INTRODUCTION

The deep inferior epigastric perforator (DIEP) flap has become the first choice in autologous breast reconstruction, as excellent aesthetic results can be obtained with minimal donor site morbidity. However, rates of fat necrosis for DIEP breast reconstruction may be as high as 35%, which not only reduces the aesthetic result but also causes uncertainty due to lump formation in the reconstructed breast.1,2 Fat necrosis is a result of inadequate flap perfusion. Selection of a suitable perforator is critical for decreasing the rate of postoperative fat necrosis and minimizing perfusion-related flap complications.3 A thorough understanding of the vascular anatomy and flow characteristics of a selected perforator is essential to balance flap design and adequate flap perfusion. The angiosome concept by Taylor and Palmer has greatly contributed to our knowledge on flap perfusion.4 Saint-Cyr et al introduced in 2009 the perforasome theory and defined a perforasome as the territory perfused by a single perforator vessel of a named artery.5 The importance of finding the most suitable perforator is reflected in the

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The primary aim of this study was to evaluate the effect of perforator dissection on the perfusion area of a selected perforator.

Indocyanine green fluorescence angiography (ICG-FA) is an invasive technique that allows for direct visualization of tissue perfusion by registration of intravascular fluorescence intensity. Infrared thermography (IRT) is a noninvasive technique that indirectly visualizes skin perfusion by measuring infrared (IR) radiation released from the skin surface. In static infrared thermography (SIRT), a single image is taken from the area of interest. In dynamic infrared thermography (DIRT), the area of interest is exposed to a mild thermal challenge, and the rate and pattern of temperature changes are recorded and analyzed. ICG-FA and IRT are well-established techniques that provide real-time assessment of skin perfusion and have been shown to provide valuable information for perforator mapping.1-12

The primary aim of this study was to evaluate the effect of perforator dissection on the perfusion area of the selected perforator in a hemi-DIEP flap by using ICG-FA and IRT intraoperatively. The secondary aim of this study was to compare the results from ICG-FA with those obtained from IRT with respect to flap perfusion.

MATERIALS AND METHODS

The study was approved by the Regional Committee for Medical and Health Research Ethics (REK Nord, approval number 2017/1641), institutional protection officer (DPO approval number 02369), and registered in ClinTrials.gov (NCT04115995). Fifteen female patients scheduled for secondary DIEP breast reconstruction were included in the study. Exclusion criteria were lactating women, smokers, body mass index (BMI) over 30 kg/m2, renal or hepatic failure and previous history of reactions on ICG or iodine contrast.

On the lower abdomen, a hemi-DIEP flap was designed on each side of the midline. Preoperative perforator mapping was performed with a $128 \times 2$-slice dual-source computed tomographic angiography (CTA-SOMATOM Definition FLASH, Siemens Healthineers, Erlangen, Germany) and hand-held unidirectional Doppler ultrasound (8 MHz, Multi Dopplex II, Humleigh Healthcare, Cardiff, UK). Arterial Doppler sound locations were marked with a permanent marker on the skin in a quadrant system overlying each rectus abdominis muscle.

Perfusion examinations (E1) of the hemi-DIEP flaps with ICG-FA and IRT were performed simultaneously after induction of general anesthesia and before surgery. Several perforators were selected for dissection based on preoperative imaging and intraoperative findings and were tested for Doppler sounds. The second examination (E2) was performed after the supra-fascial dissection of the selected perforators had been completed and with the superficial inferior epigastric vein (SIEV) open if found.

For ICG-FA, a video camera (IC-VIEW PULSION Medical Systems, Munich, Germany) was used. ICG binds exclusively to plasma proteins, is rapidly excreted into the bile, and has a plasma half-life of 3–4 minutes in healthy adults. After illumination of ICG with laser (energy $p = 0.16$ W, wavelength 7280 nm), it induces near-infrared light (NIR, max spectral range 805 nm, fluorescence emission at 835 nm), which is recorded by the camera. Before each recording, the camera was calibrated to a fluorescence reference card. For ICG-FA, a bolus of 1.0 mg/kg (5 mg ICG /1 ml saline) was injected intravenously. Recordings lasted until fluorescence was seen at the flap edge.

