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Perfusion Mapping of Flaps Using Indocyanine Green Fluorescence Angiography and Laser Speckle Contrast Imaging

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Background: Indocyanine green fluorescence angiography (ICG-FA) is often used for assessing tissue circulation in reconstructive surgery. Indocyanine green (ICG) is injected intravenously and visualized in the tissue with an infrared camera. The information is used to plan the surgery, for example, in free flap breast reconstructions. Laser speckle contrast imaging (LSCI) is another method that uses laser to assess tissue perfusion in the skin. Unlike ICG-FA, LSCI is noninvasive and may therefore have an advantaged compared with ICG-FA. The aim of this study was to evaluate the correlation between information obtained from these two techniques.

Methods: Five deep inferior epigastric perforator patients were included. The flaps were assessed with LSCI and ICG-FA. For LSCI, the perfusion was calculated in 32 regions of interest. For ICG-FA, the maximum slope and area under curve (AUC) were calculated based on average pixel intensity data.

Results: Large variations in maximum slope values could be seen between flaps, whereas AUC had lower variability within the same flap and between flaps. Pearson rank correlation comparing average perfusion (LSCI) and AUC (ICG-FA) showed a correlation between the values ($r = 0.55$, $P < 0.0001$). No significant correlation was observed between perfusion and maximum slope ($r = 0.11$, $P = 0.18$).

Conclusions: There is a significant correlation between data obtained using LSCI and ICG-FA, when ICG-FA data are presented as AUC of the ICG-FA intensity curve. Maximum slope lacks significant correlation with flap data obtained with LSCI. The study indicates that LSCI may be used in reconstructive surgery to assess tissue circulation in a way similar to ICG-FA. *(Plast Reconstr Surg Glob Open 2024; 12:e5964; doi: 10.1097/GOX.0000000000005964; Published online 26 July 2024.)*

INTRODUCTION

In reconstructive surgery, the aim is to restore function or normal appearance by reconstructing defective organs after trauma or disease. One example is reconstruction of a female breast with a free flap after breast cancer surgery, where skin, fat, and blood vessels are moved and connected to vessels on the chest wall. To ensure a successful outcome, it is crucial to maintain an adequate blood supply to the tissue. Different methods that have the ability to map both the macro- and microcirculation during surgery are therefore of great interest. Before surgery, the vessels supplying the flap are often mapped using computer tomography angiography or handheld Doppler.[1,2] Intraoperatively, the viability of the flap is an important marker in deciding which parts of the flap can be safely kept and which are at risk for ischemia and therefore should be discarded. This estimation has traditionally been based on rather subjective methods such as color of the tissue and capillary refill time, but more objective techniques might be beneficial.[3,4]

Fluorescence angiography using indocyanine green (ICG-FA) is a method for mapping the tissue circulation that has been available for some time, but it has gained ground in reconstructive surgery in the last decades.[5] The patient is given an intravenous injection of indocyanine green (ICG), a water-soluble dye that emits infrared fluorescence when illuminated with near infrared light. An infrared camera is used to visualize the ICG as it reaches the tissue. This technique produces a video showing the

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changing distribution of the dye in the tissue, which is related to the regional blood flow. This can help the surgeon in the decision to excise poorly perfused tissue in the periphery of a free flap. ICG-FA is used in many situations in reconstructive surgery and has been shown to reduce the incidence of postoperative complications after, for example, breast reconstruction with deep inferior epigastric perforator (DIEP) free flap surgery.[6,7] The technique has only few side effects and the only absolute contraindication is iodine allergy. However, for each assessment, ICG has to be administered intravenously, and the clinical assessment is to date highly subjective.

