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Technology

Correlation between Indocyanine Green Fluorescence Angiography and Laser Speckle Contrast Imaging in a Flap Model

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Background: Indocyanine green fluorescence angiography (ICG-FA) is used to assess tissue intraoperatively in reconstructive surgery. This requires an intravenous dye injection for each assessment. This is not necessary in laser speckle contrast imaging (LSCI); therefore, this method may be better suited for tissue evaluation. To determine this, we compared the two methods in a porcine flap model.

Methods: One random and one pedicled flap were raised on each buttock of six animals. They were assessed with LSCI at baseline, when raised (T_0), at 30 minutes (T_{30}) and with ICG-FA at T_0 and T_{30}. Regions of interest (ROI) were chosen along the flap axis. Perfusion, measured as perfusion units (PU) in the LSCI assessment and pixel-intensity for the ICG-FA video uptake, was calculated in the ROI. Correlation was calculated between PU and pixel-intensity measured as time to peak (TTP) and area under curve for 60 seconds (AUC_{60}).

Results: Correlation between LSCI and AUC_{60} for the ICG-FA in corresponding ROI could be seen in all flaps at all time points. The correlation was higher for T_0 (r=0.7 for random flap and r=0.6 for pedicled flap) than for T_{30} (r=0.57 for random flap and r=0.59 for pedicled flap). Even higher correlation could be seen PU and TTP (T_0: random flap r=-0.8 and pedicled flap r=0.76. T_{30}: random flap r=-0.8 and pedicled flap r=0.71)

Conclusion: There is a correlation between PU from LSCI and TTP and AUC_{60} for ICG-FA, indicating that LSCI could be considered for intraoperative tissue assessment. *(Plast Reconstr Surg Glob Open 2023; 11:e5187; doi: 10.1097/GOX.0000000000005187; Published online 22 September 2023.)*

INTRODUCTION

In reconstructive plastic surgery, different techniques are used to assess microvascular blood flow of the tissue operated on.[1–3] This is to let the surgeon make sure that the blood supply is good enough to provide optimal conditions for healing. This becomes especially important in flap surgery, where the surgeon often must rely on relatively limited blood supply via a narrow pedicle or a few individual blood vessels.

To be useful in clinical practice, the method used preferably needs to show a correct and reproducible picture of the remaining tissue microvascular blood flow throughout the tissue of interest. The technique must also be harmless to the patient, minimally invasive, and easy to handle.

At present, there is primarily one method of perfusion assessment that has gained ground in reconstructive surgery: indocyanine green fluorescence angiography (ICG-FA). Indocyanine green (ICG) is a nontoxic dye that fluoresces when illuminated by infrared light. If given intravenously, it can be followed, as it spreads into the flap vascularity, including the smallest vessels of the skin microcirculation. It is possible to give repeated injections, but for each assessment a new dose must be given, and if dye is remaining in the tissue, the data may be harder to evaluate.[4–7]

It would be preferable to use a method that gives a similar picture of the microvascular blood flow of the flap, without the need of repeated ICG injections. In

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previous studies, we have used laser speckle contrast imaging (LSCI) to assess the microvascular blood flow in deep inferior epigastric perforator (DIEP) free flaps intraoperatively.[8] The advantage of LSCI is that no intravenous injection needs to be given to make the assessment. Instead, the method relies on the laser speckle pattern phenomenon. This means that the pattern created when tissue is illuminated with coherent light contains information about the perfusion in the superficial blood vessels, which is defined as the concentration of red blood cells times their average velocity. In LSCI, the perfusion is presented as an arbitrary value (PU or perfusion units) and can easily be calculated for different regions of interest (ROI).[9–11] ICG-FA, on the other hand, is used in a more intuitive way, with the surgeon estimating the speed and degree of filling of ICG in the tissue and correlating this information to the microvascular blood flow or perfusion of the tissue.

