0 to 255. Zero is black, 255 is white, and values in between make up different shades of grey.

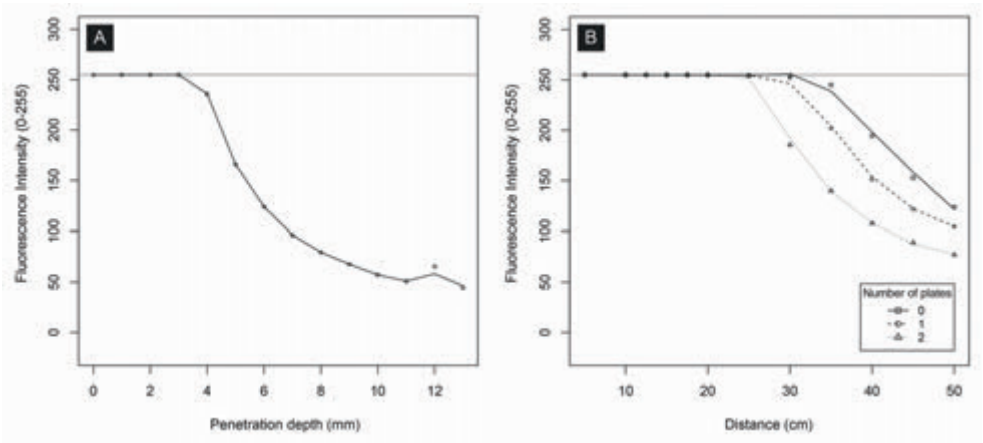

Figure 6. Results of ex vivo experiments. The graphs show fluorescence intensity for a range of (A) penetration depths (in mm) and (B) distances with various penetration depths (in number of beeswax plates). For both experiments, the indocyanine green dose was set to 10 mg. Fluorescence intensity is measured on a greyscale from 0 to 255. Zero is black, 255 is white, and values in between make up different shades of grey.

These figures illustrate that fluorescence intensity is associated with each of the factors that were independently varied in the experiment, including positioning of the imaging head in various degrees (i.e., 90°, 75°, 60°, and 45°), various distances, with or without ambient daylight and with stacking beeswax plate to mimic "penetration depth".

The curves in Figure 5A show that positioning the imaging head in an angle of 60° to 90° does not influence fluorescence intensity to any meaningful extent, exemplified by the fact they overlap almost completely. However, the fluorescence intensity for 45° does differ substantially from the other angles: the intensity is lower than any other angle over the whole range of doses. This was also substantiated by large negative regression coefficient for 45°. Compared with a 90° angle, the average difference for 75° was estimated to be −5.76 (95% confidence interval [CI]: −21.04 to 9.56; P=.460), for 60° −11.93 (95% CI: −27.21 to 3.36; P=.126), but for 45° −67.59 (95% CI: −82.88 to −52.31; P < .001).

The curves in Figure 5B show that fluorescence intensity is slightly higher in ambient light and suggest optimum fluorescence intensity reached with an ICG dose between the range of 10 and 20 mg, as the intensity is highest within this range. Figure 5C and D shows that fluorescence intensity decreases when distance increases or when penetration depth (i.e., number of beeswax plates) increases, respectively. Both figures also suggest optimum fluorescence intensity reached with an ICG dose between the range of 10 and 20 mg as all curves are at their optimum in this range. When measured from a distance of 25 cm, maximum fluorescence intensity is reached with an ICG dose of approximately 5 mg or more.

Figure 6A illustrates fluorescence intensity for different penetration depths (in mm) and Figure 6B for different distances (in cm) stratified by various penetration depths (i.e., number of beeswax plates). Both are measured with a constant dose of 10 mg of ICG. It is clear from these graphs that there is a negative association between number of plates or distance, and intensity.

Table 2 shows the regression coefficients of the curvilinear associations between dose, distance, and penetration depth, and fluorescence intensity. All 3 associations could best be described using third degree polynomials, demonstrated by the highly significant coefficients. These strong curvilinear associations are in agreement with the figures presented above. Regression coefficient for ambient light was estimated 10.17 (95% CI: 5.23 to 15.10; $P < .001$).

Table 2. Coefficients Curvilinear Associations Between Covariates and Fluorescence Intensity.

	Regression Coefficient (95% Confidence Interval)	*P*-value
Dose (mg/mL)	16.11 (15.12 to 17.11)	*<.001*
Dose (mg/mL)^2	−0.65 (−0.70 to −0.60)	*<.001*
Dose (mg/mL)^3	0.01 (0.01 to 0.01)	*<.001*
Distance (cm)	5.49 (3.55 to 7.43)	*<.001*
Distance (cm)^2	−0.35 (−0.43 to −0.27)	*<.001*
Distance (cm)^3	0.004 (0.003 to 0.005)	*<.001*
Beeswax plates (number)	−15.50 (−19.23 to −11.78)	*<.001*
Beeswax plates (number)^2	−1.82 (−2.55 to −1.09)	*<.001*
Beeswax plates (number)^3	0.13 (0.09 to 0.17)	*<.001*

DISCUSSION

The aim of this study was to provide comprehensive insight in potential factors influencing the fluorescence intensity when using ICGA to assess tissue perfusion during reconstructive flap surgery. To the authors' knowledge, the current study includes the first systematic review specifically regarding the use of ICGA protocols for evaluating tissue perfusion in reconstructive flap surgery and authors' consideration of factors that might influence fluorescence intensity during ICGA assessment. Previous review articles mainly focused on the application and the effect of ICGA in flap surgery. For example, Smit et al. recently published a systematic review and meta-analysis on intraoperative evaluation of perfusion in free flap surgery and concluded that ICGA is one of the most suitable methods to measure free flap tissue perfusion, resulting in improved flap survival.[5] Li et al. recently published a review regarding the application of ICG in flap surgery and concluded that ICGA aids in the evaluation of flap microcirculation and perfusion.[9]

In part 1 of the current study, factors that are considered important in ICGA protocols, as well as factors that might influence fluorescence intensity and are therefore considered to be important by the authors, have been reviewed based on a systematic literature search. Based on the results, it can be concluded that most ICGA protocols are insufficiently described, when concerning factors that might influence the outcome of NIRF imaging. When reported, there is no consensus on dosage of ICG, working distance, time to assessment, tissue resection and perfusion assessment, and time to and duration of assessment. Furthermore, only a few articles describe the actual consideration of potential factors of influence during ICGA.

Part 2 of this study comprises ex vivo experiments with a handheld ICGA system to identify and analyse factors that influence the fluorescence intensity. The methods for these ex vivo experiments have been previously reported using a laparoscopic NIRF imaging device with special emphasis on cholangiography.[19] In this study, these experiments were reproduced using a handheld imaging device with special emphasis on angiography; additionally, statistical analyses have been performed. Associations between dosage of ICG, working distance, angle, penetration depth, and ambient light, and fluorescence intensity have been quantified.

When concerning the dosage of ICG, fixed dose of ICG predominantly administered. Previous study by Li et al. demonstrated that there is no consensus regarding the optimal intravenous dose in flap surgery and that different groups use their own experiences to determine the dosage.[9] Furthermore, it was reported that there is no evidence showing that multiple intravenous dosages will affect the result of the quality of ICGA. Although their review included animal studies and small series, the authors agree that there is no consensus on ICGA dosage in flap surgery. Nonetheless, the ex vivo experiments suggest an optimal ICG concentration of 0.002 mg/mL to 0.004 mg/mL and demonstrate that ICG dose significantly influences fluorescence intensity. Since patients with a higher body weight have a larger blood volume, the concentration of ICG can differ between patients. Since the estimated dose is based on ex vivo experiments, which is not comparable to in vivo conditions with unique circulating plasma volumes and cardiac outputs,[9] a recommendation regarding the optimal dose cannot be given. However, consistent with van den Bos et al., the authors conclude that applying a weight-adjusted dose seems preferable over a fixed dose.[19]

With regard to working distance and ambient light, a minority of studies reported the importance of this factor. Moyer et al. previously described that fluorescence intensity, emitted by ICG, can be dependent on the distance from the camera to the skin and ambient

light in the room without reporting the relation between these factors.[57] The performed ex vivo experiments confirm that fluorescence intensity depends on distance and ambient light in the room. Analysis revealed that a higher distance significantly reduces fluorescence intensity. Since manufacturers recommend specific working distance for each available imaging device and fluorescence intensity is dependent on distance, the authors advocate not to deviate from this recommendation and to report working distances in studies regarding flap perfusion assessment.

When concerning ambient light, the observed fluorescence intensity was significantly higher when ICGA was performed in light, compared with total darkness. However, in these experiments, the surrounding objects outside the region of interest were also observed better (subjectively). Target-to-background ratios as previously reported by Schols et al. were not determined to assess differences.[76,77] However, subjective distinction between the region of interest (i.e., wells plate) and surroundings did not differ in darkness or light. Yet, the assessment is preferred to be performed in the dark when possible, since there is no in vivo evidence on the exact influence of light.

Time to and duration of assessment is only reported in a few studies as being an important factor. According to the authors' clinical experience, assessing the flap perfusion in the first minute after ICG administration is the most important period of time to assess tissue perfusion adequately.

Regarding tissue resection and perfusion assessment, the assessment of absolute value together with assessment of greyscale imaging were more frequently used than relative perfusion assessment. These 2 perfusion assessment techniques are a direct measure of the fluorescence intensity, as well as "absolute value assessments". With the results of the ex vivo experiments, the authors conclude that these measurements are influenced by dose, working distance, angle, and ambient light. Presumably, when using the relative perfusion assessment, the effects of these factors can be diminished since percentages of perfusion are compared with reference tissue during the same perfusion assessment.[57] Therefore, conclusions can be drawn that using "relative perfusion assessment" seems preferable over the other assessment methods. Furthermore, there is no consensus on cut-off values for tissue debridement in flap surgery when absolute value assessment or relative perfusion assessment is applied. Also, there is no consensus on debridement of tissue when applying greyscale imaging. For example, La Padula et al. decided to respect the representing 0 fluorescence (i.e., black area) and preserved "the hypovascularized grey area",[29] whereas Pestana et al. reported to remove all areas of "poorer perfusion".[62] Further prospective trials are warranted to determine reliable cut-off values.

When considering other factors of influence during ICGA, these factors are only described briefly in the literature. Epinephrine is the only factor that is demonstrated to negatively influence assessment of tissue perfusion when using ICGA.[47] The described ex vivo experiments have refuted the importance of the imaging head positioned perpendicular to the skin, as suggested by Yamaguchi et al.[70] The imaging head can be positioned in an angle of 60° to 90° without influencing the observed fluorescence intensity to any meaningful extent.

Furthermore, penetration depth was analysed in ex vivo experiments. The ex vivo experiments confirm that fluorescence intensity is significantly reduced when penetration depth increases. Reported penetration depth ranges from 3 mm to 1 cm.[39,57,58] In the experiments, optimum fluorescence intensity was observed up to 4 mm of depth and up to approximately 8 mm of depth was observed subjectively to distinct fluorescence intensity from the surroundings. However, this experiment is limited by beeswax plates that were used to measure penetration depth. Although the spectral scattering properties are similar to human tissue, the spectral absorption differs, so it is to be expected that the penetration depth in human tissue is different. Another possible limitation is revealed by the observation of lower fluorescence intensity with higher doses of ICG. This phenomenon was also observed by van den Bos et al.[19] Since the concentrations of ICG were diluted within 40 mg/mL of albumin in 0.9% NaCl dilution and lower concentrations were obtained by adding 40 mg/mL solution, it is possible that the absolute quantity of albumin is higher in lower concentrations of ICG. Therefore, an optimum dose cannot be given based on these ex vivo experiments.

In addition to ICGA, other imaging techniques with the ability to assess intraoperative perfusion in free flap surgery have been described in a recent systematic review.[5] These methods include the use of laser Doppler, oxygen saturation (SO_2) measurements, ultrasound, dynamic infrared thermography, venous pressure measurement, and micro dialysis. Of these methods, ICGA and laser Doppler have currently been the most objective and reliable methods to directly assess tissue perfusion, leading to improvement of flap survival.[5] Furthermore, a new imaging technique titled hyperspectral imaging has already shown promising results for physiologic tissue parameters.[78] The technique can be used in precision surgery and is already applied to guide flap reconstruction.[79] Preliminary results show a high capability for a camera to be used in perfusion measurements.[80] Hyperspectral imaging is a non-invasive technique with no risk of adverse events. One of the previously mentioned imaging technologies may ultimately replace ICGA for flap assessment, since ICGA is an invasive procedure. However, at this time ICGA is one of the most suitable methods to directly assess tissue perfusion in free flap reconstructive surgery.[5]

With regard to adverse events, Li et al. described potential adverse reactions to ICG, preoperative allergy testing, and contraindications of ICG in their systematic review.[9] The authors found a lack of reported preoperative ICG allergy tests and concluded that this may be due to the acceptance that ICG has a very low rate of allergy (1 out of 42 000 to 60 000) and does not damage blood composition and the coagulation system.[9,11] Since 2 cases of fatal ICG anaphylaxis have been previously reported, Li et al. consider preoperative iodine allergy testing a necessary precaution as iodine allergies are the most probable source of an adverse reaction to ICG. In addition to hypersensitivity to iodine, several contraindications for applying ICG are mentioned, including closed-angle glaucoma, allergic asthma, severe hypertension, hepatic and renal function failure, and pregnancy.[9]

In vivo studies would have been preferable over ex vivo experiments, including the effect on human tissue. On the other hand, measurements of ICG are confounded by in vivo fluorescence quenching, which makes it difficult to predict the precise working dose of ICG needed in the flap.[9] In addition, a half-time life of 3 to 5 minutes hinders to assess different factors (eg, distance) in a limited time frame. Nevertheless, the current ex vivo setup offered a simple and objective method to assess factors of influence when using ICGA.

In future studies, the optimal ICG dose should be standardised through large series and clinical trials. In addition, authors should consider reporting ICGA protocols comprehensively to provide reproducibility and enable comparison of used methods between studies. Fortunately, the American Association of Physicist in Medicine recently established a Task Group working toward consensus around guidelines and standards for advancing the field of fluorescence-guided surgery, by inventorying the key parameters, stakeholders, impacts, and outcomes of clinical fluorescence-guided surgery technology and its applications, to come to objective benchmarking and standardisation within this field, and is expected to stimulate innovation.[81]

CONCLUSION

In conclusion, this study identified factors that significantly influence the fluorescence intensity of ICGA, including dose, working distance, angle, penetration depth, and ambient light. Consequently, conclusions can be drawn that applying a weight adjusted ICG dose seems preferable over a fixed dose when using ICGA for tissue perfusion assessment during reconstructive flap surgery. In addition, “relative perfusion assessment” seems preferable over other assessment methods. It is advocated to use recommended working distances. Furthermore, the imaging head during ICGA can be positioned in an angle of 60° to 90° without significantly influencing the observed fluorescence intensity. All of these factors

should be considered when using ICGA for tissue perfusion assessment during reconstructive flap surgery. To work toward consensus and construct uniform guidelines, more transparency in methods in future studies is advocated.

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SUPPLEMENTARY MATERIAL

Supplementary material 1. Search syntax applied to each database

1.1 Database 1: National Library of Medicine (PubMed) database search (16.07.2018)

#	Query	Results
#11	#10 NOT ("Animals"[Mesh]) NOT "Humans"[Mesh]	227
#10	#8 AND #9	298
#9	#1 AND #2 AND #3	380
#8	#6 OR #7	752293
#7	#4 AND #5	79226
#6	(((((((("Perfusion Imaging"[Mesh]) OR "Perfusion"[Mesh]) OR perfusion[Title/Abstract]) OR "blood supply" [Subheading]) OR blood supply*[Title/Abstract]) OR Vasculari*[Title/Abstract]) OR viability[Title/Abstract]) OR vitality[Title/Abstract]	678017
#5	(((((("Monitoring, Physiologic"[Mesh]) OR monitor*[Title/Abstract]) OR measure*[Title/Abstract]) OR assess*[Title/Abstract]) OR assist*[Title/Abstract]) OR evaluat*[Title/Abstract]	7245394
#4	(("Intraoperative Period"[Mesh]) OR intraoperative*[Title/Abstract]) OR intra-operative*[Title/Abstract]	140367
#3	(((((("Angiography"[Mesh:NoExp]) OR Angiograph*[Title/Abstract]) OR Imaging[Title/Abstract]) OR Image[Title/Abstract]) OR Video[Title/Abstract]) OR Videoangiography[Title/Abstract]	1097590
#2	((((((((((("Indocyanine Green"[Mesh]) OR Indocyanine green[Title/Abstract]) OR Indocyanin green[Title/Abstract]) OR ICG[Title/Abstract]) OR Infracyanine green[Title/Abstract]) OR IFC green[Title/Abstract]) OR "Fluorescence"[Mesh]) OR "Fluorescein Angiography"[Mesh]) OR "Fluorescent Dyes"[Mesh]) OR Fluoresce*[Title/Abstract]) OR near infrared[Title/Abstract]) OR NIRF[Title/Abstract]) OR NIR[Title/Abstract])	529023
#1	((((("Surgical Flaps"[Mesh]) OR "Myocutaneous Flap"[Mesh]) OR "Perforator Flap"[Mesh]) OR "Free Tissue Flaps"[Mesh]) OR Flap*[Title/Abstract]	88152

#11
((((((((((("Surgical Flaps"[Mesh]) OR "Myocutaneous Flap"[Mesh]) OR "Perforator Flap"[Mesh]) OR "Free Tissue Flaps"[Mesh]) OR Flap*[Title/Abstract]))) AND ((((((((((("Indocyanine Green"[Mesh]) OR Indocyanine green[Title/Abstract]) OR ICG[Title/Abstract]) OR Infracyanine green[Title/Abstract]) OR IFC green[Title/Abstract]) OR "Fluorescence"[Mesh]) OR "Fluorescein Angiography"[Mesh]) OR "Fluorescent Dyes"[Mesh]) OR Fluoresce*[Title/Abstract]) OR near infrared[Title/Abstract]) OR NIRF[Title/Abstract]) OR NIR[Title/Abstract]))) AND ((((((("Angiography"[Mesh:NoExp]) OR Angiograph*[Title/Abstract]) OR Imaging[Title/Abstract]) OR Image[Title/Abstract]) OR Video[Title/Abstract]) OR Videoangiography[Title/Abstract]))) AND ((((((((((("Perfusion Imaging"[Mesh]) OR "Perfusion"[Mesh]) OR perfusion[Title/Abstract]) OR "blood supply" [Subheading]) OR blood supply*[Title/Abstract]) OR Vasculari*[Title/Abstract]) OR viability[Title/Abstract]) OR vitality[Title/Abstract]))) OR (((((("Intraoperative Period"[Mesh]) OR intraoperative*[Title/Abstract]) OR intra-operative*[Title/Abstract])) AND ((((((("Monitoring, Physiologic"[Mesh]) OR monitor*[Title/Abstract]) OR measure*[Title/Abstract]) OR assess*[Title/Abstract]) OR assist*[Title/Abstract]) OR evaluat*[Title/Abstract])))))) NOT (("Animals"[Mesh]) NOT "Humans"[Mesh])

1.2 Database 2: EMBASE (via Ovid SP) search (16.07.2018)

#	Query	Results
#11	#9 NOT (exp animal/ not human/)	265
#10	#8 AND #9	341
#9	#1 AND #2 AND #3	549
#8	#6 OR #7	850316
#7	#4 AND #5	126534
#6	perfusion.mp. or tissue perfusion/ or perfusion/ or blood supply*.mp. or vascularization/ or vasculari*.mp or viability.mp. or vitality.mp	729600
#5	monitoring/ or physiologic monitoring/ or monitoring.mp. or monitor*.mp. or measure*.mp. or assess*.mp. or assist*.mp. or evaluation/ or evaluat*.mp.	10889196
#4	intraoperative monitoring/ or intraoperative period/ or intra-operative*.mp. or intraoperative*.mp.	193821
#3	angiography/ or angiograph*.mp. or imaging/ or imaging.mp. or image.mp. or video.mp. or videoangiography.mp.	2147080
#2	indocyanine green.mp. or exp indocyanine green/ or ICG.mp. or infracyanine green.mp. or IFC.mp. or fluorescent dye/ or fluorescence imaging/ or fluorescence/ or fluorescence imaging system/ or fluorescein/ or fluoresce*.mp. or near infrared.mp. or NIR.mp.or NIRF.mp	743086
#1	surgical flaps.mp. OR exp surgical flaps/ OR myocutaneous flaps.mp. or exp myocutaneous flap/ OR perforator flap.mp. or exp perforator flap/ OR free tissue flaps.mp. or exp free tissue graft/ OR flap*.mp.	98264

#11

(((surgical flaps.mp. OR exp surgical flaps/ OR myocutaneous flaps.mp. or exp myocutaneous flap/ OR perforator flap.mp. or exp perforator flap/ OR free tissue flaps.mp. or exp free tissue graft/ OR flap*.mp.) AND (indocyanine green.mp. or exp indocyanine green/ or ICG.mp. or Infracyanine green.mp. or IFC.mp. or fluorescent dye/ or fluorescence imaging/ or fluorescence/ or fluorescence imaging system/ or fluorescein/ or fluoresce*.mp. or near infrared.mp. or NIR.mp.or NIRF.mp) AND (angiography/ or angiograph*.mp. or imaging/ or imaging.mp. or image.mp. or video.mp. or videoangiography.mp.)) AND (((intraoperative monitoring/ or intraoperative period/ or intra-operative*.mp. or intraoperative*.mp.) AND (monitoring/ or physiologic monitoring/ or monitoring.mp. or monitor*.mp. or measure*.mp. or assess*.mp. or assist*.mp. or evaluation/ or evaluat*.mp.)) OR (perfusion.mp. or tissue perfusion/ or perfusion/ or blood supply*.mp. or vascularization/ or vasculari*.mp or viability.mp. or vitality.mp))) not (exp animal/ not human/)

1.2 Database 3: Cochrane Library CENTRAL search (16.07.2018)

#	Query	Results
#10	#8 AND #9	9
#9	#1 AND #2 AND #3	11
#8	#6 OR #7	29379
#7	#4 AND #5	14683
#6	(perfusion or blood supply* or vasculari* or viability or vitality):ti,ab,kw	15159
#5	(monitor* or measur* or assess* or assist* or evaluat*):ti,ab,kw	744643
#4	(intraoperative* or intra-operative*):ti,ab,kw	19966
#3	(angiograp* or imag* or video or videoangiography):ti,ab,kw	71487
#2	(“indocyanine green” or ICG or “infracyanine green” or IFC or fluoresc* of “near infrared” or NIR or NIRF) :ti,ab,kw	949
#1	flap*:ti,ab,kw	3422

#10
((flap*:ti,ab,kw AND (“indocyanine green” or ICG or “infracyanine green” or IFC or fluoresc* of “near infrared” or NIR or NIRF) :ti,ab,kw AND (angiograp* or imag* or video or videoangiography):ti,ab,kw) AND (((intraoperative* or intra-operative*):ti,ab,kw AND (monitor* or measur* or assess* or assist* or evaluat*):ti,ab,kw) OR (perfusion or blood supply* or vasculari* or viability or vitality):ti,ab,kw))

Supplementary material 2. PRISMA 2019 checklist

Section/topic	#	Checklist item	Reported on page
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Title, p.1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Abstract, p.3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Introduction pp.4-5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Introduction, p.5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	No review protocol exists
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Methods, p.6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Methods, pp.5-6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Supplement 1-3
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Methods, p.6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Methods, p.6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Methods, p.6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	/
Summary measures	13	State the principal summary medaqdaasures (e.g., risk ratio, difference in means).	Methods, p.6 and Table 1

Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	/
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	/
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	/
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Results, p.8 and Figure 2
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	/
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	/
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	/
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	/
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	/
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Discussion, pp.14-15
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Discussion, pp.16-17
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Discussion and conclusion, p. 17
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Sources of funding, p.1

CHAPTER 3

Influence of intraoperative vasopressor use on indocyanine green angiography: first evaluation in an experimental model

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ABSTRACT

Background

Intraoperative indocyanine green (ICG) fluorescence angiography has gained popularity and acceptance in many surgical fields for the real-time assessment of tissue perfusion. Although vasopressors have the potential to preclude an accurate assessment of tissue perfusion, there is a lack of literature with regards to its effect on ICG fluorescence angiography.

Methods

An experimental porcine model was used to expose the small bowel for quantitative tissue perfusion assessment. Three increasing doses of norepinephrine infusion (0.1, 0.5, and 1.0 µg/kg/min) were administered intravenously over a 25-min interval. Time-to-peak fluorescence intensity (TTP) was the primary outcome. Secondary outcomes included absolute fluorescence intensity and local capillary lactate (LCL) levels.

Results

Five large pigs (mean weight: 40.3 ± 4.24 kg) were included. There was no significant difference in mean TTP (in seconds) at baseline (4.23) as compared to the second (3.90), third (4.41), fourth (4.60), and fifth ICG assessment (5.99). As a result of ICG accumulation, the mean and the maximum absolute fluorescence intensity were significantly different as compared to the baseline assessment. There was no significant difference in LCL levels (in mmol/L) at baseline (0.74) as compared to the second (0.82), third (0.64), fourth (0.60), and fifth assessment (0.62).

Conclusion

Increasing doses of norepinephrine infusion have no significant influence on bowel perfusion using ICG fluorescence angiography.

INTRODUCTION

Fluorescence angiography, using indocyanine green (ICG) as a contrast agent, is increasingly applied by surgeons during surgical procedures, facilitating intraoperative decision-making. This imaging technique is fast (i.e., a matter of seconds to minutes), safe, and easy to perform, and multiple assessments can be performed during a single procedure.[1] Consequently, ICG fluorescence angiography is gaining popularity and is meeting acceptance in many surgical fields,[2–5] including colorectal surgery.[6,7]

Anastomotic leakage (AL) is one of the dreaded complications in colorectal surgery, with an incidence of up to 20% of cases.[6,8] It has been associated with an increased postoperative morbidity and mortality, and even when managed, it leads to a prolonged hospital stay and to increased healthcare costs.[7,8] As adequate perfusion is essential for optimal healing and AL prevention, an insufficient blood supply at the proximal or distal end of the anastomosis is one of several factors which have been associated with a greater risk of AL in case of an intraoperatively "water-tight" anastomosis. Accordingly, the assessment of bowel perfusion and intraoperative modification of the level of resection or anastomosis in case of insufficient perfusion may contribute to a reduced risk of AL.[7]

Traditionally, bowel perfusion is evaluated by the surgeon through a direct visualization of the anastomosis, including the evaluation of the serosal and mucosal colour, bowel peristalsis, and pulsation of mesenteric arteries or bleeding at the cut edge of the bowel.[7,8] However, these subjective signs did not allow to evaluate micro perfusion and were found to be unreliable since the accuracy of AL prediction by surgeons was low.[7–9] For this reason, ICG fluorescence angiography was proposed as an objective imaging technique which allows for the real-time assessment of bowel perfusion. After intravenous administration, ICG is bound to plasma protein. When exposed to near-infrared excitation, it re-emits a fluorescent light. Bowel perfusion may be quantified by using fluorescence intensity, which is proportional to bowel vascularization.[10] Recent systematic reviews demonstrated that ICG fluorescence angiography seems to reduce AL rates as compared to conventional techniques in colorectal surgery.[7,11] Liu et al. reported an AL rate of 3.8% in the ICG group as compared to 7.8% in the non-ICG group in a meta-analysis including a total of 4,037 patients.[12]

Nevertheless, the evaluation of fluorescence intensity remains a static measure with no consideration of ICG diffusion over time. Consequently, a dynamic fluorescence videography technique, which integrates near-infrared endoscopy and specific software called fluorescence-based enhanced reality (FLER), has been developed.[13] Dynamic

fluorescence angiography allows for time-to-peak fluorescence intensity (TTP). It is found to be a promising tool for the real-time imaging of bowel perfusion in an easy and accurate way.[10,14] Additionally, FLER analyses were found to be correlated with local capillary lactate (LCL) levels, in the experimental[10] and clinical setting.[15]

While vasopressors (e.g., norepinephrine) are often used during surgery to restore and maintain blood pressure levels in case of hypotension,[16] these substances have the potential to drastically reduce blood flow via vasoconstriction. Consequently, they may potentially preclude an accurate assessment of tissue perfusion when using ICG fluorescence angiography.[17,18]

To date, there is a limited number of studies which evaluated the effect of vasopressors on tissue perfusion assessment using ICG fluorescence angiography. The aim of this study was to investigate the effect of increasing doses of norepinephrine on bowel perfusion assessment using ICG fluorescence angiography in a porcine intestinal model.

METHODS

This study, which is part of the Endoscopic Luminescent Imaging for Oncology Surgery (ELIOS) project, was performed according to the National Institutes of Health guidelines for the use of experimental animals, respecting the ARRIVE guidelines.[19]

Norepinephrine infusion

Before finalizing the study protocol, consideration was given to use either increasing boluses of norepinephrine or an increasing continuous norepinephrine infusion. Norepinephrine is a sympathomimetic amine with a primarily agonistic effect at alpha-1 and beta-1 receptors, which increases systemic vascular resistance and potentially increases cardiac output respectively.[20] Due to a short half-life of 2.5 min, an intravenous bolus injection of norepinephrine would only induce short-term hypertension and tachycardia. In this period of time, ICG fluorescence angiography could be performed. For this evaluation, and for ethical purposes, respecting the '3R' (replace, refine, reduce) principles of animal research,[21] two pilot pigs used for educational purposes without causing any intestinal damage, were utilised to assess the effect of increasing doses of bolus injections versus increasing doses of continuous infusion of norepinephrine. In the 'bolus injection pig', a very short time period of vasopressor effect was observed as blood pressure and heart rate dropped within approximately two minutes during ICG fluorescence angiography assessment. In the 'continuous infusion pig', no decrease was observed as blood pressure and heart rate were maintained during infusion. For this logistical reason, it

was decided to use increasing doses of continuous norepinephrine infusion in this study. Additionally, it was decided to use the following doses of norepinephrine of 0.1, 0.5, and 1.0 µg/kg/min, since these doses resulted in a relevant increase in blood pressure and are within the range of doses frequently used in the clinical setting.

Animal preparation

A total of five adult female large white pigs (*Sus scrofa domesticus*) were included. According to the design of the study, every animal served as its own internal control. All animals were fasted for 24 h with free access to water. A qualified anaesthesiologist (EN and SD) performed every anaesthesia-related steps of the procedure.

The animals were sedated with an intramuscular injection of zolazepam and tiletamine 10 mg/kg (Zoletil ND, Virbac, France). After general anaesthesia induction using an intravenous administration of propofol 3 mg/kg (Propofol Lipuro ND, B Braun, France) and rocuronium 0.8 mg/kg (Esmeron ND, MSD, France), the animals were intubated and mechanically ventilated in a supine position. Anesthesia was maintained by means of a continuous inhalation of isoflurane 2% (Isoflurin ND, Axience, France) and a mixture of 50% nitrous oxide in oxygen. The dose of anaesthetics was increased when necessary according to the reflex status of the animals (i.e., palpebral reflexes and jaw tone). Intravascular injections of buprenorphine (Buprecare ND, Axience, France) 0.01 mg/kg were used for analgesia. All animals were infused with Ringer's lactate intravenously at a rate of 4 mL/kg/h.

A midline laparotomy was performed to expose the small bowel (see Figure 1 for the study set-up). A 30 cm segment of the small intestine (250 cm from the pylorus) was loosely fixed to both sides of the abdominal wall with Vicryl sutures to guarantee bowel exposure during this study. No further bowel manipulation was performed. During the protocol, three increasing doses of continuous norepinephrine infusion (0.1, 0.5, and 1.0 µg/kg/min) were administered intravenously over a 25-min interval (see Figure 2 for the study flowchart). Heart rate and blood pressure were continuously monitored through an arterial line inserted into the femoral artery. At the end of the protocol, pigs were sacrificed under deep anaesthesia (isoflurane 5%) with intravenous administrations of pentobarbital 40 mg/kg (Exagon ND, Axience, France).

ICG fluorescence angiography

In this study, an ICG fluorescence videography system integrated within a near-infrared endoscope (KARL STORZ GmbH & CO. KG, Germany) was used. It could detect the ICG-emitted fluorescence signal. After exposing the small bowel, the camera was fixed to an articulated arm in order to stabilise the image and allow for repetitive assessments (e.g.,

ensuring a stable distance and angle during ICG fluorescence angiography assessments). In a recent analysis of the European registry on fluorescence image-guided surgery (EURO-FIGS), an ICG dose of 0.1 to 0.2 mg/kg was identified as the most frequently used clinical dose for near-infrared fluorescence angiography.[22] To standardise the method of ICG administration, a bolus of ICG (Infracyanine, Laboratoires Serb, Paris, France) at a dose of 0.1 mg/kg was administered intravenously through a central venous catheter in the right internal jugular vein (Vygon, arterial LeaderCath, 6 French, 15 cm).

The ICG-emitted fluorescence signal was analysed using the ER-PERFUSION software (IRCAD, Strasbourg, France), which allowed for a virtual perfusion cartography based on time-to-peak fluorescence intensity (TTP; in seconds). TTP results from the velocity of the fluorescence signal until it reaches its maximum intensity peak in a specific region of interest (ROI) within the first 40 s following ICG injection.[23] This is a relatively short period following ICG injection, as a result of ICG administration via the central venous catheter. The virtual perfusion cartography is subsequently overlapped onto the images, providing fluorescence-based enhanced reality (FLER). In this study, TTP was measured from the time when there was 25% of fluorescence intensity to 75% of fluorescence intensity using the ER-PERFUSION software. In addition, the minimum and maximum absolute fluorescence intensities were measured in four randomly chosen ROIs on the bowel surface using the FLER software. TTP and absolute fluorescence intensity were analysed as mean of the four ROIs, together with the mean difference in maximum and minimum absolute fluorescence intensities.

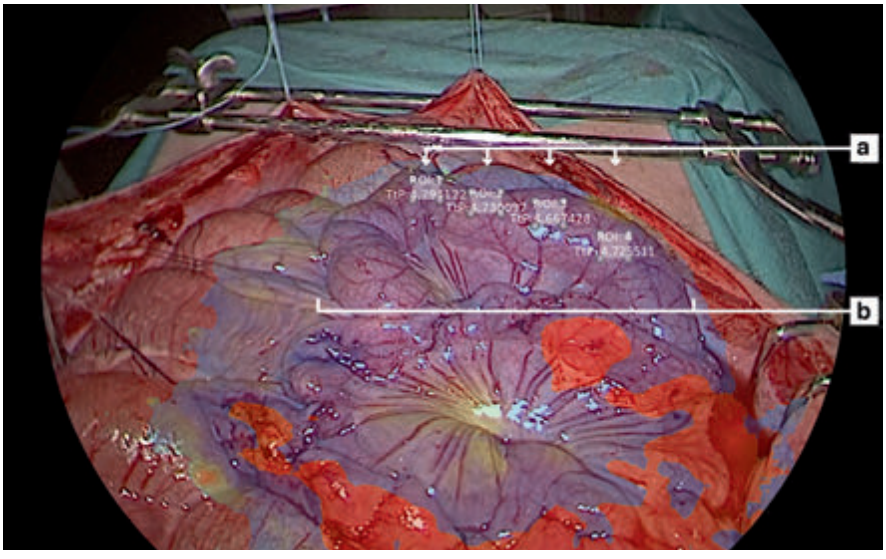

Figure 1. Study set-up with fluorescence-based enhanced reality (FLER) overlay. (a) Randomly chosen regions of interest (ROIs) and (b) small intestine parts under investigation. Images made using a near-infrared endoscope by KARL STORZ).

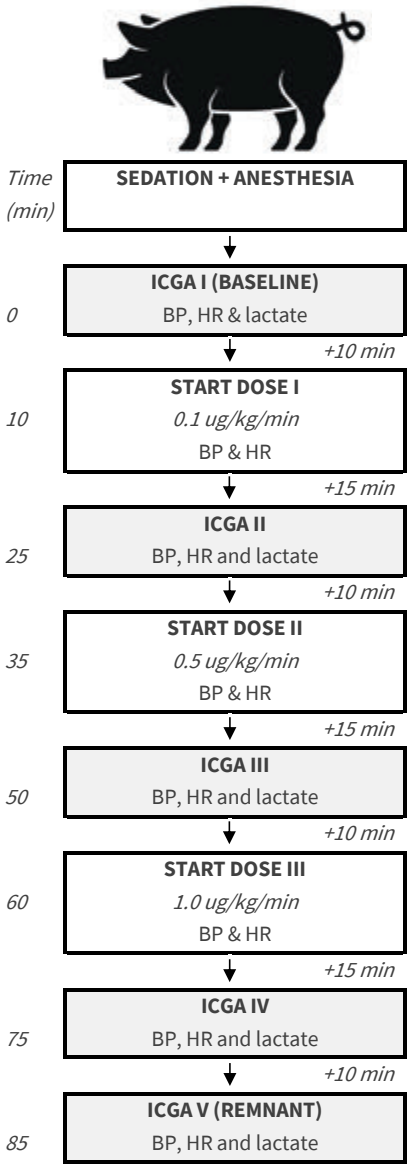

Figure 2. Study flowchart. *ICGA* indocyanine green angiography, *BP* blood pressure, *HR* heart rate.

Capillary lactate levels

Local bowel capillary lactate (LCL) levels were measured in the blood using an EDGE lactate analyser (ApexBio, Taipei, Taiwan, People's Republic of China) by puncturing the bowel serosa in the chosen ROI with a needle. LCL levels were obtained during all five fluorescence videography assessments.

Statistical analysis

Continuous variables are presented as means with standard deviation (SD). Categorical data were reported as frequency and proportion. Fisher's exact test was used to calculate *P*-values for categorical variables. The paired sample T-test was used to analyse the differences in TTP fluorescence intensity (in seconds) and mean absolute fluorescence intensity in ROIs between the different vasopressor doses. All results were analysed using IBM SPSS Statistics for Windows, version 24 (IBM Corporation, Armonk, NY, USA). A *p*-value < 0.05 was considered statistically significant.

Ethics approval

The protocol was approved by the local Ethical Committee on Animal Experimentation (ICOMETH No. 38.2020.02.003) and by the French Ministry of Superior Education and Research (MESR) under the following reference: APAFIS#8721-2017013010316298-v2. All animals used in the experimental laboratory were managed according to French laws for animal use and care and according to the directives of the European Community Council (ECC).

RESULTS

The included animals had a mean weight of 40.3 ± 4.24 kg (range: 33.0 to 44.0 kg). During the protocol, all pigs underwent five ICG fluorescence angiographies, namely once at baseline, three times following increasing doses (0.1, 0.5, and 1.0 µg/kg/min) of norepinephrine infusion, and once following discontinuation of norepinephrine infusion. In general, blood pressure and heart rate increased gradually as the infusion dose increased. After discontinuation of norepinephrine infusion, a substantial decrease in blood pressure and heart rate was observed (Tables 1 and 2).

Table 1. Measurements (BP, HR, and lactate levels) per pig over time.

	Pig 1	Pig 2	Pig 3	Pig 4	Pig 5
Weight (kg)	33.0	42.1	41.4	41.0	44.0
ICG per dose (mg)	3.3	4.2	4.1	4.1	4.4
0 min: ICGA 1 (baseline)					
Systolic BP (mmHg)	85	53	58	60	52
Diastolic BP (mmHg)	48	38	30	35	29
HR (bpm)	97	90	84	63	95
Lactate (mmol/L)	0.8	Low[a]	0.6	1.1	Low[a]

Table 1. (continued)

	Pig 1	Pig 2	Pig 3	Pig 4	Pig 5
10 min: start first NE dose					
Systolic BP (mmHg)	66	57	61	55	35
Diastolic BP (mmHg)	38	41	32	33	27
HR (bpm)	92	89	88	60	83
25 min: ICGA II (first dose)					
Systolic BP (mmHg)	89	76	91	93	70
Diastolic BP (mmHg)	60	58	48	57	38
HR (bpm)	95	76	88	82	98
Lactate (mmol/L)	Low[a]	Low[a]	Low[a]	1.3	1.0
35 min: start second NE dose					
Systolic BP (mmHg)	94	65	78	88	64
Diastolic BP (mmHg)	51	50	41	55	33
HR (bpm)	98	79	90	82	98
50 min: ICGA III (second dose)					
Systolic BP (mmHg)	155	77	130	134	89
Diastolic BP (mmHg)	97	52	60	102	46
HR (bpm)	101	95	136	105	107
Lactate (mmol/L)	0.6	Low[a]	Low[a]	Low[a]	0.8
60 min: start third NE dose					
Systolic BP (mmHg)	128	49	101	124	80
Diastolic BP (mmHg)	68	38	47	73	42
Heart rate (bpm)	92	103	125	103	125
75 min: ICGA IV (third dose)					
Systolic BP (mmHg)	127	125	128	132	81
Diastolic BP (mmHg)	65	74	62	83	45
HR (bpm)	92	146	143	120	142
Lactate (mmol/L)	Low[a]	Low[a]	Low[a]	0.6	0.6
85 min: ICGA V (remnant)					
Systolic BP (mmHg)	48	32	30	45	41
Diastolic BP (mmHg)	32	23	20	25	24
HR (bpm)	125	113	116	76	110
Lactate (mmol/L)	Low[a]	Low[a]	Low[a]	Low[a]	0.7

[a]Lactate levels between 0.1 and 0.6 mmol/L. ICGA indocyanine green angiography, BP: blood pressure, HR: heart rate, bpm: beats per minute, NE: norepinephrine.