One of the most obvious benefits of the technique is to evaluate which parts of a raised flap are viable for further reconstruction. This assessment is usually done through a visual assessment of how quickly and to what extent the dye reaches different parts of the tissue. This is a largely subjective assessment and research efforts have been made to develop methods to allow better objective quantification of the dynamics of the distribution of ICG. Some commercial systems offer built-in quantification software, but to date there is only limited data on how the arbitrary values generated by these methods can be used clinically.[8,9]

Laser speckle contrast imaging (LSCI) is a different method to assess perfusion in tissue. It is based on the so-called speckle phenomenon that occurs when a surface is illuminated with coherent light, for example, laser light. The change in contrast in the pattern correlates to speed and concentration of particles in the superficial circulation of the skin. The advantage of LSCI over ICG-FA is that LSCI is noninvasive, as no dye has to be injected into the patient before the assessment. This means that there is no risk of adverse effects of the dye, and repeated assessments can be done during the operation, as there is no risk of contamination in the signal from previous injections. LSCI does not have the same penetrance in the tissue as fluorescence angiography, but correlates to the viability of the tissue and seems to have a predictive value in detecting postoperative complications in flap surgery.[10,11]

The aim of this study was to compare the perfusion images of free flaps obtained by ICG-FA and LSCI.

Takeaways

Question: Can laser speckle contrast imaging (LSCI) be used in the same way as indocyanine green fluorescence angiography (ICG-FA) to map perfusion in deep inferior epigastric perforator (DIEP) free breast flaps?

Findings: Data from LSCI assessment of free DIEP breast flaps correlate with data from ICG-FA assessment of the same flaps.

Meaning: LSCI may be used in reconstructive surgery to assess tissue circulation in a way similar to ICG-FA.

METHODS

Patients

Five patients who underwent a unilateral deep interior epigastric perforator (DIEP) free flap breast reconstruction after breast cancer at the plastic surgical department in Linköping were included. The mean age of the patients was 55.8 ± 11.4 years, and the mean body mass index, 26.2 ± 1.5 kg per m^2. There were no exclusion criteria for the study. No patient had any comorbidity except for one patient with hypertension. No complications such as partial necrosis or complete flap loss were reported within the group.

LSCI

A laser speckle contrast imager (LSCI, Pericam PSI system, Perimed AB, Järfalla, Sweden) was used to measure skin perfusion intraoperatively. 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 perfusion units (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.[12]

ICG Fluorescence Angiography

A fluorescence imaging system (Fluobeam, Fluoptics, Grenoble, France) was used for the ICG angiography. The system uses a class I 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 a separate USB-media in the form of a MPG4-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

All assessments were done intraoperatively after the flap was completely raised, but before clipping of the pedicle. 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 by 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 3 minutes.

Image Processing

The ICG-FA movie files were imported in MATLAB (Math Works, Natick, Mass.) and converted to image stacks. For each flap, 32 regions of interest (ROIs) were chosen, and each ROI was attributed to a perfusion zone according to Hartrampf, with about eight ROIs per zone. Because the complete flaps could not be assessed in one frame either with LSCI or ICG-FA, the lateral parts of the flaps were excluded in some of the flaps. For each ROI, a curve of the merged pixel intensity of all included pixels was calculated (Fig. 2). Because we could not see a clear time to peak in most ROIs, probably due to limited recording time, we chose to look at the two other variables, the maximum slope and area under curve (AUC). Maximum slope is a technique used in different clinic and research settings to evaluate the rate of the influx of, for example, intravenous contrast, and it has been used in earlier ICG-FA studies.[13,14] AUC is also a well-established method to evaluate inflow of contrast in radiology and has been used in studies to quantify ICG influx.[15] For each curve, AUC and maximum slope was calculated for a time frame starting with the initial increase in intensity and ending 20 seconds later (Fig. 1).

By overlapping the pictures, corresponding ROIs 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.[16]

Finally, an image was created based on the ICG-FA measurement by calculating the AUC of the first 20 seconds for each point in a grid comprising 256×256 points.