For IRT, an IR camera (FLIR ThermaCAM S65 HS FLIR Systems; FLIR Systems AB, Boston, Mass.) with a sensitivity of 0.1°C was used. Thermal emissivity was set to 0.98, and the camera accuracy was regularly checked against a black body with a traceable temperature source (Model IR-2103/301, Infrared Systems Development Corp., Winter Park, Fla.).

The IR- and ICG-FA cameras were mounted beside each other on an extendable boom of a specially designed camera stand, the height of which could be electronically controlled. With this system the lenses of both cameras could be positioned above and perpendicular to the exposed abdominal surface at a distance of 40 cm and 70 cm for the ICG-FA and IRT measurements, respectively.

In E2, DIRT was performed after the SIRT and ICG-FA examinations by exposing the skin to a mild cold challenge by placing a metal plate at room temperature ($23–24 \degree C$) for 30 seconds on the skin. Skin rewarming was recorded for 3 minutes. The effect of closing SIEV or a selected perforator on flap perfusion was also evaluated. Images were digitally stored and processed using image analysis software ThermaCAM Researcher Pro 2.8 SR-1 (FLIR Systems AB, Boston, Mass.). The rate and pattern of rewarming of the skin were analyzed.

RESULTS

In 15 women, a mean age 52 years (range 32–76) and mean BMI of 26.9 kg/m2 (range 24–30) 30 hemi-DIEP flaps were designed. Six patients (40%) had been previously operated on the lower abdomen, but only a few had a scar within the quadrant system. See Table 1 for patient and surgery data. The hemodynamic conditions were stable during E1 and E2 (Figs. 1, 2), and the room temperature in the operation theater was 24°C.

| Table 1. Patient and Surgery Data |
|----------------------------------|
| **Patients:** | 15 women |
| Age, y [range]: | 52 [32–76] |
| BMI [range]: | 26.9 kg/m2 [24–30] |
| Previous surgery in lower abdomen: | 40.0% (6/15) |
| Indication for surgery: | 12 breast cancers 3 BRCA gene mutation |
| No. hemi-DIEP flaps evaluated with SIRT & ICG-FA: | 30 |

Note that 6 of the 15 patients (40%) had been previously operated in the lower abdomen with either open surgery or a laparoscopic procedure (appendectomy, salpingo-oophorectomy, laparoscopic hemicolecction, and caesarean section).
Fig. 1. Schematic presentation of the protocol for in vivo perfusion imaging of hemi-DIEP flaps in general anaesthesia before (E1) and after dissection of dominant perforators (E2). In each patient, SIRT and ICG-FA were performed simultaneously in E1 and E2. The IR- and ICG-FA cameras were mounted beside each other on the end of an extendable boom of a specially designed camera stand whose height could be electronically controlled. With this system, the lenses of both cameras could be positioned above and perpendicular to the exposed abdominal surface at a distance of 40 cm and 70 cm for the ICG-FA and IRT measurements, respectively. On the photographic images the system we used to divide the flap surface over each rectus fascia into four equal quadrants is indicated by the red (E1) and blue (E2) dashed lines. The lateral margins of the rectus muscle were marked with 3 staples in the skin (black arrows), which were easily visible on the SIRT images (white arrows) and facilitated orientation of perfusion spots after flap transfer. SIRT, static infrared thermography; ICG-FA, indocyanine green fluorescence angiography; RUL, right upper lateral; RUM, right upper medial; RLL, right lower lateral; RLM, right lower medial; LUM, left upper medial; LUL, left upper lateral; LLM, left lower medial; LLL, left lower lateral; E1, first dynamic imaging evaluations; E2, second dynamic imaging evaluations.