Table 2. Mean measurements over time.

	0 min ICGA I	10 min ICGA I	25 min ICGA II	35 min	50 min ICGA III	60 min	75 min ICGA IV	85 min ICGA V
NE dose	0	0	0.1	0.1	0.5	0.5	1.0	1.0
HR, mean SD	86 ± 14	82 ± 13	88 ± 9	89 ± 9	109 ± 16	110 ± 15	129 ± 23	108 ± 19
SBP, mean SD	62 ± 14	55 ± 12	84 ± 10	78 ± 13	117 ± 33	96 ± 33	119 ± 21	39 ± 8
DBP, mean SD	36 ± 8	34 ± 6	52 ± 9	46 ± 9	71 ± 26	54 ± 16	66 ± 14	25 ± 4
Lactate range	L—0.83	NE	L—1.15	NE	L—0.70	NE	L—0.60	L—0.70

ICGA I = baseline, II = after norepinephrine (NE, in µg/kg/min) dose 1, III = after NE dose 2, IV = after NE dose 3, V = remnant measurement following discontinuation of NE, HR: heart rate (beats per minute), SBP and DBP: diastolic and systolic blood pressure (mmHg). Lactate level range in mmol/L. L = Low.

Time-to-peak fluorescence intensity (TTP)

Mean TTP of the four regions of interest after the first (4.23 ± 0.30 s) and second dose of norepinephrine (3.90 ± 0.16 s) were slightly lower as compared to baseline (4.41 ± 0.46 s) assessment (− 0.18 and − 0.51 s respectively). Conversely, mean TTP after the third dose (4.60 ± 0.68 s) and after discontinuation of norepinephrine infusion (5.99 ± 2.07) were higher as compared to baseline (+ 0.19 and + 1.58 respectively) (see Figure 3). However, mean TTP did not significantly differ from baseline (see Table 3). In addition, mean TTP per ROI in the first, second, third dose assessment, and mean TTP in the assessment following discontinuation of norepinephrine infusion, did not significantly differ from baseline.

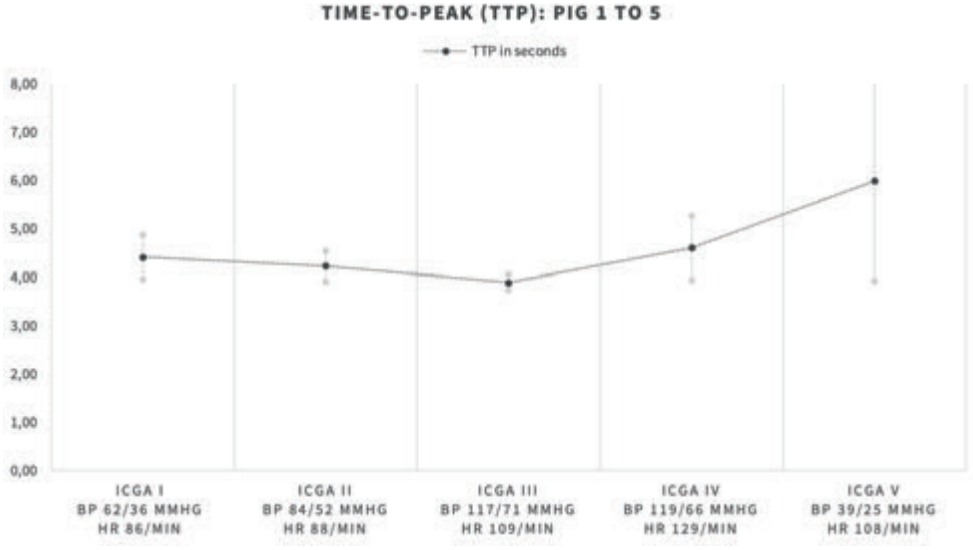

Figure 3. Mean time-to-peak (TTP) in seconds for all 5 pigs. Mean TTP (in black) with standard deviations (in grey). X-axis: ICGA assessment number, mean BP and HR for all pigs. Y-axis: mean TTP in seconds. ICGA I = baseline, II = after norepinephrine (NE) dose 1, III = after NE dose 2, IV = after NE dose 3, V = remnant measurement following discontinuation of NE), BP = blood pressure, HR = heart rate.

Table 3. Difference in mean TTP in seconds over increasing infusion doses.

	TTP, mean ± SD	TTP, mean ± SD	Mean difference	P-value
Baseline vs. NE dose 1	4.41 ± 0.46	4.23 ± 0.30	- 0.18	0.204
Baseline vs. NE dose 2	4.41 ± 0.46	3.90 ± 0.16	- 0.51	0.119
Baseline vs. NE dose 3	4.41 ± 0.46	4.60 ± 0.68	+ 0.19	0.555
Baseline vs. NE remnant	4.41 ± 0.46	5.99 ± 2.07	+ 1.58	0.110

NE: norepinephrine, ICGA: indocyanine green angiography, TTP: time-to-peak, SD: standard deviation.

Absolute fluorescence intensity

Mean minimum fluorescence intensity of the four ROIs increased during the protocol from 0 at baseline to 62.7, 98.3 (+ 57% increase as compared to previous ICG fluorescence angiography), 127.1 (+ 29%), and 134.2 (+ 6%) after the first, second, and third dose, and after discontinuation of norepinephrine infusion respectively. Mean maximum fluorescence intensity of the four ROIs also increased from 101.4 at baseline to 146.6 (+ 45%), 150.4 (+ 3%), 162.1 (+ 8%), and 159.0 (− 2%) after the first, second, and third dose, and after discontinuation of norepinephrine infusion respectively. Since the maximum intensity increased in a slower manner as compared to the minimum intensity, the mean difference decreased from 101.4 to 24.8 (see Figure 4).

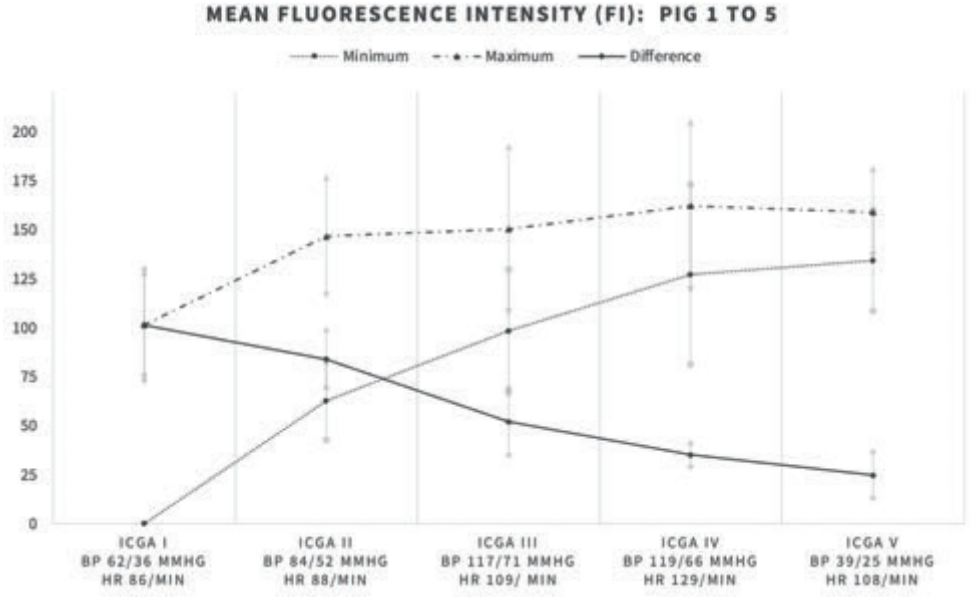

Figure 4. Mean minimum, maximum, and difference in absolute fluorescence intensity (FI) for all 5 pigs. Mean fluorescence intensity (in black) with standard deviations (in grey). X-axis: ICGA assessment number, mean BP and HR for all pigs. Y-axis: mean fluorescence intensity. ICGA I = baseline, II = after norepinephrine (NE) dose 1, III = after NE dose 2, IV = after NE dose 3, V = remnant measurement following discontinuation of NE, BP blood pressure, HR heart rate.

Mean minimum fluorescence intensity in the first ($p = 0.002$), second ($p = 0.002$), third ($p = 0.003$), and remnant assessment ($p \leq 0.001$) were significantly different as compared to the baseline assessment, as well as the maximum fluorescence intensity ($p = 0.011$, 0.016, 0.027, and 0.002 respectively).

Regarding the mean difference in fluorescence intensity, only the mean difference between fluorescence intensity in the first dose assessment did not significantly differ from baseline ($p = 0.208$, in contrast to the second ($p = 0.006$), third ($p = 0.011$), and remnant assessment ($p = 0.003$).

Local capillary lactate (LCL) levels

Local capillary lactate (LCL) levels at the time of the ICG fluorescence angiography assessment are presented in Table 3. LCL levels defined as 'low' represent a value between 0.1 and 0.59 mmol/L. In statistical analyses comparing baseline and the following ICG fluorescence angiographies, all 'low' LCL level results were regarded as 0.59 in order to prevent any underestimation bias. The subsequent LCL levels were 0.74, 0.82, 0.64, 0.60, and 0.62 from baseline to remnant assessment respectively. No significant difference in LCL levels was found.

DISCUSSION

Although there were a few previous studies which evaluated the effect of vasopressors on free flap perfusion using ICGA, this is the first study evaluating the influence of increasing doses of intraoperative vasopressor use on ICG fluorescence angiography in a standardised porcine model.[24,25] Based on this model, no difference was found in mean time-to-peak fluorescence intensity (TTP) between baseline and increasing doses of norepinephrine infusion. Additionally, a significant increase in mean minimum and maximum absolute fluorescence intensities was observed, as well as a significant decrease in mean difference between these two measurements.

In gastrointestinal surgery, an adequate intraoperative assessment of bowel perfusion is mandatory in order to prevent anastomotic leakage. Over the last decade, ICG fluorescence angiography has gained popularity for the real-time assessment of bowel perfusion.[12] Likewise, ICG fluorescence angiography is more frequently applied to plastic and reconstructive surgery to objectively evaluate flap perfusion.[2] In that surgical field, it has been suggested that pharmacologically induced vasoconstriction could preclude an accurate estimation of tissue perfusion, while an adequate regulation of systemic blood pressure is fundamental during every surgical procedure.[18] Despite a suspicion supported

by anecdotal reports in the literature, no previous study reported the effect of vasopressors on tissue perfusion assessment using ICG fluorescence angiography.

TTP as part of fluorescence-based enhanced reality (FLER) was considered the primary outcome in the current study. The measurement corresponds to the mean time elapsed for the fluorescence signal to reach its maximum intensity in a given area.[26] TTP offers two great advantages as compared to absolute fluorescence intensity values. First, TTP is independent of the distance between the ICG camera and the ROI, whereas absolute fluorescence intensity is highly dependent on distance.[1,14] In the current study, the distance between the camera and the bowel was kept constant throughout perfusion assessments. Nonetheless, it remains relevant in clinical perfusion assessments.

Theoretically, the ICG plasma half-life of approximately 3 to 5 min allows for multiple perfusion assessments throughout a surgical procedure.[1] However, in a previous preliminary test by Diana et al. concerning a series of ICG injections (0.125 mg/kg every 15 min) while focusing on a healthy small bowel loop, ICG accumulation was observed. On the other hand, the calculated TTP remained constant in each assessment.[14] As a result, a second and major advantage of TTP is that it truly allows for multiple and repetitive assessments. Only the additional signal is interpreted, and the "noise" produced by the accumulation of fluorescent dye does not affect TTP. Consequently, we derive that increasing doses of norepinephrine, as explored in this experimental porcine model, have no effect on bowel perfusion assessment using ICG fluorescence. Notably, the TTP increased from 4.60 s after dose 3 to 5.99 s after norepinephrine discontinuation ("remnant assessment"). Although this is a substantial increase, the mean difference in TTP was not significant as compared to the baseline assessment. The impaired clinical condition of the pigs, with a mean blood pressure of 39/25 mmHg at the end of the study, resulting from the termination of continuous norepinephrine infusion, contributed to this increase in TTP.

When considering absolute fluorescence intensity in the current study, an increase in mean minimum and maximum fluorescence intensity is consistent with the aforementioned study.[14] Although a 25-min interval (except between ICG fluorescence angiography assessment IV and V) was maintained to ensure ICG wash-out following the ICG injections of 0.1 mg/kg, a significant ICG accumulation was observed. Consequently, there is more reason to believe that it is better to use a dynamic fluorescence videography technique, such as FLER analysis, over absolute fluorescence intensity in case of repeat perfusion assessments throughout a surgical procedure. Notably, a decrease in the mean difference in fluorescence intensity was noted. It is likely due to approaching an absolute maximum fluorescence intensity level as a result of dye accumulation within the tissue.

Local capillary lactate (LCL) levels reflect tissue oxygenation in bowel cells, and these were previously correlated with bowel perfusion using ICG fluorescence angiography. In a previous study, it was concluded that the mean LCL level in an ischemic bowel region (5.6 ± 2.8 mmol/L) was significantly higher than LCL levels in a bowel region at 25% of perfusion on ICG fluorescence angiography (3.7 ± 1.7 mmol/L) and in a bowel region at 75% of perfusion (2.9 ± 1.3 mmol/L).[10] In the current study, low LCL levels were observed with no significant increase. This reflects the healthy state and non-ischemic condition of the bowel under investigation.

With regards to the infusion dose of norepinephrine, the effect on beta-1 adrenergic receptors may be more distinct at low doses (less than 2 µg/min), potentially leading to an increased cardiac output. In doses higher than 3 µg/min, the alpha-1 adrenergic effect may predominate, resulting in vasocontraction.[20] Since the minimum dose was 3.3 µg/min in the current study, this should result in vasoconstriction and in a dose-dependent increase in systemic vascular resistance. Due to this particular effect of norepinephrine, plastic and reconstructive surgeons have hypothesised that vasoconstriction comprises blood flow of superficial capillaries, reducing the potential of ICG fluorescence angiography in order to accurately assess flap perfusion in reconstructive surgery[17,18] The results of the current study suggest that there was no alteration in bowel perfusion based on ICG fluorescence angiography. This might be a result of an increase in blood pressure, which leads to a compensation for the vasoconstrictive effect of norepinephrine. This finding is supported by previous studies in which norepinephrine was found to preserve intestinal microcirculatory blood flow[27,28] and microcirculatory flap perfusion,[29,30] Conversely, another study found that norepinephrine decreased intestinal microcirculatory blood flow, despite significantly increased arterial blood pressure.[31] However, the design of the current study is different as compared to previous studies in which laser Doppler flowmetry (LDF) was used to evaluate microcirculatory blood flow.[27-31]

Although plastic and reconstructive surgery concerns a different surgical field, the principles of perfusion assessment remain similar. Two recent clinical studies in this particular field have evaluated the effect of vasopressor use on flap perfusion using ICGA assessment.[24,25] Anker et al. concluded that norepinephrine concentrations of 0.065 ± 0.020 µg/kg/min had no clinically significant impact on microperfusion.[24] Massaro et al. concluded that changes in mean perfusion of the free flap during the intraoperative period are nominal.[25] However, in contrast to the current study, both studies were not performed in a standardised fashion with regard to the vasopressor dose. While the results of the current standardised animal study are consistent with previous findings and suggest that increasing doses of norepinephrine may also have no effect on perfusion assessment

using ICG fluorescence angiography in other types of tissue (e.g., adipocutaneous or musculocutaneous flap perfusion in reconstructive procedures), this should be confirmed in future studies.

Despite the novelty of this first experimental study reporting on the effect of increasing doses of norepinephrine on tissue perfusion assessment using ICG fluorescence angiography, some limitations need to be addressed. Five pigs constitute a small study population and only female pigs were used.[19] However, multiple ICGA measurements were obtained without unreasonable outliers. Although one of the animals had a considerably lower total body weight of 33.0 kg compared with the mean weight of 40.3 kg, we believe that the difference in total body weight does not influence outcomes, as all animals act as their own control.

In addition, only healthy small intestines were investigated in this study. Although no difference was found in mean TTP between baseline and increasing doses of norepinephrine infusion in the current study, we are unsure about the results in diseased bowel areas. It is our intention to study this clinically relevant problem in a future experimental animal model with an ischemic bowel model by clamping the arterial supply of bowel segments in combination with the use of vasopressors. Furthermore, the study is limited by the absence of a control group in which ICG fluorescence angiography is performed without any administration of norepinephrine. Conversely, this study is strengthened by the use of two 'pilot pigs' in advance, in order to assess the effect of bolus injections versus a continuous infusion of norepinephrine. Continuous infusion was chosen over bolus injections, since an increase in blood pressure and heart rate was maintained with a continuous infusion and its administration relates more to the daily clinical practice. In addition, two previous studies concerning the effect of vasopressors on intestinal blood flow and oxygen supply also used continuous norepinephrine infusion in doses ranging from 0.01 to 2.0 µg/kg/min.[28,31]

CONCLUSION

In conclusion, increasing doses of norepinephrine (0.1, 0.2, and 0.5 µg/kg/min respectively) have no statistically significant influence on bowel perfusion assessment as time-to-peak fluorescence intensity remains constant during the FLER analysis. Secondly, ICG accumulation was observed when using absolute fluorescence intensity, which is an important finding for future studies, reflecting the need for a dynamic fluorescence videography technique over absolute fluorescence intensity in case of repeat perfusion assessments.

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CHAPTER 4

Indocyanine green angiography for preventing postoperative mastectomy skin flap necrosis in immediate breast reconstruction: a systematic review and meta-analysis

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Cochrane Database of Systematic Reviews
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ABSTRACT

Background

Breast cancer will affect one in eight women during their lifetime. The opportunity to restore the removed tissue and cosmetic appearance is provided by reconstructive breast surgery following skin-sparing mastectomy (SSM). Mastectomy skin flap necrosis (MSFN) is a common complication following SSM breast reconstruction. This postoperative complication can be prevented by intraoperative assessment of mastectomy skin flap viability and intervention when tissue perfusion is compromised. Indocyanine green fluorescence angiography is presumed to be a better predictor of MSFN compared to clinical evaluation alone.

Objectives

To assess the effects of indocyanine green fluorescence angiography (ICGA) for preventing mastectomy skin flap necrosis in women undergoing immediate breast reconstruction following skin-sparing mastectomy.

To summarise the different ICGA protocols available for assessment of mastectomy skin flap perfusion in women undergoing immediate breast reconstructions following skin-sparing mastectomy.

Search methods

We searched the Cochrane Breast Cancer Specialised Register, Cochrane Central Register of Controlled Trials (CENTRAL; Issue 3, 2019), MEDLINE, Embase, the World Health Organization's International Clinical Trials Registry Platform (ICTRP) and Clinicaltrials.gov in April 2019. In addition, we searched reference lists of published studies.

Selection criteria

We included studies that compared the use of ICGA to clinical evaluation to assess mastectomy skin vascularisation and recruited women undergoing immediate autologous or prosthetic reconstructive surgery following SSM for confirmed breast malignancy or high risk of developing breast cancer.

Data collection and analysis

Two review authors independently assessed the risk of bias of the included nonrandomised studies and extracted data on postoperative outcomes, including postoperative MSFN, reoperation, autologous flap necrosis, dehiscence, infection, haematoma and seroma, and patient-related outcomes. The quality of the evidence was assessed using the GRADE approach and we constructed two 'Summary of finding's tables: one for the comparison of ICGA to clinical evaluation on a per patient basis and one on a per breast basis.

Main results

Nine nonrandomised cohort studies met the inclusion criteria and involved a total of 1589 women with 2199 breast reconstructions. We included seven retrospective and two prospective cohort studies. Six studies reported the number of MSFN on a per breast basis for a total of 1435 breasts and three studies reported the number of MSFN on a per patient basis for a total of 573 women. Five studies reported the number of other complications on a per breast basis for a total of 1370 breasts and four studies reported the number on a per patient basis for a total of 613 patients. Therefore, we decided to pool data separately.

Risk of bias for each included nonrandomised study was assessed using the Newcastle-Ottawa Scale for cohort studies. There was serious concern with risk of bias due to the nonrandomised study design of all included studies and the low comparability of cohorts in most studies. The quality of the evidence was found to be very low, after downgrading the quality of evidence twice for imprecision based on the small sample sizes and low number of events in the included studies.

Postoperative complications on a per patient basis

We are uncertain about the effect of ICGA on MSFN (RR 0.79, 95% CI 0.40 to 1.56; three studies, 573 participants: very low quality of evidence), infection rates (RR 0.91, 95% CI 0.60 to 1.40; four studies, 613 participants: very low quality of evidence), haematoma rates (RR 0.87, 95% CI 0.30 to 2.53; two studies, 459 participants: very low quality of evidence) and seroma rates (RR 1.68, 95% CI 0.41 to 6.80; two studies, 408 participants: very low quality of evidence) compared to the clinical group. We found evidence that ICGA may reduce reoperation rates (RR 0.50, 95% CI 0.35 to 0.72; four studies, 613 participants: very low quality of evidence). One study considered dehiscence as an outcome. In this single study, dehiscence was observed in 2.2% of participants (4/184) in the ICGA group compared to 0.5% of participants (1/184) in the clinical group (P=0.372). The RR was 4.00 (95% CI 0.45 to 35.45; one study; 368 participants; very low quality of evidence).

Postoperative complications on a per breast basis

We found evidence that ICGA may reduce MSFN (RR 0.62, 95% CI 0.48 to 0.82; six studies, 1435 breasts: very low quality of evidence), may reduce reoperation rates (RR 0.65, 95% CI 0.47 to 0.92; five studies, 1370 breasts: very low quality of evidence) and may reduce infection rates (RR 0.65, 95% CI 0.44 to 0.97; five studies, 1370 breasts: very low quality of evidence) compared to the clinical group. We are uncertain about the effect of ICGA on haematoma rates (RR 1.53, CI 95% 0.47 to 4.95; four studies, 1042 breasts: very low quality of evidence) and seroma rates (RR 0.71, 95% CI 0.37 to 1.35; two studies, 528 breasts: very low quality of evidence).

None of the studies reported patient-related outcomes.

ICGA protocols: eight studies used the SPY System and one study used the Photodynamic Eye imaging system (PDE) to assess MSFN. ICGA protocols in the included studies were not extensively described in most studies.

Authors' conclusions

Although mastectomy skin flap perfusion is performed more frequently using ICGA as a helpful tool, there is a lack of high-quality evidence in the context of randomised controlled trials. The quality of evidence in this review is very low, since only nonrandomised cohort studies have been included. With the results from this review, no conclusions can be drawn about what method of assessment is best to use during breast reconstructive surgery. High-quality randomised controlled studies that compare the use of ICGA to assess MSFN compared to clinical evaluation are needed.

PLAIN LANGUAGE SUMMARY

Indocyanine green angiography versus clinical evaluation in preventing postoperative complications following breast reconstruction after mastectomy

What is the aim of this review?

The aim of this Cochrane review was to find out whether the use of a new imaging technique, called indocyanine green angiography (ICGA), during reconstructive breast surgery can reduce necrosis (cell death) of the overlying breast skin and other complications such as infections, following reconstructive breast surgery after mastectomy.

We included women who had undergone skin-sparing mastectomy (that is, where the whole breast including nipple is removed, sparing the overlying breast skin) for breast cancer or women who were at high risk of developing breast cancer (because of faulty genes). We collected and analysed all relevant studies to provide a review that will inform doctors and patients on ICGA use in reconstructive breast surgery.

Key messages

The use of ICGA during reconstructive breast surgery seems to reduce the chance of reoperations when compared to clinical evaluation only. We are uncertain about the effect of ICGA on reducing the chance of necrosis of the overlying breast skin and other post-surgery complications when compared to clinical evaluation only. The quality of studies used for this review is very low, meaning that we are not confident of the results. We need high-quality studies that have randomised women to a group of ICGA assessment or clinical evaluation alone to have a more definitive answer.

What was studied in the review?

Around 40% of women with breast cancer need to undergo mastectomy (removal of the whole breast). A skin-sparing mastectomy is a common operation in which the overlying breast skin is preserved. After skin-sparing mastectomy, women have the option to undergo reconstructive breast surgery. This operation carries some risks and complications, including an operation to correct complications (reoperation), spontaneous reopening of the surgical wound (dehiscence), infection, blood pooling outside of a blood vessel (haematoma) and a pocket filled with blood plasma underneath the skin (seroma).

Preserving the blood supply of the overlying breast skin during skin-sparing mastectomy is crucial. When the blood supply is poor, skin will not survive, and surgeons need to intervene to prevent postoperative complications. Usually, the surgeon will assess tissue colour, the

time taken for colour to return to the skin after pressure is applied (capillary refill), temperature, skin's elasticity, and bleeding of the skin.

ICGA is a new imaging technique that assesses the blood supply to the tissue. It can assess blood flow in the overlying breast skin better than clinical judgement alone. We collected studies that compared the use of ICGA to clinical evaluation by a surgeon during immediate reconstructive breast surgery after skin-sparing mastectomy. In these studies, women underwent immediate reconstructive surgery with their own tissue from another area of the woman's body or with a breast implant.

What are the main results of this review?
We found nine studies that compared the number of postoperative complications in women who had ICGA assessment of their breast skin versus clinical evaluation. Six studies were performed in the USA, two in Denmark and one in Japan. There were a total 1589 women with 2199 breast reconstructions. Studies reported the number of complications on a per patient basis or on a per breast basis. We present information based on both types of data.

The main results on a per patient analysis were that:
- ICGA may reduce reoperation rates, and
- we are uncertain as to whether ICGA has an effect on necrosis of the overlying skin of the breast, infection, haematoma and seroma rates.

The main results on a per breast basis were that:
- ICGA may reduce necrosis of the overlying skin of the breast, reoperation and infection rates, and
- we are uncertain as to whether ICGA has an effect on haematoma and seroma rates.

The evidence contributing to this review topic is considered to be very low quality. Since randomised controlled trials are found to be one of the most powerful methods in clinical research, it is a major downside that these studies are missing. We emphasise the need for randomised controlled trials to further investigate the use of ICGA in reconstructive breast surgery.

How up-to-date is this review?
Studies published up to April 2019 have been used for this review.

BACKGROUND

Description of the condition

Breast cancer is the most common malignancy among women worldwide, and will affect one in eight women during their lifetime.[1] It accounts for 25% of all cancer cases, with an estimated incidence of over two million new cases diagnosed in 2018.[2,3] Current standard surgical options are breast-conserving surgery (BCS) and mastectomy. In BCS, the tumour and an adequate surgical margin are removed to maintain a cosmetically acceptable breast. In contrast, a mastectomy is the removal of the tumour and surrounding healthy tissue. BCS has replaced mastectomy for the majority of women with breast cancer in high-income regions across the world. Many women still require a mastectomy, in the case where the tumour is multifocal, multicentric, or of significant volume relative to breast size, or in the case of recurrence, when radiotherapy is contraindicated, or when genetic susceptibility is proven or suspected.[4] In addition, women may prefer mastectomy as a consequence of fear of recurrence and perception of improved survival. Currently, approximately 40% of women with breast cancer will undergo a mastectomy.[5,6]

A skin-sparing mastectomy (SSM) is a common surgical procedure that involves the removal of all breast tissue and glands, but preserves the overlying skin of the breast (i.e., mastectomy skin flap). Since breast cancer survival rates are improving, cosmetic end-result and high quality of life among breast cancer survivors is becoming increasingly important. Therefore, the opportunity to restore the removed tissue and cosmetic appearance is provided by reconstructive breast surgery.[7] Breast reconstructive procedures following SSM can be divided into prosthetic and autologous breast reconstructions, or a combination of these two methods.[6] Prosthetic breast reconstruction remains the most performed method worldwide.[8] It involves either a one-stage immediate implant prosthesis, or a two-stage procedure, with initial placement of a tissue expander (i.e., inflatable implant designed to stretch the skin). Autologous breast reconstruction involves harvesting tissue from other areas of the woman's body, which is used to replace the breast tissue removed by the mastectomy.

Mastectomy skin flap necrosis (MSFN) is a common complication following SSM breast reconstruction. MSFN has a reported mean incidence rate of 19.4% in the literature (this rate was calculated by dividing the sum of percentage rates by the total number of included studies).[9] MSFN ensues when skin flap perfusion is compromised. Patient risk factors that have been identified to pose a greater risk of MSFN include smoking, diabetes mellitus, a body mass index (BMI) greater than 30 kg/m^2, higher mastectomy breast weight, and previous exposure to radiotherapy. Surgical risk factors include tumescent mastectomy

techniques and Wise-pattern mastectomy incision.[9] MSFN can lead to a number of challenges, including wound management problems, delayed initiation of adjuvant therapy, patient distress, aesthetic compromise, risk of infection, extrusion of breast implant or tissue expander, need for additional reconstruction, and financial loss.[9-11]

Description of the intervention

To prevent postoperative MSFN following SSM breast reconstruction, surgeons need to assess mastectomy skin flap viability intraoperatively and intervene when tissue perfusion is found to be compromised. The contemporary approach for intraoperative assessment of mastectomy skin flap perfusion is based on the surgeon's clinical judgement and subjective evaluation of tissue colour, temperature, capillary refill, turgor, and dermal edge bleeding. Clinical evaluation alone appeared to be an unreliable predictor for MSFN.[12] An innovative method capable of evaluating the flap during surgery is indocyanine green fluorescence angiography (ICGA). ICGA is a real-time imaging technique that records fluorescence images using a dedicated near-infrared camera after administering a fluorophore called indocyanine green (ICG).

ICG was originally developed by the Eastman Kodak Company for use in infrared photography. In 1962, ICG was introduced in clinical practice to assess cardiac output.[13] Techniques for acquiring fluorescence angiograms of the ocular choroid using ICG in the early 1970s have been adapted to current fluorescent angiography.[14] ICG absorbs light in the near-infrared spectrum with a maximum at 805 nanometers (nm) and emits fluorescence with a maximum at 835 nm. Fluorescent angiography uses near-infrared light projected onto the area of interest, where it penetrates into the skin and acts as an excitation light to the dye. ICG is rapidly and extensively bound to plasma protein after intravenous administration. When protein-bound ICG is exposed to near-infrared light, it emits fluorescence from blood vessels containing dye within the deep dermal plexus and subcutaneous fat. A digital camera evaluates the absorption of the ICG fluorescence and records it in real time. The plasma half-life of ICG is three to four minutes in humans. The dye is efficiently removed from the blood by the liver and excreted into the bile without any further metabolism.[15] The incidence of adverse events is low (1 out of 42,000 patients) and it has no effect on blood constituents or on the haemostatic system.[16,17]

How the intervention might work

The binding to protein confines ICG to the intravascular compartment.[17] As a consequence, ICGA is able to provide a dynamic map of dermal circulation that serves as a topographical analysis of blood flow and tissue perfusion.[18] ICGA is found to be a better predictor of MSFN than clinical evaluation alone, with 90% sensitivity and 100% specificity.[19] Postoperative

MSFN could be prevented by using intraoperative ICGA to identify mastectomy skin flap areas of insufficient perfusion (i.e., non-vital skin). These areas can be marked directly on the mastectomy skin flap. Hereafter, the surgeon can decide to excise the insufficiently perfused tissue to prevent postoperative MSFN and aim for a significant improvement in postoperative outcome in SSM breast reconstructions.

Why it is important to do this review

A number of systematic reviews have been published on the use of intraoperative ICGA during reconstructive flap surgery.[20-24] These reviews have been conducted with various other objectives. For example, Burnier et al. obtained the latest recommendations for ICG in plastic surgery.[20] The authors concluded that ICGA is an excellent tool that helps plastic surgeons in their everyday practice and that ICGA appears to be an effective tool to predict clinical outcomes such as partial and total skin necrosis for large skin paddles. Cornelissen et al. provided a comprehensive literature review on current and potential future applications of ICGA in plastic surgery, and concluded that ICGA could facilitate critical decision-making in plastic surgical procedures.[21] Li et al. summarised the applications of ICG for tissue transfer and safe dosing practices, and concluded that ICGA aids in the evaluation of flap microcirculation and perfusion.[22] Furthermore, several nonrandomised trials have been performed to evaluate ICGA in assessing mastectomy skin flap perfusion. Although most studies showed that ICGA may be beneficial in predicting postoperative MSFN, no randomised controlled trials on the utility and efficacy of ICGA in reducing MSFN during postmastectomy breast reconstructions have been performed or currently registered. We aimed to provide a detailed review of ICGA use in immediate breast reconstruction following SSM. This review has assessed and summarised the current evidence comparing the efficacy of ICGA in preventing postoperative MSFN in immediate breast reconstruction following SSM compared to clinical evaluation alone. This will assist clinicians to use ICGA with a better knowledge of the current evidence. This review has also highlighted the absence of high-quality evidence, preparing the ground for a future RCT in this field.

OBJECTIVES

The objectives are as follows:

1. To assess the effects of indocyanine green angiography (ICGA) for preventing mastectomy skin flap necrosis in women undergoing immediate breast reconstruction following skin-sparing mastectomy;

2. To summarise the different indocyanine green angiography (ICGA) protocols available for assessment of mastectomy skin flap perfusion in women undergoing immediate breast reconstruction following skin-sparing mastectomy.

METHODS

Criteria for considering studies for this review

Types of studies

First, we searched for randomised controlled trials (RCTs). When no existing RCTs for this review were found, we extended our search to include well-designed (nonrandomised) cohort or case control studies, that assessed the efficacy of indocyanine green angiography (ICGA) in immediate breast reconstruction after skin-sparing mastectomy. We did not place any restrictions on whether the studies had a prospective or retrospective design.

Types of participants

Women (aged 18 years or over) undergoing immediate autologous or prosthetic reconstructive surgery following skin-sparing mastectomy. We included women with confirmed breast malignancy, women at very high risk of developing breast cancer (i.e., breast cancer gene mutation), or both. No restrictions were placed on race, menopausal status, or breast cancer stages.

Types of interventions

Studies that compared the use of ICGA to clinical evaluation to assess mastectomy skin vascularisation, with or without debridement of suspected necrotic (i.e., non-vital) tissue. No restriction was placed on the type of ICGA system (i.e., handheld, trolley with articulating arm, and microscope-integrated ICGA system) used to prevent mastectomy skin flap necrosis (MSFN).

We considered any autologous or prosthetic breast reconstruction, provided that the mastectomy skin flap was conserved.

We excluded studies that only assessed the diagnostic value of ICGA for assessment of mastectomy skin vascularisation.

Types of outcome measures

Primary outcomes

1. Postoperative mastectomy skin flap necrosis (MSFN): we collected incidence rates.

Secondary outcomes

1. Reoperation (specified with reason for return to operating room) within 30 days after surgery;
2. Autologous flap necrosis, requiring surgical debridement with additional wound care, or reoperation within 30 days after surgery;
3. Dehiscence (e.g. wound rupture) of the incision, requiring wound care within 30 days after surgery;
4. Infection of the skin flap or implant, requiring antibiotic therapy (intravenous or oral) within 30 days after surgery;
5. Haematoma requiring surgical evacuation under general anaesthesia within 30 days after surgery;
6. Seroma requiring drainage (radiologically or surgically) within 30 days after surgery;
7. Patient-related outcomes (i.e., quality of life, participant satisfaction).

Search methods for identification of studies

Electronic searches

We searched the following databases on 8 April 2019:

1. The Cochrane Breast Cancer Group's (CBCG's) Specialised Register. Details of the search strategies used by the Group for the identification of studies, and the procedure used to code references are outlined on the Group's website (breastcancer.cochrane.org/sites/ breastcancer.cochrane.org/files/public/uploads/specialised_register_details.pdf). We extracted trials with the key words "indocyanine green", "ICG", "angiography", "infrared", "mastectomy", "postmastectomy" and considered them for inclusion in the review;
2. CENTRAL (The Cochrane Library, Issue 3, 2019). See Appendix 1;
3. MEDLINE Ovid (1946 to 8 April 2019). See Appendix 2;
4. Embase Ovid (1974 to 8 April 2019). See Appendix 3;
5. The WHO International Clinical Trials Registry Platform (ICTRP) search portal (apps.who.int/trialsearch/Default.aspx) for all prospectively registered and ongoing trials. See Appendix 4;
6. Clinicaltrials.gov (clinicaltrials.gov/). See Appendix 5.

Searching other resources

Two review authors (TP, RS) independently tried to identify further studies by reviewing the reference lists of articles retrieved by the search and the reference lists of current treatment guidelines. We obtained a copy of the full text for each reference reporting a potentially

eligible study. When this was not possible, we attempted to contact the study authors to request additional information.

Data collection and analysis

Selection of studies

Two review authors (TP, RS) independently screened the titles and abstracts retrieved by the search for eligibility for this review. We obtained the full texts for each citation reporting a potentially eligible study. Then, the aforementioned two review authors independently examined these full-text articles to exclude studies that did not meet the inclusion criteria. We did not impose language restrictions. Correspondence with the study investigators to clarify study eligibility was not required. Any disagreement about study eligibility was resolved after discussion with a third review author (SQ). We documented the study selection process in a flow chart, as recommended by the PRISMA statement, showing the number of included and excluded studies.[25]

Data extraction and management

Two review authors (TP, RS) independently extracted data for each included study using a predefined standardised data extraction form. The authors conducted pilot testing twice to identify data that were missing from the form and achieved consensus before modifying the form. The data extraction form contained details of the trial (i.e., first author, year of publication, journal, publication status, period and country of study, study design, sample size), participant characteristics (age, sex, body mass index, stage of disease, type of surgery, unilateral or bilateral reconstruction, mastectomy breast weight, tissue expander fill, and prior treatment status), details of the intervention, duration of follow-up, primary and secondary outcomes, and the definition of MSFN. We tried to collect adjusted effect estimates for primary and secondary outcomes. Since no adjusted effect measures were available, we extracted crude measures.

Any discrepancies regarding data extraction were resolved by discussion with a third review author (SQ). The most complete data set feasible was assembled by three review authors (TP, RS, SQ). For studies with more than one report, we planned to use the main report as the reference, and to derive additional details from the secondary papers. However, this was not necessary. We contacted study investigators to acquire any data of relevance that were not fully reported or unclear in the published manuscript.

Assessment of risk of bias in included studies

Two review authors (TP, SK) independently assessed the risk of bias for each eligible nonrandomised study using the Newcastle-Ottawa Scale (NOS), as described in

the *Cochrane Handbook for Systematic Reviews of Interventions*.[26] Any disagreement was resolved by discussion with a third review author (RS). The NOS is a tool for quality assessment of nonrandomised studies. It consists of a 'star system', in which a study is judged on three perspectives, including the selection of the study groups, the comparability between groups, and the ascertainment of outcomes of interest for cohort studies.[27] See Appendix 6.

Measures of treatment effect

We reported differences in dichotomous outcomes (e.g. mild mastectomy skin flap necrosis) as risk ratios (RRs), since only cohort studies were included in the review. A 95% confidence interval (CI) for each study was reported. We interpreted the effect measures as the following:

1. Effect measure > 1: the incidence in the intervention group is greater than in the control group;
2. Effect measure = 1: no difference between the intervention group and control group;
3. Effect measure < 1: the incidence in the intervention group is lower than in the control group.

We pooled the data in a meta-analysis using the pooled RR.

We planned to report the mean difference (MD) with 95% CI as the effect measure for continuous outcomes, if studies reported the exact same outcomes. If similar outcomes were reported on different scales, we planned to reported the standardised mean difference (SMD) with 95% CI. However, no continuous outcome data were collected for this review.

For ICGA protocols, we summarised the description in the 'ICGA protocols' section as we did not undertake a valid analysis.

Unit of analysis issues

It was likely that trials included women with unilateral and bilateral breast reconstructions following skin-sparing mastectomy. In the protocol, we noted that multiple observations per woman could be observed, when multiple body parts (i.e., two breasts) received the same intervention. Therefore, we analysed those outcomes that were not dependent on the other breast (such as mastectomy skin flap necrosis, reoperation, autologous flap necrosis, dehiscence, infection of the skin flap or implant, haematoma and seroma) on a per breast basis when this was possible, given the data described in the studies.

Dealing with missing data

In the case of missing data, we contacted the original investigators via email and gave them three weeks to reply to the request. We recorded the date of contact. We discussed missing data in the 'Discussion' section of the review.

Assessment of heterogeneity

We assessed between-study heterogeneity by reviewing clinical and statistical heterogeneity. We assessed clinical heterogeneity by checking the characteristics of the studies, types of participants, interventions, and outcomes. We evaluated statistical heterogeneity of treatment effects using the Cochran's Q test, with significance set at $P < 0.01$. We used the I^2 statistic as an approximate guide to interpret the magnitude of heterogeneity. We have interpreted the I^2 as follows[26]:

1. 0% to 40%, might not be important;
2. 30% to 60%, may represent moderate heterogeneity;
3. 50% to 90%, may represent substantial heterogeneity;
4. 75% to 100%, considerable heterogeneity.

We used forest plots to show the original results of the included studies and the variation in the results of the studies. We planned to study heterogeneity by performing a moderator analysis. However, we decided not to perform moderator analysis due to the low number of studies included in this review and absence of considerable heterogeneity. Although this was mentioned in the review protocol, the choice between a fixed-effect and a random-effect meta-analysis was not made on the basis of the statistical test for heterogeneity. See Data synthesis.