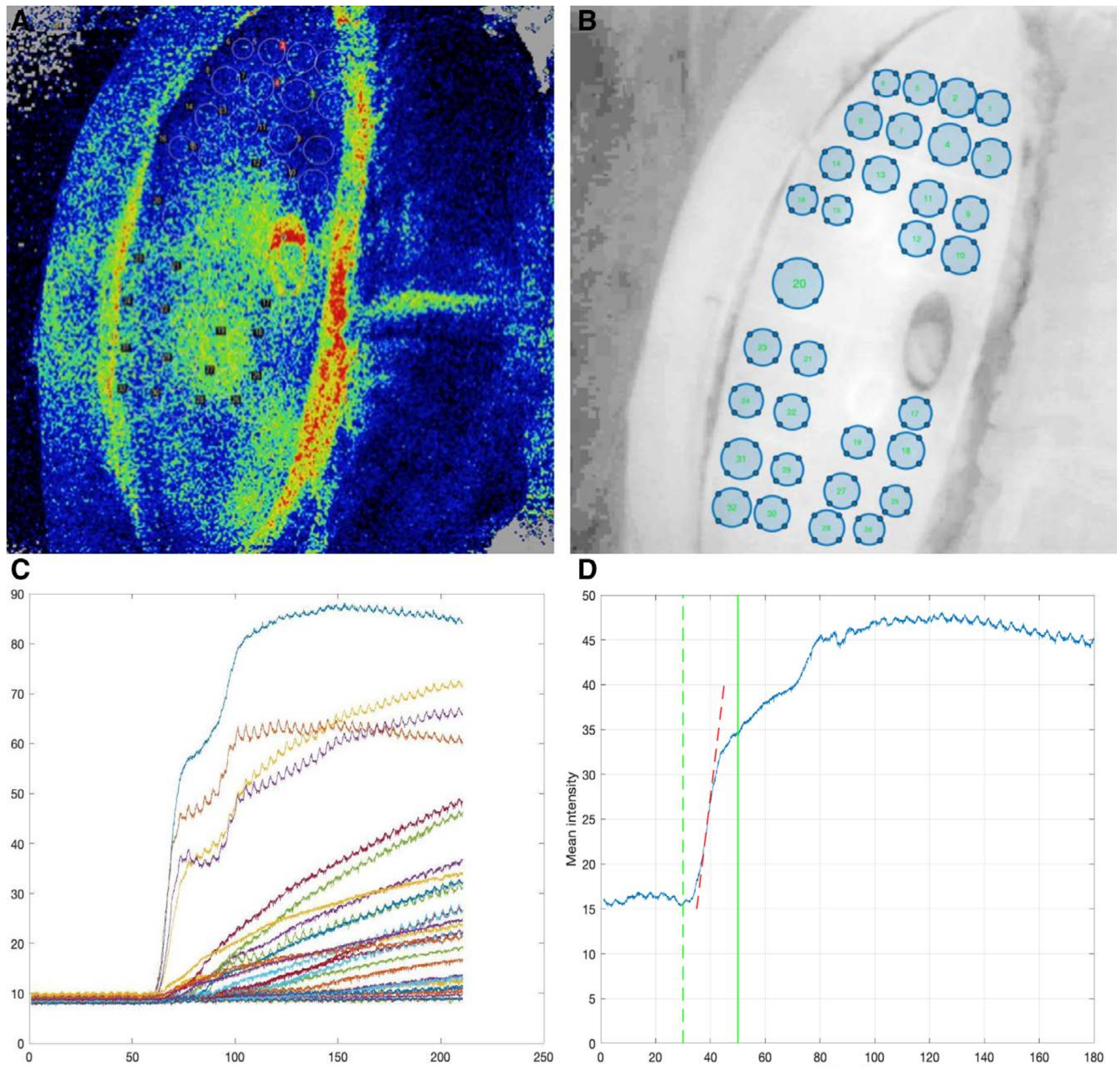

Fig. 1. Methods. A, LSCI measurements of a DIEP free flap. The perforator is situated under the green area in the right medial zone. B, The ICG-FA assessment of the same flap. The circles indicate the different ROIs. C, The pixel intensity in the 32 different ROIs of the ICG-FA assessment seen above. The steeper curves represent the ROI situated above the perforator.D, For each ROI, the AUC for 20 seconds from the start of the increase of intensity and the maximum slope for the same time span was calculated.