If LSCI is to be used in a similar way as ICG-FA, it is reasonable to ask whether the techniques provide comparable information, especially because they use very different methods to evaluate the microvascular blood flow. To determine this, the ICG-FA assessments also need to be quantified. This is not as straightforward as in the case of LSCI because there is no generally accepted method for quantification of ICG-FA assessments, and many systems do not offer built-in software for this purpose.[12,13]

The aim of this study was, therefore, to evaluate if the information regarding the perfusion obtained from ICG-FA assessment correlates with information obtained from LSCI assessment of the same tissue.

Two parallel porcine flap models were chosen in each experimental animal to compare ICG-FA and LSCI. One pedicle-based flap was based on the CGAP flap, as previously described by Zötterman et al, and one adjacent random flap, based on a nondefined vessel and a broad proximal skin pedicle.[14,15] The length of all flaps was intentionally kept longer than the pedicle could provide for, so that in a controlled environment, we could study flaps with both well-perfused areas and peripheral areas where perfusion would be insufficient for tissue survival.[16,17]

Takeaways

Question: Indocyanine green fluorescence angiography (ICG-FA) is used to map tissue perfusion intraoperatively. The laser-based technology laser speckle contrast imaging (LSCI) has the advantage that the examiner does not need to inject ICG for each assessment. Can LSCI replace ICG-FA for intraoperative flap planning?

Findings: We have used LSCI and ICG-FA to assess flaps in a porcine animal model. We correlated the data and concluded that the techniques provide similar information about the perfusion pattern.

Meaning: LSCI may replace or supplement ICG-FA in intraoperative flap planning.

METHODS

Animals

Six mixed breed pigs (mean age 4 months, 45 kg, Swedish Landrace pigs) were used in the study.

LSCI

A laser speckle contrast imager (LSCI, Pericam PSI system, Perimed AB, Järfalla, Sweden) was used to assess the flaps. The system uses a near-infrared laser with a wavelength of 785 nm. The speckle pattern created by the laser light on the assessed surface is captured by a digital camera. From this pattern, a perfusion value is calculated, given as PU, an arbitrary unit proportional to the concentration and mean velocity of red blood cells. The theoretical principles of LSCI are further described by Briers et al.[10]

ICG-FA

A fluorescence imaging system (Fluobeam, Fluoptics, Grenoble, France) was used for the ICG angiography. The system uses a class 1 laser as the excitation light source and a near-infrared sensitive camera for the video uptake. The system offers no built-in software for quantification of the data, but the video sequences can be exported to separate USB media in the form of an MPEG4 file. Verdye Indocyanine Green (Diagnostic Green GmbH, Aschheim-Dornach, Germany) was used for the assessments. One 5-mg ampulla was diluted with 10 mL saline solution.

Protocol

The pigs were preanesthetized with Dexdomitor 0.1 mg per kg, Zoletil 5 mg per kg, and atropine 0.05 mg per kg. Anesthesia was maintained with pentobarbital sodium 8 mg per kg per hour and fentanyl 0.5 μg per kg per hour dissolved in Ringer's acetate given continuously intravenously with motorized infuser along with crystalloid fluids (Ringer acetate). Body temperature, blood pressure, heart rate, and oxygen saturation were monitored during the whole procedure.

Two 10 × 20 cm fasciocutaneous flaps were raised on each buttock of the pigs: one pedicled flap based on the CGAP and one random flap directly adjacent to the first. The flaps were dissected along the surface of the muscle, including skin, subcutaneous tissue, and muscle fascia. The pedicle containing the perforator artery along with comitant veins was isolated on the pedicled flap (Fig. 1).

Assessment of the flap area was done before surgery (only LSCI), directly after the flaps were raised (T_0), and 30 minutes after surgery (T_{30}). First, an LSCI video sequence was recorded for 10 seconds. During measurements the distance between the LSCI camera and the flap was kept between 25 and 35 cm. The field of view was set to 25 × 20 cm. The point density was set to normal, resulting in a spatial resolution of 0.05 mm per pixel. The frame rate was set to 21 images per second, and 10 consecutive images were averaged, yielding an effective frame rate of 2.1 images per second. Then, 2 mL of the ICG dilution was injected intravenously at the same time as the assessment with the Fluobeam equipment started. Monitoring with the infrared camera continued for about 10 minutes.