Assessment of reporting biases

Reporting biases arise when the dissemination of the research findings is influenced by the nature and direction of results. Reporting bias can be classified into publication bias, time lag bias, multiple publication bias, location bias, citation bias, language bias, and outcome bias. We planned to explore potential publication bias by generating a funnel plot, and using a statistical test for publication bias using Egger's test if there were at least 10 studies identified. In this review, we included nine studies and a funnel plot was not generated.[26]

In order to minimise reporting biases, we did not place any restrictions on language. We planned to include data from studies with multiple publications only once in the review when these were identified. In this review, no studies with multiple publications were included.

Data synthesis

Two review authors (TP, SK) analysed the extracted data according to the recommendations of the *Cochrane Handbook for Systematic Reviews of Interventions*[26] using Review Manager 5.[28] We presented meta-analyses of outcome data, concerning data per patient and per breast.

The decision to pool data in a meta-analysis depends on the availability of outcome data and the assessment of between-trial heterogeneity. We did not pool data where heterogeneity was ≥ 75%. The decision to perform a fixed-effect or random-effect meta-analysis was based on the expectation of whether the intervention effects were truly identical, preferring a fixed-effect model (Mantel-Haenszel) if this was likely and the random-effect model[29] if this was unlikely.[30]

For all outcomes (i.e., dichotomous outcomes), we presented the summary estimate as RR with 95% CI.

We presented a narrative overview of the included trials in order to summarise ICGA protocols for assessment of mastectomy skin flap perfusion in women undergoing immediate breast reconstructions following skin-sparing mastectomy.

'Summary of findings' table

Two review authors (TP and SK) independently applied methods following the GRADE approach to assess the certainty of the evidence associated with specific outcomes.[31] A third review author (RS) resolved any disputes.

We planned to construct two ‘Summary of findings’ tables to illustrate the main outcomes for randomised and nonrandomised studies separately. In this review, only nonrandomised studies were included. However, two 'Summary of findings' table were constructed using the GRADEproGDT software [32]: one 'Summary of findings' table for complications reported on a per patient basis and one 'Summary of findings' table for complications reported on a per breast basis.

The GRADE approach appraises the certainty of a body of evidence for each outcome, based on five domains: (1) limitations of detailed design and execution of the included studies (i.e., risk of bias criteria), (2) inconsistency of results across studies (i.e., heterogeneity), (3) indirectness (i.e., applicability), (4) imprecision (i.e., number of events and confidence intervals), and (5) publication bias. We rated the certainty of the evidence for each outcome as high, moderate, low, or very low. We justified all decisions in footnotes.

We listed the following six outcomes of importance in 'Summary of findings for the main comparison 1' and 'Summary of findings 2' and assessed the certainty of the evidence:

1. Postoperative mastectomy skin flap necrosis;
2. Reoperation within 30 days after surgery;
3. Dehiscence of the incision within 30 days after surgery;
4. Infection of the skin flap or implant within 30 days;
5. Haematoma within 30 days after surgery;
6. Seroma within 30 days after surgery.

Subgroup analysis and investigation of heterogeneity

If there were sufficient trials of adequate size, it may have been possible to conduct subgroup analyses. The ability to conduct subgroup analysis also depended on whether or not the required information was retrieved from the included trials. We considered performing subgroup analyses using the following characteristics:

1. Current smoking status (yes versus no);
2. Diabetes mellitus (yes versus no);
3. BMI (under 30 kg/m^2; 30 kg/m^2 and over);
4. Mastectomy breast weight (0 to 500 grams; 501 to 1000 grams; over 1000 grams);
5. Previous exposure to radiotherapy (yes versus no);
6. Tumescent mastectomy technique (yes versus no);
7. Wise-pattern mastectomy incision (yes versus no).

Subgroup analysis was not performed in this review because the required information was not available in the included studies.

Sensitivity analysis

We planned to perform sensitivity analysis for the primary outcome by comparing results with or without studies of high methodological quality (i.e., low risk of bias), if a sufficient number of high-quality studies were identified. When we would have obtained similar results and drawn similar conclusions from the analysis, the results of the review would have been regarded with a higher degree of certainty.

Sensitivity analysis was not performed in this review because only studies with a low methodological quality (i.e., high risk of bias) were identified.

RESULTS

Description of studies

Results of the search

The search was conducted in April 2019. We found 232 records from the following databases: Cochrane Breast Cancer Group's (CBCG's) Specialised Register (8), CENTRAL (5), MEDLINE (79), Embase (133), WHO ICTRP (7), Clinicaltrial.gov (0). After removing duplicate records, we screened the titles and abstracts of 167 records.

An additional search across references of published studies resulted in no new records.

After screening the titles and abstracts, we identified 18 potentially eligible articles. We obtained a copy of the full text for the 18 articles. We excluded a total of nine studies, because they did not meet the inclusion criteria.[16,19,33-39] See Characteristics of excluded studies table. Nine reports contributed to this review.[40-48] No randomised clinical trials were included. In addition, there were no relevant ongoing studies identified from the clinical trial registry searches.

A study flow diagram summarising the study selection process is shown in Figure 1.

Included studies

We included seven nonrandomised, retrospective cohort studies.[40-46] and two nonrandomised, prospective cohort studies.[47-48]

The nine studies included a total of 1589 women with 2199 breast reconstructions, with a mean of 177 women and 244 breast reconstructions in each study. Six studies reported the number of MSFN on a per breast basis for a total of 1435 breasts.[40,43-45,47,48] and three studies reported the number of MSFN on a per patient basis for a total of 573 women.[41,42,46] Regarding the other complications, five studies reported the number of complications on a per breast basis for a total of 1370 breasts.[40,43-45,47] and four studies reported the number of complications on a per patient basis for a total of 613 patients.[41,42,46,48]

Six studies were conducted in the United States of America (USA),[41,42,45-48] two in Denmark[40,44] and one in Japan.[43] Eight studies performed ICGA with the SPY system (LifeCell Corp., Branchburgm, NJ)[40-42,44-48] and one study used the Photodynamic Eye imaging system (PDE; Hamamatsu Photonics, K.K., Hamamatsu, Japan).[43]

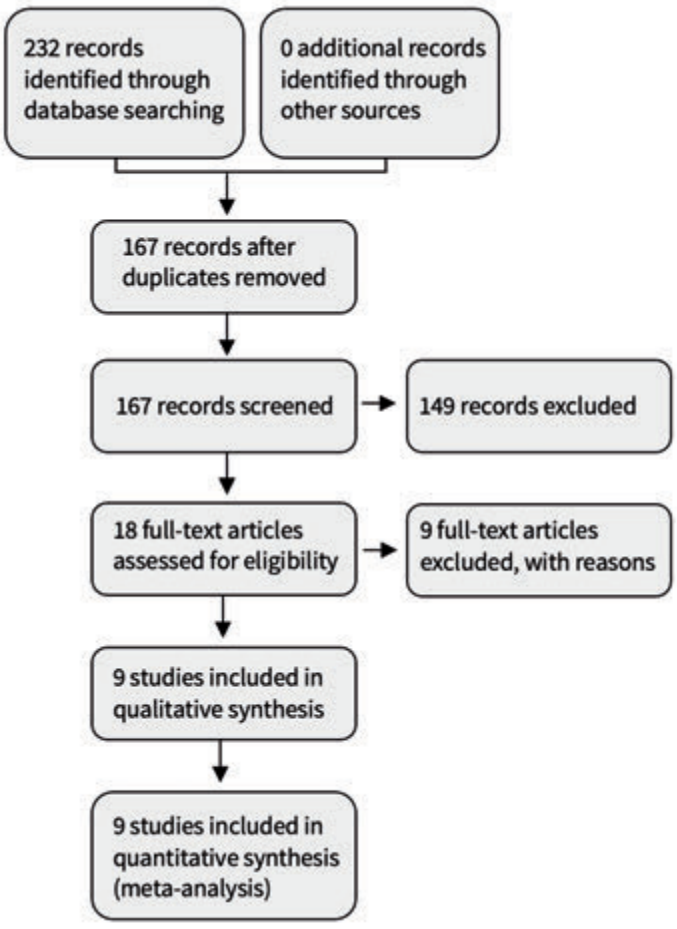

Figure 1. Study flow diagram.

Summaries of the nine studies are given below. All included studies compared the use of ICGA with clinical evaluation to assess mastectomy skin vascularisation during breast reconstruction following mastectomy. For further details, see the Characteristics of included studies tables.

Alstrup et al.[40] conducted a single-institution retrospective review and included 191 women (mean age 51 years) who underwent immediate or delayed, unilateral or bilateral pedicled autologous flap breast reconstructions in Denmark. Events occurring within six months of the initial procedure were evaluated. Complications were recorded on a per breast basis.

Diep et al.[41] conducted a single-institution retrospective review and included 114 women (no mean age reported) who underwent a total of 145 unilateral or bilateral mastectomies

for invasive breast cancer, carcinoma in situ, or prophylaxis in Minneapolis, USA. Events occurring within 90 days of the initial procedure were evaluated. Complications were recorded on a per patient basis.

Duggal et al.[42] conducted a retrospective review and included 368 consecutive women (mean age 50 years) who underwent a total of 477 skin-sparing mastectomies and breast reconstructions in Atlanta, USA. Mean follow-up was 8.3 months in the ICGA group and 24.7 months in the clinical group. Complications were recorded on a per patient basis.

Gorai et al.[43] conducted a single-institution retrospective review and included 181 women (mean age not reported) who underwent a total of 184 total mastectomies and 1-stage immediate breast reconstructions in Japan. Mean follow-up was not reported. Complications were recorded on a per breast basis.

Hammer-Hansen et al.[44] conducted a single-institution retrospective review and included 92 women (mean age 44.6 years in the ICGA group and 46.7 years in the clinical group) who underwent a total of 128 implant-based skin-sparing immediate breast reconstructions with a porcine acellular dermal matrix (ADM) in Denmark. The follow-up period was predetermined to be 90 days postoperatively. Complications were recorded on a per breast basis.

Harless et al.[45] conducted a single-institution retrospective review and included 269 consecutive women (mean age 50 years in the ICGA group and 48 years in the clinical group) who underwent a total of 467 implant-based breast reconstructions in Minnesota, USA. Mean follow-up was 4.6 months in the ICGA group and 16.9 months in the clinical group. Complications were recorded on a per breast basis.

Sood et al.[46] conducted a single-institution retrospective review and included 91 women (mean age of 51 in the ICGA group and 52 in the clinical group) who underwent a total of 142 immediate breast reconstructions with prostheses following skin-sparing mastectomies in New Jersey, USA. Reconstructions with tissue expander and/or implants were included. All reconstructions were performed using human acellular dermal matris (HADM). Information on follow-up period for each treatment group was not provided. Complications were recorded on a per breast basis.

Mirhaidari et al.[47] compared complications in a two-institution cohort (ICGA group) to a retrospective cohort (clinical group) and included a total of 243 women (mean age 51.4 years in the ICGA group and 52.8 years in the clinical group) who underwent a total of 400

breast reconstructions in Ohio, USA. Breast reconstruction was performed either as a direct to implant procedure, a tissue expander, or autologous reconstruction. The ICGA group consisted of 126 women who underwent 206 immediate breast reconstructions and the clinical group consisted of 117 women who underwent 194 breast reconstructions. ADM was used in every case of implant-based reconstruction. Participants in the prospective study were followed for 90 days to document complications. Information on follow-up period for each treatment group was not provided. Complications were recorded on a per breast basis.

Rinker et al.[48] compared complications in a single-institution prospective cohort (ICGA group) to a retrospective cohort and included a total of 60 women who underwent a total of 99 skin-sparing mastectomies and immediate reconstructions in Kentucky, USA. Breast reconstruction was performed with either tissue expanders or muscle-sparing free transverse rectus abdominis musculocutaneous (TRAM) flaps. Mastecomy skin flap viability was assessed either visually (20 women, 30 breasts), with fluorescein dye and Wood's lamp imaging (20 women, 34 breasts), or by ICGA (20 women, 35 breasts). For this review, only women in the clinical group and ICGA group are included. The mean age was 47.2 years in the ICGA group and 50.4 years in the clinical group. Women were followed for a minimum of six months. Mean follow-up was 10 months. The complication rate for mastectomy flap necrosis was recorded on a per breast basis. The complication rate for other complications was recorded on a per patient basis.

Excluded studies

We excluded eight studies because they used a study design different from our inclusion criteria[33, 35-39,18-19] and one study because it did not report any of the predefined study outcomes[34] (see 'Characteristics of excluded studies' table).

Risk of bias in included studies

Since no RCTs were included in this review, the Cochrane 'Risk of bias' tool was not applicable. If, in the future, RCTs are identified on this topic, we will use Cochrane's 'Risk of bias' tool. Risk of bias for each included nonrandomised study (NRS) was assessed using the Newcastle-Ottowa Scale (NOS) for cohort studies[27] (see Appendix 6). Summaries of our 'Risk of bias' assessment of the included NRSs, using the NOS, are given below. For the NOS quality assessment presented as stars, see Table 1. The justification for the NOS quality assessment for each included study is provided in the risk of bias tables, see Characteristics of included studies table. However, please ignore the 'Authors' judgement' column listed as 'unclear' in all of the risk of bias tables as this column could not be removed and is not applicable to the NOS. Information relating to each assessment using the Newcastle-Ottawa Scale is provided below.

Table 1. Newcastle-Ottawa Scale

Study	Selection				Comparability	Outcomes		
	Representative of the exposed cohort	Selection of the non-exposed cohort	Exposure ascertainment	Outcome not present at start	Comparability of cohorts	Assessment	Follow-up length	Follow-up adequacy
Alstrup et al. (2018)[40]	★	★	★	★	★★	★	★	★
Diep et al. (2016)[41]	★	★	-	★	-	-	★	-
Duggal et al.(2014)[42]	★	★	-	★	-	-	★	-
Gorai et al. (2017)[43]	★	★	-	★	-	-	-	-
Hammer-Hansen et al. (2017)[44]	★	★	★	★	-	★	★	★
Harless et al. (2016)[45]	★	★	★	★	-	★	★	-
Mirhaidari et al. (2018)[47]	★	★	-	★	-	-	★	-
Rinker et al. (2016)[48]	★	★	★	★	★★	★	★	-
Sood et al. (2013)[46]	★	★	-	★	-	-	-	-

-: study did not report information relating to this outcome

Selection

Representativeness of the exposed cohort

All nine studies scored one star (out of one). Seven studies selected a truly representative cohort, including participants who underwent immediate autologous or prosthetic reconstructive surgery following skin-sparing mastectomy[40-42,44-46,48] Two studies selected a somewhat representative cohort, one study including participants who underwent immediate prosthetic reconstructive surgery following total mastectomy[43] and one study including participants who underwent immediate breast reconstruction following nipple-sparing and nipple-sacrificing mastectomies.[47]

Selection of the non-exposed cohort

All nine studies acquired one star (out of one) since all studies selected a truly or somewhat representative cohort, including participants undergoing similar breast reconstructions compared to the exposed cohort.

Ascertainment of exposure

Four studies acquired one star (out of one).[40,44,45,48] Alstrup et al.[40], Hammer-Hansen et al.[44] and Harless et al.[45] extracted data from patient medical records and Rinker et al.[43] reported collecting data from personnel interviews and reviews of the patient's chart. The other five studies acquired no stars since no information on ascertainment of exposure had been described.[41-43,46,46]

Demonstration that outcome of interest was not present at start of study

All studies acquired one star (out of one) because the outcomes of interest did not occur prior to a breast operation.

Comparability

Comparability of cohorts based on the design or analysis controlled for confounders

Seven studies acquired no stars for comparability of cohorts (out of a maximum of 2 stars) because no information on the analysis to control for confounders was reported.[41-45,46,47] See the Risk of bias in included studies table for more information. Two studies acquired two stars. Alstrup et al.[40] performed a logistic regression analysis using total and major complications, as dependent variables. The primary independent variables were ICGA assessment and timing of reconstruction. The analysis also included age, body mass index (BMI), prior smoking, chemotherapy, radiation, diabetes and hypertension. Rinker et al.[48] compared participant factors for homogeneity, analysed variation across the groups and performed a multivariable regression analysis to identify independent predictors for mastectomy skin flap necrosis.

Outcomes

Assessment of outcome

None of the studies reported independent blind assessment of outcomes. Four studies acquired one star (out of one) since outcome assessment was done by extracting data from medical records.[40,44,45,48] The other five studies acquired no stars, since no information on the assessment of outcomes was provided.[41-43,46,47]

Was follow-up long enough for outcomes to occur?

Six studies acquired one star (out of one). The reported time from the initial procedure to the evaluation of occurrence of complications ranged from 90 days in three studies[41,44,47] to six months in two studies.[40,46] In two studies, no exact time for outcome assessment was reported. However, the minimum follow-up time of one month[45] and a reported follow-up period of 8.3 months[42] were considered long enough for outcomes to occur. Two studies acquired no stars because no information on the time for outcome assessment (follow-up) was reported.[43,46]

Adequacy of follow-up of cohorts

Two studies acquired one star (out of one): Alstrup et al.[40] reported that complication data were available six months after surgery for 191 of 230 enrolled cases (83%) and Hammer-Hansen et al.[44] explicitly reported that there were no missing data for the included participants. The other seven studies reported no statement concerning the completeness of follow-up (missing data).[41-43,45-48]

Effects of interventions

See: **Summary of findings for the main comparison 1:** ICGA compared to clinical evaluation for preventing complications (on a per patient basis), **Summary of findings 2:** ICGA compared to clinical evaluation for preventing complications (on a per breast basis).

GRADE Working Group grades of evidence (in Summary of findings tables)

High certainty:	We are very confident that the true effect lies close to that of the estimate of the effect
Moderate certainty:	We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low certainty:	Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low certainty:	We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

SUMMARY OF FINDINGS

Table 2. Summary of findings for the main comparison (per patient basis).
Indocyanine Green Angiography (ICGA) compared to clinical evaluation for preventing complications (on a per patient basis) in immediate breast reconstruction following mastectomy.

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with clinical evaluation	Risk with Indocyanine Green Angiography (ICGA)				
Mastectomy skin flap necrosis (number of women with mastectomy skin flap necrosis within 30 days after surgery) Mean follow-up: ICGA: range 90 days to 8.3 months, clinical: range 90 days to 24.7 months	Study population 215 per 1.000	177 per 1.000 (98 to 299)	RR 0.79 (0.40 to 1.56)	573 (3 studies)	⊕⊕⊕⊖ VERY LOW [1,2]	The mean follow-up range was based on all studies except for Sood 2013.
Reoperation (number of women that were operated again within 30 days after surgery) Mean follow-up: ICGA: range 90 days to 10 months, clinical: range 90 days to 24.7 months	Study population 239 per 1.000	136 per 1.000 (99 to 185)	RR 0.50 (0.35 to 0.72)	613 (4 studies)	⊕⊕⊕⊖ VERY LOW [1,2]	The mean follow-up range was based on all studies except for Sood 2013.
Dehiscence (number of women with dehiscence within 30 days after surgery). Mean follow-up: ICGA: 8.3 months, clinical: 24.7 months	5 per 1000	22 per 1000 (2 to 193)	RR 4.00 (0.45 to 35.45)	368 (1 study)	⊕⊕⊕⊖ VERY LOW [1,2]	Dehiscence rate was only reported by Duggal 2014.

(Continued)

[1] We downgraded twice based on the nonrandomised characteristics of the studies and low comparability of the cohorts.

[2] We downgraded twice based on the small sample sizes and low number of events.

Infection (number of women with infection of the skin flap or implant within 30 days after surgery) Mean follow-up: ICGA: range 90 days to 10 months, clinical: range 90 days to 24.7 months	Study population		RR 0.91 (0.60 to 1.40)	613 (4 studies)	⊕⊕⊖⊖ VERY LOW[1,2]	The mean follow-up range was based on all studies except for Sood 2013.
	120 per 1.000	110 per 1.000 (75 to 118)				
Haematoma (number of women with haematoma within 30 days after surgery) Mean follow-up: ICGA: 8.3 months, clinical: 24.7 months	Study population		RR 0.87 (0.39 to 2.53)	459 (2 studies)	⊕⊕⊖⊖ VERY LOW[1,2]	The mean follow-up range was only based on Duggal 2014.
	26 per 1.000	23 per 1.000 (8 to 63)				
Seroma (number of women with seroma within 30 days after surgery) Mean follow-up: ICGA: range 8.3 to 10 months, clinical group: range 10 to 24.7 months	Study population		RR 1.68 (0.41 to 6.86)	408 (2 studies)	⊕⊕⊖⊖ VERY LOW[1,2]	
	88 per 1.000	149 per 1.000 (38 to 397)				

The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio.

Table 3. Summary of findings 2 (per breast basis).

Indocyanine Green Angiography (ICGA) compared to clinical evaluation for preventing complications (on a per breast basis) in immediate breast reconstruction following mastectomy.

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of breasts (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with clinical evaluation	Risk with Indocyanine Green Angiography (ICGA)				
Mastectomy skin flap necrosis (number of breasts with mastectomy skin flap necrosis within 30 days after surgery) Mean follow-up: ICGA: range 90 days to 10 months, clinical: range 90 days to 16.9 months	Study population 171 per 1.000	113 per 1.000 (90 to 145)	RR 0.62 (0.48 to 0.82)	1435 (6 studies)	⊕⊖⊖⊖ VERY LOW [1,2]	The mean follow-up range was based on all studies except for Gorai 2017.
Reoperation (number of breasts that needed to be operated again within 30 days after surgery) Mean follow-up: ICGA: range 90 days to 6 months, clinical: range 90 days to 16.9 months	Study population 190 per 1.000	133 per 1.000 (99 to 162)	RR 0.65 (0.47 to 0.92)	1370 (5 studies)	⊕⊖⊖⊖ VERY LOW [1,2]	The mean follow-up range was based on all studies except for Gorai 2017.
Dehiscence - not reported	-	-	-	-	-	Dehiscence rates were not reported in the included studies on a per breast basis.

(Continued)

[1] We downgraded twice based on the nonrandomised characteristics of the studies and low comparability of the cohorts.

[2] We downgraded twice based on the small sample sizes and low number of events.

Infection (number of breasts with infection within 30 days after surgery) Mean follow-up: ICGA: range 90 days to 6 months, clinical: range 90 days to 16.9 months	Study population		RR 0.65 (0.44 to 0.97)	1370 (5 studies)	⊕⊖⊖⊖ VERY LOW [1,2]	The mean follow-up range was based on all studies except for Gorai 2017.
	80 per 1.000	53 per 1.000 (37 to 78)				
Haematoma (number of breasts with haematoma within 30 days after surgery) Mean follow-up: ICGA: range 90 days to 6 months, clinical: range 90 days to 16.9 months	Study population		RR 1.53 (0.47 to 4.95)	1042 (4 studies)	⊕⊖⊖⊖ VERY LOW [1,2]	Haematoma rates were only reported for a subgroup of participants (immediate reconstructions: 47/191 breast) in Alstrup 2018.
	22 per 1.000	34 per 1.000 (11 to 102)				
Seroma (number of breast with seroma within 30 days after surgery) Follow-up: 90 days	Study population		RR 0.71 (0.37 to 1.35)	528 (2 studies)	⊕⊖⊖⊖ VERY LOW [1,2]	Events occurring within 90 days following operation were considered as complications.
	78 per 1.000	57 per 1.000 (30 to 103)				

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio.

ICGA versus clinical evaluation for the prevention of complications

The first objective of this review was to assess the effects of indocyanine green angiography (ICGA) for preventing mastectomy skin flap necrosis (together with other outcomes) in women undergoing immediate breast reconstruction following skin-sparing mastectomy. Since some studies reported complications on a per patient basis and other studies reported complications on a per breast basis, we decided to present and pool data separately. See Included studies section. In addition, separate 'Summary of Findings' tables were developed: 'Summary of findings Table for the main comparison 1' for ICGA compared to clinical evaluation for preventing complications in immediate breast reconstruction following mastectomy, on a per patient basis; 'Summary of findings 2' for ICGA compared to clinical evaluation for preventing complications in immediate breast reconstruction following mastectomy, on a per breast basis.

For all of the outcomes described below, there was serious concern with the risk of bias due to the study designs used by all of the included studies (that is, cohort studies) and the majority of studies described cohorts of low comparability. There was no concern relating to inconsistency or indirectness, but we downgraded the quality of evidence twice for imprecision based on the small sample sizes and low number of events in the included studies. We did not suspect a high probability of publication bias. The quality of the evidence was not upgraded, since the effects were not large, there was no effect of all plausible residual confounding, and there was no presence of a dose-response gradient. Overall, the quality of the evidence was very low.

For all outcomes described below, except for 'dehiscence of the incision' (reported in only one study), we decided to perform a random-effect meta-analysis, since we expected that the intervention effects across the included studies were unlikely to be truly identical.

Postoperative mastectomy skin flap necrosis (MSFN)

We are uncertain about the effect of ICGA on MSFN rates on a per patient basis. On a per breast basis, ICGA may reduce MSFN rates. See table 4.

Table 4. Comparison 1 Mastectomy skin flap necrosis

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Per patient	3	573	Risk Ratio (M-H, Random, 95% CI)	0.79 [0.40, 1.56]
2 Per breast	6	1435	Risk Ratio (M-H, Random, 95% CI)	0.62 [0.48, 0.82]

Per patient basis: we are uncertain about the effect of ICGA on MSFN given the very low quality of evidence. Based on three studies, the pooled analysis reported: RR 0.79 (95% CI 0.40 to 1.56; 573 participants; very low quality of evidence; Figure 2). The I^2 was 64%, that may represent substantial heterogeneity.

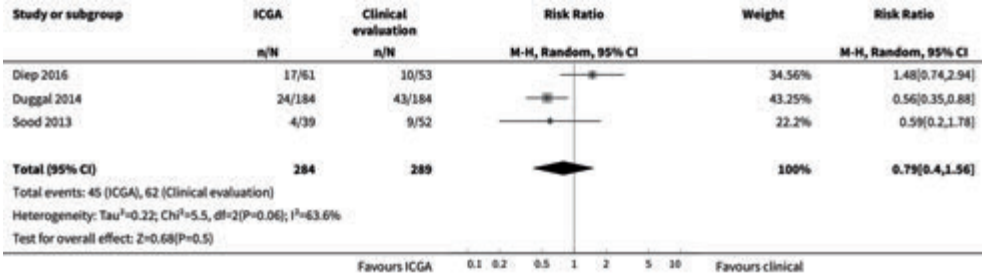

Figure 2. Comparison 1 Mastectomy skin flap necrosis, Outcome 1 Per patient.

Per breast basis: ICGA may reduce MSFN compared to clinical evaluation. Based on three studies, the pooled analysis showed RR 0.62 (95% CI 0.48 to 0.82; 1435 breasts, very low quality of evidence; Figure 3). The I^2 was 2%, representing heterogeneity that might not be important.

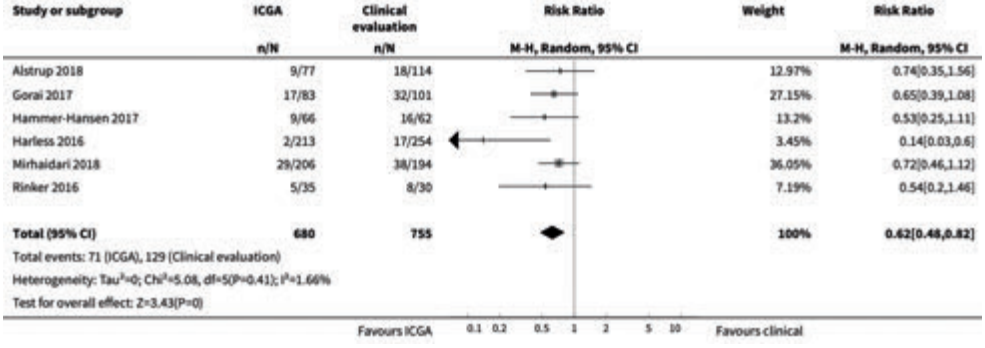

Figure 3. Comparison 1 Mastectomy skin flap necrosis, Outcome 2 Per breast.

Reoperation within 30 days after surgery

On a per patient and per breast basis, ICGA may reduce reoperation rates compared to clinical evaluation. See table 5.

Table 5. Comparison 2 Reoperation.

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Per patient	4	613	Risk Ratio (M-H, Random, 95% CI)	0.50 [0.35, 0.72]
2 Per breast	5	1370	Risk Ratio (M-H, Random, 95% CI)	0.65 [0.47, 0.92]

Per patient basis: ICGA may reduce reoperation rates compared to clinical evaluation. Based on four studies, the pooled analysis showed RR 0.50 (95% CI 0.35 to 0.72; 613 participants; very low quality of evidence; Figure 4). The I^2 was 0%, representing heterogeneity that might not be important.

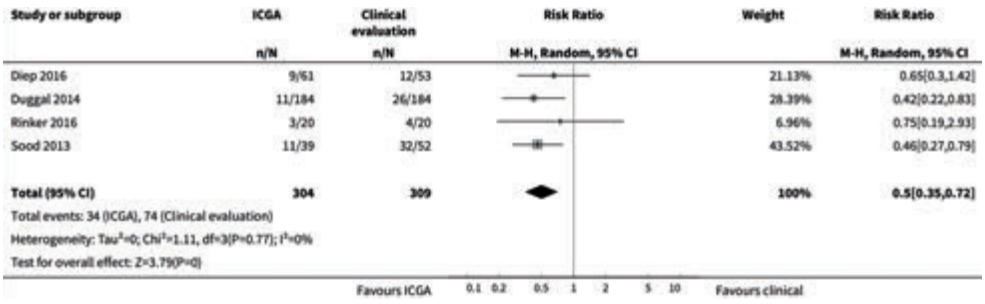

Figure 4. Comparison 2 Reoperation, Outcome 1 Per patient.

Per breast basis: similar to the analysis by per patient basis, ICGA may reduce reoperation rates compared to clinical evaluation. Based on five studies, the pooled analysis showed RR 0.65 (95% CI 0.47 to 0.92; 1370 breasts; very low quality of evidence; Figure 5). The I^2 was 41%, representing moderate heterogeneity.

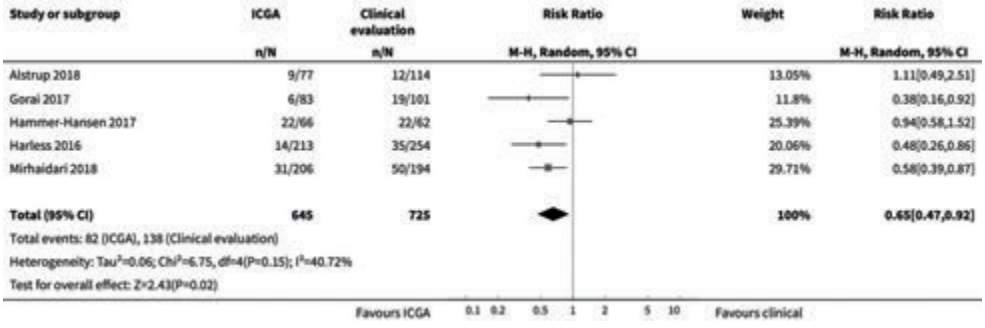

Figure 5. Comparison 2 Reoperation, Outcome 2 Per breast.

Infection of the skin flap or implant, requiring antibiotic therapy within 30 days after surgery

We are uncertain about the effect of ICGA on infection rates on a per patient basis. On a per breast basis, ICGA may reduce infection rates. See table 6.

Table 6. Comparison 3 Infection.

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Per patient	4	613	Risk Ratio (M-H, Random, 95% CI)	0.91 [0.60, 1.40]
2 Per breast	5	1370	Risk Ratio (M-H, Random, 95% CI)	0.65 [0.44, 0.97]

Per patient basis: we are uncertain about the effect of ICGA on infection rates given the very low quality of evidence. The RR was 0.91 (95% CI 0.60 to 1.40; four studies; 613 participants; very low quality of evidence; Figure 6). The I^2 was 0%, representing heterogeneity that might not be important.

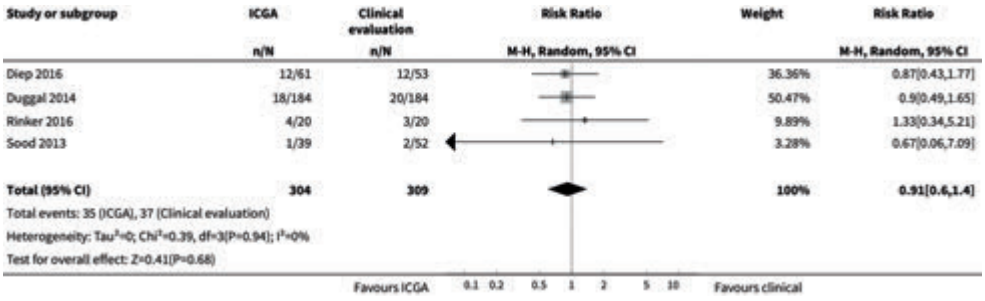

Figure 6. Comparison 3 Infection, Outcome 1 Per patient

Per breast basis: ICGA may reduce infection rates compared to clinical evaluation. The RR was 0.65 (95% CI 0.44 to 0.97; five studies; 1370 breasts; very low quality of evidence; Figure 7). The I^2 was 0%, representing heterogeneity that might not be important.

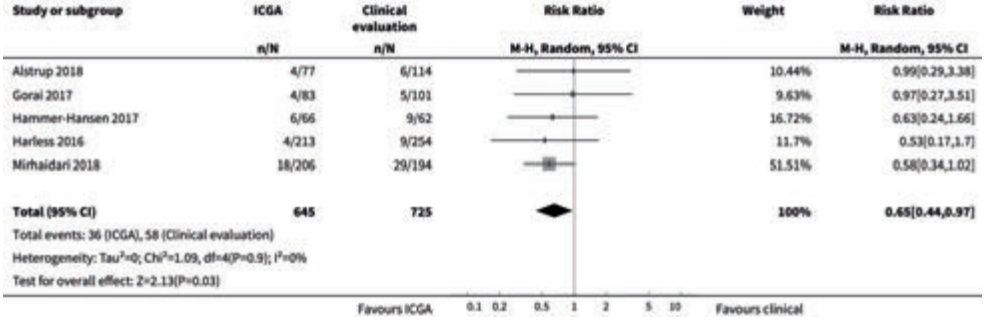

Figure 7. Comparison 3 Infection, Outcome 2 Per breast

Haematoma requiring surgical evacuation under general anaesthesia within 30 days after surgery

We are uncertain of the effect of ICGA on haematoma rates on a per patient and on a per breast basis. See table 7.

Table 7. Comparison 4 Haematoma.

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Per patient	2	459	Risk Ratio (M-H, Random, 95% CI)	0.87 [0.30, 2.53]
2 Per breast	4	1042	Risk Ratio (M-H, Random, 95% CI)	1.53 [0.47, 4.95]

Per patient basis: we are uncertain of the effect of ICGA on haematoma rates given the very low quality of evidence. The RR was 0.87 (95% CI 0.30 to 2.53; two studies; 459 participants; very low quality of evidence; Figure 8). The I^2 was 0%, representing heterogeneity that might not be important.

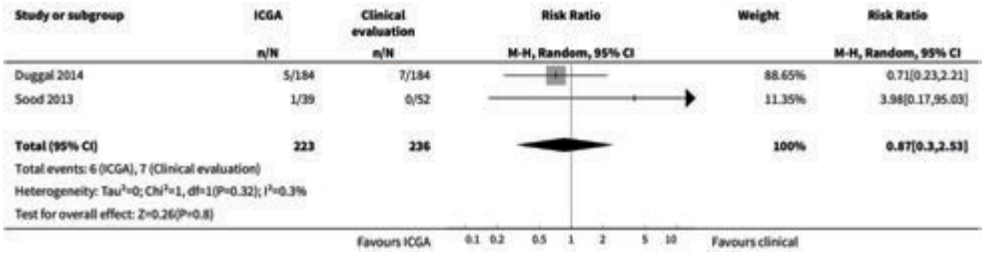

Figure 8. Comparison 4 Haematoma, Outcome 1 Per patient

Per breast basis: similar to the per patient analysis, we are uncertain of the effect of ICGA on haematoma rates (RR 1.53; 95% CI 0.47 to 4.95; four studies; 1042 breasts; very low quality of evidence; Figure 9). In one study, haematoma rates were only reported for a subgroup of participants (immediate reconstructions), representing 47 of the total 191 breasts.[40] The I^2 was 40%, that may represent moderate heterogeneity.

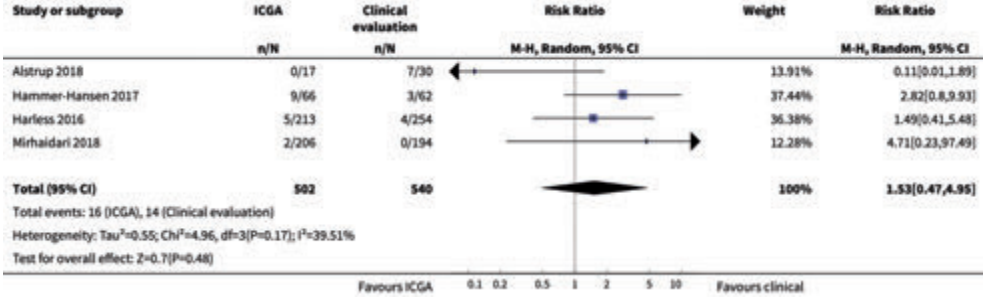

Figure 9. Comparison 4 Haematoma, Outcome 2 Per breast

Seroma requiring drainage within 30 days after surgery

We are uncertain of the effect of ICGA on seroma rates on a per patient and on a per breast basis. See table 8.

Table 8. Comparison 5 Seroma

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Per patient	2	408	Risk Ratio (M-H, Random, 95% CI)	1.68 [0.41, 6.80]
2 Per breast	2	528	Risk Ratio (M-H, Random, 95% CI)	0.71 [0.37, 1.35]

Per patient basis: we are uncertain about the effect of ICGA on seroma rates within 30 days after surgery given the very low quality of evidence. The RR was 1.68 (95% CI 0.41 to 6.80; two studies; 408 participants; very low quality of evidence; Figure 10). The I^2 was 51%, representing substantial heterogeneity.

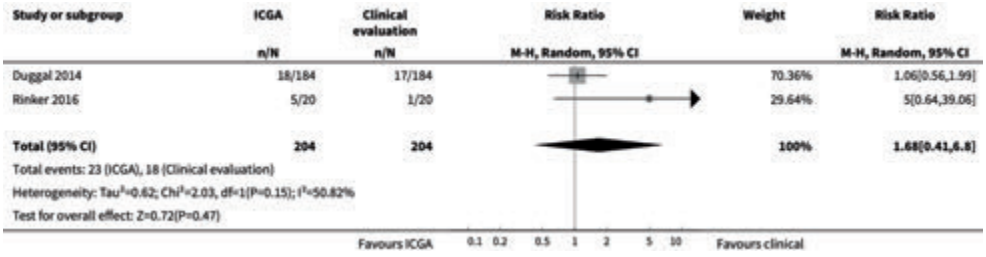

Figure 10. Comparison 5 Seroma, Outcome 1 Per patient

Per breast basis: similar to the per patient analysis, we are uncertain of the effect of ICGA on seroma rates. The RR was 0.71 (95% CI 0.37 to 1.35; two studies; 528 breasts; very low quality of evidence; Figure 11). The I^2 was 0%, representing heterogeneity that may not be important.

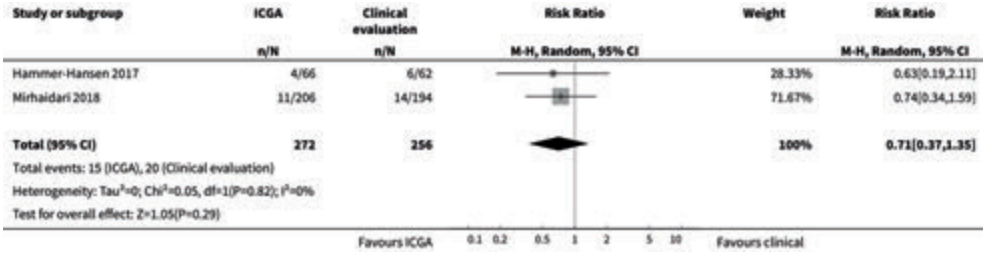

Figure 11. Comparison 5 Seroma, Outcome 2 Per breast

Autologous flap necrosis, requiring surgical debridement with additional wound care or reoperation within 30 days after surgery

Two studies considered autologous flap necrosis as an outcome.[40,42]

Alstrup et al.[40] defined autologous flap necrosis as necrosis of most (that is, two-thirds) of the latissimus dorsi (LD) musculocutaneous flap, muscle-sparing LD flap and the LD flap with or without addition of an implant as well as the transverse rectus abdominal musculocutaneous (TRAM) flap that were used for breast reconstruction. Partial flap loss was observed in 10.4% of breasts in the ICGA group (8/77 breasts) compared to 7% in the clinical group (8/114 breasts; P=0.435).

Duggal et al.[42] reported total flap loss for TRAM flaps, although LD flaps and deep inferior epigastric perforator (DIEP) flaps were also performed for breast reconstruction. Total TRAM flap loss was observed in 1.4% of participants in the ICGA group (1/71 TRAM flaps) compared to 3.4% of participants in the clinical group (2/59 TRAM flaps; *P*=0.237).