Fig. 2. Boxplots of hemodynamic values and body temperature at E1 and E2 during SIRT and ICG-FA examinations for all 15 patients. E1, SIRT and ICG-FA in general anesthesia but before surgery; E2, SIRT and ICG-FA in general anesthesia and after perforator dissection of hemi-DIEP flaps; SaO₂, Oxygen saturation.
First Examination (E1) with SIRT and ICG-FA

Thermographic images showed skin surface areas with continuous high IR radiation, called “hotspots.” ICG-FA gave a short-lasting image of first appearing fluorescence spots and their subcutaneous branching network connecting associated fluorescence spots, before the dye rapidly reached the subdermal plexus and was distributed into the entire flap surface. The locations of IR hotspots and patterns of increased IR radiation were comparable with locations of fluorescence spots and their associated fluorescence patterns. Both imaging modalities showed a similar large variability in number, size, and locations of the fluorescence and IR patterns between both hemi-DIEP flaps for each patient, but also between patients. In contrast to SIRT, ICG-FA visualized more clearly a branching network between fluorescence spots and showed the dynamic changes within this network. On the other hand, SIRT visualized hotspots with a clearer border, especially in the lateral parts of the flap, where ICG always appeared less pronounced due to limited spread of the laser beam (Figs. 3–6).

Second Examination (E2) with SIRT and ICG-FA

After perforator dissection, both the patterns of the IR hotspots with IR radiation and fluorescence spots with their branching network changed dramatically compared with E1. Each remaining perforator was associated with the new perfusion pattern of fluorescence and IR radiation. The location of the first appearing fluorescence spot and high IR hotspot was always associated with the perforators exit point through the rectus abdominis fascia. IR radiation patterns associated with the selected perforators were clearly marked from each other by areas with lower IR radiation. Similarly, the fluorescence spots and branching network were surrounded by a clearly hypo-fluorescent bordered area. IRT confirmed increase in the size of the hotspot and ICG-FA visualized more clearly fluorescence accumulation in the localized remaining perfusion areas on E2 (Figs. 3, 4). ICG-FA rapidly showed a clear branching pattern that could not be seen on IRT, which stabilized into an IR radiation area of a certain size only after a few minutes. While the IRT pattern remained over time, the fluorescence pattern only lasted for a short time until ICG reached the subdermal...
plexus and thereafter drained through the open end of the SIEV. High IR radiation from blood draining at the end of SIEV was also seen on IRT. In patients with abdominal scars, there were areas with no or minimal fluorescence uptake that went laterally from the scars after perforator dissection and remained hypo-fluorescent even when the dye reached the SIEV. In these areas, IR radiation was low but higher than room temperature (Figs. 5, 6).

**DIRT and Clamping of Perforators**

DIRT allowed for a continuously real-time monitoring of IR radiation from the flap surface. Clamping of a selected perforator followed by release of the clamp made it easy to identify the area and pattern of increased IR radiation associated with this perforator. Each perforator had a specific rewarming pattern that returned after release of the clamp. It was thus possible to identify the first appearing IR hotspot and associated IR hotspots. Clamping of a perforator had a variable effect on the rate and patterns of rewarming of the flap. In some cases, clamping of a lateral row perforator resulted in a disappearance of the associated hotspot and an increase in the rate and pattern of rewarming of the remaining perforators and visa versa (Figs. 7, 8). However, in other cases, clamping of a perforator did not result in a change in the overall thermal pattern. If DIRT showed high IR from the open end of SIEV, clamping the superficial vein resulted in a change of the pattern and rate of rewarming of the IR hotspots in the flap surface (Fig. 7).

**DISCUSSION**

This study shows for the first time in vivo the effect perforator dissection has on the flow characteristics and vascular territory of the selected perforator in a hemi-DIEP flap. Another important finding is that the noninvasive technique IRT and invasive technique ICG-FA provide comparable information on the dynamic changes that occur in a hemi-DIEP flap after perforator dissection.