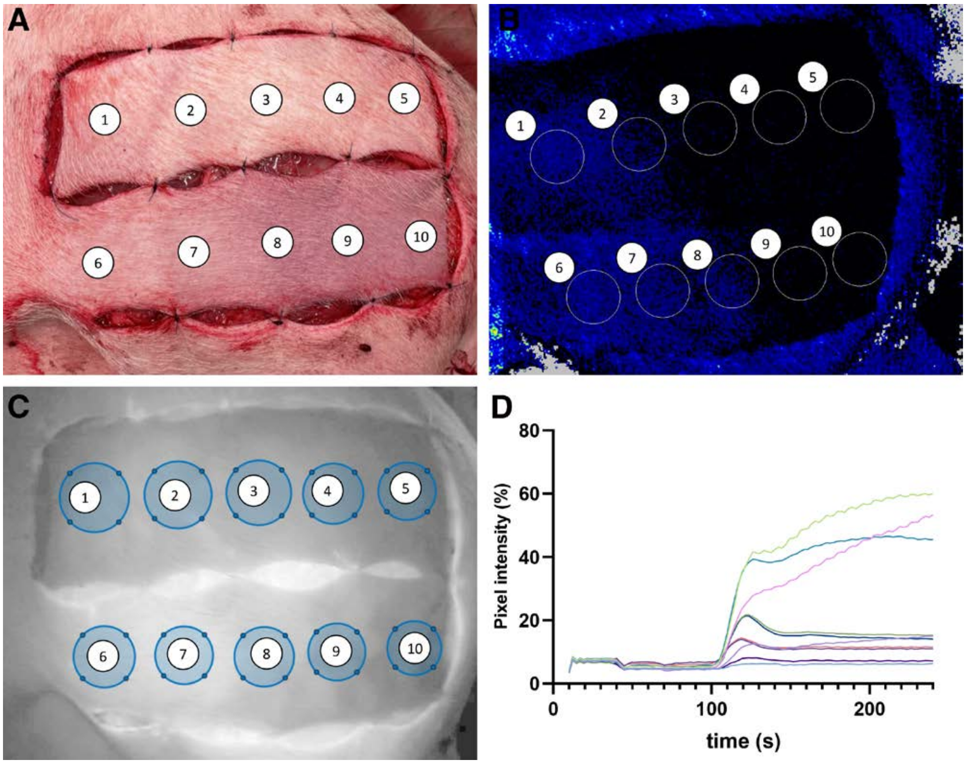

Fig. 1. Assessment setup. A, The flaps were raised on the buttocks of the pig. The superior flap in this case is a pedicled flap and the inferior, a random flap. The flaps were assessed with LSCI (B) and ICG-FA (C). ROI were chosen along the axis of each flap (1–10). D, The ICG-FA pixel-intensity curves for one of the flaps. Different colors represent different ROI.

Image Processing

The ICG-FA movie files were imported in MATLAB (Math Works, Natick, Mass.) and converted to image stacks. For each flap, five ROI were chosen along the central axis of the flap. For all ROI, a curve of the merged pixel-intensity of all included pixels was calculated (Fig. 2). For each curve, the area under curve (AUC) for a time frame starting with the initial increase in intensity and ending 60 seconds later (AUC_{60}) and time to peak (TTP) was calculated (Fig. 1).

By overlapping the pictures, corresponding ROI in the LSCI pictures could be calculated in PimSoft (Perimed AB, Järfalla, Sweden). The LSCI frame with the lowest average PU was considered the most relevant according to earlier methodological studies[18] (Fig. 1).