Dehiscence of the incision, requiring wound care within 30 days after surgery

One study considered dehiscence as an outcome. Duggal et al.[42] defined assumed dehiscence to be related to ischaemia of the mastectomy skin flap. Per patient basis: we are uncertain about the effect of ICGA on infection rates given the very low quality of evidence. Dehiscence was observed in 2.2% of participants in the ICGA group (4/184) compared to 0.5% of participants in the clinical group (1/184; *P*=0.372). The RR was 4.00 (95% CI 0.45 to 35.45; one study; 368 participants; very low quality of evidence).

Patient-related outcomes

None of the studies reported patient-related outcomes.

ICGA protocols

The second objective of this review was to summarise the different ICGA protocols available for assessment of mastectomy skin flap perfusion in women undergoing immediate breast reconstructions following skin-sparing mastectomy. The aim of this summary was to present data on the ICGA system, dose of ICG and methods for mastectomy-skin flap perfusion used in the included studies, including timing of ICGA assessment during the breast reconstruction, timing from ICGA injection to assessment and the period of time of assessment, debridement of mastectomy skin flap tissue and other factors that were taken into account during ICGA assessment.

ICGA system

Eight studies used the SPY System (SPY Imaging: LifeCell Corp, Branchburg, NJ) to assess mastectomy skin flap perfusion.[40-42,44-48] One study used the Photodynamic Eye imaging system (PDE; Hamamatsu Photonics, K.K., Hamamatsu, Japan).[43]

ICG dose

Five studies reported an ICG dose. Two studies reported a dose of 7.5 mg of ICG,[40,47] two studies reported a dose of 25 mg of ICG.[43,48] Rinker et al.[48] reported a 10 mL bolus of ICG (25 mg/mL), which reflects a total dose of 250 mg. Since we expected the dose to be 25 mg, we checked this information with the study investigators. They confirmed that an ICG dose of 25 mg was used. One study reported a 3 cc dose of ICG without mentioning the concentration (mg/mL).[46] Four studies reported no ICG dose.[41,42,44,45]

Method: timing of ICGA assessment during breast reconstruction

Gorai et al.[43] performed ICGA assessment before closure of the skin (e.g. at the end of reconstruction). Hammer-Hansen et al.[44] performed ICGA assessment for a minimum of three times during breast reconstruction. The first evaluation was performed after mastectomy, the second evaluation was performed with an implant sizer and the third evaluation was performed after the implant had been placed and the dermis of the mastectomy flaps had been sutured. Harless et al.[45] performed ICGA assessment twice; once after mastectomy and once following placement of the implant or expander (with or without expansion). Mirhaidari et al.[47] performed ICGA assessment after temporarily closing the skin after placement of a tissue expander or implant (the expander was inflated to surgeon's estimated desired postprocedural volume). After excision of mastectomy skin flap tissue or removal of tissue expander volume, a second ICGA assessment was performed. Rinker et al.[48] performed ICGA assessment once after completion of the mastectomy. Four studies did not describe the timing of ICGA assessment.[40-42,46]

Method: timing from ICGA injection to assessment and the period of time of assessment

Four studies reported the timing from ICGA injection to assessment and the period of time for assessment.[40,43,47,48] Alstrup et al.[40] described recording for a period of two minutes, directly upon injection of ICG into a peripheral venous line. Gorai et al.[43] described starting the measurement when the monitor showed the first pass of ICG into the breast skin. Then, images were recorded for a period of two minutes. Mirhaidari et al.[47] reported that ICG was injected and after a 3-minute latency period, images of the mastectomy skin flap were taken. Rinker et al.[48] imaged the skin over a 1-minute interval after ICG was injected through the participant's peripheral intravenous line. Five studies did not report timing from ICGA injection to assessment and the period of time of assessment.[41,42,44-46]

Method: tissue debridement

Two studies reported using a relative perfusion assessment (i.e., percentage of perfusion).[40,44] Alstrup et al.[40] reported that skin flap perfusion was measured as the intensity of fluorescence, scored and consequently defined as a relative measurement of a known well-perfused area outside the surgical field, which was set to 100% perfusion (i.e., lower sternal border). Poorly perfused areas of the flap, defined as perfusion below 33%, were excised. Hammer-Hansen et al.[44] reported that areas showing perfusion below 33% were excised. However, when excision was not possible due to the extent of the diminished perfusion, the surgeon converted the reconstruction to a two-staged procedure using a tissue expander.

Seven studies did not report a method of perfusion assessment.[41-43,45-48] Diep et al.[41] reported that ischaemic tissue was resected when ischaemia 'was noted' on ICGA. Duggal et al.[42] reported debridement of mastectomy skin flaps based on a combination of clinical assessment and SPY perfusion analysis, without explanation of the method of analysis. Gorai et al.[43] reported that the non-enhanced areas were marked according to the image on the monitor. Before trimming the mastectomy skin flap, the surgeon examined the mastectomy site for tension that might complicate wound closure. In such cases the volume of saline injected in the tissue expander was reduced to eliminate skin tension. If the skin tension was too strong, portions of low-intensity areas were left untrimmed. No definition of 'low-intensity areas' was reported. Harless et al.[45] reported that reconstruction was aborted when mastectomy flaps were globally hypoperfused. In the case of isolated peri-incisional areas of the flap being underperfused, these areas were resected. However, no definition of 'hypoperfusion' or 'underperfusion' was reported. Mirhaidari et al.[47] made the decision to excise additional skin, remove fluid from the expander or change the implant size, or to not intervene based on the ICGA images and the surgeon's clinical judgement without reporting the method of perfusion assessment. Rinker et al.[48] reported that surgeons marked and excised all non-perfusion areas after completion of the mastectomy. No definition of 'non-perfusing areas' was reported. Sood et al.[46] reported that the surgeon noted areas of low fluorescence and performed manoeuvers to improve perfusion according to his judgement and preference, including resection of skin and/or reduction of implant volume. Although they reported that low fluorescence indicated limited perfusion of the flap, no definition or method was reported.

Methods: other factors

Three studies reported other factors that were taken into account during ICGA assessment.[40,43,48] Alstrup et al.[40] performed ICGA assessment with a mean systolic blood pressure above 100 mmHg and doppler confirmed that flow through the pedicle was maintained during assessment. Gorai et al.[43] ported that the surgeon did not use epinephrine injections into the surgical site. The imaging system was positioned over the breast at 30 cm from the skin. Rinker et al.[48] placed laparotomy sponges in the breast pocket to fill the dead space. This allowed the mastectomy skin flap to lie flat without areas of redundancy, but also without stretch. The imaging system was positioned over the breast at approximately 20 cm from the skin. The other six studies reported no other factors.[41,42,44-47]

DISCUSSION

Summary of main results

We included a total of nine nonrandomised cohort studies, including seven retrospective studies and two prospective studies. The studies involved a total of 1589 women who underwent 2199 breast reconstructions following skin-sparing mastectomy. In all studies, the use of ICGA was compared to clinical evaluation for preventing postoperative complications. The majority of included studies (six studies) reported the number of mastectomy skin flap necrosis on a per breast basis in order to allow bilateral reconstructions to be evaluated separately. Accordingly, five studies reported the number of other complications on a per breast basis. All studies reported rates of mastectomy skin flap necrosis, reoperation and infection. Six studies reported rates of haematoma and four studies reported rates of seroma. Multiple meta-analyses on a per patient and a per breast basis were performed to analyse the effect of ICGA on preventing mastectomy skin flap necrosis, reoperation, infection, haematoma and seroma compared to clinical evaluation. One study reported the combination of haematoma and seroma.[41] We received no response after contacting the authors twice via email to retrieve the separate number of complications. Therefore, this study was not included in the meta-analysis for haematoma and seroma. Only two studies considered autologous flap necrosis as a postoperative outcome and only one study considered dehiscence as an outcome. Therefore, no meta-analyses were performed to compare these outcomes. None of the included studies considered patient-related outcomes.

The comparison of ICGA versus clinical evaluation indicated that ICGA may reduce reoperation rates (on a per patient and per breast basis) and may reduce mastectomy skin flap necrosis and infection rates compared to clinical evaluation alone (based on per breast basis only). On a per patient basis, we were uncertain about the effect of ICGA on mastectomy skin flap necrosis, infection, haematoma and seroma; on a per breast basis, we were uncertain about the effect of ICGA on haematoma and seroma rates.

The ICGA protocols reported in the nine included studies were summarised. The majority of studies used the SPY System (SPY Imaging: LifeCell Corp, Branchburg, NJ) to assess mastectomy skin flap perfusion (eight studies). We found that the ICGA protocols in the included studies were not extensively described. The dose of ICG was reported in four studies and ranged from 7.5 mg to 25 mg. Four studies did not describe the timing of ICGA assessment. The majority of studies did not report timing from ICGA injection to assessment and the period of time of assessment (five studies) and did not report a method of perfusion assessment (seven studies). Only three studies reported that other factors were taken into

account during ICGA assessment, including the positioning distance of the imaging system from the skin. Since dose and working distance are two of the factors that significantly influence fluorescence intensity of ICGA, it is important to consider these factors when using ICGA for mastectomy skin flap perfusion.[49]

Overall completeness and applicability of evidence

To date, no randomised controlled trials have been conducted to assess the utility and efficacy of ICGA in reducing postoperative complications following mastectomy breast reconstructions. For this reason, only retrospective (seven studies) and prospective (two studies) nonrandomised cohort studies have been included in this review. All studies were conducted in high-income countries (predominately in the USA, six studies). We do not believe that this could influence the effect of the intervention. All women underwent immediate autologous or prosthetic reconstructive surgery, or a combination of the two, following a skin-sparing mastectomy. Outcomes reported in each study were reported as the number of complications without the need for standardised scales. All studies provided data on mastectomy skin flap necrosis, reoperations and infections. However, the included studies were not sufficient to address all of the objectives of this review, since there was a paucity of data concerning haematoma, seroma, autologous flap necrosis, dehiscence and patient-related outcomes. Therefore, it was not possible to generalise about the effect of ICGA versus clinical evaluation on these postoperative complications and patient-related outcomes. Although all studies compared perfusion assessment using ICGA to clinical evaluation, not all studies reported an extensive ICGA protocol and/or a clear definition of clinical evaluation. Since ICGA protocols and clinical evaluations may vary per study, we should be careful in generalising the effect of ICGA versus clinical evaluation on all postoperative outcomes.

Quality of the evidence

As presented in 'Summary of findings table for the main comparison 1 per patient basis)' and 'Summary of findings 2 (per breast basis)', all of the included studies had methodological shortcomings. There was serious concern with risk of bias due to the study design and the low comparability of cohorts described in the majority of studies. In our opinion, there was no inconsistency of the results. The main reasons for downgrading the quality of evidence were the nonrandomised characteristics of the studies, low comparability of the cohorts, the small sample sizes and the low number of events. Therefore, we concluded that the overall quality of the body of evidence was very low, which means that we are very uncertain about the reported results and the effect of ICGA on postoperative outcomes.

Potential biases in the review process

We conducted extensive searches, were careful and systematic in our screening process, and minimised the risk of bias, as we strictly followed the recommendations in the *Cochrane Handbook for Systematic Reviews of Interventions* on searching, study selection, data collection, and data analysis.[26] However, it is possible that we may have failed to identify studies, especially those that were unpublished. We were unsuccessful in our attempts to obtain further information on outcome data from the authors of one of the included studies.[41] In addition, no assessment of publication bias through funnel plot analysis was performed because fewer than 10 studies were included in the meta-analyses. Furthermore, no subgroup and sensitivity analysis were performed.

Agreements and disagreements with other studies or reviews

At the time the protocol for this review was published, no systematic review with meta-analysis had been conducted to assess the current evidence comparing the efficacy of ICGA in preventing postoperative MSFN in immediate breast reconstruction following mastectomy compared to clinical evaluation alone. After publication of the protocol, one systematic review was published involving all studies that reported on the use of the SPY system to assess perfusion of postmastectomy skin flaps from January 1, 1960, to March 1, 2018.[50] This review included seven studies that reported on the use of the SPY system (six studies) and PDE system (one study), including a total of 902 participants. Meta-analysis showed that ICGA was associated with a significantly reduced rate of postoperative mastectomy skin necrosis, reoperation and overall complications. For these three outcomes, the weighted odds ratio favoured the ICGA group: MSFN (OR 0.56; 95% CI 0.35 to 0.89; P=0.02, I^2=40%), reoperation (OR 0.32; 95% CI: 0.21 to 0.49; $P \leq 0.00001$; I^2=19%) and overall operative complications (OR 0.62; 95% CI 0.41 to 0.94; P=0.06; I^2=54%). Our review is in agreement with Liu's review showing benefit in preventing mastectomy skin flap necrosis (in the per breast analysis) and reoperation (in the per patient and per breast analysis). Although the seven studies in Liu et al.[50] were included in the current review, not all the numbers of mastectomy skin flap necrosis and reoperations extracted from the included studies were similar. For example, concerning the number of operations, Liu et al.[50] extracted only the perfusion-related reoperations while we included all reoperations. Furthermore, concerning the number of cases of mastectomy skin flap necrosis, Liu et al.[50] extracted only the number of cases of 'clinical necrosis' (defined by: necrosis treated solely with conservative treatment in outpatient clinic) without extracting the number of cases of necrosis requiring revision surgery from one study.[44] The major difference between the review by Liu et al.[50] and this Cochrane review was that we performed separate meta-analyses on a per patient basis and a per breast basis. Postoperative complications have been reported on a per patient basis in three studies, and included in the review by Liu et

al.[50] and our review.[41,42,46] Since women can develop complications in both breasts when a bilateral reconstruction is performed, these were not reported separately in contrast to reporting complications on a per breast basis.

AUTHORS' CONCLUSION

Implications for practice

The certainty of evidence in this review is very low, since included studies regarding mastectomy skin flap perfusion assessment using ICGA are nonrandomised cohort studies representing low-quality evidence. Although ICGA may reduce some postoperative complications compared to clinical evaluation, we are very uncertain about the effect. The current view in reconstructive breast surgery is that ICGA is a helpful tool to assess perfusion intraoperatively, however, there is a lack of high-quality evidence. With the results from this Cochrane review, we cannot draw conclusions about what method of assessment is best to use during breast reconstructive surgery.

Implications for research

Considering the high incidence of breast cancer and the low quality of evidence available on the use of ICGA in breast reconstructive surgery, high-quality randomised controlled trials that compare the use of ICGA to assess mastectomy skin flap perfusion compared to clinical evaluation appear to be needed. Large multicentre clinical trials are possible, since there are several institutions specialising in breast cancer care where ICGA devices are available to use during breast reconstructive surgery.

A C K N O W L E D G E M E N T S

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CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Alstrup et al. (2018)[40]	
Methods	A single-institution retrospective review was performed at Aarhus University Hospital (Denmark). Events occurring within 6 months of the initial procedure were evaluated. Information of follow-up period for each treatment group was not provided.
Participants	191 women (mean age 51 years) who underwent a total of 191 immediate or delayed, unilateral or bilateral pedicled autologous flap breast reconstructions between January 2013 and September 2016. No information on participant distribution over time was provided. Women receiving breast reconstructions with the latissimus dorsi (LD) musculocutaneous flap, muscle sparring (MS) LD flap and the LD flap with or without addition of an implant as well as the transverse rectus abdominal musculocutaneous (TRAM) flap were included. Patients with recurrent breast cancer, smoking within 4 weeks before the operation and not able to read and understand Danish were excluded.
Interventions	**ICGA group:** 77 women underwent perioperative ICGA evaluation with the SPY Elite System, after transposition of the flap to the recipient site, evaluating the perfusion and predicted viability of the flap. A clear ICGA protocol was described. **Clinical group:** 114 women underwent perioperative evaluation by the surgeon, observing capillary refill, colour of the flap and dermal edge bleeding.
Outcomes	No distinction between primary and secondary outcomes was made. Complications were categorised as being minor or major. The rate of complications was measured per breast, allowing bilateral reconstructions to be evaluated separately. Minor complications comprised events requiring minimal surgical interventions or prolonged wound care. These were defined as clinical sign of infection, fat necrosis and prolonged wound healing. Prolonged wound healing was defined as wounds requiring dressing changes assisted by health personnel. Major complications comprised events requiring revision surgery in general anaesthesia; these were categorised as necrosis of most of the flap (two-thirds), total flap loss and surgically evacuated haematoma. Complications regarding fat and skin necrosis were defined as related to the autologous skin paddle.
Notes	Study approval: *"The study was approved by the Danish Data Protection Agency and Scientific Ethical Committee of the Central Denmark Region and carried out in agreement with the Declaration of Helsinki II. Informed consent was acquired for all participants".* Study registration: Registration at clinicaltrials.gov, ID number NCT03069261

Source of support: Information not reported.
Competing interest: *"No potential conflict of interest was reported by the authors".*
Analysis on a per patient or per breast basis: For 191 women, the complication rate was recorded on a per breast basis for a total of 191 breasts.

Risk of Bias	Authors' judgement	Support for judgement
Representativeness of the exposed cohort	Unclear risk	Please ignore the 'Authors' judgement' column listed as 'unclear' in all of the risk of bias tables as this column could not be removed and is not applicable to the NOS. A truly representative cohort was selected, including immediate or delayed, unilateral or bilateral pedicled autologous flap reconstructions, with or without addition of an implant, between January 2013 and September 2016 (one star).
Selection of the non-exposed cohort	Unclear risk	A truly representative nonexposed cohort was selected, including participants undergoing similar breast reconstructions (one star).
Ascertainment of exposure	Unclear risk	Data were extracted from patient records (one star).
Demonstration that outcome of interest was not present at start of study	Unclear risk	There was no need to demonstrate that the outcomes of interest were not present at start of study, since outcomes of interest do not occur prior to a breast operation (one star).
Comparability of cohorts on the basis of the design or analysis	Unclear risk	A logistic regression analysis was performed, using total and major complications as dependent variables. The primary independent variables were ICGA assessment and timing of reconstruction. Also, the analysis included age, BMI, prior smoking, chemotherapy, radiation, diabetes and hypertension (two stars).
Assessment of outcome	Unclear risk	Data were extracted from patient medical records (one star).
Was follow-up long enough for outcomes to occur?	Unclear risk	Events occurring within 6 months of the initial procedure were evaluated (one star).
Adequacy of follow-up of cohorts?	Unclear risk	For 191 of 230 enrolled cases (83%) complication data 6 months after surgery were available (one star).

Diep et al.(2016)[41]		
Methods	A single-institution retrospective review was performed at the University of Minnesota (Minneapolis, USA). Events occurring within 90 days of the initial procedure were evaluated. Information of follow-up period for each treatment group was not provided.	
Participants	114 women (no mean age reported) who underwent a total of 145 unilateral or bilateral mastectomies and breast reconstructions for invasive breast cancer, carcinoma in situ, or prophylaxis between September 2009 and December 2013 were included. No information on participant distribution over time was provided. Patients who underwent delayed reconstruction and those who received autologous tissue flap reconstructions were excluded.	
Interventions	**ICGA group:** 61 women underwent intraoperative ICGA evaluation using SPY Imaging. No ICGA protocol was described. **Clinical group:** 53 women underwent intraoperative clinical evaluation only. No description of clinical evaluation was provided.	
Outcomes	No distinction between primary and secondary outcomes was made. The postoperative complications recorded were flap necrosis, unexpected returns to the operating room, cellulitis, and other complications. Other complications included haematoma and seroma. Mastectomy flap necrosis was further classified as mild (no intervention required), moderate (requiring in-office debridement), or severe (requiring operative debridement).	
Notes	Study approval: *"Approval was obtained from the local Institutional Review Board."* Study registration: Information not reported. Source of support: Information not reported. Competing interest: *"Dr. Cunningham (one of the authors) is a consultant for LifeCell Corpand Mentor Corp".* Analysis on a per patient or per breast basis: The complication rate was recorded on a per patient basis for a total of 114 women.	
Risk of bias	**Authors' judgement**	**Support for judgement**
Representativeness of the exposed cohort	Unclear risk	Please ignore the 'Authors' judgement' column listed as 'unclear' in all of the risk of bias tables as this column could not be removed and is not applicable to the NOS. A truly representative cohort was selected, including all mastectomy patients who underwent immediate tissue expander-based reconstruction between Sepember 2009 and December 2013 (one star).

Selection of the non-exposed cohort	Unclear risk	A truly representative nonexposed cohort was selected, including participants undergoing similar breast reconstructions (one star).
Ascertainment of exposure	Unclear risk	Information on the ascertainment of exposure was not described (no star).
Demonstration that outcome of interest was not present at start of study	Unclear risk	There was no need to demonstrate that the outcomes of interest were not present at start of study, since outcomes of interest do not occur prior to a breast operation (one star).
Comparability of cohorts on the basis of the design or analysis	Unclear risk	Univariate analysis were performed to identify the possible factors associated with severe flap necrosis, including BMI, age, smoking status, breast weight, tumescence, prior breast surgery, history of prior radiation therapy, and receipt of nipple-sparing mastectomy. However, participants in the clinical group underwent reconstruction between September 2009 and March 2012 (only one participant underwent surgery after March 2012). Participants in the ICGA group underwent reconstruction between March 2012 and December 2013. The ICGA cohort was younger (P=0.04) and had higher rates of prior breast surgery (P=0.03). All of the mastectomies were performed by two surgical oncologists. ICGA evaluations were performed by two plastic surgeons. There was no information on clinical evaluation and number of surgeons in the clinical group. No information on analysis to control for confounders was reported (no star).
Assessment of outcome	Unclear risk	Information on assessment of outcome was not described (no star).
Was follow-up long enough for outcomes to occur?	Unclear risk	A complication was defined as an event occurring within 90 days of the mastectomy (one star).
Adequacy of follow-up of cohorts?	Unclear risk	No statement regarding the completeness of follow-up (missing data) was reported (no star).

Duggal et al.(2014)[42]	
Methods	A single-institution retrospective review was performed at Emory University Hospital (Atlanta, USA). Mean follow-up was 8.3 ± 6.2 months in the ICGA group and 24.7 ± 11.8 months in the clinical group. Follow-up period for outcome assessment was not reported.
Participants	368 consecutive women (mean age of 49.91 ± 10.08 years in the ICGA group and 50.02 ± 10.15 in the clinical group) who underwent a total of 477 skin-sparing mastectomies and breast reconstructions between October 2007 and December 2011 were included. 184 women underwent breast reconstruction with ICGA between April 2019 and December 2011. 184 women underwent breast reconstruction with clinical evaluation between October 2007 and April 2009. No inclusion or exclusion criteria were applied.
Interventions	**ICGA group:** 184 women underwent intraoperative ICGA evaluation using SPY Imaging. Debridement of mastectomy skin flaps was completed based on a combination of clinical evaluation as well as SPY perfusion. No clear ICGA protocol was described. **Clinical group:** 184 women underwent intraoperative clinical evaluation, including assessment of colour, capillary refill, and dermal edge bleeding of the mastectomy skin flap.
Outcomes	No distinction between primary and secondary outcomes was made. The postoperative complications recorded were mastectomy skin necrosis, flap necrosis, fat necrosis, flap loss, seroma, haematoma, infection, and implant exposure. Mastectomy skin necrosis was defined clinically at postoperative visits and divided into 3 categories: mild (skin necrosis healing in less than 1 month and requiring no intervention), moderate (healing in 1-3 months and requiring in-office debridement), and severe (requiring operative debridement and local flap or skin graft coverage). Fat necrosis was clinically diagnosed on physical examination at follow-up appointments. Unexpected reoperations were recorded.
Notes	Study approval: Information not reported Study registration: Information not provided Source of support: Information not reported Competing interest: Disclosures: *"Dr Losken is a paid speaker for LifeCell Corporation."* Funding: *"The authors received no financial support for the research, authorship, or publication of this article."* Analysis on a per patient or per breast basis: The complication rate was recorded on a per patient basis for a total of 368 women.

Risk of bias	Authors' judgement	Support for judgement
Representativeness of the exposed cohort	Unclear risk	Please ignore the 'Authors' judgement' column listed as 'unclear' in all of the risk of bias tables as this column could not be removed and is not applicable to the NOS. A truly representative cohort was selected, including all mastectomy patients who underwent immediate tissue expander-based reconstruction between October 2007 and December 2011 (one star).
Selection of the non-exposed cohort	Unclear risk	A truly representative nonexposed cohort was selected, including participants undergoing similar breast reconstructions (one star).
Ascertainment of exposure	Unclear risk	Information on the ascertainment of exposure was not described (no star).
Demonstration that outcome of interest was not present at start of study	Unclear risk	There was no need to demonstrate that the outcomes of interest were not present at start of study, since outcomes of interest do not occur prior to a breast operation (one star).
Comparability of cohorts on the basis of the design or analysis	Unclear risk	184 women who underwent breast reconstruction from April 2009 (ICGA introduction) to December 2011 were included. A historical cohort for comparison was created, including 184 women who underwent breast reconstruction between October 2007 and April 2009. All mastectomies were performed by three different breast surgical oncologists and breast reconstructions were performed by one plastic surgeon. Only chi-square tests were undertaken to compare demographics between the ICGA group and clinical group for comparison of proportions and 2-sample T-tests for continuous measures. No information on analysis to control for confounders was reported (no star).
Assessment of outcome	Unclear risk	No information on assessment of outcome was provided (no star).
Was follow-up long enough for outcomes to occur?	Unclear risk	The time for outcome assessment was not specifically reported. However, the reported follow-up period was long enough for outcomes to occur (one star).
Adequacy of follow-up of cohorts?	Unclear risk	No statement regarding the completeness of follow-up (missing data) was reported (no star).

Gorai et al.(2017)[43]		
Methods	A single-institution retrospective review was performed at Shizuoka Cancer Centre Hospital (Shizuoka, Japan). Mean follow-up was not reported.	
Participants	181 women (mean age not reported) who underwent a total of 184 total mastectomies and 1-stage immediate breast reconstructions between April 2006 and August 2014 were included. 83 breast reconstructions (81 women) were performed with ICGA between April 2012 and August 2014. 101 breast reconstructions (100 women) were performed with clinical evaluation between April 2006 and March 2012. The indication for immediate tissue expander reconstruction was limited to stage II disease. In all cases, total mastectomy was performed by a simple ellipse incision. Cases of nipple-sparing mastectomy were excluded from this study.	
Interventions	**ICGA group:** 81 women underwent intraoperative ICGA evaluation using a Photodynamic Eye imaging system (PDE) before skin closure. A clear ICGA protocol was described. **Clinical group:** 100 women underwent intraoperative clinical evaluation, according to the surgeon's judgement. No description of clinical evaluation was provided.	
Outcomes	No distinction between primary and secondary outcomes was made. The postoperative complications recorded were wound necrosis, tissue expander infection and tissue expander removal. Wound necrosis was divided into 3 groups according to the depth of necrosis: grade I (epidermal necrosis), grade II (necrosis reaching to the dermis) and grade III (necrosis reaching to the subcutaneous fat layer). Treatment outcome after conservative or surgical wound treatment was also reported.	
Notes	Study approval: Information not reported. Study registration: Information not reported. Source of support: *"The authors have no financial interest to declare in relation to the content of this article."* Competing interest: Information not provided. Analysis on a per patient or per breast basis: For 181 women, the complication rate was recorded on a per breast basis for a total of 184 breasts.	
Risk of bias	**Authors' judgement**	**Support for judgement**
Representativeness of the exposed cohort	Unclear risk	Please ignore the 'Authors' judgement' column listed as 'unclear' in all of the risk of bias tables as this column could not be removed and is not applicable to the NOS. A somewhat representative cohort was selected, including total mastectomy patients who underwent immediate tissue expander

		breast reconstruction between April 2006 and August 2014 (one star).
Selection of the non-exposed cohort	Unclear risk	A somewhat representative nonexposed cohort was selected, including participants undergoing similar breast reconstructions (one star).
Ascertainment of exposure	Unclear risk	Information on the ascertainment of exposure was not described (no star).
Demonstration that outcome of interest was not present at start of study	Unclear risk	There was no need to demonstrate that the outcomes of interest were not present at start of study, since outcomes of interest do not occur prior to a breast operation (one star).
Comparability of cohorts on the basis of the design or analysis	Unclear risk	83 breast reconstructions (81 women) between April 2012 (ICGA introduction) and August 2014 were included. A historical cohort for comparison was created, including 101 breast reconstructions (100 women) between April 2006 and March 2012. Surgery was performed by residents and the specialist team. "The chief surgeon in the breast surgery department did not change throughout the study period". Only Pearson's chi-square test was used to compare background factors. No information on analysis to control for confounders was reported (no star).
Assessment of outcome	Unclear risk	No information on assessment of outcome was provided (no star).
Was follow-up long enough for outcomes to occur?	Unclear risk	No time for outcome assessment was reported (no star).
Adequacy of follow-up of cohorts?	Unclear risk	No statement regarding the completeness of follow-up (missing data) was reported (no star).

Hammer-Hansen et al. (2017)[44]	
Methods	A single-institution retrospective review was performed at Aarhus University Hospital (Denmark). The follow-up period was predetermined to be 90 days postoperatively. Information on follow-up period for each treatment group was not provided.
Participants	92 women (mean age of 44.6 ± 10.1 years in the ICGA group and 46.7 ± 8.7 years in the clinical group) underwent a total of 128 implant-based skin-sparing immediate breast reconstructions with a porcine acellular dermal matrix (ADM) from March 2012 to October 2015. All implant reconstructions were performed using partial subpectoral implant positioning. 66 breast reconstructions (46 women) were performed with ICGA and 62 breast reconstructions (46 women) were performed with clinical evaluation. No information on participant distribution over time was provided.
Interventions	**ICGA group:** 46 women (66 breast reconstructions) underwent intraoperative ICGA evaluation using the SPY-Elite-system minimum of three times during the surgical period. Areas showing perfusion below 33% using the contour-mode were excised when possible. **Clinical group:** 46 women (62 breast reconstructions) underwent intraoperative clinical evaluation only. No description of clinical evaluation was provided.
Outcomes	No distinction between primary and secondary outcomes was made. The postoperative complications recorded were necrosis requiring revision surgery, clinical necrosis, infection, haematoma, seroma, implant exchange and reconstructive failure. Clinical necrosis was defined as necrosis treated solely with conservative treatment in the outpatient clinic. Infection was defined as clinically suspected infections where the participant was treated with additional intravenous antibiotics. Haematoma was defined as haematoma requiring surgical evacuation. Seromas were registered when verified and treated by ultrasound-guided needle puncture. Reconstructive failure was defined as removal of implant, being the definitive silicone gel-filled implant or the tissue expander with the need for conversion to autologous breast reconstruction.
Notes	Study approval: *"The study was approved by Danish Data Protection Agency (case number: 1–16-02–204-15). The Central Denmark Regional Ethics board under the Danish National Committee on Biomedical Research Ethics assessed the specific study and deemed it as exempt from need of approval and accepted to be carried out (96/2015)."* Study registration: Information not reported. Source of support: Information not reported. Competing interest: *"No potential conflict of interest was reported by the authors."* Analysis on a per patient or per breast basis: The complication rate was recorded on a per breast basis for a total of 128 breast in 92 patients.

Risk of bias	Authors' judgement	Support for judgement
Representativeness of the exposed cohort	Unclear risk	Please ignore the 'Authors' judgement' column listed as 'unclear' in all of the risk of bias tables as this column could not be removed and is not applicable to the NOS. A truly representative cohort was selected, including participants who underwent implant-based skin-sparing immediate breast reconstruction with porcine ADM between March 2012 and October 2015 (one star).
Selection of the non-exposed cohort	Unclear risk	A truly representative nonexposed cohort was selected, including participants undergoing similar breast reconstructions (one star).
Ascertainment of exposure	Unclear risk	Data were extracted from the electronic patient record (one star).
Demonstration that outcome of interest was not present at start of study	Unclear risk	There was no need to demonstrate that the outcomes of interest were not present at start of study, since outcomes of interest do not occur prior to a breast operation (one star).
Comparability of cohorts on the basis of the design or analysis	Unclear risk	128 breast reconstructions (92 women) between March 2012 and October 2015 were included. 62 breast reconstructions were performed before implementation of ICGA and 66 after the implementation. No date of ICGA implementation was reported. The ICGA and clinical group varied in regards to the weight of the removed breast, with a significantly higher mastectomy weight in the clinical group (P=0.002) and significantly more participants in the clinical group received hormone therapy (P=0.013) or chemotherapy (P=0.004). No information on analysis to control for confounders was reported (no star).
Assessment of outcome	Unclear risk	Data were extracted from the electronic patient record (one star).
Was follow-up long enough for outcomes to occur?	Unclear risk	Follow-up period was predetermined to be 90 days postoperatively (one star).
Adequacy of follow-up of cohorts?	Unclear risk	There were no missing data present for the included 128 breast reconstructions (one star).

Harless et al.(2016)[45]		
Methods	A single-institution retrospective review was performed at the Mayo Clinic in Rochester (Minnesota, USA). Mean follow-up was 4.6 (range 1 to 13) months in the ICGA group and 16.9 (range 1 to 52) months in the clinical group.	
Participants	269 consecutive women (mean age of 50 ± 11 years in the ICGA group and 48 ± 9.8 years in the clinical group) underwent a total of 467 implant-based breast reconstructions between 2008 and 2013. 213 breast reconstructions (120 women) were performed with ICGA between 2008 and mid-2011. 254 breast reconstructions (149 women) were performed with clinical evaluation between mid-2011 and 2013.	
Interventions	**ICGA group:** 120 women (213 breast reconstructions) underwent intraoperative ICGA evaluation using the SPY Elite system. **Clinical group:** 149 women (254 breast reconstructions) underwent intraoperative clinical evaluation only.	
Outcomes	The primary complication was mastectomy flap skin necrosis requiring operative intervention. Secondary outcomes included flap salvage, implant extrusion, bleeding, infection, implant explantation, and implant deflation. Mastectomy flap skin necrosis, implant extrusion, infection, bleeding, and deflation were only noted if operative intervention was required. *"Patients requiring flap salvage were defined as those patients in whom planned implant-based reconstruction alone was unattainable due to complications experienced".*	
Notes	Study approval: *"Prior to review of all charts, institutional review board approval was obtained"*. Study registration: Information not reported. Source of support: *"The authors received no financial support for the research, authorship, or publication of this article."* Competing interest: *"Dr. Jacobson is a consultant for Novadaq."* Analysis on a per patient or per breast basis: The complication rate was recorded on a per breast basis for a total of 467 breasts in 269 women.	
Risk of bias	**Authors' judgement**	**Support for judgement**
Representativeness of the exposed cohort	Unclear risk	Please ignore the 'Authors' judgement' column listed as 'unclear' in all of the risk of bias tables as this column could not be removed and is not applicable to the NOS. A truly representative cohort between 2008 and 2013 was selected (one star).

Selection of the non-exposed cohort	Unclear risk	A truly representative nonexposed cohort was selected, including participants undergoing similar breast reconstructions (one star).
Ascertainment of exposure	Unclear risk	Data were extracted by reviewing all patient charts (one star).
Demonstration that outcome of interest was not present at start of study	Unclear risk	There was no need to demonstrate that the outcomes of interest were not present at start of study, since outcomes of interest do not occur prior to a breast operation (one star).
Comparability of cohorts on the basis of the design or analysis	Unclear risk	467 breast reconstructions (269 women) between 2008 and 2013 were included. 254 breast reconstructions were performed before implementation of ICGA in June 2011, and 213 breast reconstructions after the implementation. Participant demographics and comorbidities were compared before and after implementation of ICGA assessment. The ICGA and clinical group varied in regards to prior radiation, with a significantly higher percentage of women who underwent radiation in the ICGA group. No information on analysis to control for confounders was reported (no star).
Assessment of outcome	Unclear risk	Data were extracted from patient charts (one star).
Was follow-up long enough for outcomes to occur?	Unclear risk	No exact time for outcome assessment was reported, however, the minimum follow-up time of 1 month is long enough for outcomes to occur (one star).
Adequacy of follow-up of cohorts?	Unclear risk	No statement regarding the completeness of follow-up (missing data) was reported (no star).

Mirhaidari et al.(2018)[47]	
Methods	A prospective review was performed at two institutions, including Summa Health System, Akron and Akron General Medical Centre, Akron (Ohio, USA) from January 2014 to January 2015. The prospectively collected data were compared with the senior surgeon's experience of retrospectively collected data from 194 consecutive immediate reconstructions in the period before the use of ICGA. Participants in the prospective study were followed for 90 days to document complications. Information on follow-up period for each treatment group was not provided.
Participants	243 women (mean age of 51.4 ± 11.99 years in the ICGA group and 52.8 ± 12.17 years in the clinical group) underwent a total of 400 breast reconstructions. 126 women underwent 206 immediate reconstructions in the ICGA (prospective) group between January 2014 to January 2015. Breast reconstruction was performed either as a direct-to-implant procedure, a tissue expander, or autologous reconstruction by 1 of 3 plastic surgeons. Acellulair dermal matrix was used in every case of implant-based reconstruction. 117 women underwent 194 immediate reconstructions in the period before the use of ICGA. No exact period of time was reported.
Interventions	**ICGA group:** 126 women (206 breast reconstructions) underwent intraoperative ICGA evaluation using the SPY Elite System. Based on ICGA and the surgeon's clinical judgement, the decision was made to excise additional skin, remove fluid from the expander, change implant size, or not intervene. **Clinical group:** 117 women (194 breast reconstructions) underwent intraoperative clinical evaluation only based on surgeon's clinical judgement.
Outcomes	No distinction between primary and secondary outcomes was made. The postoperative complications recorded were full-thickness necrosis, partial-thickness necrosis, seroma, haematoma, infection, or implant loss. Full-thickness necrosis was defined as a loss of epidermis and dermis with exposure of subcutaneous fat, muscle, acellular dermal matrix, or implant. Partial-thickness necrosis was defined as the loss of epidermis, partial dermal loss, and/or eschar formation that does not expose subcutaneous fat. Seroma was defined as a fluid collection after drain removal with or without intervention to drain the fluid. A haematoma was defined as any collection of blood with or without intervention to drain the blood. Infection was defined as documented erythema, fever, abscess, or purulent drainage requiring treatment with antibiotics or surgical intervention, regardless of whether or not positive cultures were obtained.
Notes	Study approval: The study was approved by the institutional review board and written consent was obtained from all patients. Study registration: Information not reported.

Source of support: Information not reported.
Competing interest: *"Dr. Wagner is a Speaker for LifeCell".*
Analysis on a per patient or per breast basis: The complication rate was recorded on a per breast basis for a total 400 breast in 243 women.

Risk of bias	Authors' judgement	Support for judgement
Representativeness of the exposed cohort	Unclear risk	Please ignore the 'Authors' judgement' column listed as 'unclear' in all of the risk of bias tables as this column could not be removed and is not applicable to the NOS. A somewhat representative cohort was selected, including mastectomy patients who underwent immediate reconstruction following nipple-sparing and nipple-sacrificing mastectomies (one star).
Selection of the non-exposed cohort	Unclear risk	A somewhat representative nonexposed cohort was selected, including participants undergoing similar breast reconstructions (one star).
Ascertainment of exposure	Unclear risk	Information on the ascertainment of exposure was not described (no star).
Demonstration that outcome of interest was not present at start of study	Unclear risk	There was no need to demonstrate that the outcomes of interest were not present at start of study, since outcomes of interest do not occur prior to a breast operation (one star).
Comparability of cohorts on the basis of the design or analysis	Unclear risk	400 breast reconstructions (243 women) were included. 194 breast reconstructions were performed in the period before implementation of ICGA, however no period of time was reported. 206 breast reconstructions were performed after implementation of ICGA between January 2014 and January 2015. The ICGA and clinical group varied significantly in regards to mastectomy type and reconstruction method. No information on analysis to control for confounders was reported (no star).
Assessment of outcome	Unclear risk	Data that were prospectively collected for the ICGA group were compared to retrospectively collected data in the clinical group, however no information on assessment of outcome was provided (no star).
Was follow-up long enough for outcomes to occur?	Unclear risk	Participants were followed for 90 days (one star).
Adequacy of follow-up of cohorts?	Unclear risk	No statement regarding the completeness of follow-up (missing data) was reported (no star).

Rinker et al. (2016)[48]	
Methods	A single-institution prospective cohort study was performed at the University of Kentucky, Lexington (Kentucky, USA) over an 18-month period. Women were followed postoperatively for a minimum of 6 months. Mean follow-up was 10 (range 6 to 34) months.
Participants	60 women (99 breast reconstructions) underwent skin-sparing mastectomy and immediate reconstruction with either tissue expanders or muscle-sparing free transverse rectus abdominis musculocutaneous (TRAM) flaps. Mastecomy skin flap viability was assessed either visually (20 women, 30 breasts), with fluorescein dye and Wood's lamp imaging (20 women, 34 breasts), or by ICGA (20 women, 35 breasts). The mean age was 47.2 ± 7.7 years in the ICGA group and 50.4 ± 7.6 years in the clinical group. Patients undergoing delayed reconstruction were excluded.
Interventions	**ICGA group:** 20 women (35 breasts) underwent intraoperative ICGA evaluation using the SPY Elite System. All non-perfusing areas were marked and excised. **Clinical group:** 20 women (30 breasts) underwent intraoperative clinical evaluation only, including mastectomy skin flap examination for thickness, colour, edge bleeding, and capillary refill. Any areas of questionable viability were excised.
Outcomes	No distinction between primary and secondary outcomes was made. The postoperative complications recorded were mastectomy flap necrosis, infection, seroma, or unexpected reoperation. Mastectomy flap necrosis was defined as any size area of full-thickness skin necrosis. Partial-thickness epidermolysis did not qualify.
Notes	Study approval: *"This study was approved by the sponsoring institution's review board for research involving human subjects."* Study registration: Information not reported. Source of support: Information not reported. Competing interest: *"The author had no financial interest in any of the products or devices mentioned in this article."* Analysis on a per patient or per breast basis: The complication rate for mastectomy flap necrosis was recorded on a per breast basis for a total of 65 breasts. The complication rate for reoperation, seroma and infection were recorded on a per patient basis for a total 40 patients. We only used two arms ('visually' and 'fluorescein dye') of this three-arm study.