Studies have shown that ICG-FA and IRT can be used for perforator mapping. In our study, the locations of fluorescence spots were associated with locations of IR hotspots in both E1 and E2. Following perforator dissection (E2), first appearing fluorescence spots were associated with first appearing IR hotspots on DIRT and the perforator’s exit point at the rectus abdominis fascia. Onoda et al found a good correlation between perforator mapping with CTA and ICG-FA. Perstana and Zenn...
did not find a correlation between the localization of skin blush from ICG-FA and perforator mapping with CT angiography. A plausible explanation could be that they did not differentiate between fluorescence area and first appearing fluorescence spot which appears before the “blush.” Studies have shown that blood vessels can be outlined at a depth of nearly 2 cm from the skin surface by applying ICG-FA. In our study, E1 and E2 showed a subcutaneous vascular network between the first appearing fluorescence spot and associated fluorescence spots. The flow within this network reveals that the perforator has a divergent branching network producing other fluorescence spots followed by fluorescence in the subdermal plexus. This network produces the IR hotspots and IR area associated with the first appearing hotspot.

Although the patterns of ICG-FA and IRT were comparable in our study, the areas of intensity were slightly different in size. In ICG-FA, fluorescence of plasma bound ICG is registered and provides direct information on intravascular perfusion. On the other hand, IRT is an indirect method to monitor perfusion and may result in an overestimation of tissue perfusion as it registers the convective heat produced by transport of warm blood. This can explain the differences in area between both imaging modalities. The rapid washout of ICG-FA could even result in a slight underestimation of the perfusion area. In 2 experimental studies evaluating skin perfusion using both imaging modalities, Miland et al came to the same conclusion.

The flow characteristics of the selected perforator changed after the number of perforators was reduced. This was especially evident with IRT, which showed an increased IR radiation at the hotspots of the selected perforator. ICG-FA also showed increased fluorescence with a clearer border of the branching network (Figs. 3–7). This may be caused by an increased flow through the selected perforator from the source artery after other perforators have been cut and sealed.

Selecting the largest perforator with the most optimal 3-dimensional branching within the subcutaneous fat has

Fig. 5. The effect of perforator dissection revealed by real time perfusion images in a 47-year old female who had scars on the lower abdomen after appendectomy and laparoscopic surgery. Each dominant perforator selected during flap dissection is identified with a white number and an abbreviation (dashed line framed box in right corner). Left box: The results from E1. The locations of arterial Doppler sounds on both hemi-DIEP flaps were marked with black dots on the skin. The areas of increased fluorescence on ICG-FA and IR radiation on SIRT are highlighted with white dashed lines. Note the lack of fluorescence uptake in the McBurney scar (blue arrows). Based on her medical history and results from E1, a left sided hemi-abdominal flap was initially planned for her unilateral breast reconstruction. Right box: Radical changes in perfusion patterns after perforator dissection in both hemi-DIEP flaps. Due to such findings, the surgical plan was changed intraoperatively to select the right hemi-DIEP flap for the breast reconstruction. Areas with less ICG uptake and low IR radiation (E2) were trimmed off the flap and no perfusion related complications were reported in this patient. The perfusion patterns of both modalities were comparable, except in the lateral parts of the ICG-image (blue dashed line) due to limited spread of the laser beam. Selected periumbilical perforators after dissection (on E2) were draining into the SIEV. E1, evaluation before surgery; E2, evaluation after perforator dissection; Doppler US, Doppler ultrasound; ICG-FA, fluorescence angiography of indocyanine green; SIRT, static infrared thermography; SIEV, superficial inferior epigastric vein.
been suggested to optimize perforator flap surgery outcomes. Both ICG-FA and IRT could provide this information by, respectively, the vascular network shown by ICG-FA, and the perfusion area with associated hotspots on IRT when selected perforators are clamped. The clearly bordered area of fluorescence and IR radiation produced by the selected perforator is much smaller compared with the results of anatomical studies reported by Wong et al. They showed that medial row perforators supply a larger vascular territory of tissue of 296 cm² compared with lateral row perforators with 196 cm². Lee et al explained that anatomical studies likely illustrate the maximum potential capacity of these vessels, while clinical studies incorporate hormonal or neural stimulation, which is present only in living tissue. Blood vessels are of greater measurable caliber in cadaveric specimens than in the living.