RESULTS

Before the flaps were raised, there was no significant difference in mean perfusion measured as PU with LSCI between the ROI analyzed, either for the pedicled or the random flaps. Directly (T_0) after the flaps were raised and 30 minutes later (T_{30}) there were statistically significant differences and a negative trend (one-way ANOVA and

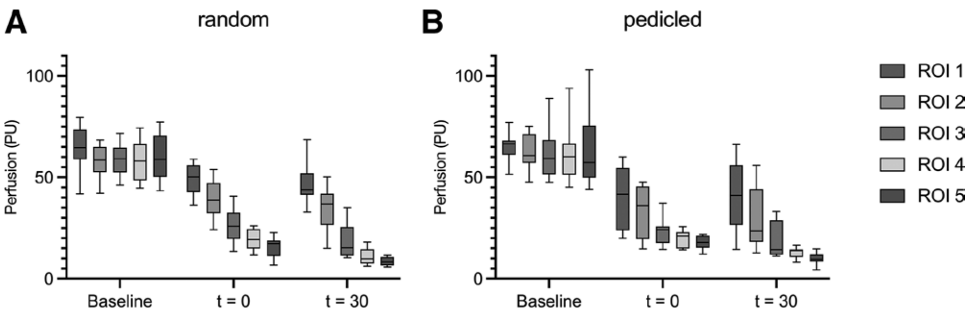

Fig. 2. Mean perfusion in respective ROI measured as PU (LSCI) in random (A) and pedicled (B) flaps. No difference could be seen between the different ROI in the LSCI assessment before the flaps were raised (baseline). At T_0 and T_{30}, there were statistically significant differences and negative trend (one way ANOVA and test for linear trend, p<0,0001) with lower PU in the more distal ROI in both pedicled and random flaps.

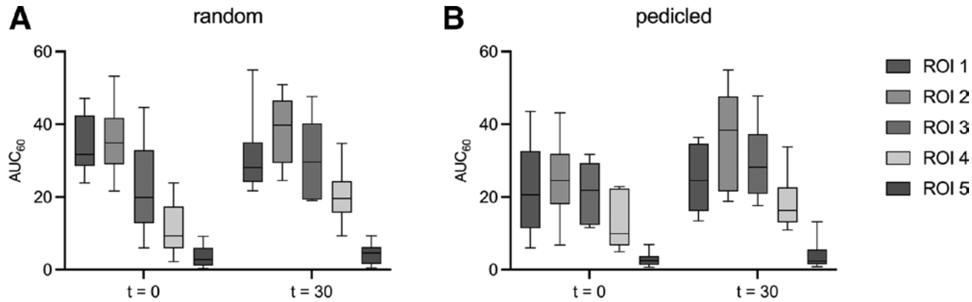

Fig. 3. Mean perfusion in random (A) and pedicled (B) flaps in respective ROI measured as AUC_{60}. At T_0 and T_{30}, there were statistically significant differences and negative trend (one way ANOVA and test for linear trend, p<0,0001) with lower values in the more distal ROI in both pedicled and random flaps.

test for linear trend, $P < 0.0001$) between all ROI with lower PU in the more distal ROI in both types of flaps (Fig. 2).

ICG-FA assessment was not performed before the flaps were raised. When the ICG assessment data were expressed as AUC for the initial 60 seconds (AUC_{60}), a significant difference and negative trend (one-way ANOVA and test for linear trend, $P < 0.0001$) could be seen in the pedicled flap from the proximal to the distal ROI (Fig. 3).

When the ICG assessment data were expressed as TTP, a significant difference and positive trend (one-way ANOVA and test for linear trend, $P < 0.0001$, except from pedicled flap at T_0, p = 0.019 and T_{30}, p = 0.0002) could be seen in the pedicled flap from the proximal to the distal ROI (Fig. 4).

A correlation between perfusion measured as PU with LSCI and measured as AUC_{60} for the pixel-intensity curve of the ICG in corresponding ROI could be seen in all flaps at all time points. The correlation was higher for T_0 (r = 0.7 for random flap and r = 0.6 for pedicled flap) than for T_{30} (r = 0.57 for random flap and r = 0.59 for pedicled flap) (Fig. 5).