Risk of bias	**Authors' judgement**	**Support for judgement**
Representativeness of the exposed cohort	Unclear risk	Please ignore the 'Authors' judgement' column listed as 'unclear' in all of the risk of bias tables as this column could not be removed and is not applicable to the NOS.

		A truly representative cohort was selected, including skin-sparing mastectomy patients who underwent immediate reconstruction with either tissue expander or muscle-sparing free TRAM flaps over a period of 18 months (one star).
Selection of the non-exposed cohort	Unclear risk	A truly representative nonexposed cohort was selected, including participants undergoing similar breast reconstructions (one star).
Ascertainment of exposure	Unclear risk	Data were collected from personnel interview and review of patient charts (one star).
Demonstration that outcome of interest was not present at start of study	Unclear risk	There was no need to demonstrate that the outcomes of interest were not present at start of study, since outcomes of interest do not occur prior to a breast operation (one star).
Comparability of cohorts on the basis of the design or analysis	Unclear risk	65 breast reconstructions (40 women) in the ICGA and clinical group were included. All reconstructions were performed over an 18-month period. 35 breast reconstructions were performed with ICGA and 30 breast reconstructions with clinical evaluation. Participants factors were compared for homogeneity. Variation across the groups was analysed. A multivariable regression analysis was performed to identify independent predictors for mastectomy skin flap necrosis (two stars).
Assessment of outcome	Unclear risk	Data from personnel interview and review of the patient chart for demographics and operative variables were collected. In all participants, the area of excision was measured and recorded (one star).
Was follow-up long enough for outcomes to occur?	Unclear risk	Participants were followed for a minimum of 6 months (one star).
Adequacy of follow-up of cohorts?	Unclear risk	No statement regarding the completeness of follow-up (missing data) was reported (no star).

Sood et al. (2013)[46]	
Methods	A single-institution retrospective review was performed at Philadelphia College of Osteopathic Medicine, Cherryhill (New Jersey, USA). Information on follow-up period for each treatment group was not provided.
Participants	91 women (mean age of 51 ± 8.6 years in the ICGA group and 52 ± 7.9 years in the clinical group) underwent a total of 142 immediate breast reconstructions with a prosthesis following skin-sparing mastectomy from January 2009 to April 2012. Reconstructions with tissue expander and/or implants were included. 52 women underwent breast reconstruction with ICGA between April 2011 and April 2012. 39 women underwent breast reconstruction with clinical evaluation between January 2009 and April 2011. All reconstructions were performed using human acellular dermal matrix (HADM). Delayed procedures or flap-based reconstructions were excluded.
Interventions	**ICGA group:** 52 women (80 breast reconstructions) underwent intraoperative ICGA evaluation using SPY Imaging. Areas of low fluorescence were noted and manoeuvres, including resection of skin and/or reduction of implant volume, were performed to improve perfusion. **Clinical group:** 39 women (62 breast reconstructions) underwent reconstruction without ICGA evaluation. No description of the intervention was provided.
Outcomes	No distinction between primary and secondary outcomes was made. The postoperative complications recorded were flap necrosis, capsular contracture, cellulitis, haematoma, extrusion of tissue expander, displacement of tissue expander and repeat operation room visits.
Notes	Study approval: *"This study was granted exemption from IRB review based on its retrospective chart-design".* Study registration: Information not reported. Source of support: Information not reported. Competing interest: *"Dr. Glat is on the Speakers' Bureau for LifeCell Corp".* Analysis on a per patient or per breast basis: The complication rate was recorded on a per patient basis for a total of 91 women.

Risk of bias	**Authors' judgement**	**Support for judgement**
Representativeness of the exposed cohort	Unclear risk	Please ignore the 'Authors' judgement' column listed as 'unclear' in all of the risk of bias tables as this column could not be removed and is not applicable to the NOS. A truly representative cohort was selected, including consecutive participants undergoing immediate breast reconstruction with a prosthesis following mastectomy between January 2009 and April 2012 (one star).

Selection of the non-exposed cohort	Unclear risk	A truly representative nonexposed cohort was selected, including participants undergoing similar breast reconstructions (one star).
Ascertainment of exposure	Unclear risk	Information on ascertainment of exposure was not described (no star).
Demonstration that outcome of interest was not present at start of study	Unclear risk	There was no need to demonstrate that the outcomes of interest were not present at start of study, since outcomes of interest do not occur prior to a breast operation (one star).
Comparability of cohorts on the basis of the design or analysis	Unclear risk	142 breast reconstructions (91 women) in the ICGA and clinical group between January 2009 and April 2011 were included. 52 women underwent breast reconstruction before implementation of ICGA from January 2009 to April 2011. 39 women underwent reconstruction after ICGA implementation from April 2011 to April 2012. There were no significant differences between the two groups with regard to baseline characteristics and reconstructive modality. No information on analysis to control for confounders was reported (no star).
Assessment of outcome	Unclear risk	No information on assessment of outcome was reported (no star).
Was follow-up long enough for outcomes to occur?	Unclear risk	No time for outcome assessment was reported (no star).
Adequacy of follow-up of cohorts?	Unclear risk	No statement regarding the completeness of follow-up (missing data) was reported (no star).

Abbreviations:
ADM: acellular dermal matrix
BMI: body mass index
HADM: human acellular dermal matrix
ICGA: indocyanine green angiography
LD: latissimus dorsi
MS: muscle sparing
PDE: photodynamic eye
TRAM: transverse rectus abdominis musculocutaneous

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Diep et al. (2019)[33]	Wrong study design: no comparison of ICGA with clinical evaluation to assess mastectomy skin flap vascularisation
Griffiths et al. (2016)[18]	Wrong study design: this was a literature review.
Komorowska-Timek et al. (2010)[34]	Wrong outcome data: only overall total complication rates were reported.
Mattison et al. (2016)[35]	Wrong study design: the diagnostic value of ICGA for assessment of mastectomy skin flap vascularisation was assessed.
Munabi et al. (2014)[36]	Wrong study design: the diagnostic value of ICGA for assessment of mastectomy skin flap vascularisation was assessed.
Newman et al. (2010)[37]	Wrong study design: the diagnostic value of ICGA for assessment of mastectomy skin flap vascularisation was assessed.
Phillips et al. (2012)[19]	Wrong study design: the diagnostic value of ICGA for assessment of mastectomy skin flap vascularisation was assessed.
Wang et al. (2018)[38]	Wrong study design: ICGA assessment of the NAC perfusion to discover the relationship between incision methods and NAC circulation
Zenn et al. (2018)[39]	Wrong study design: this was a review article.

Abbreviations:
ICGA: indocyanine green angiography
NAC: nipple-areola complex

APPENDICES

Appendix 1. Search in CENTRAL

#1MeSH descriptor: [Mastectomy] explode all trees
#2(mastectom*):ti,ab,kw
#3(postmastectom*):ti,ab,kw
#4(post-mastectom*):ti,ab,kw
#5#1 OR #2 OR #3 OR #4
#6MeSH descriptor: [Indocyanine Green] this term only
#7((indocyanine green)):ti,ab,kw
#8(ICG):ti,ab,kw
#9(IFCG):ti,ab,kw
#10((Infracyanine green)):ti,ab,kw
#11#6 OR #7 OR #8 OR #9 OR #10
#12#5 AND #11

Appendix 2. Search in MEDLINE

1. exp mastectomy/
2. mastectom*.mp.
3. postmastectom*.mp.
4. post-mastectom*.mp.
5. or/1-4
6. (indocyanine adj3 green).mp.
7. indocyanine green/
8. ICG.mp.
9. (Infracyanine adj3 green).mp.
10. IFCG.mp.
11. or/6-10
12. and/5,11

Appendix 3. Search in EMBASE

1. exp mastectomy/
2. mastectom*.mp.
3. postmastectom*.mp.
4. post-mastectom*.mp.
5. or/1-4
6. indocyanine green/
7. indocyanine green angiography/
8. (indocyanine adj3 green).mp.
9. ICG.mp.
10. (Infracyanine adj3 green).mp.
11. IFCG.mp.
12. or/6-11
13. and/5,12

Appendix 4. Search in WHO ICTRP

Basic search

Mastectom* AND indocyanine green

Advanced search:

Condition: Mastectom*

Intervention: indocyanine green

Recruitment status: ALL

Appendix 5. Search in Clinicaltrials.gov

Basic searches

Mastectom* AND indocyanine green

Advanced search:

Condition or disease:

Mastectom* OR breast*

Other terms:

indocyanine green

Appendix 6. Newcastle-Ottawa Scale (NOS) quality assessment form for cohort studies

Note: A study can be awarded a maximum of one star for each numbered item within the selection and outcome categories. A maximum of two stars can be given for comparability.

Selection

1) Representativeness of the exposed cohort

1. Truly representative (one star)
2. Somewhat representative (one star)
3. Selected group
4. No description of the derivation of the cohort

2) Selection of the nonexposed cohort

1. Drawn from the same community as the exposed cohort (one star)
2. Drawn from a different source
3. No description of the derivation of the non exposed cohort

3) Ascertainment of exposure

1. Secure record (e.g. surgical records) (one star)
2. Structured interview (one star)
3. Written self report
4. No description

4) Demonstration that outcome of interest was not present at start of study

1. Yes (one star)
2. No

Comparability

1) Comparability of cohorts on the basis of the design or analysis controlled for confounders

1. Study controls for smoking status, diabetes mellitus, BMI, mastectomy breast weight, previous exposure to radiotherapy, tumescent mastectomy technique, and Wise-pattern mastectomy incision(one star)
2. Study controls for any additional factor (one star)

Outcome

1) Assessment of outcome

1. Independent blind assessment (one star)
2. Record linkage (one star)
3. Self report
4. No description

2) Was follow-up long enough for outcomes to occur

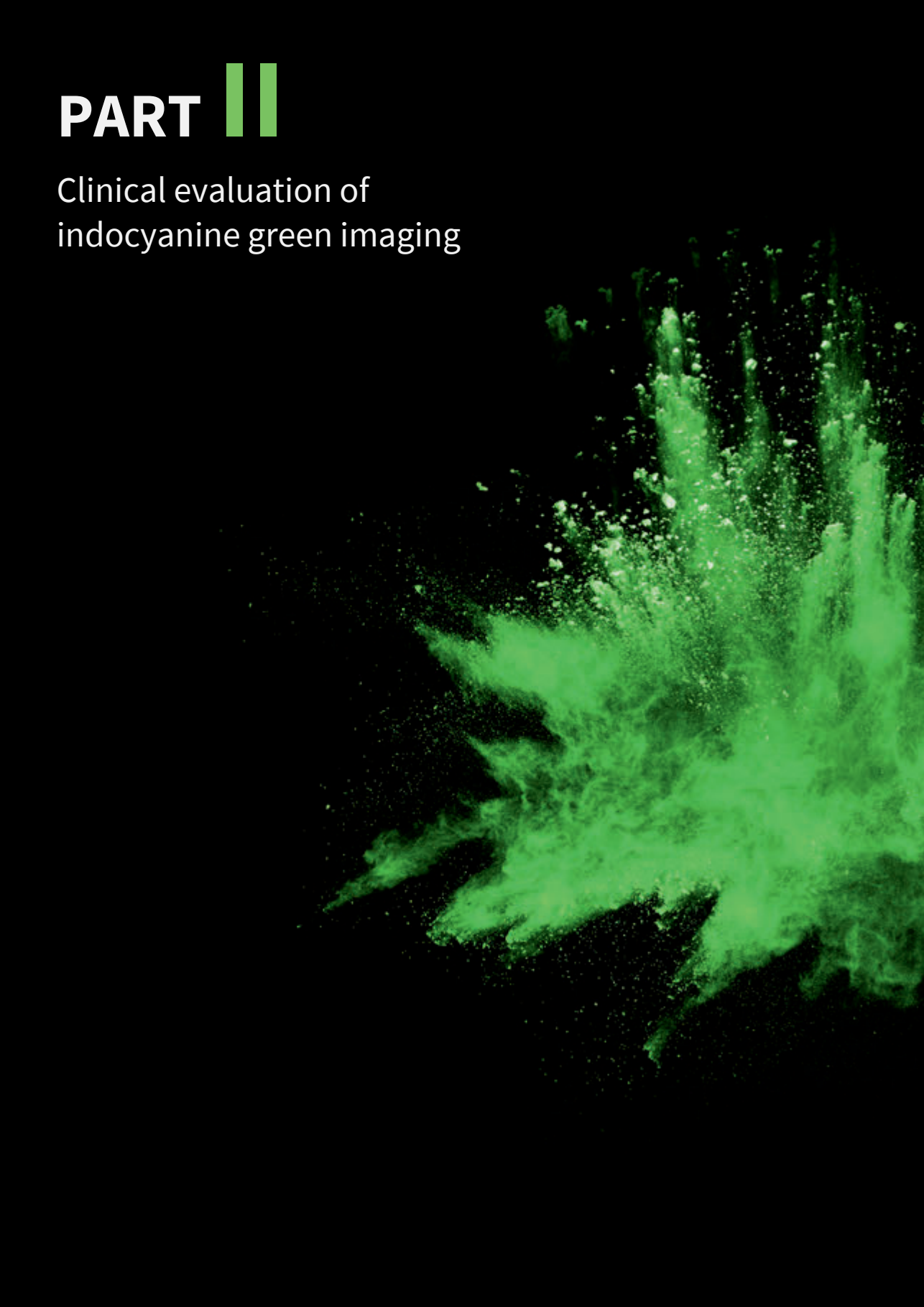
1. Yes (one star)
2. No

3) Adequacy of follow up of cohorts

1. Complete follow-up - all subjects accounted for (one star)
2. Subjects lost to follow-up unlikely to introduce bias - number lost less than or equal to 20%, or description provided of those lost suggested no different from those followed (one star)
3. Follow-up rate less than 80% and no description of those lost
4. No statement

PART II

Clinical evaluation of indocyanine green imaging



CHAPTER 5

The impact of using the internal mammary artery as a recipient vessel on medial mastectomy skin flap perfusion in autologous breast reconstruction: an observational study using indocyanine green

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Submitted

ABSTRACT

Background

The internal mammary artery (IMA) is considered the preferred recipient vessels for micro anastomosis in immediate autologous breast reconstruction following skin-sparing mastectomy (SSM). Although the IMA accounts for approximately 60 percent of the blood supply to the breast, the exact contribution to the mastectomy skin flap perfusion is unclear. The aim of this observational study was to investigate the impact of using the IMA as recipient vessel on medial mastectomy skin flap perfusion assessed using indocyanine green angiography (ICGA).

Methods

This observational study is conducted on ten consecutive women who underwent immediate autologous breast reconstructions following SSM. Two intraoperative ICG assessments were required to assess perfusion of the upper and lower part of the medial mastectomy skin flap: the first following SSM and the second after clamping the IMA. During a 120 seconds video-angiography, three additional images were made at 60, 90 and 120 seconds. Indocyanine green (ICG) inflow time and mean, minimum and maximum fluorescence intensities (FIs) were obtained.

Results

Four unilateral and six bilateral autologous breast reconstructions were included. There was no difference in tissue perfusion when comparing the inflow time (24.1s vs 23.0s, P=0.348), the mean FI (131.4 vs 124.0, P=0.126), min FI (28.6 vs 33.4, P=0.086) and max FI (253.1 vs 247.6, P=0.166) before and after clamping the IMA.

Conclusion

According to this study, the preparation and use of the IMA as recipient vessel, does not reduce medial mastectomy skin flap perfusion in patients undergoing immediate autologous breast reconstructions following SSM.

INTRODUCTION

Although breast-conserving surgery has replaced mastectomy for the majority of patients presenting with breast cancer in high-income regions across the world, nearly 40 percent of women will still undergo a mastectomy.[1] Skin-sparing mastectomy (SSM) provides removal of the breast tissue, preserving the overlying skin of the breast (i.e., mastectomy skin flap) giving the possibility of performing immediate breast reconstruction with benefits in patients' body image, self-esteem, sexuality and quality of life.[2]

According to the NABON Breast Cancer Audit (NBCA), 26.0 percent of the invasive breast cancer patients and 49.7 percent of the ductal carcinoma in situ patients underwent immediate breast reconstruction in the Netherlands in 2015, involving implant-based reconstruction, autologous tissue reconstruction or a combined type of breast reconstruction.[3] While implant-based reconstruction remains the most performed method in the Netherlands and worldwide, autologous breast reconstructions are gaining popularity.[3,4]

Although preserving the mastectomy skin flap during SSM improves the cosmetic end-result following immediate breast reconstruction, mastectomy skin flap necrosis (MSFN) is a common complication with a reported incidence ranging from 5 to 30 percent, with a mean incidence rate of 19.4% in the literature.[5,6] Several patient and surgery related risk factors for MSFN have been identified including smoking, diabetes mellitus, a body mass index greater than 30 kg/m^2, higher mastectomy weight, previous exposure to radiotherapy, tumescence mastectomy techniques, wise-pattern mastectomy, and the surgeons' level of experience.[5,7] In order to prevent postoperative MSFN, the intraoperative use of indocyanine green angiography (IGCA) has been suggested.[8] ICGA is a real-time imaging technique that records fluorescence images using a near-infrared camera following intravenous administration of a fluorophore called indocyanine green (ICG). Results of a recent Cochrane review comparing the efficacy of ICGA in preventing MSFN in immediate breast reconstruction following SSM to clinical evaluation alone were inconclusive due to lack of high-quality evidence. However, ICGA was proposed as a good imaging technique to assess mastectomy skin flap perfusion.[8]

When concerning the mastectomy skin flap, its blood supply depends on the subdermal plexus, which is in communication with the deeper vessels supplying the breast parenchyma. The breast parenchyma is predominately vascularised by the internal thoracic (internal mammary) artery over the lateral thoracic (external mammary) artery in 68 to 74% of patients.[9] The medial part of the breast and the anterior chest wall are supplied by blood

via perforating branches of the internal mammary artery (IMA) and anterior intercostal arteries.[10,11] Branches of the lateral thoracic artery and pectoral branches of the thoraco-acromial artery, both originating from the axillary artery, are responsible for the upper outer part of the breast. The remainder is supplied by branches of the posterior intercostal arteries.[10,12]

During immediate autologous breast reconstruction utilizing free flaps following SSM, the internal mammary vessels are the preferred recipient vessels for micro anastomosis.[13,14] Although the internal mammary artery (IMA) accounts for approximately 60 percent of the blood supply to the breast, the exact contribution of the IMA to the mastectomy skin flap perfusion is unclear. Whether or not the use of IMA can cause a possible MSFN is not described in the literature. In other disciplines such as cardiothoracic surgery, harvesting the complete IMA during coronary artery bypass grafting (CABG) resulted in reduced sternal and parasternal skin perfusion.[15,16] Consequently, it could be hypothesised that using the IMA as recipient vessel during immediate breast reconstruction might pose a risk factor for mastectomy skin flap necrosis.

The aim of the current observational study is to investigate the impact of using the IMA as recipient vessel on the perfusion of the medial mastectomy skin flap in patients undergoing autologous breast reconstructions using ICGA.

METHODS

This observational study was conducted on ten consecutive women who underwent unilateral or bilateral skin-sparing mastectomy followed by immediate breast reconstruction utilizing autologous free flaps at Maastricht University Medical Centre between December 2020 and July 2021. Delayed autologous reconstruction, previous radiotherapy to the axillary or breast region, and allergy or hypersensitivity to ICG or iodine were exclusion criteria. All procedures performed in this study were in accordance with the ethical standards of the institutional research committee (Medical Ethics Committee in Maastricht: METC 2019-1165-A-10) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Preoperative patient characteristics were obtained from the medical records of the included patients. Variables obtained included age, weight, Body Mass Index (BMI), smoking status, patients' history (e.g., Diabetes Mellitus or previous chemotherapy). The following intraoperative features were registered: the incision used for mastectomy (i.e., around the nipple-areolar complex (NAC) and/or including a vertical incision to the inframammary

fold), intercostal space level used to expose the recipient vessel for anastomosis (i.e., second or third), timing of the two ICGA assessments, the use and doses of systemic vasopressor (e.g., dobutamine), and location of the peripheral infusion system (e.g., left or right arm or foot). Furthermore, the patients' blood pressure, heart rate, oxygen saturation, and body temperature, as well as the room temperature were registered. To correlate the intraoperative ICGA assessment with the clinical evaluation, postoperative mastectomy skin flap necrosis (MSFN) was assessed within 30 days following surgery.

ICGA protocol

In bilateral breast reconstructions, only one breast was assessed using ICGA in order to ensure ICG washout, without unnecessarily prolong the operation time. For logistical reasons, the second amputated breast was measured. During this study, a handheld near-infrared fluorescence angiography camera (Fluobeam 800, Fluoptics, Grenoble, France) was used. The imaging-head was fixated to a stable and mobile construction, together with laser range finder (Zamo, Robert Bosch Power Tools GmbH, Stuttgart, Germany) to ensure a constant distance above the medial mastectomy skin flap for each measurement and each patient. The distance was set to 25 centimetres allowing the autofocus to function adequately and to visualize the upper and lower part of the medial mastectomy skin flap. Specifically, this region included the midsternal skin to the border of the NAC in a predefined area of 18 x 13 cm. In order to obtain two identical ICGA images, staples were placed at the following landmarks: the jugular notch of the sternum, 18 cm caudal to the jugular notch, from here 13 cm laterally and the second and third intercostal space. During ICGA assessment, the mastectomy skin flap was positioned in a tension-free position over the chest wall with three moist gauzes (45 x 70 cm, folded) underneath the flap to prevent measuring fluorescence originating from the chest wall.

The first ICGA assessment was acquired at 15 to 60 minutes following the skin-sparing mastectomy. The second ICGA assessment was performed at 10 minutes after placing a microvascular clamp on the internal mammary artery (IMA) to mimic the situation following anastomosis. An interval of at least 20 minutes was maintained between the two ICGA assessments to ensure ICG wash-out. Real-time greyscale video-angiography was obtained for 120 seconds per assessment, starting directly following intravenous injection of 0.1 mg/kg of ICG (Diagnostic Green, Aschheim-Dornach, Germany). ICGA images were made using the photo-option at 60, 90, and 120 seconds during the videography. Inflow time was obtained from the ICG videography's and defined as the time (in seconds) from ICG administration to first observation of fluorescence. The mean, minimum and maximum fluorescence intensity (FI) were obtained from the ICGA images in a predefined region of interest (ROI) and measured on a greyscale from 0 (black) to 255 (white) using ImageJ

software (Version 1.53a, ImageJ, National Institutes of Health, USA). Values in between are considered different shades of grey.

Statistical Analysis

Continuous variables were reported as mean with standard deviation (SD) and minimum and maximum for normally distributed data. Categorical data were reported as frequencies and proportions. Outcome data before and after clamping the IMA were compared using the paired samples-T test. The repeated measures analysis of covariance (ANCOVA) was used to assess the influence of the intercostal space (IC) as a covariate. Data were analysed using IBM SPSS Statistics for Mac, version 25 (IBM Corp., Armonk, NY, USA). A p-value <0.05 was considered statistically significant.

RESULTS

Ten women with a mean age of 47 ± 8 years underwent immediate autologous breast reconstruction following SSM, four unilateral and six bilateral. The majority of patients underwent deep inferior epigastric artery perforator (DIEP) flap breast reconstruction (n=9), four unilateral and five bilateral. In one case, a bilateral diagonal upper gracilis (DUG) flap reconstruction was performed. Table 1 summarises the main characteristics of the patients. The smokers temporarily stopped smoking one week (n=1) and four weeks (n=2) prior to the operation. Chemotherapy treatments were administered more than 2 years (n=2) and 4 months (n=1) prior to surgery. Relevant intraoperative features are summarised in Table 2.

Table 1. Main characteristics of the patients

	Mean, SD	N
Patients		10
Age	47 ± 8	
Weight (kg)	79.5 ± 13.3	
BMI (kg/m^2)	27.2 ± 4.1	
ASA classification		
I		1
II		8
III		1
Smoking status		
Yes*		3
No		7
Diabetes		
Yes		1
No		9
Previous chemotherapy		
Yes		3
No		7

BMI: Body Mass Index. *Currently smoking or recently (<4 weeks) stopped.

Figure 1 shows an example of an ICGA image before and after clamping the IMA at 120 seconds following ICG administration in the predefined ROI.

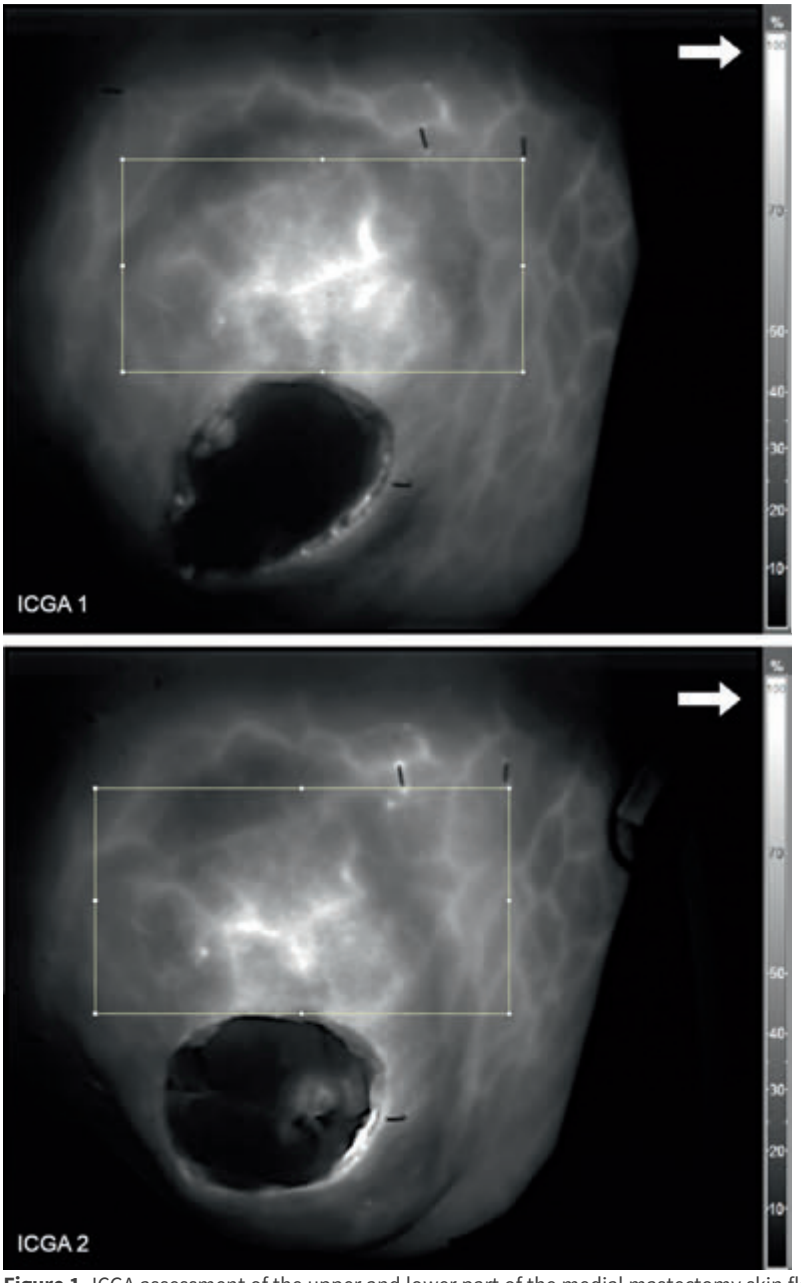

Figure 1. ICGA assessment of the upper and lower part of the medial mastectomy skin flap of the left breast. ICGA image before clamping the IMA (ICGA 1) and an ICGA image after clamping the IMA (ICGA 2) at 120 seconds following ICG administration. Grey rectangle: region of interest: medial mastectomy skin flap. Arrow indicating the cranial side.

Table 2. Intraoperative features

	Mean, SD	N	*P*-value
Free flap breast reconstructions			
Unilateral		4	
Bilateral		6	
Incision for mastectomy			
Round		7	
Round with vertical extension		3	
Intercostal space			
Second		5	
Third		5	
Location of ICG administration			
Dorsum hand		1	
Dorsum foot		9	
Weight of the free flap (gram)	635.5 ± 278.4		
Time from ICGA 1 to ICGA 2	49.5 ± 32.7		
Dobutamine use, dose (mcg/kg/min)			*0.009*
ICGA 1	0.4 ± 0.6	4	
ICGA 2	0.7 ± 0.5	9	
Systolic blood pressure (mmHg)			*NS*
ICGA 1	110 ± 9		
ICGA 2	109 ± 6		
Diastolic blood pressure (mmHg)			*< 0.001*
ICGA 1	63 ± 5		
ICGA 2	58 ± 5		
Heart rate (bpm)			*NS*
ICGA 1	70 ± 9		
ICGA 2	70 ± 10		
Saturation (%)			*NS*
ICGA 1	98 ± 1		
ICGA 2	98 ± 1		
Patients' temperature (°C)			*0.007*
ICGA 1	36.3 ± 0.5		
ICGA 2	36.5 ± 0.5		
Room temperature (°C)			*NS*
ICGA 1	20.6 ± 0.5		
ICGA 2	20.5 ± 0.5		

DIEP: Deep Inferior Epigastric Artery Perforator, SSM: Skin-sparing mastectomy, ICGA: indocyanine green angiography, MSFN: mastectomy skin flap necrosis, bpm: beats per minute.

The mean difference in inflow time, mean, minimum and maximum FI obtained from the three ICGA images (i.e., at 60, 90 and 120 seconds) of the pre-clip and post-clip phase ICGA are presented in Table 3. The repeated measures ANCOVA showed that intercostal space (i.e., IC2 or IC3) was not of significant influence when comparing the mean differences for the primary and secondary outcomes of both the pre-clip and post-clip phase.

Table 3. ICGA 1 (before clamping IMA) versus ICGA 2 (after clamping IMA).

	ICGA 1 (n=10) Mean ± SD	ICGA 2 (n=10) Mean ± SD	Mean difference	*P*-value
Inflow time	24.1 ± 6.4	23.0 ± 7.2	- 1.1	*0.348*
Mean FI	131.4 ± 14.9	124.0 ± 21.9	- 7.4	*0.126*
Min FI	28.6 ± 20.9	33.4 ± 21.4	+ 4.8	*0.086*
Max FI	253.1 ± 4.0	247.6 ± 9.72	- 5.5	*0.166*

Inflow time: time from ICG administration to first observation of fluorescence, Mean, Min and Max FI: mean, minimum and maximum fluorescence intensity (0 to 255) the region of interest at 60, 90 and 120 seconds (mean of the three moments). Analysis: Paired-samples-T-test

The mean, maximum and minimum FI for the 60, 90 and 120 second images are presented separately in supplementary material 1. Additionally, pre-clip and post-clip inflow time and mean, minimum and maximum FI values were presented per patient in supplementary material 2.

Regarding the postoperative complications, one patient was reoperated to stop a bleeding and evacuate hematoma. After a mean follow-up period of 14 ± 9 (range 5 to 30) weeks, there were no cases of infection and/or seroma. Superficial MSFN in a small region of 2 mm on both sides of the vertical incision was observed in two patients. Both cases were resolved following conservative wound therapy.

DISCUSSION

The IMA is the preferred recipient vessel in autologous breast reconstruction. It allows easy access and provides the best calibre match to the donor vessel and offers good flap positioning.[13,14] This observational study confirmed its safety, as the results showed that using the IMA as a recipient vessel in autologous breast reconstruction does not affect the medial mastectomy skin flap perfusion.

In this study, the mean ICG inflow time before and after clamping the IMA were comparable for all ICGA assessments. Therefore, the reliability to compare both ICGA assessments was considered high. The mean minimum FI was higher in the post-clip phase, when compared to the pre-clip phase. While there was no statistically significant difference, this FI increase may be the result of ICG accumulation.[17] Although the mean FI and maximum FI were both slightly lower in the post-clip phase when compared to the pre-clip phase, suggesting a reduction in tissue perfusion, these mean differences were not found to be statistically significant.

The current findings were in line with recent work by our group, reporting no difference in medial mastectomy skin flap perfusion before and after clamping the IMA in autologous breast reconstruction.[18] In this study, a non-invasive imaging technique called Hyperspectral Imaging (HSI) was used to assess mastectomy skin flap perfusion in ten consecutive patients who underwent immediate unilateral autologous breast reconstruction. HSI was found to be successful in correctly identifying MSFN in all three patients who developed MSFN during follow-up. Additionally, no difference was found in medial mastectomy skin flap perfusion before and after clipping the IMA.[18] While the use of HSI in breast reconstructive surgery is relatively novel and needs to be further investigated, current study confirmed these results with ICGA, which already proved its clinical utility for the intraoperative assessment of mastectomy skin flap perfusion.[19]

When concerning mastectomy skin flap perfusion, the IMA with its perforating branches is found to be the most important and reliable vessel in breast vascularization with anastomoses to the lateral thoracic arteries in 50% of cases.[11] This collateral vascularization should ensure vascularization in case the blood supply from the IMA is disrupted by surgical interventions (e.g., CABG). However, two previous studies concluded that removal of the LIMA leads to a reduction in presternal and parasternal perfusion.[15,16] Hence, it was hypothesised that using the IMA as recipient vessel would poses a risk factor for medial mastectomy skin flap necrosis. However, there are major differences between previous studies and the current study, including the proportion of the IMA that is affected and removal of breast tissue and associated vascularization. In CABG surgery, the complete LIMA is harvested including the IMA perforators originating from the 1st to 5th intercostal space, without interfering the blood supply from the lateral thoracic arteries.[20] In autologous breast reconstructive surgery, the perforators raising from the anterograde and retrograde internal mammary vessels are spared, while most of the lateral thoracic artery perforators are sacrificed during mastectomy. The IMA perforators, which originate from the internal mammary vessel and pass through the breast tissue on their way to supply the skin, are also (partially) damaged during removal of the breast.[21,22]

Additionally, the perforators around the already visualised internal mammary vessels are often ligated during the standard exposure and preparation of the recipient vessels.[23] In the current study, medial mastectomy skin flap perfusion assessments were performed once prior to IMA preparation, and once after preparation (including IMA perforator ligation) and clamping of the IMA. With the results of this study, it could be concluded that medial mastectomy skin flap perfusion is not affected by the preparation and use of the IMA as a recipient vessel.

We, therefore, agree with Lee et al. who reported that MSFN is found to be a complication that plastic surgeons cannot completely control and it may be dependent on patients' risk factors, the mastectomy method and the surgeons' level of experience.[7] In the current study, superficial MSFN was observed in two patients. In both cases, necrosis was observed 2 mm around the vertical incision. One of the patients had three known risk factors for MSFN: a BMI of 31.8 kg/m^2, a positive smoking status, and vertical incision to the inframammary fold (IMF).[6,7,24] The latter risk factor also applies to the second patient. Moreover, these mastectomy skin flaps might have been thinner as the mastectomies were performed by different oncological surgeons.[25] Unfortunately, both cases of MSFN were undiscovered by ICGA assessment as the selected region of interest (i.e., medial part) did not comprise the vertical scar. Although there were no clinical signs of reduced perfusion intraoperatively, the size of the region of interest is considered a limitation of this study. However, enlarging the region of interest could lead to indistinct (i.e., blurry) images.

Despite the encouraging results of the current study, some other limitations need to be addressed, including the small sample size. Furthermore, the maximum and mean FI values could be overestimated as it was not possible to correct for ICG accumulation when recording in greyscale. Although multiple ICGA parameters were obtained without unreasonable outliers, enlarging the sample size would result in a more reliable data analysis. Moreover, this study would be strengthened by simultaneous assessment of the contralateral breast with and without clipping the IMA in patients undergoing bilateral mastectomy. However, this is logistically difficult given the maximum working distance of the ICG device.

CONCLUSION

In conclusion, the results of this observational study using ICGA showed that the preparation and use of the IMA as a recipient vessel did not reduce medial mastectomy skin flap perfusion in patients undergoing autologous breast reconstruction.

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SUPPLEMENTARY MATERIAL

Supplementary material 1. Mean, minimum and maximum fluorescence intensities (FI) for ICGA 1 (before clamping IMA) versus ICGA 2 (after clamping IMA) at 60, 90, and 120 seconds following ICG administration.

	ICGA 1 (n=10) Mean ± SD	ICGA 2 (n=10) Mean ± SD	Mean difference	*P*-value
Mean FI				
60 sec	130.0 ± 17.8	119.3 ± 25.5	- 10.7	0.098
90 sec	131.2 ± 15.0	126.8 ± 23.1	- 4.4	0.438
120 sec	133.5 ± 15.2	125.9 ± 18.9	- 7.6	0.109
Minimum FI				
60 sec	30.3 ± 21.9	35.2 ± 22.7	+ 4.9	0.246
90 sec	28.4 ± 22.0	32.0 ± 22.2	+ 3.6	0.303
120 sec	27.9 ± 20.8	33.0 ± 20.5	+ 5.1	0.033*
Maximum FI				
60 sec	250.6 ± 9.4	246.8 ± 17.4	- 3.8	0.582
90 sec	254.1 ± 2.8	246.1 ± 14.6	- 8.0	0.127
120 sec	254.6 ± 1.3	250.0 ± 7.6	- 4.6	0.104

Supplementary material 2. ICGA assessments pre-clip and post-clip.

Patient	ICGA	Inflow time (s)	Mean FI	Min FI	Max FI
1	ICGA 1	21	151.7	32.7	255.0
	ICGA 2	20	137.6	45.0	255.0
2	ICGA 1	23	136.6	15.3	244.3
	ICGA 2	20	146.7	26.0	250.7
3	ICGA 1	27	159.8	75.0	246.7
	ICGA 2	21	163.8	85.0	255.0
4	ICGA 1	21	141.5	45.3	255.0
	ICGA 2	22	125.7	39.7	230.3
5	ICGA 1	21	119.2	45.7	255.0
	ICGA 2	23	106.0	40.7	235.3
6	ICGA 1	17	123.3	14.7	255.0
	ICGA 2	19	113.5	22.7	252.0
7	ICGA 1	27	116.9	14.0	255.0
	ICGA 2	28	124.7	23.7	255.0
8	ICGA 1	17	121.8	12.3	255.0
	ICGA 2	14	91.7	7.0	252.0
9	ICGA 1	38	122.1	7.7	255.0
	ICGA 2	41	129.4	21.7	255.0
10	ICGA 1	29	122.9	26.0	255.0
	ICGA 2	22	101.2	22.7	236.0

ICGA 1: more than 10 min following mastectomy before clipping the IMA, ICGA 2: after clipping the IMA, Inflow time: time from ICG administration to first observation of fluorescence, Mean, Min and Max FI: mean, minimum and maximum fluorescence intensity (0 to 255) in a predefined region of interest in the 60, 90 and 120 seconds ICGA images.

CHAPTER 6

Fasciocutaneous anterolateral thigh flaps for complex abdominal wall reconstruction after resection of enterocutaneous fistulas and the role of indocyanine green angiography

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ABSTRACT

Background

No previous study reported the use of a fasciocutaneous anterolateral thigh (ALT) flap combined with a biological mesh for abdominal wall reconstruction (AWR) after enterocutaneous fistula (ECF) in a single-staged procedure and the use of Indocyanine Green Angiography (ICGA) intraoperatively. The purpose of this study was to determine the feasibility and safety of this procedure and to examine the added value of ICGA in minimizing postoperative complications.

Methods

A single-institution review of a prospectively maintained database was conducted at Maastricht University Medical Centre. To evaluate the feasibility and safety of this procedure, early (≤ 30 days) and late (> 30 days) postoperative complications were assessed. ECF recurrence was considered the primary outcome. To examine the added value of ICGA, complications in the ICGA group and the non-ICGA group were compared descriptively.

Results

Ten consecutive patients, with a mean age of 66.7 years, underwent a single-staged AWR with fasciocutaneous ALT flaps. Mean follow-up was 17.4 months (4.3-28.2). Two early ECF recurrences were observed. Both restored without the need for reoperation. A lower rate of early complications was observed in the ICGA group compared to the non-ICGA group.

Conclusion

The combination of a biological mesh and fasciocutaneous ALT flap is feasible and safe in AWR after ECF repair in a single-staged approach, with an acceptable complication rate in a cohort of complex patients operated in a dedicated centre. ECF closure was achieved in all patients. ICGA seems to be of great added value in minimizing postoperative complications during AWR.