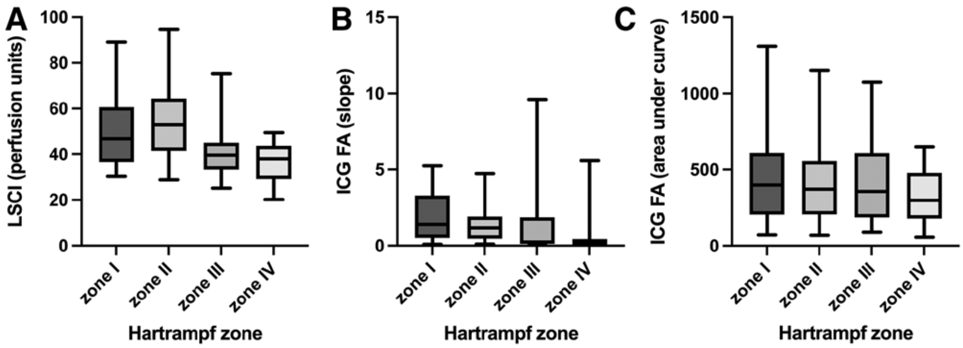

Fig. 2. LSCI data from all five flaps (A) showed significant differences between zone I and III ($P = 0.0043$); zone I and IV ($P = 0.0001$); zone II and III ($P < 0.0001$) and zone II and IV ($P < 0.0001$). In the ICG-FA data from all flaps, presented as slope (B), multiple comparisons only showed significant difference between zone I and IV ($P = 0.0115$). ICG-FA data from all flaps, presented as AUC (C), showed no significant differences.

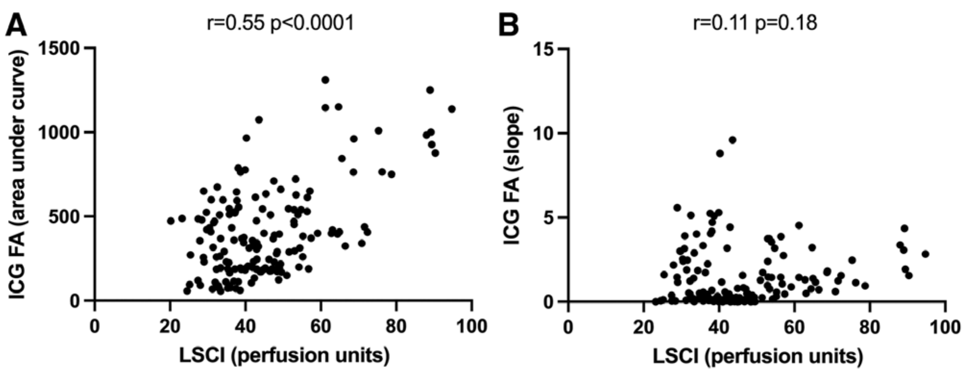

Fig. 3. Scatter plots of ICG-FA data presented as AUC and PU values from LSCI measurements (A) and ICG-FA data presented as maximum slope and PU values from LSCI measurements (B). A significant correlation can only be seen in the former.

These were then assigned a false color, where high values were indicated in red and low values in blue (Fig. 1).

Hartrampf Zones

The tissue of DIEP free flaps is often divided into four zones, according to its position relative to the supplying perforator. The one right above the perforator, zone I, is considered the safest with the lowest risk of postoperative complications such as necrosis. Zone II is on the contralateral side closest to zone I; zone III, on the ipsilateral side; and zone IV, the least safe zone, is lateral of zone II.[17,18]

RESULTS

Measurements with both LSCI and ICG-FA showed predominant higher perfusion values in areas close to the perforators as expected, although this was not true for all flaps. A large spread in maximum slope values could be seen between flaps. When data were presented as AUC, the data were more cohesive, both within the same flap and between flaps (Fig. 2).

When performing a Pearson rank correlation comparing LSCI and ICG-FA perfusion data presented as AUC from all five flaps, we could see a significant correlation between the values ($r = 0.55$, $P < 0.0001$). However, when data presented as maximum slope were compared, no significant correlation could be seen ($r = 0.11$, $P = 0.18$).

To further investigate how the assessed perfusion distribution differs between the methods, the different ROI were divided in groups according to the four Hartrampf perfusion zones. Figure 3 shows the LSCI perfusion data and the ICG-FA perfusion data presented as both maximum slope and AUC, divided into Hartrampf zones.

One-way analysis of variance showed significant differences between Hartrampf zones in LSCI data and ICG-FA data presented as maximum slope, but not as AUC. Tukey's multiple comparisons of the LSCI data showed significant differences between zone I and III ($P = 0.0043$); zone I and IV ($P = 0.0001$); zone II and III ($P < 0.0001$); and zone II and IV ($P < 0.0001$). In the ICG-FA data presented as slope, multiple comparisons showed significant difference only between zone I and IV ($P = 0.0115$; Figs. 1–3).