An even higher correlation could be seen between corresponding ROI when ICG measurements were expressed as TTP (r = -0.8 for random flap and r = 0.76 for pedicled flap at T_0 and r = -0.8 for random flap and r = 0.71 for pedicled flap at T_{30}) (Fig. 6).

DISCUSSION

ICG-FA is probably the most widely used method for intraoperative evaluation of tissue microvascular blood flow in reconstructive plastic surgery today.[6,7,19–23] The method is primarily used as a visual aid and decision support when raising free flaps or evaluating skin flaps in breast surgery. Unfortunately, like many other new surgical methods and tools, ICG-FA has been introduced without being evaluated to the extent desired. The lack of reliable methods for quantifying the measurements may have contributed to the difficulty in conducting good comparative studies, but it is still a fact that relatively few studies with a high level of evidence indicate that there is a clear clinical advantage of using the method. LSCI is, on the other hand, a method of tissue perfusion assessment that is sparsely used clinically, despite the advantage of offering better interassessment comparability. A further advantage of LSCI is that there is no need for injection of ICG, which makes it more convenient to make repeated assessments. This study was therefore designed to evaluate if the data obtained from LSCI measurements could offer the same information as ICG-FA data and thereby become

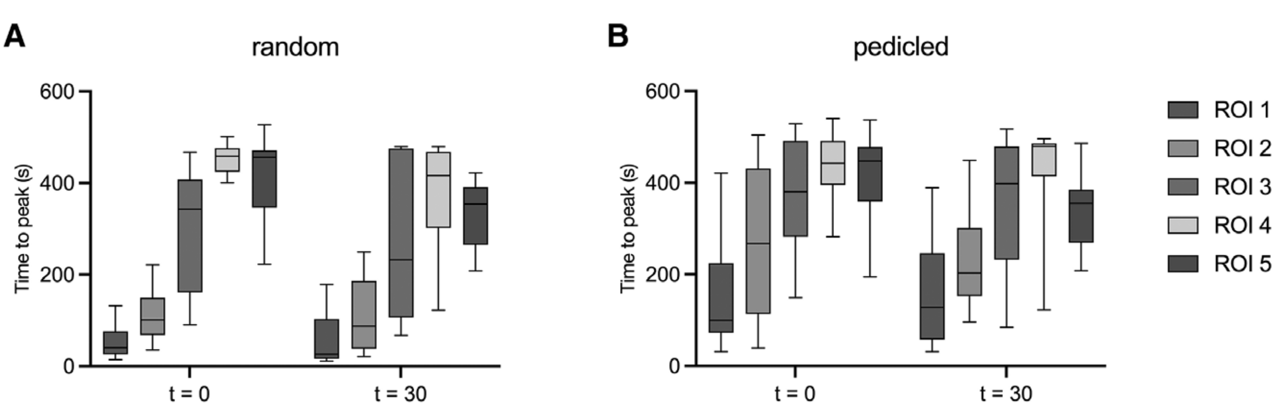

Fig. 4. Mean perfusion in random (A) and pedicled (B) flaps in respective ROI measured as time to peak. At T_0 and T_{30}, there were statistically significant differences and positive trend (one way ANOVA and test for linear trend, p<0.0001, except from pedicled flap at T_0, p=0.019 and T_{30}, p=0.0002) with higher values in the more distal ROI in both pedicled and random flaps.