INTRODUCTION

An enterocutaneous fistula (ECF) is defined as an unnatural communication between the gastrointestinal tract and the skin. ECFs allow intestinal contents to leak and can result in electrolyte abnormalities, fluid imbalances, malnutrition and sepsis.[1] Conservative management alone, including optimal nutritional and metabolic support, wound care and antimicrobial therapy, results in ECF closure of approximately one-third of the cases over a period of three to six months.[1,2] Lower fistula output volume (low < 200 ml/day, moderate 200–500 ml/day and high > 500 ml/day), is associated with a higher chance of closure.[1,2]

In case of a persistent ECF, surgical intervention is needed. The purpose of surgery is to resect the fistula complex, which includes the bowel segment giving rise to the fistula, re-establishing intestinal continuity and providing definitive abdominal wall reconstruction (AWR). Since ECFs often coexist with major abdominal wall defects,[3] creating a new abdominal wall might represent a surgical challenge. Current preferred reconstructive options include component separation and local flaps, combined with or without a biological mesh to create coverage of the abdominal cavity.[3,4] A biological mesh consists of an extracellular collagen matrix that is gradually replaced with a collagen framework. This allows for cellular regeneration, neovascularization and remodulation of the mesh into a neofascia which should withstand mechanical forces. Evidence suggests that biological meshes are less sustainable and are associated with higher abdominal hernia and bulging rates when compared to synthetic material.[4] However, biological meshes are associated with higher salvage rates in cases of infection.[5] Hernia and fistula recurrence rates reaching up to 41.7% have been reported after component separation techniques in combination with biological mesh.[6] Moreover, component separation may not be sufficient to restore abdominal integrity in large abdominal wall defects. Therefore, autologous tissue flaps are recommended in these cases.[4,7,9]

Anterolateral thigh (ALT) flaps have become increasingly popular in reconstructive surgery since their use was first reported in 1984.[10] Although the ALT flap is well described and is found to be a feasible autologous tissue flap for reconstruction of various abdominal defects,[11,14] there is a paucity in the literature concerning ALT flaps in AWR after ECF resection.[3,7,15-17] Since a biological mesh has the advantage of reconstructing abdominal fascia in a contaminated area and an ALT flap has the advantage of restoring a complex and large abdominal wall defect, the combination of both seems feasible in these complex ECF cases. The combination of a musculocutaneous ALT flap and a biological mesh has recently been reported in a single-staged AWR after ECF resection.[3] In our opinion, a fasciocutaneous ALT flap combined with a biological mesh, instead of a musculocutaneous ALT flap seems a

more attractive reconstructive option, taking into account the lower probability of donor-site morbidity. To the best of our knowledge, no study reported the use of a fasciocutaneous ALT flap and a biological mesh in single-staged surgery.

To minimize postoperative complications, it is important to assess tissue perfusion intraoperatively as immediate debridement of insufficiently perfused portions of both the ALT flap and abdominal skin edges might reduce postoperative wound complications. Current evaluation of tissue perfusion depends highly on the surgeon's experience and is found to be an unreliable predictor of perfusion.[18] Therefore, the use of Indocyanine Green Angiography (ICGA) is suggested to improve postoperative outcomes in flap surgery.[19] ICGA uses Indocyanine Green (ICG) as a contrast dye with fluorescent characteristics. After intravenous administration, ICG is bound to plasma protein. When exposed to near-infrared excitation, it reemits fluorescent light, which enables in-depth visualization of blood vessels. This innovative technique allows for real-time imaging of tissue perfusion and aids in performing accurate excision of non-vital tissue intraoperatively.[18,19]

The purpose of the current study was twofold. First, to determine the feasibility and safety of single-stage AWR using a fasciocutaneous ALT flap combined with a biological mesh after ECF resection and second, to examine the added value of ICGA in minimizing postoperative complications.

METHODS

A single-institution review of a prospectively maintained database was conducted on ten consecutive patients who underwent single-staged ECF repair and AWR with a fasciocutaneous ALT flap at Maastricht University Medical Centre between July 2017 and September 2018. All procedures performed in this study were in accordance with the ethical standards of the institutional research committee (Medical Ethics Committee in Maastricht: METC 2018-0941) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All ALT flaps were combined with a non-cross-linked porcine-derived biological mesh (Strattice™, LifeCell Corp., Branchburg, NJ, USA).

Preoperative patient characteristics were obtained from the medical records of the included patients and from a prospectively maintained database from the Department of Plastic and Reconstructive Surgery. Variables obtained included age, Body Mass Index (BMI), smoking status, diabetes, previous abdominal surgeries, ECF classification and time from ECF onset to surgery. Surgical characteristics, including free or pedicled ALT flap, operating time, hospital stay, and follow-up were registered. To evaluate feasibility and

safety of this procedure, postoperative complications were assessed. Complications were categorised into early (within 30 days following surgery) and late (after 30 days) complications.

ECF recurrence was considered the primary outcome, proven by the clinical presence of fistula fluid, or confirmed by a computed tomography (CT) scan. Secondary outcomes included abdominal hernia and bulging, flap loss, partial flap necrosis, wound dehiscence, mesh exposure, surgical site infection (SSI), seroma and hematoma. Abdominal hernia was defined as dehiscence of the fascial closure, confirmed by a CT scan and abdominal bulging was defined as any asymmetrical abdominal bulge. Flap loss was defined as the removal of the ALT flap and partial flap necrosis was defined as loss of a part of the flap. Wound dehiscence was defined as any wound rupture over the incision line. Mesh exposure was defined as exposure of (part of) the mesh. We defined SSI as documented erythema, abscess, or purulent drainage requiring treatment with antibiotics or surgical intervention, regardless of whether positive cultures were obtained. Seromas were defined as a fluid collection, verified and treated by ultrasound-guided needle puncture. A hematoma was defined as any collection of blood with or without the need for intervention.

Patients were split into two groups: the ICGA and non-ICGA group. In the non-ICGA group, perfusion of both the ALT flap and abdominal skin edges were clinically evaluated by the plastic surgeon, by determining color and temperature of the skin, capillary refill and dermal edge bleeding. In the ICGA group, intraoperative ICGA was performed with Fluobeam™ (Fluoptics, Grenoble, France) to evaluate perfusion. In both groups, excision of ALT flap and abdominal skin edges was performed when perfusion seemed insufficient. Rates of postoperative complications in the two groups were described.

Continuous variables were reported as mean with standard deviation and minimum and maximum for normally distributed data and reported as median with minimum and maximum for not normally distributed data. Categorical data were reported as frequencies and proportions. Data were analysed using IBM SPSS Statistics for Mac, version 25 (IBM Corp., Armonk, NY, USA). We decided not to perform statistical analysis to compare patients and surgical characteristics, and complications between the ICGA and non-ICGA group, due to a lack of statistical power in this small sample size.

Surgical procedure

The general surgeon, specialised in gastrointestinal surgery, started the procedure. First, extensive adhesiolysis was performed to detect and expose the ECF. The fistula complex, including all bowel segments involved, was resected. Hereafter, intestinal continuity was

re-established with a side-to-side stapled anastomosis, and the complete intestine was screened for unnoticed serosal injuries.

After resection of the ECF complex, the biological mesh 20 × 30 cm in size was cut to the defect size, positioned underlay with an overlap of at least 4 cm and fixed to the remaining the abdominal musculofascial layer with interrupted Prolene® Polypropylene sutures (Ethicon Inc. Somerville, NJ, USA) size 2-0 in an outer and inner ring to reconstruct the fascia of the rectus abdominis muscles in all patients. See Figure 1a.

Following fascial closure, a free or pedicled ALT flap was harvested as a fasciocutaneous flap and raised on the descending branch of the lateral femoral circumflex artery. See Figure 1b and 1c. In case of a pedicled ALT flap, the flap was tunnelled under the rectus femoris muscle to reach the defect in the abdomen.

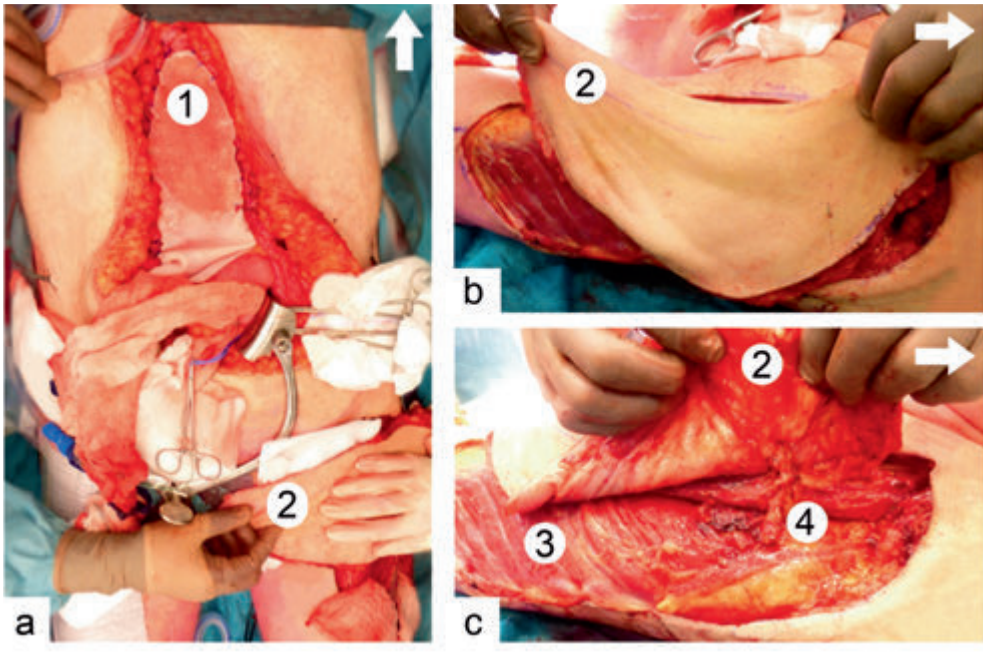

Figure 1. Intraoperative overview of the abdominal wall reconstruction (arrow indicating the cranial side). **a** Intraoperative aspect of the abdominal wall after suturing the biological mesh (1) to reconstruct the fascial defect and the fasciocutaneous ALT flap (2) that was harvested. **b** Intraoperative aspect of the fasciocutaneous ALT flap with intact underlying musculus vastus lateralis (3). **c** ALT flap raised on its vascular pedicle (4)

In the case of a free ALT flap, a pedicle of approximately 10–12 cm was harvested and the anastomosis was performed underneath the rectus abdominis muscle to the inferior epigastric vessels.[20] The size of the flap was determined according to the defect of the abdominal wall after suturing the biological mesh. For the defects at the level of the umbilicus or lower, a pedicled flap was used whereas for defects located more cranially, a free ALT flap was preferred. The fascia and subcutaneous tissue of the ALT flap were sutured to the remaining muscular fascia of the abdominal wall. The skin island of the ALT flap was placed above the biological mesh, covering it in its totality. The abdominal skin was sutured to the skin island of the ALT flap, after de-epithelization of the edges of the flap. See Figure 2a and 2b for the preoperative and postoperative aspect of the abdomen, respectively. The donor-site wound on the anterolateral thigh was approximated on both the cranial and caudal side without undue tension, and the remaining defect was covered with a split skin graft.

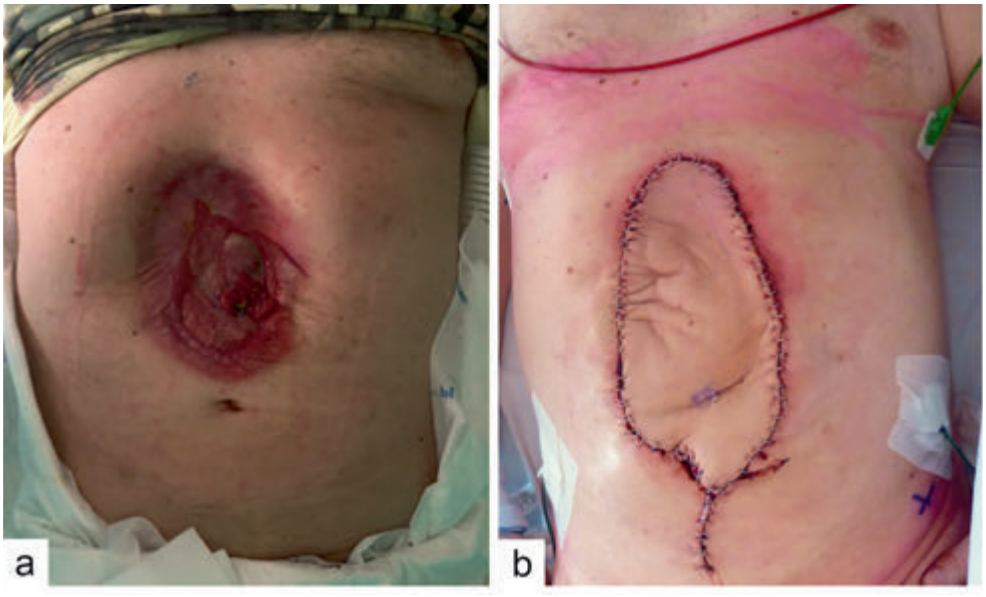

Figure 2. a Preoperative aspect of the abdominal wall. **b** Postoperative result of the abdominal wall reconstruction

ICGA protocol

Vascularization of the ALT flap and abdominal skin edges were checked after flaps harvesting. The handheld imaging-head was manually positioned approximately 20–25 cm above the skin. Real-time greyscale video-angiography was obtained for 120 s, starting 10 s after intravenous injection of 0.1 mg/kg of ICG (Diagnostic Green, Aschheim-Dornach, Germany).

Fluorescent regions (i.e., white area) indicated areas of well perfused, whereas no fluorescence (i.e., black area) and less fluorescence (i.e., grey area) indicated areas of insufficiently perfused tissue and these were removed during surgery. No additional relative perfusion assessment or absolute flow value assessment using software were used. See Online Resource 1 for an intraoperative ICG video angiography with a description of the images over time.

RESULTS

Patient characteristics

A total of ten consecutive patients underwent single-staged AWR with a fasciocutaneous ALT flap combined with biological mesh after ECF resection. Table 1 summarises the main characteristics of the sample size. Median follow-up was 17.4 months (4.3–28.2). One patient died by suicide 4.3 months after surgery. This patient was known with psychiatric complaints that were not related to the abdominal wall reconstruction. Follow-up measurements of this patient were excluded from the 'late complication' analysis. Two patients were operated a second time within the first 30 days after initial surgery to revise the arterial anastomosis ($n = 1$) and to explore a suspected bowel leakage ($n = 1$).

Secondary outcomes

One patient was diagnosed with an abdominal hernia confirmed by a CT scan, 3 months after surgery. Abdominal bulging was observed in three patients. None of these cases required reoperation as symptoms of discomfort and abdominal pain dissolved by wearing abdominal binders.

No ALT flaps were lost. Early partial ALT flap necrosis occurred in two patients. In one patient, necrosis was first observed 2 days after surgery. The necrotic part was debrided in the patient ward, followed by vacuum-assisted wound therapy. In the other patient, necrosis was observed 18 days after surgery. After debridement and vacuum-assisted wound therapy, the remaining defect was closed with a rhomboid flap 66 days after surgery.

Wound dehiscence was observed in five patients (four early and one late cases). Two out of five patients experienced wound dehiscence following ALT flap necrosis as described above. The remaining three patients were treated with conservative wound therapy with ($n = 2$) or without ($n = 1$) vacuum-assisted therapy. Mesh exposure as a result of wound dehiscence was observed in two out of six of the aforementioned patients. The biological mesh was covered with granulation tissue in both patients in a few days and no patient required explantation of the biological mesh.

Table 1 Patient and surgical characteristics

Variable	Statistics
Total patients	10
Age (years)	
Mean (SD)	66.7 (9.6)
Min–max	53–78
Body Mass Index (kg/m^2)	
Mean (SD)	26.4 (4.7)
Min–max	19.3–35.3
Diabetes, *n* (%)	4 (40)
Smoking, *n* (%)	0 (0)
Previous surgeries	
Median[a]	4
Min–max	2–15
ECF classification, [b] *n* (%)	
Low	5 (50)
Moderate	2 (20)
High	3 (30)
Fistula onset to surgery (days)	
Mean (SD)	185 (83)
Min–max	56–313
ALT, *n* (%)	
Free	7 (70)
Pedicled	3 (30)
Operating time (min)	
Mean (SD)	634 (89)
Min–max	434–780
Hospital stay (days)	
Median[b]	28
Min–max	14–105
Follow-up (months)	
Mean (SD)	17.4 (7.3)
Min-max	4.3-28.2

[a]The data from previous surgeries and hospital days were not normally distributed

[b]Low output < 200 ml/day, moderate output 200–500 ml/day, high output > 500 ml/day.[1]

Table 2. Patient and surgical characteristics ICGA versus non-ICGA

Variable/statistics	ICGA	Non-ICGA
Total patients	5	5
Age (years)		
Mean (SD)	66.2 (9.5)	67.2 (10.8)
Min–max	54–77	53–78
Body mass index (kg/m^2)		
Mean (SD)	26.6 (5.8)	26.1 (3.9)
Min–max	19.3–35.3	21.9–32.1
Diabetes, *n* (%)	1 (20)	3 (60)
Smoking, *n* (%)	0 (0)	0 (0)
Previous surgeries		
Median[a]	4	4
Min–max	2–7	3–15
ECF classification, *n* (%)		
Low	3 (60)	2 (40)
Moderate	1 (20)	1 (20)
High	1 (20)	2 (40)
Fistula onset to surgery (days)		
Mean (SD)	227 (69)	142 (78)
Min–max	146–313	56–221
ALT, *n* (%)		
Free	3 (60)	4 (80)
Pedicled	2 (40)	1 (20)
Operating time (min)[b]		
Mean (SD)	638 (130)	630 (32)
Min–max	434–780	577–658
Hospital stay (days)		
Median[a]	22	32
Min–max	14–105	23–98
Follow-up (months)		
Mean (SD)	14.4 (2.5)	20.3 (9.6)
Min–max	12.6–18.6	4.3–28.2

[a]The data from previous surgeries and hospital days are not normally distributed

[b]Total operation time (i.e., surgical and reconstructive time)

Six patients experienced an early SSI. One patient was treated with antibiotics only for a superficial SSI and five patients were treated with a combination of ultrasound-guided aspiration and antibiotics for deep SSIs. Three patients experienced early seroma and were treated with ultrasound-guided aspiration, seven and fifteen days after surgery, respectively. No hematomas were observed during this study.

Indocyanine green angiography

Intraoperative perfusion assessment with ICGA was performed in five patients. ICGA was performed by the plastic surgeon when the ICGA device was available. Table 2 summarises the main characteristics of patients in the ICGA group and patients in the non-ICGA group. To examine the added value of ICGA, postoperative complications in the ICGA group versus the non-ICGA group were compared. See Table 3.

Table 3. Early and late postoperative complications in total group, ICGA-group and non-ICGA group

Complication	Total (*n* = 10)	ICGA (*n* = 5)	Non-ICGA (*n* = 5)
Early complications *n* (%)[a]			
ECF recurrence	2 (20)	1 (20)	1 (20)
Partial flap necrosis	2 (20)	0 (0)	2 (40)
Wound dehiscence	4 (40)	1 (20)	3 (60)
Mesh exposure	2 (20)	0 (0)	2 (40)
Infection	6 (60)	4 (80)	2 (40)
Seroma	3 (30)	1 (20)	2 (40)
Any early complication	8 (80)	3 (60)	5 (100)
Total, *n* (% of complications)	19	7 (37)	12 (63)
Late complication, *n* (%)[a]	Total (*n* = 9)[b]	ICGA (*n* = 5)	Non-ICGA (*n* = 4)
Abdominal hernia	1 (11)	0 (0)	1 (25)
Abdominal bulge	3 (33)	2 (40)	1 (25)
Wound dehiscence	1 (11)	1 (20)	0 (0)
Any late complication	5 (56)	3 (60)	2 (50)
Total, *n* (% of complications)	5	3 (60)	2 (40)

[a]Early complication: onset within 30 days following operation. Late complication: onset after 30 days following operation

[b]Follow-up measurements of patient who died by suicide were excluded from the 'late complication' analysis

DISCUSSION

Reconstruction of a large and complex abdominal wall defect after ECF resection is challenging and requires surgical creativity. The combination of an ALT flap and biological mesh is a potential solution to reconstruct large abdominal wall defects with the potential advantage of lowering the chance of ECF recurrence and postoperative abdominal hernia and bulging rates.[7,16] We reviewed our experience with single-staged AWR after ECF resection, utilizing fasciocutaneous ALT flaps combined with biological mesh, to determine the feasibility and safety in the face of ECF recurrence and other postoperative complications.

Two ECF recurrences have been observed early after the operation, representing a 20% ECF recurrence rate in this study. This is comparable to the reported ECF recurrence rates ranging from 9% to 41.7% in studies concerning ECF repair and AWR with biological mesh reinforcements techniques without the use of ALT flaps.[6,8,22] For instance, Connolly et al. reviewed 61 patients who underwent ECF repair and simultaneous AWR with component separation technique. After stratification for closure technique, they found a 41.7% ECF recurrence rate after suture repair, combined with a porcine collagen mesh during a median follow-up time of 29 months.[6] Krpata et al. and Atema et al. reported substantially lower ECF recurrence rates of 10.8% after a mean follow-up of 20 months and 9% after a mean follow-up of 7 months, respectively.[8,22] However, 25% and 57% of patients who experienced ECF recurrence after ECF repair and AWR in their series, respectively, were in need of reoperation. In the current study, both recurrent ECFs were closed definitively with conservative treatment only. Importantly, no ECF recurrences have been observed during long-term follow-up in any of the included patients.

Likewise, rates of abdominal herniation and bulging ranging from 13% to 50.6% have been reported in previous studies concerning contaminated AWR, with reoperation rates reaching 50%.[6,8,22-26] For instance, Kaufmann et al. conducted a multicentre cross-sectional cohort study of 77 patients and reported an abdominal hernia rate of 28.6% and an abdominal bulging rate of 50.6% after a mean follow-up of 22.2 months. Of those patients, 45% of patients underwent reoperation.[26] Although Atema et al. reported a low rate of abdominal hernia of 13%, half of these patients underwent reoperation during follow-up.[22] In our study, lower rates of abdominal hernia and bulging were observed and most importantly, no patient required reoperation. All four patients were treated conservatively by wearing abdominal binders, without experiencing physical complaints.

Single-staged AWR with ECF repair is known to be associated with a high rate of postoperative infections. The SSI rate in our series of complex patients was 60%, which falls within the range of previously described rates following AWR after ECF repair (27.3–65%).[6,8,24,26] In contrast to the safety of a biological mesh to endure exposure and SSI,[8] biological mesh removal rates as high as 6.5% have been reported in recent studies.[25,26] Although a mesh exposure rate of 20% was observed in the current study, no explantation of a biological mesh was required.

In the current study, we utilised an ALT flap with a biological mesh instead of current preferred reconstructive options (e.g., component separation techniques and local flaps) to create coverage of the abdominal wall. The additive benefit of an ALT flap is that AWR of large defects can be performed without undue tension on the abdominal skin, which would have been inevitable with component separation or local flaps. Another benefit is that the ALT flap is thicker compared to the native abdominal skin and thus provides more strength to the abdominal wall once the biological mesh is remodeled into neofascia over approximately 6 months.[27] The results obtained in this series showed promising (i.e., comparable to better) long-term outcomes in comparison with the existing literature. Consequently, we demonstrated that the combination of a biological mesh and fasciocutaneous ALT flap in AWR in a single-staged procedure is feasible and safe with an acceptable complication rate. During follow-up, no mortality related to the surgical procedure was observed. The majority of complications were considered minor complications since, in most cases, no surgical reintervention was needed after a long follow-up.

The ability to compare our data with other published series concerning ALT flaps in single-staged ECF repair is limited. To the best of our knowledge, this pilot study comprised the first series that aimed to evaluate single-staged AWR using fasciocutaneous ALT flaps combined with a biological mesh after ECF resection. Only few authors have reported their early experience using this procedure.[3,7,15-17]

Two independent case reports described the use of a pedicled ALT flap to successfully close an abdominal defect after resection of a malignant ECF[16] and to close an abdominal defect with an untraceable ECF.[15] Lambe et al. reported a retrospective review on seven patients who had undergone massive extensive enteroatmospheric fistulation with a pedicled subtotal lateral thigh flap including the vastus lateralis muscle without a biological mesh.[17] Although no ECF recurrences were observed in this study, a higher rate of abdominal herniation of 14% was observed.[17] Walia et al. also reported a retrospective review on seven

patients who underwent a pedicled ALT flap for AWR. However, no single-staged ECF repair and AWR were performed since the authors favored a staged approach for ECF patients.[7]

More recently, another author reported a series of eighteen patients who had undergone single-staged ECF repair and AWR with a musculocutaneous ALT flap and biological mesh. During a mean follow-up of 24 months, no ECF recurrences were observed and abdominal bulging was observed in only one patient.[3] The lower rate of abdominal hernia and bulging compared to the rates in current study, could be due to the fact that the addition of vastus lateralis included in musculocutaneous flaps might provide extra fascial strength in comparison to a fasciocutaneous ALT flap.[3,7,17] On the other hand, musculocutaneous flaps can potentially increase donor-site morbidity.[3] In our experience, there is a low risk of donor-site morbidity after harvesting a fasciocutaneous ALT flap and covering the donor-site defect with a split skin graft. No patient experienced donor-site morbidity in this study. In our opinion, the additive benefit of a biological mesh over fascia or muscle component of an ALT flap is that a mesh is easier to be customised (i.e., cut to size) to cover the size of the defect. Furthermore, a mesh is easier to be fixed in an underlay position in the abdominal defect, before positioning the ALT flap.

ICGA is previously found to be a more reliable predictor of tissue perfusion compared to clinical assessment.[28] In this pilot study, a lower rate of early complications was observed in the ICGA group compared to the non-ICGA group. In particular, rates of perfusion related complications including partial flap necrosis and wound dehiscence were lower in the ICGA group (0–40%, respectively) compared to the non-ICGA group (40–60%, respectively). Resection of insufficiently perfused abdominal skin and portions of the ALT flap in the ICGA group, resulted in a necrosis rate of 0%. This observation is consistent with results reported by Wormer et al. who performed a randomised controlled trial evaluating ICGA in reducing wound complications in complex AWR. Although they found a 6.1% rate of skin necrosis in the non-ICGA group versus 2.2% in the ICGA group, this difference was not statistically significant.[29] In addition to the assessment of abdominal skin and ALT flap perfusion, ICGA serves as an effective tool to assess anastomotic bowel perfusion.[30] Moreover, it could have a role in reducing anastomotic leakage rates following ECF resection, which reflects the potential for implementation of ICGA in reconstructive abdominal wall surgery.

Several limitations of the present study need to be addressed. Due to the limited number of patients included in the current study, no statistical analyses were performed. Furthermore, the retrospective design may have resulted in missing data and selection bias. However, accurate outcome data were entered into a prospectively maintained database so this risk should be minimised. The results of this study cannot be generalised to every hospital, since

all surgical procedures are performed in a dedicated centre, experienced in abdominal wall surgery and reconstructive surgery. Conversely, this study is strengthened by the fact that that all AWRs are performed by the same team and using the same surgical strategy.

As the number of operated patients increases, more data will become available. In the future we will collect more data on this specific cohort of complex ECF patients to gain more expertise and improve the proposed surgical procedure.

CONCLUSION

The combination of a biological mesh and fasciocutaneous ALT flap is feasible and safe in AWR after ECF repair in a single-staged approach, with an acceptable complication rate in a cohort of complex patients operated in a dedicated centre. ECF closure was achieved in all patients. Since a lower number of early complications were observed in the ICGA group, ICGA seems to be of great added value. Further research using the same procedure in the context of prospective clinical trials is needed to confirm the role of ICGA in AWR.

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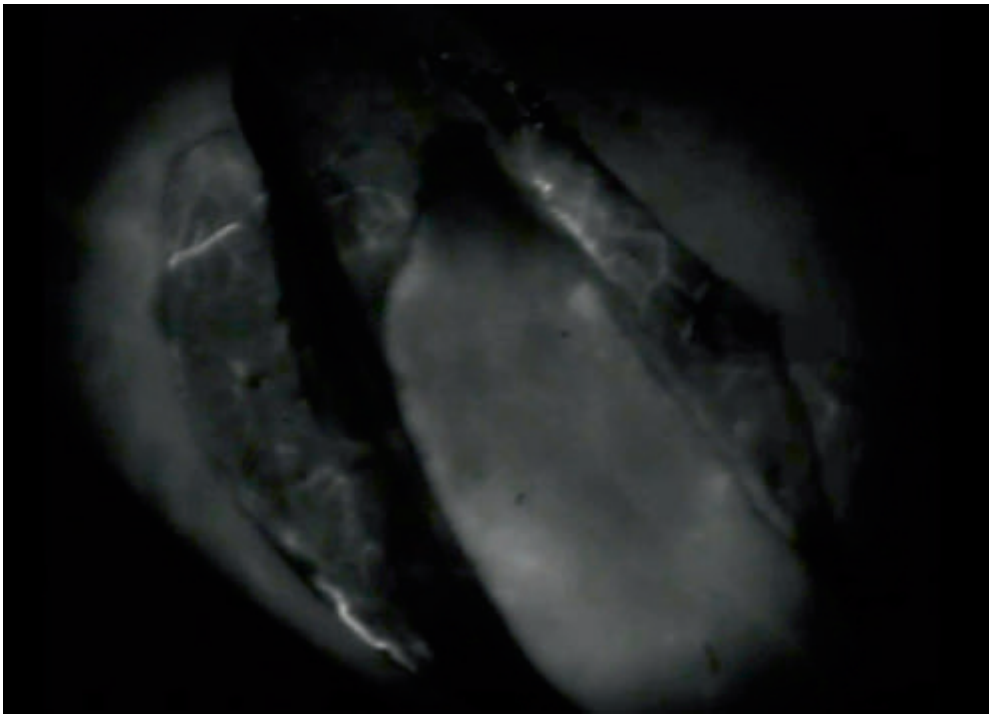
SUPPLEMENTAL DIGITAL CONTENT

Online resource 1. Intra-operative ICG video angiography during abdominal wall reconstruction. 0:00 to 0:08: start of arterial inflow of fluorescent ICG bound to albumin. 0:09 to 0:19 (cranial up, caudal down): slowly increasing fluorescence intensity during assessment of the abdominal wall). 0:20 to 0:57 (proximal right, distal left): assessment and marking of the ALT flap with a clear difference in fluorescence intensity between the distal and proximal portion of the ALT flap. The region of no fluorescence (i.e., black area) is marked and removed in this patient. 0:58 to 1:42 (cranial up, caudal down): during assessment of the abdominal skin the region of less fluorescence (i.e., grey area) is marked and removed. The remaining abdominal skin is sufficiently perfused, indicated by bright fluorescence regions.



Video available online:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8055571/bin/10029_2020_2167_MOESM1_ESM.mp4

CHAPTER 7

Long-term outcomes following lymphaticovenous anastomosis (LVA) for 100 cases of lymphedema and the role of indocyanine green lymphography

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ABSTRACT

Background

Lymphedema is a debilitating condition that significantly affects patient's quality of life (QoL). The aim of this study was to assess the long-term outcomes after lymphaticovenous anastomosis (LVA) for extremity lymphedema.

Methods

A single-centre prospective study on upper and lower extremity lymphedema patients was performed. All LVA procedures were preceded by outpatient Indocyanine Green (ICG) lymphography. Quality of life measured by the Lymph-ICF was the primary outcome. Limb circumference, use of compression garments, and frequency of cellulitis episodes and manual lymphatic drainage (MLD) sessions were secondary outcomes. All patients underwent preoperative ICG lymphography during the outpatient appointment. The lymphatic vessels suitable for LVA surgery and the incision site for anastomosis were marked with a regular skin marker, captured in colour pictures and used during the operation.

Results

One hundred consecutive patients, predominantly experiencing upper extremity lymphedema following breast cancer (n = 85), underwent a total of 132 LVAs. During a mean follow-up of 25 months, mean Lymph-ICF score significantly decreased from 43.9 preoperative to 30.6 postoperative, representing significant QoL improvement. Decrease in upper and lower limb circumference was observed in 52% of patients with a mean decrease of 6%. Overall mean circumference was not significantly different. Percentage of patients that could reduce compression garments in the upper and lower extremity group was 65% and 40%, respectively. Number of cellulitis episodes per year and MLD sessions per week showed a mean decrease of respectively 0.6 and 0.8 in the upper extremity and 0.4 and 1.0 in the lower extremity group.

Conclusion

LVA resulted in significant QoL improvement in upper and lower extremity lymphedema patients. Limb circumference did not significantly improve but good results concerning compression garments, cellulitis episodes, and MLD sessions were obtained. Additionally, a simple and patient-friendly method for outpatient ICG lymphography is presented.

INTRODUCTION

Lymphedema is a chronic, debilitating condition, characterised by abnormal accumulation of subcutaneous protein-rich fluid due to failure of the lymphatic drainage system.[1-4] It can affect any part of the body but is predominantly observed in the upper and lower extremities.[5] Lymphedema causes physical morbidity as it can lead to pain, skin tightness, heaviness, recurrent periods of cellulitis, and decreased range of motion.[3-7] Moreover, it affects psychological and emotional well-being instigating body image disturbances, anxiety, and depression.[5,8] Consequently, lymphedema will significantly affect quality of life (QoL) and the ability to work and participate in social activities.[8]

Treatment of lymphedema traditionally begins with complex decongestive therapy, consisting of a combination of skin care, exercise, compression therapy, and manual lymphatic drainage (MLD).[5,9] This treatment is time-consuming, and the effectiveness largely depends on the patient's compliance.[3] Although this may result in enough symptomatic relief, none of the therapies will cure lymphedema, thus lifelong time-consuming therapy appointments and continuous use of compression garments are necessary, with a significant practical impact.[2,3,8]

Therefore, over the last decades several surgical procedures have been proposed for the treatment of lymphedema, including lymphaticovenous anastomosis (LVA).[2,10] LVA is a minimal invasive method that redirects excessive lymph fluid from the oedematous limb into the venous system, by anastomosing lymphatic vessels to subdermal venules.[1] Although LVA surgery was already proven to be a valuable procedure in the 1970s,[11,12] it gained popularity after the introduction of supermicrosurgical techniques by Koshima et al. and the availability of indocyanine green (ICG) lymphography.[13-16]

ICG lymphography is an innovative imaging technique that combines the administration of the fluorescent dye ICG with a near-infrared camera. This imaging modality enables direct visualization of the lymphatic system and is therefore used to determine the stage of lymphedema and evaluate the functionality of lymphatic vessels.[10,15,17,18] In addition, ICG lymphography can guide surgeons during surgery by facilitating real-time decision-making, leading to more reliable and improved outcomes following LVA.[1]

Over the years, numerous studies have investigated the efficacy of LVA as a treatment for lymphedema, demonstrating promising results.[6,9,18-25] Recent systematic reviews have demonstrated that limb circumference significantly decreased and QoL significantly increased following LVA surgery.[2,3] However, most studies involved small sizes and reported

short follow-up periods. In addition, a minority of previous studies have reported the effects of LVA on discontinuation of compression garments [9,17,22-24] and episodes of cellulitis.[1,4,6,26]

The aim of the current study was to assess the effect of LVA surgery in a large cohort of 100 lymphedema patients during a 24-month follow-up period. Special attention was paid to patients' QoL, limb circumference, use of compression garments, the number of cellulitis episodes, and the number of MLD sessions.

METHODS

A single-institution prospective cohort study on 100 consecutive patients who underwent LVA procedures for primary and secondary lymphedema was performed at Maastricht University Medical Centre between June 2015 and June 2018. Approval of the institutional review board was obtained (METC 2018-0869). Written informed consent was obtained from all included patients.

Patient selection

Patients were eligible for LVA if they experienced subjective complaints of a confirmed unilateral upper or lower limb lymphedema, stage I to III according to the International Society of Lymphology (ISL) classification, and having undergone complex decongestive therapy for at least 3 months.[27] Additionally, patients were required to have patent lymphatic collecting vessels as visualised by preoperative ICG lymphography.[15] Exclusion criteria were as follows: active recurrent disease or metastasis in patients with history of malignancy, and the presence of an active skin infection. No limits were set on the time from the onset of lymphedema. Preoperative patient characteristics were obtained from the medical records of the included patients and from a prospectively maintained database. Variables obtained included the following: age, Body Mass Index (BMI), location (arm or leg), side and aetiology of lymphedema, ISL stage, and ICG stage. The preoperative ISL and ICG staging were frequently, but not always, defined by the operating microsurgeon (SQ).

ICG lymphography

Although there is no substantial difference in the practical use of ICG lymphography, the time when to perform ICG lymphography is novel in this study. All patients underwent preoperative ICG lymphography during the outpatient appointment. A standard protocol was used for ICG lymphography: 0.02 ml (5 mg/ml) of ICG (PULSION® 25 mg for solution, PULSION Medical Systems SE, Feldkirchen, Germany) was subcutaneously injected in the second and fourth web spaces of the hand or foot. Patients were asked to wait in the waiting room for approximately 20 min, where they were able to drink a cup of coffee and complete

the Lymph-ICF. Patients were regularly monitored by a nurse, despite the low incidence of side effects of ICG.[28] Meanwhile, the plastic surgeon was able to perform another consult before conducting the ICG lymphography. The fluorescence signal was mapped and recorded using a handheld near-infrared camera (Fluobeam®, Fluoptics, Grenoble France). Subsequently, the lymphatic vessels suitable for LVA surgery and the incision site for anastomosis were marked with a regular skin marker. These markings and relevant anatomical landmarks were captured in colour pictures that were used during the operation to determine the place of incision (see Figure 1).

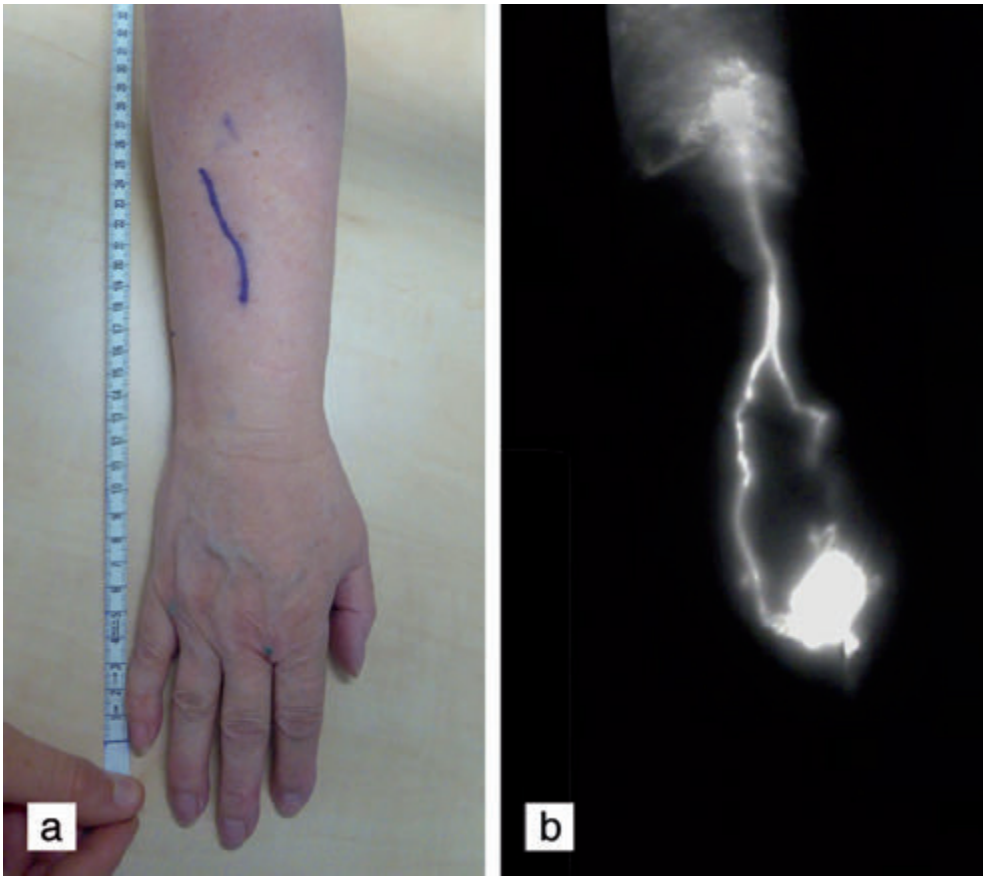

Figure 1. Outpatient ICG lymphography. Preoperative planning using ICG lymphography in the outpatient clinic (a) Lymph vessels visualised by ICG lymphography; linear pattern to stardust pattern and (b) corresponding markings for incision site, based on ICG lymphography

To assess the severity of upper and lower extremity lymphedema, dermal backflow was categorised into six stages according to Narushima et al. Briefly, in stage 0, no dermal backflow pattern is seen. In stage I, a splash pattern is seen around the axilla or in the groin

region. In stage II to IV, progressive stardust patterns are observed, and stage V represents a diffuse pattern in the whole limb.[15]

Surgical procedure

The LVA procedures were performed under local anesthesia (bupivacaine hydrochloride 5 mg/ml with adrenaline 5 µg/ml) using the technique described by Koshima et al.[13] All procedures were performed by one microsurgeon (SQ), within three to four months following the outpatient ICG lymphography. The incision was performed at the level of the lymphatic collecting vessel, as located using the aforementioned preoperative ICG lymphography. Using a microscope (ZEISS OPMI PENTERO 900; × 25 to × 50 magnification), one or more lymphaticovenous anastomoses were completed between a suitable lymphatic collecting vessel and a subcutaneous vein. In general, the anastomoses were performed in an end-to-end fashion using Ethilon 11-0. End-to-side anastomoses were created when the recipient vein was substantially larger than the lymphatic collecting vessel. Finally, the "milk test" was performed to evaluate anastomotic patency and evidenced lymphatic flow into the venules by gently stroking the lymph vessel. Hereafter, the skin was closed. All surgeries were performed within a maximum duration of 120 min. If not all potential lymphaticovenous anastomoses could be created within 120 min, a second or third procedure was planned. Figure 2 illustrates a completed LVA intra-operatively.