The results from our study were obtained during the first 2 hours of a DIEP breast reconstruction and may also explain this difference. Before surgery, perforators contribute to the perfusion of the blood vessels in the watershed zones between their perforasomes and create a pressure equilibrium that becomes disrupted with flap dissection. Vessel manipulation and local accumulation of blood following ligation of perforators may further temporarily influence the flow characteristics of the selected perforator. Anatomical studies have described the existence of direct and indirect linking vessels between perforasomes. Dahr and Taylor reported on the anatomic changes that occur at the level of the reduced-caliber choke vessels between adjacent vascular territories of a pedicled flap. Initially the choke vessels showed a vasoconstriction in the first 3 hours, but they returned to a diameter comparable to the control between 3 and 24 hours. Thereafter, the choke vessels underwent progressive sequential dilation that was most prominent between 48 and 72 hours. A similar phenomenon could exist between perforasomes within the same angiosome. The pressure gradient may also be the cause of an earlier perfusion of perforasomes within the angiosome compared with perforators of adjacent angiosomes. In the present study when using DIRT, clamping of a perforator resulted in an expansion of IR of an adjacent perforator to the location of the clamped perforator, indicating direct linking vessels. However, in other
cases, no changes were monitored, which could mean the existence of indirect linking vessels (Figs. 7, 8). With the use of thermography, CTA and results from cadaveric angiographic studies, Chubb et al were able to show the robustness of interconnections between perforators. A study on the perfusion dynamics of DIEP flaps showed a rapid expansion of IR hotspots within the same angiosome that could be related to the previous locations of perforators, whereas there was a clear delay in the appearance of IR hotspots in the adjacent angiosomes. Such may indicate that the vascular resistance of vessels linking perforasomes within an angiosome is less than in choke vessels between angiosomes due to caliber difference. The same study also revealed a stepwise progression of perfusion of the subdermal plexus and subcutaneous tissue, each with its own time sequences. Both ICG-FA and DIRT indicate that the subdermal plexus becomes perfused after the selected perforator but before the subcutaneous network of adjacent perforators.

Another interesting finding was the lack of ICG uptake in the scar and nearby lateral area in patients previously operated on in the abdomen. IR radiation was also low in these areas, but higher than the room temperature. This was more prominent on E2 and easier to distinguish on ICG-FA, which may help in intraoperative flap planning (Figs. 5, 6).

A perforasome is the vascular territory perfused by a perforator of a known source artery. However, adequate perfusion of a perforasome also relies on venous drainage. The DIEP flap can have a dual venous system: the deep inferior epigastric vein (DIEV) and the SIEV with connecting vessels between them. One of the veins might be the dominant route to drain the DIEP flap. Obstruction of this dominant vein may influence the perfusion area of the selected perforator and contribute to flap perfusion problems. Our results show that DIRT can highlight the dynamic changes in the DIEP flap when the dominant venous route is obstructed (Fig. 8).

The limitations of our study are that only the effect of perforator dissection on skin perfusion within a hemi-DIEP flap was evaluated. However, a previous study using DIRT reported on the perfusion dynamics of DIEP flaps and SIEA flaps across the midline. Another limitation of
our study is that ICG-FA and DIRT provide information on skin perfusion and less on perfusion of the subcutaneous tissue, which is important for evaluation of fat necrosis. The branching network and associated fluorescence spots, as well as the pattern of associated hotspots on IRT provide some information on subcutaneous perfusion. Previous studies have reported significantly lower incidence of fat necrosis and partial necrosis in a DIEP flap when ICG-FA was applied for deciding safe tissue margins.\textsuperscript{7,31,32}

**CONCLUSIONS**

Perforator dissection causes dynamic changes in perforasome perfusion in a hemi-DIEP flap. These changes cannot be registered by clinical signs as skin color inspection. ICG-FA and IRT can provide the surgeon valuable information during surgery on the flow characteristics and the perforasome territory of the selected perforator after dissection, as long as the limitation and strength of both dynamic imaging tools are taken into consideration. Our study shows that evaluation of flap perfusion with the direct method ICG-FA and the indirect method IRT produce comparable and reproducible results.

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