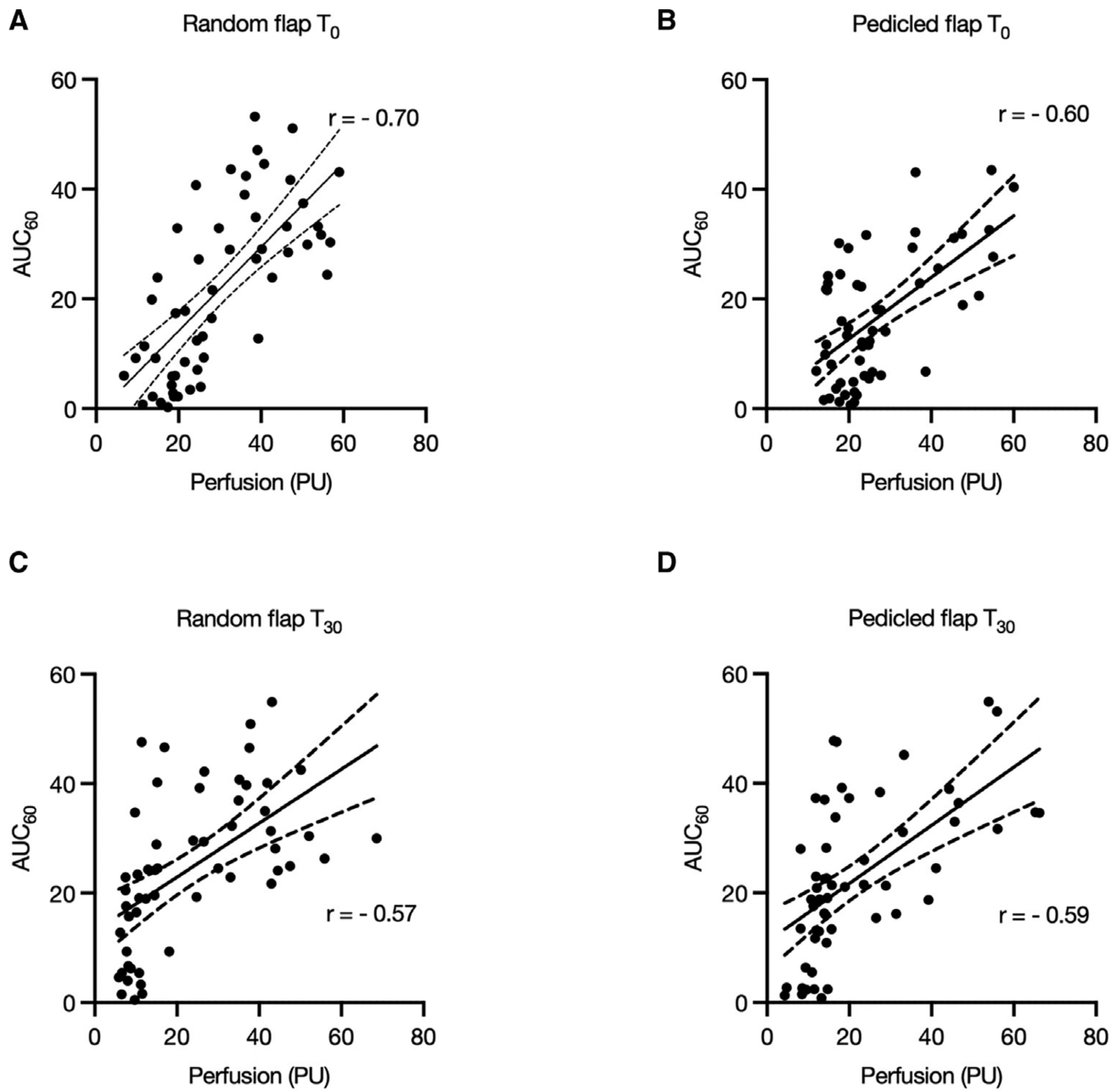

Fig. 5. Correlation between AUC_{60} of the ICG-FA assessment and perfusion assessed with LSCI. A, Random flap at T_0. B, random flap at T_{30}. C, Pedicled flap at T_0. D, Pedicle flap at T_{30}.

an alternative method for intraoperative tissue evaluation in reconstructive surgery.