DISCUSSION

The use of LSCI instead of ICG-FA in intraoperative evaluation of tissue viability would offer many advantages, such as faster and multiple assessment. With the exception of our earlier LSCI study on DIEP free flaps, few studies have evaluated LSCI in reconstructive microsurgery.[11,19] ICG-FA, on the other hand, has been evaluated in multiple clinical studies, and has gained widespread clinical use. For example, ICG-FA has been shown to perioperatively predict flap necrosis with relatively high specificity and sensitivity, and the risk for postoperative complications can be minimized in DIEP surgery, when an affected perioperative ICG intensity is seen.[7,20]

One step in the evaluation of the usefulness of LSCI in reconstructive surgery would be to ascertain that the information from LSCI assessments offers similar information as ICG-FA in a clinical setting, such as perioperative evaluation of viability of DIEP free flaps. One major question, however, is how these two methods are best compared. ICG-FA is mainly used clinically in a more intuitive way and seems to fulfill its purpose without the need to analyze and quantify individual ROIs, and most ICG-FA studies have focused on endpoints such as postoperative necrosis and flap failure, not looking at specific quantifiable ICG values. However, to be able to compare LSCI with ICG-FA in a reliable way, it is necessary to quantify the data obtained from the ICG-FA assessment. This can be done in multiple ways. The most direct way is to use a graphic editor to compare pixel intensity in different parts of the tissue in the obtained images. The SPY Elite from Stryker, one of the more widely used ICG-FA systems, offers a built-in software for quantification, based on the pixel intensity. The data are presented as fluorescence intensity or increase of the intensity per second. This system, called SPY-Q, has, for example, been used to perform qualitative and quantitative analysis of flap perfusion in DIEP free flaps.[9]

Other systems do not offer this possibility. Why many studies, therefore, use custom-designed statistical methods and software for analysis. For example, Nerup et al showed a strong correlation between the slope of the curve of the pixel intensity in ICG-FA assessments of porcine intestines and microsphere-measured regional blood flow. The slope was calculated as a quota of the difference between minimum and maximum pixel intensity, and the time to peak, defined as the time span between the start of the increasing curve to the point of plateau intensity. Wada et al and Son et al also used time to peak and maximum intensity to calculate a slope in similar setups.[21–23] In these studies, the assessments were done on porcine intestine serosa and in all three studies, a clear peak in the intensity with a following decrease could be seen in most ROIs. This could not be seen as clearly on the assessments on the skin of the DIEP flaps in this study, where the intensity in many ROIs continued to increase during the whole assessment (Fig. 1). This is likely to be explained by the differences in the properties of the different types of tissue and microvascular properties, as well as blood flow differences in the resting state. The porcine serosa is thin and has a high resting blood flow, compared with human skin, where the reticular dermis is relatively thick and the resting microvascular blood flow is low. These differences, as well as a partial venous stasis in the raised flaps, could contribute to the accumulation of ICG in the tissue and a slower wash-out of ICG in the skin compared with intestine. The consequence of the lack of a clear ICG intensity peak in most of the ROIs in the DIEP flaps is that time to peak could not be used as a factor in the quantification of the ICG-FA assessment. Instead, two different values were chosen, maximum slope during 10 seconds from the start of the increase of intensity and AUC for the same period. Both these values are commonly used in many other situations, but not validated in ICG-FA quantification. LSCI is not used in clinical settings for intraoperative evaluation of tissue viability in reconstructive surgery, with the exception of a few clinical studies.[11,24,25] A step toward being able to use LSCI in the same way as ICG-FA in reconstructive surgery would be to evaluate whether the perfusion pattern presented by the LSCI equipment corresponds to what is seen in ICG-FA. This has been done in a few preclinical studies in other fields than reconstructive surgery. For example, in 2019, Rønn et al conducted an in vivo experimental study, comparing LSCI and ICG-FA for assessment of perfusion in porcine intestines and, in 2012, Towle et al compared the two modalities for evaluating perfusion in cortex in rodent cortex.[26,27] Because both these studies assess tissue with other vascular and microcirculatory properties than skin, the results are hard to translate to a reconstructive surgical setting.