Postoperative protocol

The postoperative protocol was followed as previously described.[17] In brief, patients were not allowed to wear compression garments or receive MLD in the first four weeks after surgery to minimize the chance of damaging the newly formed, fragile anastomosis. After this period, patients could choose, in consultation with the plastic surgeon and skin therapist, to restart compression garments and/or MLD sessions, depending on the presence of subjective complaints and the presence of swelling in the limb.

Outcomes

Patients' quality of life (QoL) was considered the primary outcome in this study. Secondary outcome measures included limb circumference, use of compression garment, annual episodes of cellulitis, and weekly MLD sessions. Patients' QoL and circumference measurements were obtained preoperatively and postoperatively, presented in different follow-up periods: less than 2 months, 2 to 6 months, 6 to 12 months, 12 to 24 months, and > 24 months following LVA surgery. The final QoL or circumference measurement at the last outpatient appointment for each patient was also obtained. Similarly, the other outcomes (e.g., episodes of cellulitis) were evaluated during the last outpatient appointment per patient.

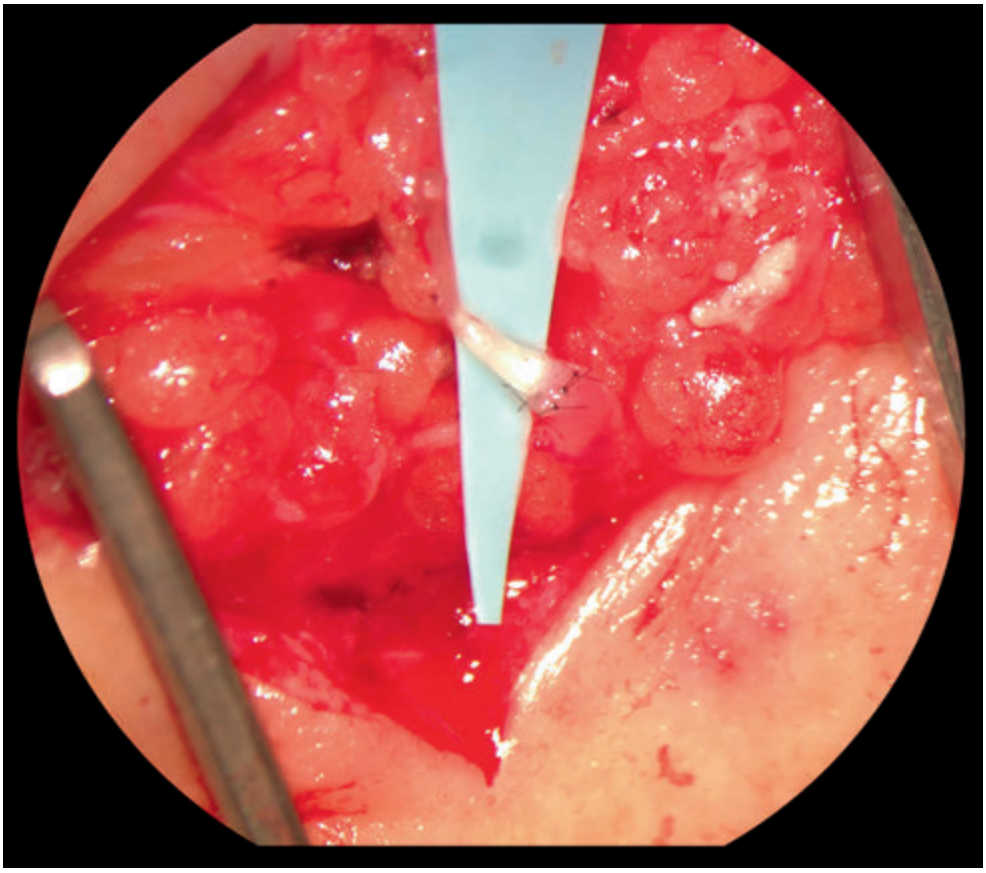

Figure 2. Intraoperative picture of an end-to-end lymphaticovenous anastomosis

Quality of life

Disease-specific QoL was measured by the Dutch Lymphedema Functioning, Disability, and Health questionnaire (Lymph-ICF).[29] This is a validated questionnaire to evaluate limb specific symptoms in lymphedema patients using a Visual Analogue Score (VAS), with the advantage of a wide score range and high sensitivity.[30,31] There are two versions: one for upper extremity and one for lower extremity lymphedema. Both comprise five domains: physical function, mental function, household activities, mobility activities, and life and social activities. The scores range from 1 to 100: a lower score on the questionnaire represents a better QoL. A decrease in VAS of more than 10 in the total score was considered to be statistically significant ($p < 0.05$).[29]

Limb circumference

Circumference change in the operated arm and leg were calculated with the Upper and Lower Extremity Lymphedema index (UEL- and LEL-index).[32] Limb circumference was

measured at standardised landmarks on the arm or leg, and together with the patient's BMI, the UEL- or LEL-index was calculated.[32] Patients had to remove their compression garment 24 h prior to the follow-up moment in order to achieve a more reliable measurement.[17]

Statistical analysis

Continuous variables were reported as mean with standard deviation. Categorical data were reported as frequency and proportion. To examine the effect of LVA, differences in preoperative and postoperative means were analysed using the Paired Samples T-Test. Differences in proportions were analysed using the Chi-squared test or McNemar test for independent and dependent proportions, respectively. Differences in change scores (preoperative minus postoperative) between the operated limb and non-operated limb were tested using linear regression analyses. To deal with loss to follow-up, postoperative outcome measurements were compared to the related preoperative outcome measurements for the specific number of patients.

The relationship between the number of LVAs, ICG stage (0–3 vs. 4–5), circumference difference, follow-up months, compression garment (no versus yes), and success on QoL was computed using linear regression analysis and quantified as unstandardised beta (B) with a 95% confidence interval (CI).

Results were analysed using IBM SPSS Statistics for Windows, version 24 (IBM corp. ®, Armonk, N.Y, USA). A *P*-value < 0.05 was considered to be statistically significant.

Demographics

One hundred consecutive patients with a mean age of 57.1 years underwent a total of 132 operations, in which 270 anastomoses were completed. Since some patients had more potential LVAs than could be performed in 120 min, the total number of LVAs was split over two or three procedures. The majority of patients ($n = 70$) underwent a single operation with a mean number of 2.7 LVAs. Twenty-eight patients underwent 2 operations with a mean number of 3.93 LVAs and only 2 patients underwent 3 operations with a mean number of 7.5 LVAs. Mean follow-up was 25.0 months. LVAs were predominantly performed in women with unilateral upper limb lymphedema following breast cancer treatment ($n = 85$), classified as ISL stage IIA or ICG stage 3. Table 1 summarises the main characteristics of the patients included in the current study.

Table 1. Demographics and clinical information

		Mean, SD	*N*
Patients			100
Operations			132
LVAs			270
Gender	Female		94
	Male		6
Age (years)		57.1 ± 10.6	
BMI (kg/m^2)		26.3 ± 4.9	
Location of lymphedema	Arm		85
	Leg		15
Etiology of lymphedema	Primary		6
	Secondary		94
Affected side	Left		46
	Right		54
ISL stage[a]	I		4
	IIA		69
	IIB		25
	III		2
ICG stage[b]	1		1
	2		19
	3		45
	4		26
	5		9
Follow-up (months)		25.0 ± 10.9	
Number of OR per patient		1.3 ± 0.5	
	1 operation		70
	2 operations		28
	3 operations		2
Number of LVAs per patient		2.7 ± 1.4	
	1 LVA		16
	2 LVAs		39
	3 LVAs		21
	4 LVAs		15
	5 LVAs		5
	6 LVAs		1
	7 LVAs		2
	8 LVAs		1

[a]International Society of Lymphology

[b]Stage according to ICG lymphography

Quality of life

After a mean follow-up of 25 months, the mean total lymph-ICF score showed a decrease of 13.3 ($p < 0.001$); 43.9 ± 19.0 preoperative to 30.6 ± 20.2 postoperative ($n = 100$). See Figure 3.

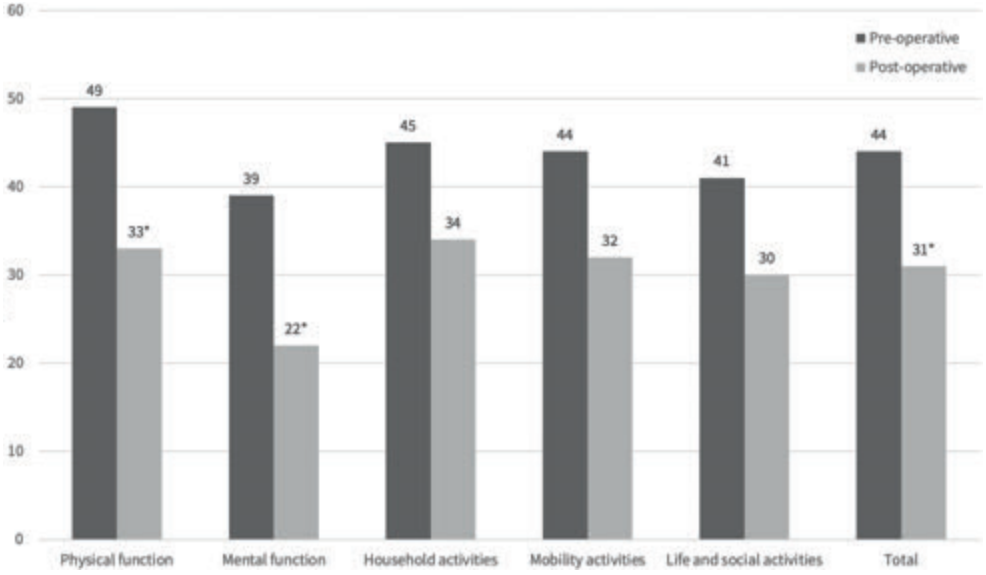

Figure 3. Mean Lymph-ICF preoperatively and postoperatively. Final Lymph-ICF measurement at last outpatient appointment for each patient ($n = 100$) over a mean follow-up 24.5 ± 10.9 months. Analysis: Paired Samples *T*-Test. *Statistically significant difference: A decrease of 10, 15, 12, 23, 15, and 14 in total score, physical function, mental function, household activities, mobility activities, and life and social activities were considered a statistically significant difference ($p < 0.05$).[29]

This decrease was independent of duration of follow-up. The response rate in the different follow-up periods ranged from 53 to 67%. Of all 100 consecutive patients, only 56 patients had an end-point available at 24 months follow-up as not all patients returned to the outpatient appointment in each follow-up period and the questionnaire was not always filled in completely. See Tables 2 and 3.

When concerning the final Lymph-ICF measurement at the last outpatient appointment for each patient ($n = 100$), a lower postoperative total score in each domain of the Lymph-ICF was observed. However, only in domain 'physical function' and 'mental function' a decrease of more than 10 was observed, representing a statistically significant decrease ($p < 0.05$).[29] See Figure 3.

Following LVA surgery, a lower Lymph-ICF score was observed in 84% of patients, with a mean decrease of 17.7 ± 14.0. A decrease of more than 10 in Lymph-ICF score was observed

in 51% of patients, with a mean decrease of 25.8 ± 12.1 ($p < 0.05$). No relationship between Lymph-ICF score and preoperative ICG stage, difference in limb circumference, follow-up period, and the need to use compression garment was found. However, the number of LVAs was related to a decrease in Lymph-ICF, representing a better QoL (B − 2.89, 95% CI − 5.29 to − 0.50, $p = 0.018$). See Table 3.

Table 2. Preoperative versus postoperative total Lymph- ICF score for upper and lower extremities

FU period	FU (months) Mean ± SD	Lymph-ICF score[a]		Mean Difference	*P*-value
		Preoperative Mean ± SD	Postoperative[b] Mean ± SD		
< 2 months ($n = 67$)[c]	1.3 ± 0.5	46.8 ± 17.6	27.3 ± 18.1	- 19.5	*<0.001*
2–6 months ($n = 56$)[c]	3.7 ± 1.0	43.0 ± 18.4	29.5 ± 19.6	− 13.5	*<0.001*
6–12 months ($n = 53$)[c]	8.0 ± 1.9	43.0 ± 18.8	26.4 ± 18.0	- 16.6	*<0.001*
12–24 months ($n = 59$)[c]	16.0 ± 3.6	45.3 ± 16.6	31.3 ± 18.6	- 14.0	*<0.001*
> 24 months ($n = 56$)[c]	32.1 ± 6.4	39.6 ± 19.4	27.5 ± 20.6	- 12.0	*<0.001*

[a]Calculated using the Paired Samples *T*-Test

[b]Mean difference in total Lymph-IC score between preoperative and postoperative score for the reported number of patients

[c]The number of patients included in the analysis

Table 3. Linear regression analysis with Lymph-ICF difference as dependent variable

Independent variable	Lymph-ICF score difference[a]		
	B[b]	95% CI	*P*-value
Number of LVAs	− 2.89	− 5.29 to − 0.50	*0.018*
ICG stage (1–3 vs 4–5)	− 3.22	− 10.20 to 3.75	*0.361*
Circumference difference	− 0.09	− 0.34 to 0.15	*0.448*
Follow-up (months)	0.03	− 0.28 to 0.34	*0.852*
Compression garment (no vs yes)[c]	− 5.24	− 11.98 to 1.50	*0.126*

[a]Lymph-ICF score difference is calculated by subtracting the post-OR lymph-ICF score from the pre-OR lymph-ICF score

[b]Unstandardised beta (B):Calculated using linear regression analysis. A negative value means a decrease in Lymph-ICF, representing an increase in Quality of Life

[c]No: discontinuation of compression garment, Yes: partial discontinuation and continuation of compression garment

Limb circumference

A decrease in circumference was observed in 52.1% of all patients ($n = 50$). When analysed separately, a decrease in UEL-and LEL-index was observed in 53% ($n = 43$) and 46.7% ($n = 7$) of patients, respectively, with a mean decrease of both UEL- and LEL-index of 6%.

The mean difference in UEL-index during the last outpatient appointment per patient ($n = 81$) was + 0.5 ($p = 0.686$): 122.9 ± 19.9 preoperative to 123.4 ± 22.3 postoperative. Four patients were excluded from circumference analyses, since they were wearing compression garments during follow-up moments. The mean difference in UEL-index of the operated arm and the non-operated arm was + 0.5 and − 0.4, respectively ($p = 0.420$). The mean differences in preoperative and postoperative UEL-indices over the different follow-up periods are presented in Table 4.

The mean difference in LEL-index during the last outpatient appointment per patient (n = 15) was + 2.3 ($p = 701$): 265.9 ± 54.2 preoperative to 268.2 ± 56.7 postoperative. The mean difference in LEL-index of the operated leg and the non-operated leg was + 2.3 and + 7.3, respectively ($p = 0.961$).

Table 4. Preoperative versus postoperative upper extremity lymphedema (UEL) indices ($n = 85$)

FU period	FU (months) Mean ± SD	Circumference (cm)[a]			
		Preoperative circumference Mean ± SD	Postoperative[b] Circumference Mean ± SD	Difference Mean ± SD	*P*-value
< 2 months ($n = 73$)[c]	1.34 ± 0.5	122.1 ± 20.5	122.4 ± 22.2	+ 0.3 ± 10.7	*0.760*
2–6 months ($n = 52$)[c]	3.6 ± 0.9	124.4 ± 20.6	123.2 ± 21.7	− 1.2 ± 8.8	*0.334*
6–12 months ($n = 39$)[c]	8.1 ± 2.0	121.4 ± 17.7	123.4 ± 19.8	+ 2.0 ± 9.7	*0.207*
12–24 months ($n = 40$)[c]	14.4 ± 3.0	122.1 ± 17.5	121.7 ± 21.1	− 0.4 ± 8.7	*0.787*
> 24 months ($n = 18$)[c]	27.5 ± 4.3	119.8 ± 13.8	116.7 ± 15.0	− 3.1 ± 8.7	*0.144*

[a]Calculated using the Paired Samples *T*-Test

[b]Mean difference in UEL-index preoperative and postoperative score for the reported number of patients

[c]The number of patients included in the analyses

Other outcomes

The majority of patients with upper and lower extremity lymphedema experienced a positive effect of the LVA procedure. Overall, 43% of all patients completely discontinued the use of compression garments at the last outpatient appointment. Eighteen percent of all patients reported the use of compression garments only during some activities (e.g., sports, gardening). The continuation rate was 35.5% in patients with upper extremity lymphedema, in contrast to 60% in patients with lower extremity lymphedema.

The proportion of patients experiencing episodes of cellulitis was lower in both groups ($p < 0.01$). Although the mean decrease in number of cellulitis episodes was lower in patients with upper (− 0.6) and lower extremity lymphedema (− 0.8), the mean difference in the lower

extremity group was not found to be statistically significant ($p = 0.492$). This was probably due to the small sample size ($n = 15$).

A mean decrease in MLD sessions per week in patients with upper (− 0.4) and lower extremity lymphedema (− 1.0) was observed ($p < 0.01$). See Table 5 for differences between the arm and leg group.

Table 5. Compression garments and patient-reported outcomes

	Total	*P*-value	Arm	*P*-value	Leg	*P*-value
Compression garments (*n*)	*100*		*85*		*15*	
Discontinuation (%)	43		47.1		20	
Partial discontinuation (%)	18		17.6		20	
Continuation (%)	39	*NE*	35.3	*NE*	60	*NE*
Positive effect[b] (*n*)[a]	*100*		*85*		*15*	
Yes (%)	80		77.6		93.3	
No (%)	20	*< 0.001*	22.4	*< 0.001*	7.6	*0.005*
Patients experiencing cellulitis[a] (*n*)[c]	*98*		*83*		*15*	
Before operation (%)	38.8		41		26.7	
After operation (%)	23.5	*0.001*	26.5	*0.007*	6.7	*0.031*
Cellulitis episodes per year[a] (*n*)[d]	*98*		*83*		*15*	
Before operation (Mean ± SD)	1.1 ± 1.9		1.0 ± 1.6		1.4 ± 3.3	
After operation (Mean ± SD)	0.5 ± 1.3	*0.006*	0.4 ± 1.0	*0.001*	0.6 ± 2.3	*0.492*
MLD sessions per week[a] (*n*)[d]	*82*		*70*		*12*	
Before operation (Mean ± SD)	1.3 ± 1.0		1.2 ± 0.8		1.9 ± 19	
After operation (Mean ± SD)	0.8 ± 0.8	*< 0.001*	0.8 ± 0.71	*< 0.001*	0.9 ± 1.4	*0.005*

[a]Calculated using the Chi-Square test
[b]Outcome reported by patient
[c]Calculated using the McNemar test
[d]Calculated using the Paired Samples *T*-Test

DISCUSSION

This prospective cohort study comprising 100 upper and lower extremity lymphedema patients showed significant QoL improvement after LVA surgery. Two-thirds of extremity lymphedema patients were able to reduce (i.e., partially or completely discontinue) the use of compression garments. Moreover, the postoperative number of cellulitis episodes and MLD sessions decreased for both types of lymphedema. Additionally, a simple and patient-friendly method for outpatient ICG lymphography is presented.

Significant mean QoL improvements after LVA surgery were reported with consistent results in all follow-up periods until mean follow-up of 32 months. A recent systematic review on QoL following surgical treatment of lymphedema revealed that the majority of previous studies report QoL improvement solely based on the patient's feelings.[7] The reported proportions (range 57–100%) are consistent with the 80% of patients experiencing a positive effect in the current study.[7] The other studies did use validated tools to asses QoL. However, these are studies with small sample sizes (range 10–74 patients) and relatively short follow-up (range 6–12 months).[10,22,23,33-35] To the best of our knowledge, the current study may cover the largest population with the longest follow-up, assessing QoL improvement over different periods of time. Moreover, the Lymph-ICF was used, which has recently been assessed as one of the most complete and accurate questionnaires to assess QoL in lymphedema patients.[30] Improvement in QoL was related to the total number of LVAs performed per patient in this study. This could be explained by previous observation that a higher of anastomoses could be associated with a better patency rate and the suggestion of a positive correlation between a patent anastomosis and clinical improvement.[17]

The overall mean limb circumference did not improve. This result is consistent with previous findings.[17,22] In the current study, a decrease in limb circumference in terms of UEL- and LEL-index was observed in half the patients, with a mean decrease of 6%. The difference with a previous systematic review, reporting a weight mean circumference reduction of 8.5%, could be due to the high heterogeneity of patient population and assessment modalities in previous studies.[3] Although one may conclude that LVA treatment was not effective when minimal decrease in circumference is observed, lymphedema progression may be ceased due to the procedure.[10]

Only 35% of upper extremity lymphedema patients needed to continue their compression garments after LVA, compared to 60% of lower extremity lymphedema patients. These results are in line with previous studies reporting continuation rates (range 15–66%).[9,17,22,23,36] Since a majority of these studies report a maximum follow-up of 12 months, more research similar to the current study is recommended to confirm the long-term effects of LVA surgery on compression garment usage. The difference between continuation rates for upper and lower extremity lymphedema patients supports the finding by Chang et al., who concluded that LVA in the lower extremity was not as effective compared to the upper extremity.[18]

A reduction of more than 50 percent in mean cellulitis episodes was observed for both upper and lower extremity lymphedema cases. This is an important finding, since 23–35% of lymphedema patients experience recurrent and progressive cellulitis, and it has a

tremendous impact in their quality of life.[5,6,37] Cellulitis leads to a vicious cycle of lymphatic vessel destruction, lymphedema, and recurrent cellulitis episodes. Few studies have shown that LVA can interrupt this cycle and reduce the number of cellulitis episodes.[1,4,6,26] The current study underlines these findings, with the positive observation that this is also the case for the longer term.

Regarding the need for MLD, 20% of patients were able to cease MDL sessions, while the remaining patients continued MLD to a lesser degree (51%) or at the same frequency (29%), resulting in a significantly lower mean number of MLD sessions for all patients. Although previous studies concerning MLD as an outcome measurement are scarce, these results are in line with previous research and confirm a consistent longer-term result.[17]

In this study, a simple and patient-friendly method for outpatient ICG lymphography is presented. Previously, intraoperative mapping of lymphatics was reported with the subsequent disadvantage of lengthening duration of the operation.[18,19,21,23,38,39] Since all ICG injections were well tolerated, patients experienced the method as patient-friendly, knowing that these would not be different in the operation room. Moreover, patients felt safe, as they were observed for side effects. Additionally, we believe that the presented method can save time in the operation room, since all lymphatic vessels were identified exactly at the incision site that was marked and photographed in outpatient clinic making intraoperative ICG lymphography redundant. However, the exact time saving effect remains to be investigated.

In the current study, clinical heterogeneity was low as predominantly female patients with secondary upper limb lymphedema with a small standard deviation for age and BMI were included. However, three-quarters and two-thirds of the included patients presented with early ISL stages and low ICG stages, respectively. These factors could have been a beneficial factor since LVA might have been less effective in patients with advanced lymphedema and patients presenting with ICG stage IV or more.[17] Nevertheless, no relationship between ICG stages 1 to 3 and QoL improvement was observed in this study. Although not assessed in the current study, the use of an experienced microsurgeon who operated all patients using the same surgical technique may have affect the outcome. Furthermore, the postoperative treatment protocol in the current study differs from studies in which patients directly start wearing compression garments following surgery.[23] However, there is currently no evidence on the optimal conservative treatment protocol following LVA surgery.[39]

The limitations that are worthy to mention in the current study are the following: the number of patients included in each individual follow-up moment is limited. Patients were

unfortunately lost to follow-up as patients found it not useful to return to the outpatient clinic a long time after the operation and patients needed to travel a long distance as patients from all over the country visit our institution for lymphedema treatment. Nonetheless, this is still the largest prospective cohort study evaluating multiple relevant outcomes following LVA procedure. In current study, no correlation between patency and QoL improvement after LVA was explored. However, previous work by our group on 25 patients, who were also included in current study, showed that 76% of patients had at least one patent anastomosis after 12 months, and a positive correlation between a patent anastomosis and clinical improvement was observed.[17] Furthermore, due to the maximum operation duration of 120 min, one-third of patients required a second or third operation to perform all potential LVAs. This may entail financial burdens for patients, since LVA surgery is not reimbursed by every health care insurance companies over the world.

Another remark, which also applies to previous studies, is that the possible effect of arm dominance on patients' QoL was not taken into account. Notwithstanding the promising results, randomised controlled studies are required to provide higher evidence for the effectiveness of LVA surgery.[40]

CONCLUSION

LVA resulted in significant QoL improvement of upper and lower extremity lymphedema patients. Limb circumference did not significantly improve, but good results concerning discontinuation of compression garments (especially for the upper extremity lymphedema group), decrease in cellulitis episodes, and MLD sessions were observed. Additionally, a simple and patient-friendly method for outpatient ICG lymphography is presented which facilitates preoperative decision-making.

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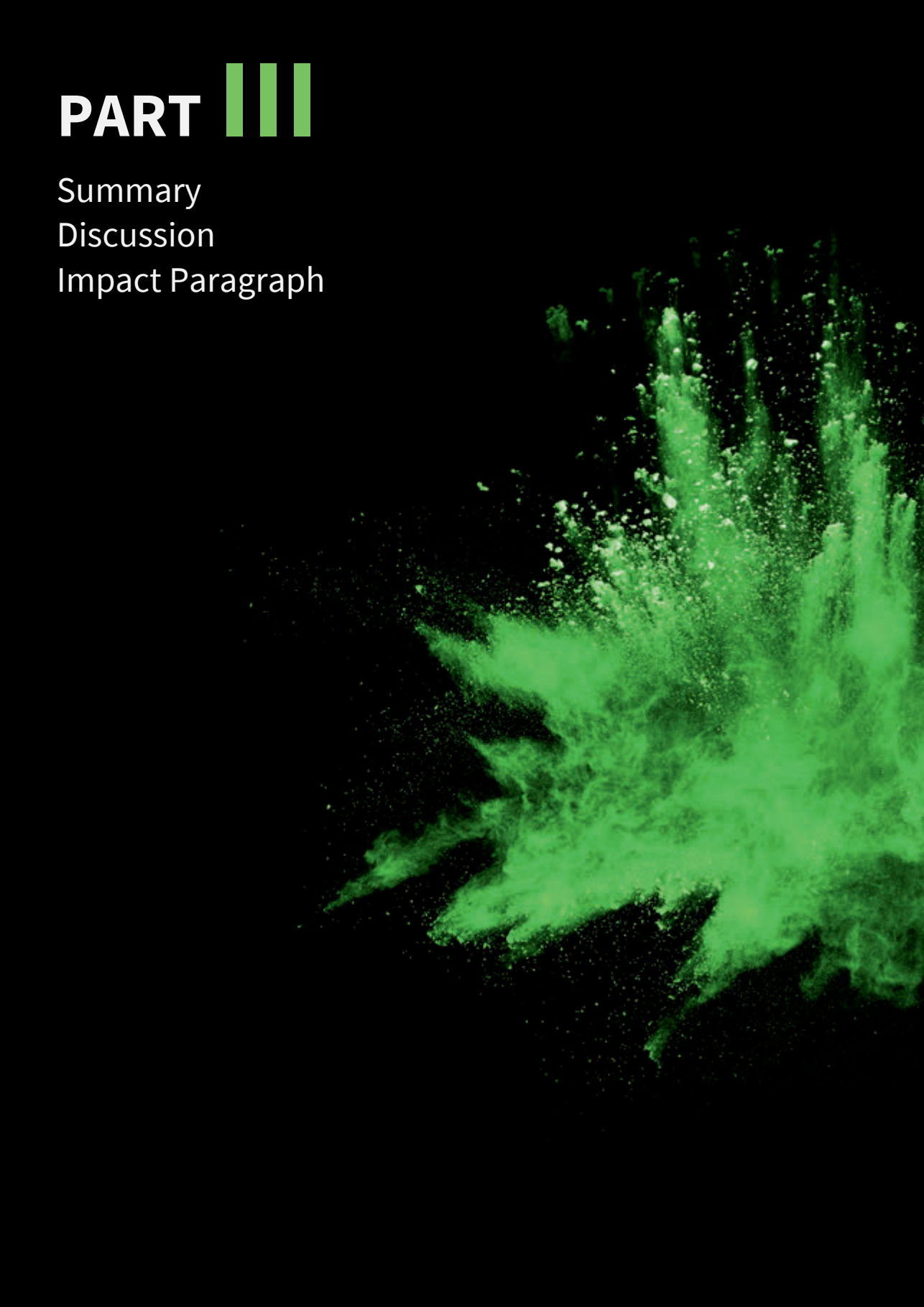
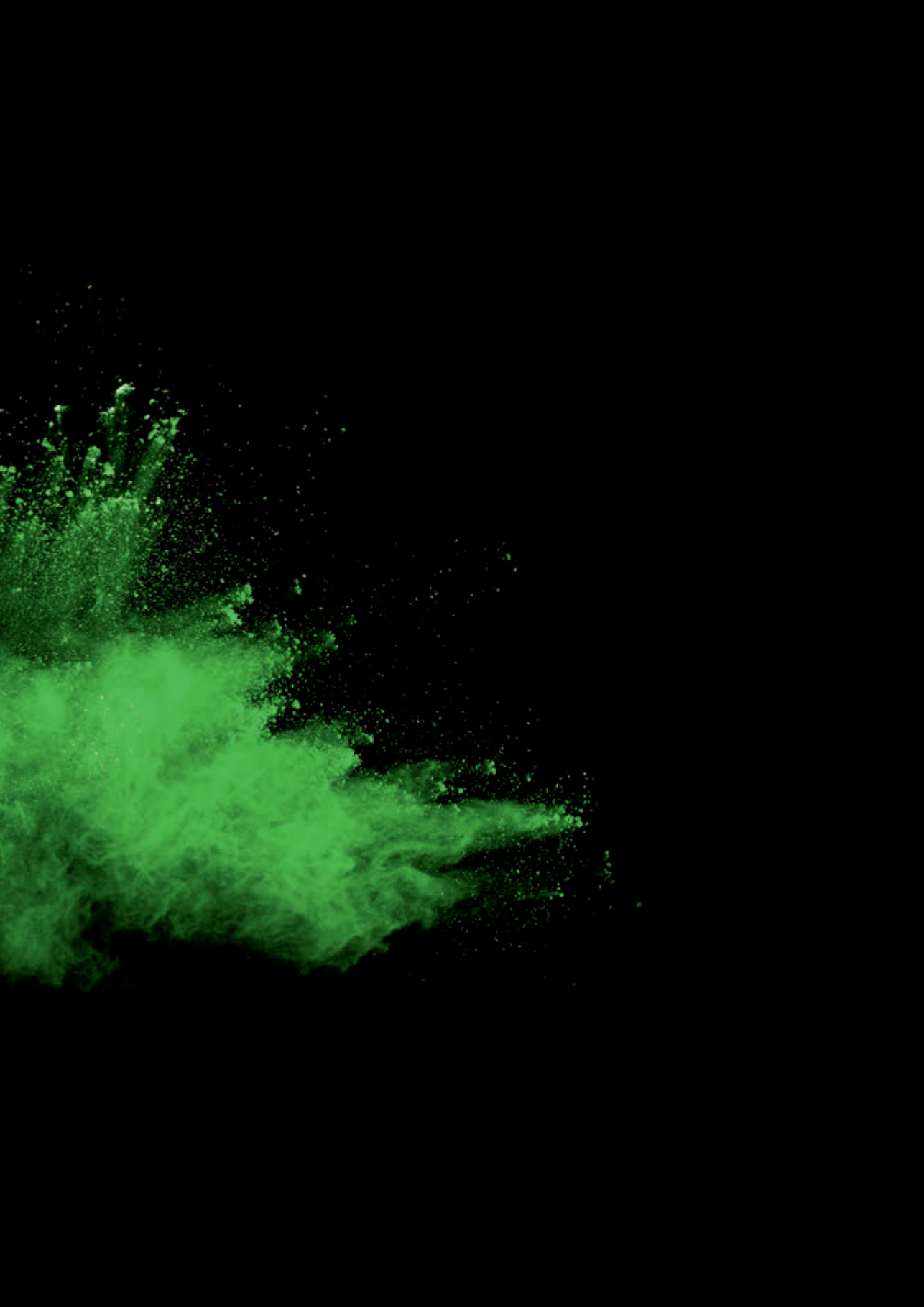
PART III

Summary
Discussion
Impact Paragraph





CHAPTER 8

Summary, general discussion and future perspectives

DISCUSSION

Over the last decades, indocyanine green imaging has gained popularity in plastic and reconstructive surgery as it is more frequently applied in the assessment of perfusion in reconstructive flap surgery and in the assessment of lymphatic vessels in lymphedema surgery.[1,2] Indocyanine green imaging, with its unique properties and the ability to provide detailed anatomical information, has proven to be useful in intraoperative decision making to increase surgical precision and improve postoperative outcomes in several clinical applications.[3] However, there is lack of consensus on imaging protocols regarding factors that influence fluorescence intensity, and the imaging technique is not yet implemented as standard of care in plastic and reconstructive surgery. Consequently, more research is needed to benefit the further development and implementation of indocyanine green imaging. Therefore, the first aim of this thesis was to better understand the factors that influence indocyanine green imaging in order to optimize the use of indocyanine green imaging. The second aim of this thesis was to evaluate the possible applications of indocyanine green imaging in plastic and reconstructive surgery in order to facilitate preoperative and intraoperative decision-making and minimize postoperative complications.

Factors of influence during indocyanine green imaging

As already highlighted in the introduction of this thesis, the factors that might influence fluorescence intensity during indocyanine green imaging are only briefly described in the current literature.[4-6] Although recommendations of use have been reported,[7] there is no consensus about the technical use of indocyanine green imaging during reconstructive surgery. Since these factors potentially preclude accurate assessment of indocyanine green, it is important to identify and confirm these factors.

For this reason, **Chapter 2** provides a comprehensive insight in potential factors influencing fluorescence intensity when using indocyanine green imaging.[8] In the first part of this chapter, a systematic review was conducted. Forty-nine articles regarding the use of indocyanine green imaging in reconstructive flap surgery were selected, including a total of 1996 flaps. This review provided a comprehensive overview of currently used protocols and revealed that most protocols were insufficiently described and even when reported, there was no consensus on dosage of ICG, working distance, timing and duration of perfusion assessment, and timing of tissue resection. Other factors that might have influenced tissue perfusion assessment (i.e., vasopressor use[5,7] or systolic blood pressure[9]) have only been discussed in the minority of included articles.

In the second part of this chapter, ex vivo experiments were performed to further investigate the practical influence of potentially relevant factors. Except for using a handheld ICG system, the same methods were used as described by van den Bos et al. who used a laparoscopic indocyanine green imaging device.[10] Similar to the outcomes presented in their article, we concluded that weight-adjusted ICG dose seems preferable over a fixed dose. In addition, we advocated to use the recommended working distance and to position the imaging head in an angle of 60 to 90 degrees in order not to influence fluorescence intensity.

Furthermore, the maximum fluorescence intensity was measured up to 8 mm of depth when using beeswax plates with spectral scattering properties similar to human skin. However, since the spectral absorption differs, the penetration depth in human could be different. This is confirmed by a previous study describing a maximum depth of 2 cm from the skin surface.[11] Although in vivo studies were preferable, these would have been limited by in vivo fluorescence quenching, which makes it difficult to predict the precise working dose of ICG needed in the flap.[12] Moreover, the half-life of ICG and the maximum ICG dose per day would hinder to assess different factors in a limited time frame in human beings.[13-15]

Multiple factors that potentially influence fluorescence intensity during indocyanine green imaging emerged from **Chapter 2**, including the intraoperative use of vasopressors. While vasopressors (e.g., norepinephrine) are often used during surgery to restore and maintain blood pressure levels in case of hypotension,[16] it is hypothesized that vasoconstriction comprises blood flow of superficial capillaries, reducing the potential of ICG fluorescence angiography in order accurately assessment of tissue perfusion in reconstructive surgery.[5,7] To test this hypothesis, **Chapter 3** described the first, well-standardized, study to investigate the effect of increasing doses of norepinephrine on bowel perfusion assessment using indocyanine green angiography in a porcine intestinal model.[17] A total of five adult pigs were included and the following three increasing doses of continuous norepinephrine infusion were evaluated over a 25-minute interval: 0.1, 0.5 and 1.0 µg/kg/min. An ICG fluorescence videography system integrated within a near-infrared endoscope (KARL STORZ SE & Co. KG, Germany) was used. The ICG-emitted fluorescence signal was transposed to a virtual perfusion cartography based on time-to-peak fluorescence intensity (TTP), which resulted from the velocity of the fluorescence signal until it reaches its maximum intensity peak within the first 40 seconds following ICG injection. The TTP was measured from the time when there was 25% of maximum fluorescence intensity to 75% of maximum fluorescence intensity using specific software called fluorescence-based enhanced reality (FLER).[18] The mean TTP in the first (4.23 sec), second (3.90 sec) and third (4.60 sec) assessment did not significantly differ from baseline (4.41) assessment.

Therefore, it was concluded that increasing doses of norepinephrine have no effect on bowel perfusion assessment using indocyanine green angiography. In addition, a significant increase in absolute fluorescence intensity was observed, while a 25-interval was used to prevent accumulation of ICG. However, the advantages of TTP compared to absolute fluorescence intensity, is that TTP truly allows for multiple and repetitive assessments as the 'noise' produced by the accumulation of fluorescent dye does not affect TTP.[19] Consequently, it was concluded that it is better to use a dynamic fluorescence videography technique over absolute fluorescence intensity in case of repeat perfusion assessments throughout a surgical procedure.[17]

The results of this animal study are in line with previous clinical studies that were performed in a non-standardized fashion[20,21] and suggest that increasing doses of norepinephrine may also have no effect on tissue perfusion assessment using indocyanine green angiography in plastic and reconstructive surgery. However, since this study included only five pigs with healthy intestines, the results should be confirmed in larger studies, also including diseased tissues.

As previously described in **Chapter 2**, the currently reported protocols regarding indocyanine green imaging are insufficiently described, with no consensus on factors that might influence tissue perfusion. Since indocyanine green imaging is gaining popularity in reconstructive breast surgery, several studies reported on the efficacy of indocyanine green imaging in reducing postoperative complications. However, the lack of consensus in study protocol and outcomes, makes it difficult to interpret the data. In order to summarize and simplify this data **Chapter 4** describes a systematic *Cochrane* review that aimed to evaluate the available literature on indocyanine green angiography protocols for mastectomy skin flap perfusion assessment and to perform a meta-analysis to assess the effect of indocyanine green angiography for preventing postoperative complications in breast reconstructive surgery.[22]

Breast reconstruction following a skin-sparing mastectomy for breast cancer provides the opportunity to restore the removed breast tissue and cosmetic appearance.[23] Unfortunately, mastectomy skin flap necrosis is a common complication following skin-sparing breast reconstruction.[24] However, this postoperative complication can be prevented by intraoperative assessment of mastectomy skin flap viability and intervention when tissue perfusion is compromised. Since indocyanine green angiography is presumed to be a better predictor of mastectomy skin flap necrosis compared to clinical evaluation alone and, it is more frequently used.[25]

After conducting a comprehensive literature search that yielded 232 hits, nine studies with a combined number of 1589 women with 2199 breast reconstructions were included. Concerning the indocyanine green angiography protocols, these were insufficiently described, which is in line with the previous observations reported in **Chapter 2** of this thesis.

Regarding the complications, six of the included studies reported the number of complications on a per breast basis and the other three studies on per patient basis. Therefore, we decided to pool data separately for the meta-analysis. On a per breast basis, the results suggested that indocyanine green angiography led to fewer cases of postoperative mastectomy skin flap necrosis (Risk Ratio (RR): 0.62), reoperation (RR: 0.65), infection (RR: 0.65), and seroma (RR: 0.71). Conversely, the use of indocyanine green angiography led to more cases of postoperative hematoma (RR: 1.53). On a per patient basis, the results suggested that indocyanine green angiography led to fewer cases of postoperative mastectomy skin flap necrosis (RR: 0.79), reoperation (RR: 0.5), infection (RR: 0.91) and hematoma (RR: 0.87). Conversely, indocyanine green angiography led to more cases of seroma (RR: 1.68) and wound dehiscence (RR: 4.00).