Quantification of ICG-FA is normally not used to any extent in clinical settings but is necessary to be able to directly compare the technique with other methods for tissue evaluation. Different methods have been proposed and are used for quantification of ICG-FA. These can roughly be divided into two categories: methods that use the rate of the inflow of ICG in the tissue, usually defined as TTP or maximum slope of the intensity curve over time, or methods that use the pixel-intensity of the assessed region of interest, either directly or relative to a baseline.[24–26] In a not yet published study, we compared ICG-FA and LSCI in deep inferior epigastric perforator surgery. We saw a correlation between the indirect measures of microvascular blood flow values that the two methods provided, but that the correlation differed depending on which method was used for quantification of the ICG-FA values. We chose to compare maximum slope with the AUC for the ICG-FA data and saw a better correlation between LSCI and ICG-FA measured as AUC. We then reasoned, based on the hypothesis that AUC gave a measure of both the rate of the inflow and the pixel-intensity of the assessed region of interest, that it gave a more precise prediction of the local perfusion. However, pixel-intensity is prone to differ between assessments due to environmental factors such as distance to camera and ambient light, and hemodynamic

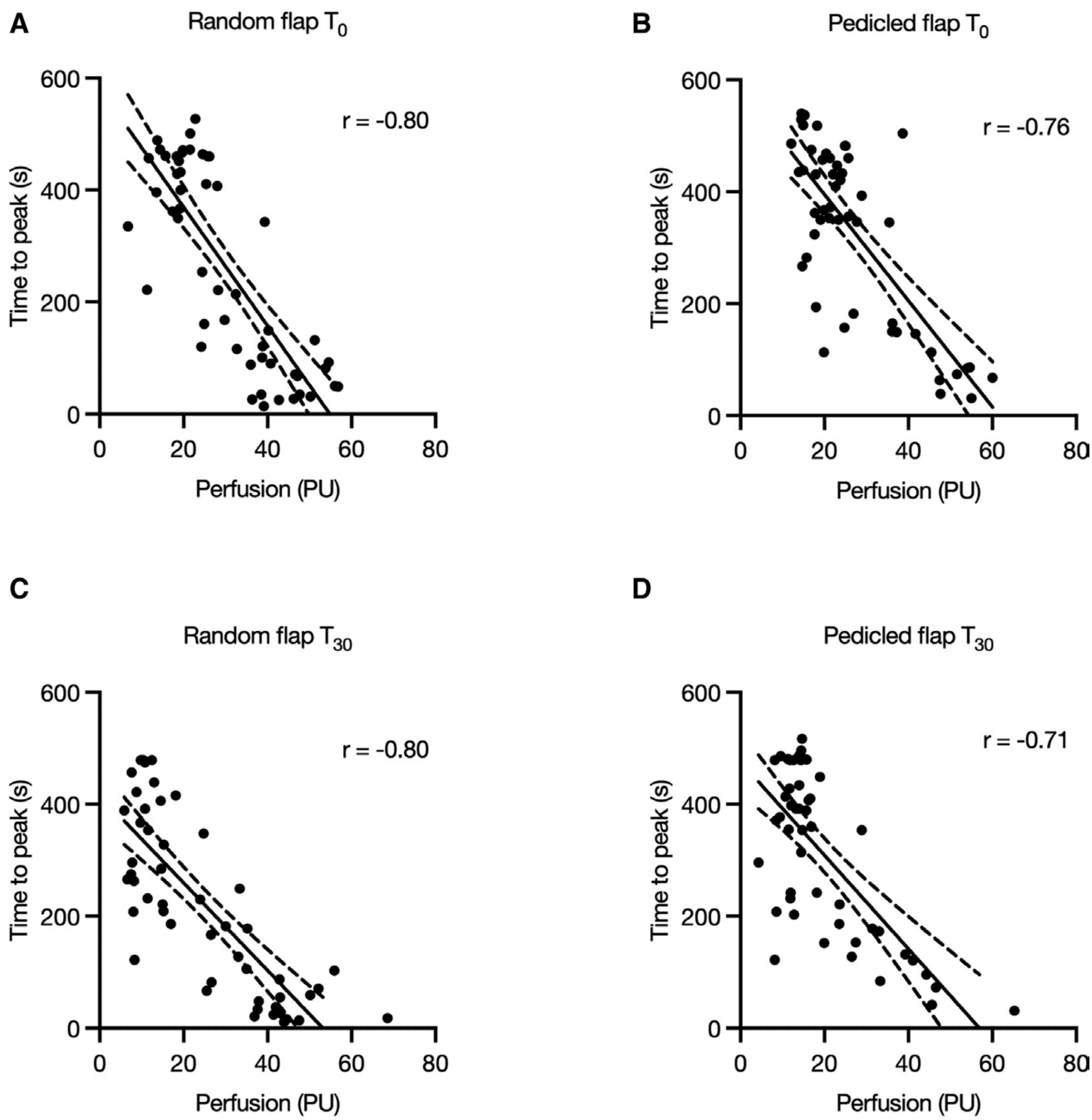

Fig. 6. Correlation between TTP for the ICG-FA assessment and perfusion assessed with LSCI. A, Random flap at T_0. B, Random flap at T_{30}. C, Pedicled flap at T_0. D, Pedicle flap at T_{30}.

factors such as blood pressure and blood volume. These sources of error can to some extent be compensated for by normalizing the pixel-intensity, but the rate of the inflow might still be a more reliable factor to use for quantification. This was also the conclusion drawn in a study by Lütken et al in 2021.[27] They reviewed thirteen studies using ICG-FA to predict anastomotic leakage after intestinal resection. The studies using intensity parameters (maximum intensity and relative maximum intensity) could not identify patients with anastomotic leakage, whereas studies using inflow parameters (TTP, slope, and t1/2max) were able to predict anastomotic leakage. In the current study, the authors therefore chose to compare correlation of perfusion measured with LSCI with both AUC and TTP. A better correlation between LSCI and TTP was seen, which might reflect the problem with the intensity factor in AUC. Intuitively, the information obtained from TTP also seems closer to the clinical evaluation of ICG-FA assessment in the operating room, as the surgeon probably places more emphasis on how quickly the tissue fills than brightness of the area assessed.

When designing the study, the authors chose to use two different types of flaps on each pig, a pedicled flap based on a CGAP and, directly adjacent to it, a random flap of the same size. This made it possible to exclude that the correlation between the methods would depend on the choice of flap, and data from the different flaps were therefore reported separately. It was assumed that the use of two different types of flaps would not significantly affect the result of this study. As expected, no noticeable difference between the flap types was seen, regarding either correlation between ICG-FA measured as TTP and LSCI measured as PU or ICG-FA measured as AUC and LSCI measured as PU.

In the LSCI assessment the perfusion decreased evenly along the central axis of the flaps with the highest perfusion proximal and lower distal. This is in accordance with the results from our previous studies.[28] However, when the AUC for the ICG-FA results were analyzed, we saw a dip in the measured values for the most proximal ROI for both pedicled and random flaps was seen. This might be explained by a faster flush out of ICG in the proximal parts of the flaps, which further emphasizes the problem with using intensity parameters for the quantification of ICG-FA. A corresponding increase in TTP for the ICF-FA curves in the proximal ROI was not seen.

It is difficult to conclude from this study that LSCI could replace ICG-FA for intraoperative evaluation of tissue microvascular blood flow in reconstructive surgery. Although LSCI has been known as a method for perfusion imaging for many years, it is still a rather immature technique with few commercial systems on the market. In this study, a system from Perimed was used, which is not optimal for use in a clinical setting, at least not in an operating room. The device is rather bulky and requires a skilled user. The cost for the system is around 70.000 € which is about the same as the ICG system. A smaller form factor and a more easy-to-use user interface are needed to make the device more suitable for intraoperative monitoring of tissue perfusion. Also, further clinical studies would be needed to evaluate to a greater extent the possibility of predicting and preventing adverse outcomes such as tissue necrosis and flap loss using LSCI perfusion assessment.

CONCLUSIONS

There is a correlation between PU obtained from the LSCI assessment and TTP and AUC_{60} calculated from the pixel-intensity of the video uptake of ICG-FA assessment, indicating that LSCI could be considered for intraoperative tissue assessment. Further clinical studies are needed to determine the clinical usefulness of the system.

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DISCLOSURE

The authors have no financial interest to declare in relation to the content of this article.

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