The results of this study are in agreement with a similar review and meta-analysis by Liu et al. showing the benefit of the SPY system (which is a specific indocyanine green angiography system) in preventing mastectomy skin flap necrosis and reoperation.[26] Although eight of the included studies in our review used this SPY system, Liu et al. included only seven studies. Moreover, not all the numbers of mastectomy skin flap necrosis and reoperations extracted from the studies were described similarly. Although women can develop complications in both breasts when a bilateral reconstruction is performed, these were not reported separately in the included studies. The choice to pool the data separately in the current study is a major difference and benefit of the current study.

Although the results of this *Cochrane* review suggest that indocyanine green angiography is associated with a lower risk of postoperative complications, there were serious concerns with risk of bias of the included studies due to the nonrandomized study design of all included studies and the low comparability of cohorts in most studies. Therefore, it was concluded that while indocyanine green is found to be a helpful tool to assess perfusion intraoperatively, the effect in reducing postoperative complications compared to clinical evaluation, is uncertain. High-quality randomized controlled trials are needed to compare indocyanine green imaging to clinical evaluation.

The role of indocyanine green imaging in flap surgery

Part II of this thesis comprises clinical studies regarding the application of indocyanine green imaging. **Chapter 4** demonstrated that indocyanine green imaging is a helpful tool to assess mastectomy skin flap perfusion intraoperatively. For this reason, the imaging technique was used in free flap breast reconstructions in **Chapter 5.**

During autologous breast reconstructions, the internal mammary vessels are used as the preferred recipient vessels for micro-anastomosis in autologous breast reconstructions.[27] Previous studies regarding coronary artery bypass grafting in cardiothoracic surgery demonstrated that harvesting the complete left internal mammary artery resulted in reduced sternal and parasternal skin perfusion.[28] As the internal mammary artery is one of the most important blood vessels to the breast tissue, it was hypothesized that using this artery would pose a risk factor for mastectomy skin flap necrosis. In this observational study, indocyanine green imaging was used to evaluate the impact of using the internal mammary artery as a recipient vessel on mastectomy skin flap perfusion in autologous breast reconstruction. A total of 10 women underwent unilateral (n=4) or bilateral (n=6) skin sparing mastectomy and immediate autologous reconstruction with a DIEP flap breast reconstruction (n=9) and diagonal upper gracilis (DUG) flap reconstruction (n=1).

During surgery, two indocyanine green angiographies of 120 seconds with additional images at 60, 90, and 120 seconds were acquired: once before and once after temporarily clamping the internal mammary artery. The mean inflow time (in seconds) in the pre-clamp group and post-clamp showed no statistically significant difference (24.1 versus 23.0 seconds, P=0.348). No statistically significant mean differences were found for the mean (131.4 vs 124.0, P=0.126), minimum (28.6 vs 33.4, P=0.086) and maximum (253.1 vs 247.6, P= 0.166) fluorescence intensity obtained from the 60, 90, and 120 second images. As the fluorescence intensity was comparable before and after temporarily clamping the internal mammary artery, it was concluded that the preparation and use of the IMA as a recipient vessel does not reduce medial mastectomy skin flap perfusion in patients undergoing autologous breast reconstruction.

Although this was the first study that used indocyanine green for this purpose, it was limited by the size of the region of interest, as well as the small sample size, and the use of absolute fluorescence intensity values (e.g. grayscale imaging) without the possibility to correct for possible ICG accumulation with specific software[18] as described in **Chapter 3**. However, the results of this study were in line with previous work by our group reporting no difference in mastectomy skin flap perfusion before and after temporarily clipping the IMA during

autologous breast reconstruction using another innovative imaging technique, called hyperspectral imaging (HSI).[29]

Although indocyanine green imaging is increasingly applied in the assessment of mastectomy skin flap perfusion and free flaps in breast reconstructive surgery, the role of indocyanine green imaging in free flap surgery for abdominal wall reconstruction has not been extensively described in the literature. **Chapter 6** describes the application of indocyanine green imaging in complex abdominal wall reconstruction following resection of enterocutaneous fistula using fasciocutaneous ALT flaps.[30] An enterocutaneous fistula (ECF) is defined as an unnatural communication between the gastrointestinal tract and the skin. Since these fistulas often coexist with major abdominal wall defects[31], abdominal wall reconstruction after resection of an ECF is challenging and requires surgical creativity. In this study, the feasibility and safety of single-stage abdominal wall reconstruction using anterolateral thigh (ALT) flaps combined with biological mesh after ECF resection was evaluated, together with the effect of indocyanine green imaging on postoperative complications. ECF recurrence was the primary outcome of interest. Two early ECF recurrences were observed during follow-up. This represented a 20% ECF recurrence rate, which is comparable to the reported ECF recurrence rates ranging from 9% to 41.7% in previous studies concerning abdominal wall reconstruction with biological mesh without the use of ALT flaps after ECF resection.[32-34] Importantly, both recurrent ECFs were closed definitively with conservative treatment only, which is superior when compared with the previous studies reporting reoperation rates up to 57%.[32]

Likewise, the rate of other postoperative complications, including (partial) flap necrosis, abdominal herniation and bulging, postoperative infection, wound dehiscence, mesh exposure, seroma, and hematoma were acceptable and comparable to previous studies. Consequently, we found that the combination of an ALT flap with biological mesh is a feasible and safe surgical procedure for single-stage abdominal wall reconstruction after ECF resection. Moreover, indocyanine green angiography was found to be of great added value, as the total early complication rate and the rates of perfusion related complications, including partial flap necrosis and wound dehiscence, were lower when ALT flaps were assessed with indocyanine green imaging, compared to clinical evaluation alone. However, due to the limited number of ten patients in this study, we were not able to perform statistical analysis to compare indocyanine green imaging to clinical evaluation of the ALT flap perfusion. Furthermore, the results of this study could not be generalized to every hospital around the globe, since all surgical procedures were performed in a dedicated centre, experienced in complex abdominal wall surgery and reconstructive surgery.

The role of indocyanine green imaging in lymphedema surgery

As previously described in the introduction of this thesis, lymphedema is a debilitating condition that significantly affects patient's quality of life.[35,36] **Chapter 7** describes the role of indocyanine green lymphography in the treatment of lymphedema.[37] In this single-institution prospective cohort study, 100 consecutive lymphedema patients were included to assess the effect of LVA surgery on lymphedema during a 24-month follow-up period. Furthermore, the role of indocyanine green lymphography to facilitate pre-operative decision was evaluated.

The primary outcome in this study was quality of life, measured with a to a validated questionnaire called the Lymph-ICF.[38] After a mean follow-up period of 25 months, the mean total lymph-ICF score showed a decrease of 13.3 (43.9 preoperative to 30.6 postoperative), representing a significant improvement in patient's quality of life after LVA surgery. Overall, a lower ICF-score was observed in 84% of patients, with a mean decrease of 17.7. Furthermore, good results were obtained concerning the secondary outcomes, including the use of compression garments, the frequency of cellulitis episodes and manual lymphatic drainage (MLD) sessions. For example, two-third of the lymphedema patients were able to reduce (i.e., partially or completely discontinue) the use of compression garments which was in line with previous studies reporting a maximum follow-up of 12 months.[39-43] Although a decrease in limb circumference was observed in only 52.1 percent of patients, the overall mean limb circumference did not significantly improve. Accordingly, one may conclude that LVA treatment was not effective. On the other hand, it could be concluded that progression of lymphedema is ceased due to the procedure.[44]

Regarding indocyanine green lymphography, we presented a simple, fast and patient-friendly method for preoperative mapping of the lymphatic vessels using indocyanine green lymphography in the outpatient clinic. All ICG injections were well tolerated, and patients felt safe, as they were regularly monitored by a nurse. Although we believe that this method can save time in the operation room, since no intraoperative lymphography was needed in comparison to previous literature[39,45-49], the exact time saving effect remains to be investigated.

Although this study was the largest prospective cohort study evaluating multiple relevant outcomes following LVA procedure, the follow-up is limited as patient were loss to follow-up as patient needed to travel a long distance from all over the country to visit our institution for lymphedema treatment. Notwithstanding the promising results of this study, future randomized controlled studies will provide higher evidence for the effectiveness of LVA surgery.[50]

FUTURE PERSPECTIVES

In conclusion, multiple potential factors that might impact the adequacy of indocyanine green imaging have been identified in this thesis. Although the use of vasopressors did not significantly impact the adequacy of indocyanine green imaging, it was demonstrated that other factors significantly influence fluorescence intensity. These include: ICG dose, working distance, angle, penetration depth and ambient light. In addition, it seemed preferable to use relative perfusion assessment or a dynamic fluorescence videography technique over absolute fluorescence intensity analysis to prevent influence of ICG accumulation. These factors should be considered when using indocyanine green imaging as they contribute to the reliability of indocyanine green imaging in every future clinical application. We would like to encourage future researchers to describe their study methods with more transparency. This will help in reaching consensus and eventually construct uniform international guidelines in order to facilitate surgeon's confidence and education in the use of indocyanine green imaging.

Furthermore, the work in this thesis confirmed that indocyanine green imaging is a useful adjunct when applied for angiography and lymphography in plastic and reconstructive surgery. Indocyanine green angiography was found to be of great added value in lowering the postoperative complications, when compared to clinical evaluation. Despite the positive findings, we also highlighted the absence of high-quality evidence. For this reason, we advocate for more and larger studies, preferably randomized controlled trials or Delphi studies to confirm the real impact of indocyanine green imaging and build consensus on the application in plastic and reconstructive surgery.

To our expectation, the global use of indocyanine green imaging will increase over the next decade, and eventually it will become standard of care in plastic and reconstructive surgery. Consequently, more expertise regarding indocyanine green imaging will be obtained. When surgical precision will further enhance and postoperative outcomes for patients will improve, this may lead to a reduction in health care costs.

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CHAPTER 9

Dutch summary | Nederlandse samenvatting

Hoofdstuk 1. Inleiding en opzet van het proefschrift

Indocyanine groen (ICG) beeldvorming, ook wel 'ICG imaging' genoemd, is een innovatieve, beeldvormende techniek waarbij gebruik wordt gemaakt van een contrastmiddel (indocyanine groen) en nabij-infrarood licht. Met behulp van ICG imaging kan de doorbloeding van verschillende weefsels worden beoordeeld en kan informatie over anatomische structuren worden verkregen. Deze techniek kan de preoperatieve, intra-operatieve en postoperatieve besluitvorming van de (plastisch) chirurg vergemakkelijken en de chirurgische precisie verbeteren. Dit leidt tot betere patiëntveiligheid en postoperatieve resultaten. ICG imaging wordt steeds vaker gebruikt in de plastische en reconstructieve chirurgie, maar is in veel opzichten nog in ontwikkeling. Om alle mogelijke toepassingen te realiseren, is meer onderzoek nodig.

Dit proefschrift had onder andere als doel om een beter begrip te verschaffen van de factoren die van invloed zijn op ICG imaging. Hiernaast werd een aantal van de toepassingen van ICG imaging binnen de plastische en reconstructieve chirurgie geëvalueerd. Voorbeelden hiervan zijn ICG-angiografie om doorbloeding van vrije lappen te beoordelen en ICG-lymfografie om lymfebanen te beoordelen.

Hoofdstuk 2. Optimalisatie van ICG-angiografie bij reconstructieve lapchirurgie: een systematische review en ex vivo experimenten

In hoofdstuk 2 hebben we factoren geïdentificeerd die de fluorescentie-intensiteit van de ICG imaging kunnen beïnvloeden. Dit hoofdstuk bestaat uit twee delen.

In het eerste deel hebben we een systematische review uitgevoerd om een overzicht te krijgen van de protocollen die momenteel worden gebruikt voor ICG imaging bij reconstructieve lapchirurgie. De nadruk werd gelegd op ICG-dosering, de timing van zowel toediening van ICG als de timing en duur van de beoordeling van de fluorescentie-intensiteit, de werkafstand en andere factoren die mogelijk van invloed konden zijn. Negenenveertig artikelen, met in totaal 1996 geopereerde lappen, werden geselecteerd voor deze review. We vonden geen consensus over de eerder genoemde factoren.

In het tweede deel van dit hoofdstuk voerden we ex-vivo experimenten uit om de invloed van de verschillende factoren te onderzoeken. We concludeerden dat een ICG-dosis op basis van gewicht de voorkeur lijkt te hebben boven een vaste dosis. Om de fluorescentie-intensiteit niet te beïnvloeden, pleiten we voor het gebruik van de door de fabrikant aanbevolen werkafstand en het plaatsen van de ICG-camera in een hoek van 60 tot 90 graden boven het weefsel.

Hoofdstuk 3. Invloed van intra-operatief vasopressor gebruik op ICG-angiografie: de eerste evaluatie in een experimenteel model

In hoofdstuk 3 hebben we ons gericht op één van de factoren die het fluorescentiesignaal tijdens ICG imaging zou kunnen beïnvloeden, namelijk noradrenaline (vasopressor). Om dit te onderzoeken, hebben we gebruikt gemaakt van een varkensmodel. In dit model hebben we de doorbloeding van darmweefsel gemeten met ICG-angiografie. Tijdens dit onderzoek werd de invloed van oplopende doseringen noradrenaline op de ICG-beoordeling geëvalueerd.

In totaal werden vijf varkens geïncludeerd. Drie noradrenaline doseringen werden toegediend in een interval van 25 minuten: continue infuussnelheid van 0,1, 0,5 en 1,0 µg/kg/min. Een nabij-infrarood endoscoop werd gebruikt om fluorescentiebeelden te verkrijgen. De 'tijd-tot-piek fluorescentie-intensiteit', in het hoofdstuk genoemd als: 'time-to-peak fluorescence intensity' (TTP), was de primaire uitkomstmaat. De TTP werd berekend met behulp van de snelheid van het fluorescentiesignaal om de maximale piekintensiteit te bereiken, binnen 40 seconden na injectie van ICG. De TTP stond gelijk aan de gemeten tijd tussen 25% van de maximale piekintensiteit tot 75% van de maximale piekintensiteit. Op deze manier werd rekening gehouden met de invloed van ICG-stapeling in het weefsel.

De TTP-waarden voor de oplopende noradrenaline doseringen verschilden niet significant van de baseline-beoordelingen. Hieruit hebben we geconcludeerd dat oplopende noradrenaline doseringen geen effect hebben op de beoordeling van darmweefseldoorbloeding met ICG-angiografie.

Hoofdstuk 4. ICG-angiografie ter preventie van postoperatieve mastectomiehuidlap-necrose tijdens directe borstreconstructie: een systematische review en meta-analyse

In hoofdstuk 4 hebben we ons gericht op de beoordeling van mastectomiehuidlap-doorbloeding en hebben we de effectiviteit van ICG-angiografie in het vóórkomen van postoperatieve complicaties in directe borstreconstructies geanalyseerd. Voor deze uitgevoerde *Cochrane* review en meta-analyse werd een systematische zoektocht uitgevoerd, waarna negen studies werden geïncludeerd. In deze negen studies waren 1589 vrouwen met een totaal van 2199 borstreconstructie betrokken. De resultaten uit deze studies werden verzameld en geanalyseerd om een uitgebreide samenvatting te geven van de beschikbare literatuur. Er werden meerdere meta-analyses uitgevoerd, zodat het aantal complicaties per borst en per patiënt kon worden berekend. De primaire uitkomstmaat was 'postoperatieve mastectomiehuidlapnecrose'.

De resultaten van de meta-analyses suggereren dat het gebruik van ICG-angiografie tijdens directe borstreconstructie leidt tot minder gevallen van postoperatieve mastectomiehuidlapnecrose in vergelijking met klinische evaluatie van weefsel-doorbloeding, met een risicoratio (RR) van 0,62 op basis van de analyse per borst en 0,71 op basis van de analyse per patiënt.

Echter, alle negen geïncludeerde studies betroffen niet-gerandomiseerde cohortonderzoeken, waardoor de kwaliteit van het bewijs laag is. Hierdoor blijft het echte effect van ICG-angiografie onzeker.

Hoofdstuk 5. Beoordeling van de mastectomiehuidlapdoorbloeding tijdens autologe borstreconstructie met behulp van ICG-angiografie: een observationele studie
In hoofdstuk 5 onderzochten we de invloed van het gebruik van de arteria mammaria interna (IMA, slagader tussen de ribben) voor autologe borstreconstructie (eigen weefsel reconstructie) op de doorbloeding van de mastectomiehuidlap. Eerdere studies lieten namelijk zien dat de huid boven en naast het sternum minder van bloed werd voorzien, ná het verwijderen van de gehele IMA bij coronaire bypassoperaties (hartoperatie).

Een observationeel onderzoek werd uitgevoerd om na te gaan of deze verminderde doorbloeding ook wordt gezien bij het gebruik van de IMA als aanvoerend bloedvat bij een directe autologe borstreconstructie.

In totaal ondergingen 10 vrouwen een unilaterale (n=4) of bilaterale (n=6) huid-sparende mastectomie met directe autologe reconstructie. Tijdens de operatie werden twee ICG-video's van 120 seconden gemaakt met aanvullende foto's op 60, 90 en 120 seconden: eenmaal vóór en eenmaal ná het afklemmen van de IMA.

De gemiddelde ICG-instroomtijd toonde geen verschil in beide groepen. Er werd geen verschil gevonden in de gemiddelde, maximale en minimale ICG fluorescentie-intensiteit. Hieruit werd geconcludeerd dat de mastectomiehuidlapdoorbloeding bij autologe borstreconstructie niet vermindert als de IMA wordt gebruikt.

Hoofdstuk 6. Fasciocutane 'anterolateral thigh (ALT) flap' voor complexe buikwand-reconstructie na resectie van enterocutane fistels en de rol van ICG-angiografie
In hoofdstuk 6 onderzochten we de toegevoegde waarde van ICG-angiografie tijdens reconstructieve chirurgie met vrije en gesteelde lappen. We verzamelden en analyseerden informatie uit de database van één universitair medisch centrum.

Tien patiënten met een enterocutane fistel (ECF: een verbinding tussen darm en huid) en een groot buikwanddefect werden geïncludeerd. Zij ondergingen een specifieke, uitgebreide operatie waarin in één operatie de ECF werd verwijderd en direct de buikwand werd gereconstrueerd met behulp van een biologisch matje (mesh) gecombineerd met een (vrije of gesteelde) fasciocutane anterolateral thigh (ALT) lap. Dit is een weefselverplaatsing vanuit de buitenzijde van het bovenbeen naar de buik. In de helft van de groep patiënten werd ICG-angiografie gebruikt om de doorbloeding van de ALT-lap te beoordelen. In de andere helft van de groep werd de doorbloeding beoordeeld op basis van klinische evaluatie (onder andere: kleur en temperatuur).

Deze studie had als doel de geschiktheid en veiligheid van deze operatie te evalueren. Hiernaast werd onderzocht of het gebruik van ICG-angiografie, tijdens deze operatie van toegevoegde waarde was om het aantal postoperatieve complicaties te verminderen. De primaire uitkomstmaat was een recidief ECF. Hoewel twee vroege fistel recidieven werden waargenomen, herstelden deze allebei zonder een nieuwe operatie.

We concludeerden dat deze specifieke operatie geschikt en veilig was voor patiënten met een ECF en een groot buikwanddefect. Bovendien leek ICG-angiografie van grote toegevoegde waarde te zijn. Het gebruik van ICG-angiografie leidde namelijk tot een lager percentage van 'vroege complicaties' in vergelijking met de groep waarin de ALT-lap werd beoordeeld op basis van klinische evaluatie.

Hoofdstuk 7. Lange termijn uitkomsten na lymfaticoveneuze anastomose (LVA) in 100 lymfeoedeem patiënten en de rol van ICG-lymfografie

Hoofdstuk 7 gaat over lymfechirurgie en de rol van ICG-lymfografie. In deze studie werd ICG-lymfografie gebruikt om het besluitvormingsproces voorafgaande aan een lymfe-operatie (LVA) te vergemakkelijken. Het nut van ICG-lymfografie werd geëvalueerd. Deze lymfe-operatie betrof lymfaticoveneuze anastomose (LVA). Tijdens deze operatie wordt een microchirurgische verbinding gemaakt tussen een lymfevat en een ader om de afvoer van lymfe te herstellen.

Een prospectief onderzoek werd uitgevoerd onder 100 patiënten met lymfeoedeem. In totaal werden 132 LVA's uitgevoerd. Voorafgaande aan elke operatie ondergingen alle patiënten eenmaal een ICG-lymfografie om de lymfevaten te beoordelen en geschikte lymfevaten te markeren. Dit onderzoek werd uitgevoerd op de polikliniek tijdens een reguliere afspraak. Er werden foto's gemaakt van de markeringen. Omdat deze foto's tijdens

de operatie werden gebruikt, hoefde niet opnieuw een ICG-lymfografie uitgevoerd te worden.

De primaire uitkomstmaat van de lymfe-operatie was kwaliteit van leven. Een significante verbetering van de kwaliteit van leven werd waargenomen na een gemiddelde follow-up periode van 25 maanden. Poliklinische ICG-lymfografie werd daarnaast gezien als eenvoudig, patiëntvriendelijk en effectief om het preoperatieve besluitvormingsproces voorafgaande aan LVA te vergemakkelijken. Hiernaast is het een geschikte methode die het gebruik van ICG-lymfografie tijdens de operatie overbodig maakt.

Hoofdstuk 8, 9 en 10. Samenvatting, discussie en impact.
De samenvatting en discussie in hoofdstuk 8 reflecteren op de bevindingen en beperkingen van de individuele onderzoeken in dit proefschrift. Hiernaast worden voorstellen voor toekomstig onderzoek gedaan in het onderdeel ‘future perspectives’. Hoofdstuk 9 bent u nu aan het lezen. Hoofdstuk 10 beschrijft een reflectie op de wetenschappelijke impact van de resultaten uit dit proefschrift.

CHAPTER 10

Impact paragraph

IMPACT PARAGRAPH

The main theme of this thesis is the application of indocyanine green imaging in plastic and reconstructive surgery. As extensively described in this thesis, it is of utmost importance that a surgeon is able to adequately assess tissue perfusion intraoperatively during reconstructive flap surgery, and to effectively assess lymph vessels in order to stage and treat lymphedema. Clinical evaluation alone is found to be an unreliable predictor, and previous imaging techniques were found to be expensive, complex, time-consuming and unreliable. Therefore, Indocyanine green imaging is introduced in plastic and reconstructive surgery. Over the last decades, this imaging technique is increasingly used to improve postoperative outcome by lowering postoperative complications rates of tissue necrosis and to improve surgical precision by mapping lymphatic vessels. For example, the use of indocyanine green imaging can possibly lead to a shorter operation time and reduce rates of reoperation, reduce length of hospital stay, or decrease postoperative outpatient clinic visits. From a societal point of view, when indocyanine green imaging is used in an adequate manner, it can prevent additional health care costs. However, indocyanine green imaging is still in a developing phase with a lack of knowledge to fully realize its potential. Consequently, there is no consensus on use of the imaging technique in plastic and reconstructive surgery.

The work of Part I of this thesis provides a better understanding of the factors that influence indocyanine green imaging. If the identified factors regarding the dose of ICG, working distance, the angle of the imaging head, ambient light and the use of intraoperative vasopressors are considered and reported when using indocyanine green in future applications, it will optimize the use and improve the reliability of indocyanine green imaging. Furthermore, transparency in reporting methods will provide information for constructing uniform guidelines and lead towards consensus on the use of indocyanine green imaging. Eventually, this will assist clinicians in a better knowledge of the current evidence and lead to the implementation as a standard of care.

Postoperative complications related to tissue perfusion include partial flap necrosis or total flap failure, wound dehiscence, and infection. These complications may lead to longer hospital stay and more frequent outpatient clinic visits, which increases patient's discomfort. Although surgical techniques are continuously improving, these postoperative complications will always occur after surgery to a greater or lesser degree. As described in this thesis, there is a need to objectively assess tissue perfusion using indocyanine green imaging in order to minimize the risk of these complications and immediately intervene during surgery. This need for tissue assessment is not only confined to plastic and reconstructive surgery. Tissue debridement in trauma surgery and assessment of bowel

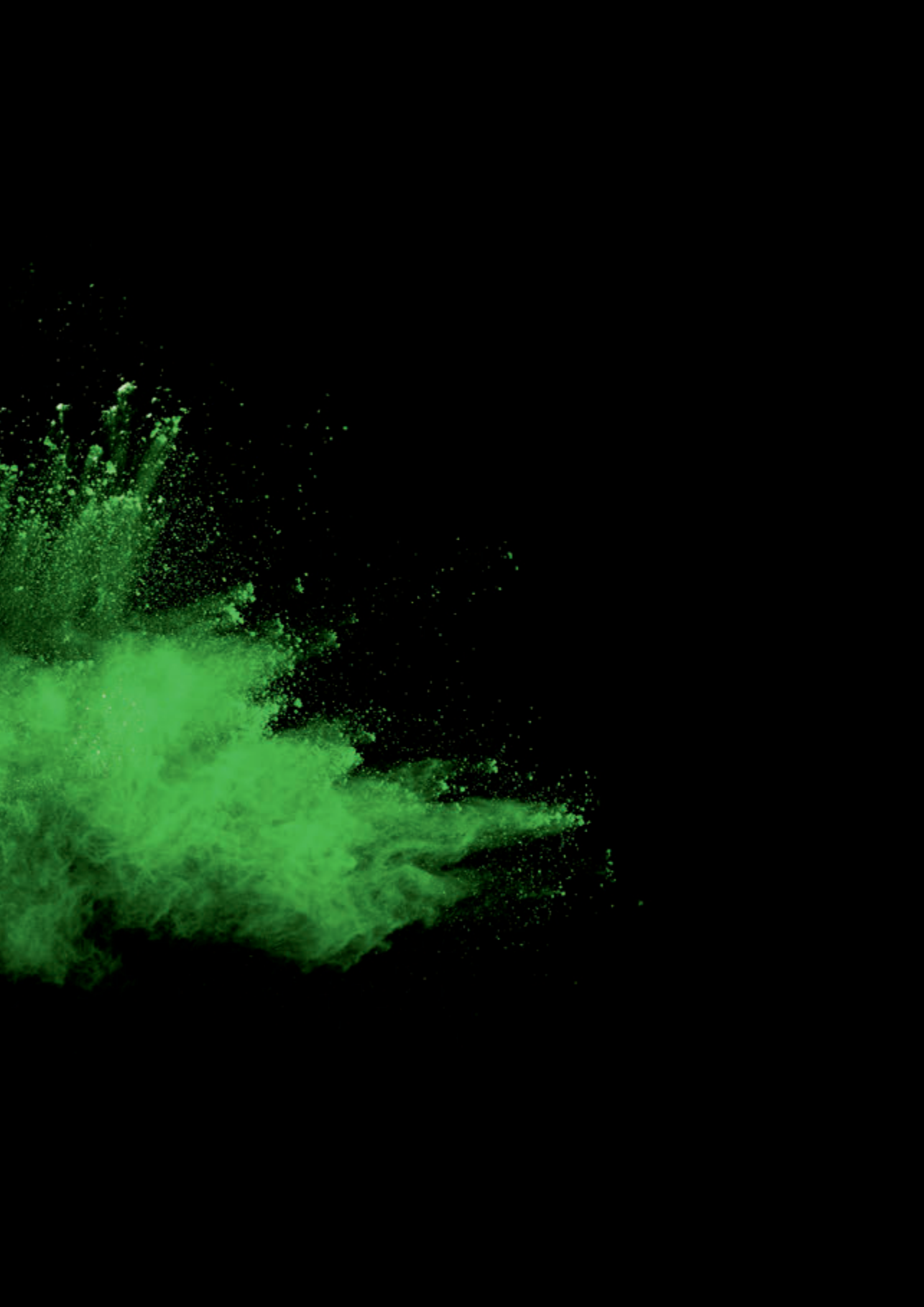
perfusion in gastrointestinal surgery are other examples of surgical procedures in which adequate tissue perfusion assessment is important.

The work of Part II of this thesis describes the feasibility of indocyanine green angiography to objectively assess tissue perfusion of the mastectomy skin flap, and free and pedicled flaps. The results of the performed studies suggest that the use of indocyanine green imaging in breast reconstructive surgery and abdominal wall reconstruction can reduce postoperative complications. This may lead to a shorter hospital stay, less frequent outpatient visits for wound problems and reoperations for necrosis or dehiscence. All of this could lead to an increase in comfort for both the patient and the physician. Consequently, it has a positive impact on both the patient, as well as the health care itself.

However, since the currently performed studies are predominantly small, non-randomised cohort studies, there is a lack of high evidence. For this reason, future high-quality randomised controlled trials that compare the use of indocyanine green imaging to clinical evaluation are needed to confirm the real impact of indocyanine green imaging.

PART IV

Appendices



CHAPTER 11

Acknowledgments | Dankwoord

“I thank you in advance for the great round of applause I'm about to get.”

- Bo Diddley –

DANKWOORD

Dit proefschrift kon niet tot stand komen zonder de support van de mensen om mij heen. Daarom wil ik iedereen bedanken die op een of andere manier een bijdrage heeft geleverd aan dit proefschrift. Een aantal mensen zou ik graag in het speciaal willen bedanken voor de gegeven hulp en steun.

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We zijn nu dik zeven-en-een-halfjaar jaar samen en in deze tijd hebben we al mooie herinneringen mogen maken. Van onze eerste ontmoeting en smalltalk op de studentenvereniging, tot het delen van lief en leed. Van samenwonen op 20m2 (inclusief bad -en slaapkamer), tot het kopen van ons eerste huis in Maastricht. Van onze eerste vakantie in Parijs, tot een onvergetelijke reis naar Indonesië. Van het verjagen van een huismuis en zijn vrienden, tot het aanschaffen van twee lieve, maar luie, harige viervoeters.

In deze mooie tijd samen, heb je ook de periode van mijn eerste publicatie, tot het einde van mijn promotietraject mogen meemaken. Tijdens de combinatie van mijn ANIOS-schap en onderzoek hadden we best wat te verduren. Ik heb het jou namelijk niet makkelijk gemaakt met mijn manier van plannen en mijn irrealistische besef van tijd. Doordeweekse avond- en nachturen, weekenduren en regelmatig tussendoor “nog even snel wat doen”. In mijn onderzoeksbubbel was vrije tijd soms ver te zoeken. Mijn persoonlijkheid met perfectionistische en ADHD-achtige trekjes gaven een extra dimensie aan deze interessante en leerzame periode van vallen en opstaan.

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De wijze, Engelse schrijver en dichter Aldous Huxley zei ooit: “Several excuses are always less convincing than one”. (Meerdere excuses zijn altijd minder overtuigend dan één). Daarom laat ik het bij één excuus en ik beloof dat we na dit promotietraject weer meer gaan genieten van de vrije tijd samen. Genieten van elkaar en de wereld ontdekken!

CHAPTER 12

Sientific output

LIST OF OF PUBLICATIONS

1. Schols RM, Dip F, Lo Menzo E, Haddock NT, Landin L, Lee BT, Malagón P, Masia J, Mathes DW, Nahabedian MY, Neligan PC, Newman MI, Phillips BT, Pons G, **Pruimboom T**, Qiu SS, Ritschl LM, Rozen WM, Saint-Cyr M, Song SY, van der Hulst RRWJ, Venturi ML, Wongkietkachorn A, Yamamoto T, White KP, Rosenthal RJ. Delphi survey of intercontinental experts to identify areas of consensus on the use of indocyanine green angiography for tissue perfusion assessment during plastic and reconstructive surgery. Surgery, Volume 172, Issue 6, Supplement, 2022, Pages S46-S53.
2. **Pruimboom T**, Tuinder S, Qiu SS, Keuter X, van der Hulst RRWJ. The impact of using the internal mammary artery as a recipient vessel on mastectomy skin flap perfusion in autologous breast reconstruction: an observational study using indocyanine green angiography [submitted]
3. **Pruimboom T**, Lindelauf AAMA, Felli E, Sawor JH, Deliaert AEK, van der Hulst RRWJ, Al-Taher M, Diana M, Schols RM. Perioperative Hyperspectral Imaging to Assess Mastectomy Skin Flap and DIEP Flap Perfusion in Immediate Autologous Breast Reconstruction: A Pilot Study. Diagnostics (Basel). 2022 Jan 13;12(1):184.
4. Ishizawa T, McCulloch P, Muehrcke D, Carus T, Wiesel O, Dapri G, Schneider-Koriath S, Wexner SD, Abu-Gazala M, Boni, L, Cassinotti E, Sabbagh, C, Cahill R, Ris F, Carvello M, Spinelli A, Vibert E, Terasawa M, Takao M, Hasegawa K, Schols RM, **Pruimboom T**, Murai Y, Matano F, Bouvet M, Diana M, Kokudo N, Dip F, White K, Rosenthal RJ. Assessing the development status of intraoperative fluorescence imaging for perfusion assessments, using the IDEAL framework. BMJ Surgery, Interventions, & Health Technologies 2021;3:e000088. doi: 10.1136/bmjsit-2021-000088
5. Al-Taher M, **Pruimboom T**, Schols RM, Okamoto N, Bouvy ND, Stassen LPS, van der Hulst RRWJ, Kugler M, Hostettler A, Noll E, Marescaux J, Diemunsch S, Diana M. Influence of intraoperative vasopressor use on indocyanine green fluorescence angiography: first evaluation in an experimental model. Sci Rep. 2021 May 6;11(1):9650.
6. Qiu SS, **Pruimboom T**, Cornelissen AJM, Schols RM, van Kuijk SMJ, van der Hulst RRWJ. Outcomes following lymphaticovenous anastomosis (LVA) for 100 cases of lymphedema: results over 24-months follow-up. Breast Cancer Res Treat. 2020 Nov;184(1):173-183.
7. **Pruimboom T**, Schols RM, Van Kuijk SM, Van der Hulst RR, Qiu SS. Indocyanine green angiography for preventing postoperative mastectomy skin flap necrosis in immediate breast reconstruction. Cochrane Database Syst Rev. 2020 Apr 22;4(4):CD013280.
8. **Pruimboom T**, Ploegmakers IBM, Bijkerk E, Breukink SO, van der Hulst RRWJ, Qiu SS. Fasciocutaneous anterolateral thigh flaps for complex abdominal wall reconstruction after resection of enterocutaneous fistulas and the role of indocyanine green angiography: a pilot study. Hernia. 2021 Apr;25(2):321-329.

9. **Pruimboom T**, van Kuijk SMJ, Qiu SS, van den Bos J, Wieringa FP, van der Hulst RRWJ, Schols RM. Optimizing Indocyanine Green Fluorescence Angiography in Reconstructive Flap Surgery: A Systematic Review and Ex Vivo Experiments. Surg Innov. 2020 Feb;27(1):103-119.
10. **Pruimboom T**, Scheltinga MR. Keloid Formation due to Repetitive Mammographies. Case Rep Dermatol. 2018 Nov 28;10(3):257-262.
11. **Pruimboom T**, Schols RM, Qiu SS, van der Hulst RRWJ. Potential of near-infrared fluorescence image-guided debridement in trauma surgery. Case Reports Plast Surg Hand Surg. 2018 Jun 28;5(1):41-44.
12. **Pruimboom T**, Scheltinga M. Massive Buttock Necrosis Following Aortobifemoral Bypass Surgery. Eur J Vasc Endovasc Surg. 2018 May 11. pii: S1078-5884(18)30250-8.
13. **Pruimboom T**, Hubens LPMA, Dercksen MW, Scheltinga, M. Slecht doorbloede voet (2017). Medisch Contact; Week 14, pagina 29.
14. **Pruimboom T**, Hubens LPMA, Dercksen MW, Helmich FA, Scheltinga M (2017). Bovenbeenamputatie ten gevolge van arteriële occlusie na therapie met dalteparine. Medisch Journaal. Máxima Medisch Centrum; Jaargang 2017, nr. 1, pagina 30-32

LIST OF PRESENTATIONS

1. “Influence of intraoperative vasopressor use on indocyanine green fluorescence angiography. Video presentatie. 17th IFSES World Congress of Endoscopic Surgery. Spanje, Barcelona, 26 november 2022.
2. “Het gebruik van ICG imaging in de Plastische Chirurgie, Maastricht UMC+”. Werkgroep bijeenkomst Dutch Fluorescence guided Surgery Group (DFGS). Presentatie via ZOOM. Nederland, Maastricht, 5 maart 2022.
3. “Weke delen bedekking: een vrije lap?!” Presentatie. Refereeravond Chirurgie, thema: Nonunion. Nederland, Maastricht, 3 juni 2021.
4. “Outcomes of anterolateral thigh flaps in complex abdominal wall reconstruction after resection of enterocutaneous fistulas and the role of indocyanine green angiography.” Presentatie. 10th Congress of World Society for Reconstructive Microsurgery (WSRM 2019): Bologna, Italië, 15 Juni 2019.
5. “Feasibility of ALT flaps for complex abdominal wall reconstruction after takedown of enterocutaneous fistulas and the role of indocyanine green angiography.” Poster presentatie. Vereniging Chirurgie voor Medisch Studenten (VCMS) Symposium: Utrecht, Nederland, 26 januari 2019.
6. “Future Application of Indocyanine Green (ICG) Imaging in Plastic and Reconstructive Surgery”. Presentatie. Pélerin Wetenschapssymposium: Maastricht, Nederland, 16 juni 2018

CHAPTER 13

About the author

“Life isn’t about finding yourself. Life is about creating yourself.”

- George Bernard Shaw –

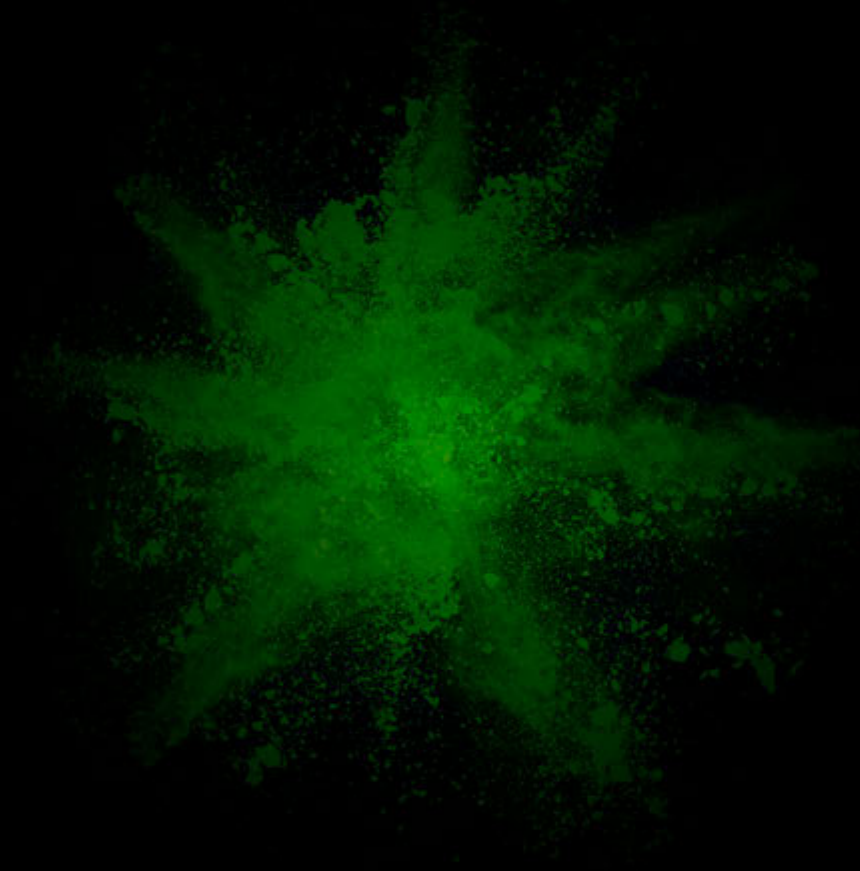
ABOUT THE AUTHOR

Tim Pruimboom was born on Saturday March 14, 1992 in Eindhoven and raised in Best, Noord-Brabant, the Netherlands. He obtained his Atheneum degree at Heerbeeck College in Best in 2010.

After being eliminated by central lottery and decentralised selection at Radboud University, he started the bachelor in Nursing at Avans University of Applied Sciences in Den Bosch. After obtaining his bachelor's degree and almost completing the second year of Nursing, he still wanted to become a doctor. Therefore, he decided to sign in for the decentralised selection in Maastricht and started his medical study at the Faculty of Medicine at the Maastricht University in 2012.

Before starting the internships in the master phase of Medicine (in Dutch: coschappen), he was quite sure to become an oncologist as he felt he was not a 'surgery-type'. However, at the Laurentius Hospital in Roermond, he discovered his interest and enthusiasm for surgery. Later in the master phase, he had the opportunity to start studying the application of Indocyanine Green in the department of Plastic and Reconstructive Surgery. After receiving his medical doctor degree in August 2018, he started as a medical doctor not in training (in Dutch: ANIOS) at the department of Plastic and Reconstructive Surgery in Zuyderland Medical Center, location Heerlen for one year. Hereafter, he worked in Zuyderland Medical Center, location Sittard for one year, VieCuri Medical Center for three months and Maastricht University Medical Center for six months. During his fulltime job in these hospitals, he was also engaged in PhD research.

In October 2021 he was admitted to the training as a specialist in plastic and reconstructive surgeon. As part of his plastic surgery training, he completed his residency in general surgery at Maastricht University Medical Center under the supervision of prof. dr. Stassen and dr. Melenhorst in July 2023. Since then, he continued his training to become a plastic and reconstructive surgeon at Maastricht University Medical Center.



"Imagination is more important than knowledge."

- Albert Einstein -