When comparing the use of the two methods in reconstructive surgery, one must be aware that, although both offer information about concentration and movement of blood in the assessed tissue, the data obtained from the two methods still differ considerably. ICG-FA gives a dynamic picture of the blood flow in the flap, and the intensity in the picture will first increase in the area above the perforator, which, therefore, in some cases will be very clearly visualized. LSCI, on the other hand, gives a more static picture, and the perforator is, therefore, sometimes not as easily identified.

Another difference is measurement depth; fluorescence angiography using ICG has a measurement depth of approximately 3 mm.[28] This means that, depending on body localization and skin thickness, both the deep and superficial capillary plexus, and to some extent the subcutaneous vessels, will contribute to the pixel intensity in the picture. In LSCI, on the other hand, only the concentration and speed of blood cells in the uppermost 0.5 mm of the skin will affect the obtained perfusion value, which means that only the superficial capillary plexus is assessed. This may give misleading information of the viability of thick flaps like DIEP flaps because the perfusion of the deep tissue may differ from the more superficial tissue. This could occur if there is a peripheral vasoconstriction, for example, if the flap is cold.

A disadvantage of LSCI is that there are few studies where the method is used clinically on plastic surgery patients. It is therefore difficult to assess the extent to which the method can reliably identify areas threatening ischemia in free flaps in a clinical setting. In a study from 2019, we examined 23 DIEP patients intraoperatively with LSCI. Three of these experienced partial necrosis, and we could see in retrospect that they had relatively low measured perfusion in the relevant areas; however we could not clearly determine a threshold value for the perfusion.

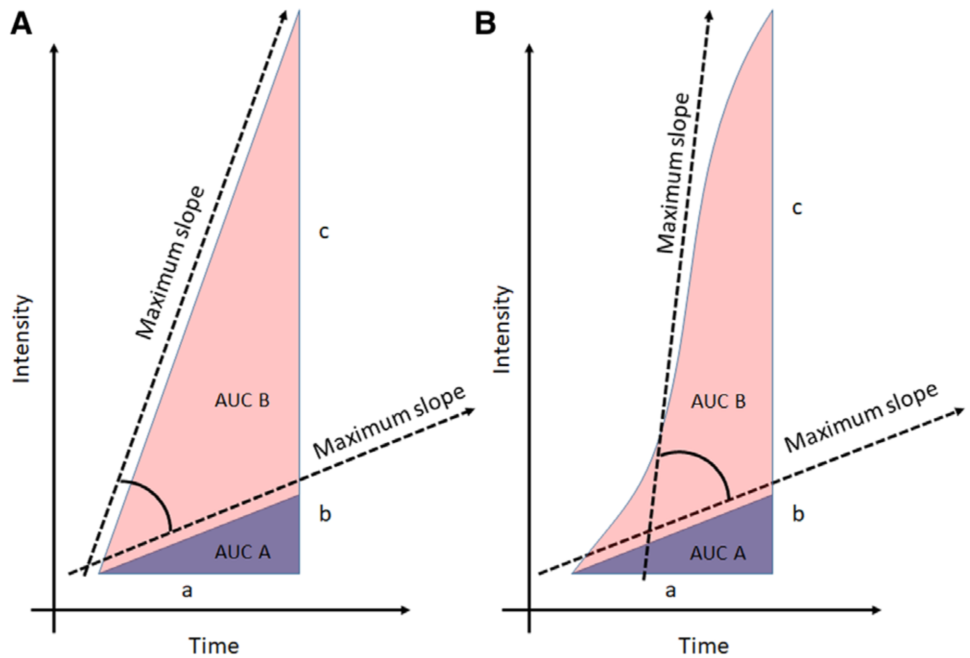

Fig. 4. In two right-angled triangles with the same base (A) but different height (B–C), the ratio between the two areas (AUC A and B) will be the same as the ratio between the slopes. However, in an uneven curve, the spread of the values of the slope may be larger than the spread of the values of the AUC. This might explain the poorer correlation between the LSCI measurements and the ICG-FA data presented as maximum slope.

On the other hand, there are few well-designed prospective studies correlating measured quantified perfusion values measured by ICG-FA with flap necrosis (Fig. 4).

In this study, a correlation could be seen between the LSCI measurements and ICG-FA measurements presented as AUC but not as slope. It seems like perfusion measured with LSCI gradually decreases from the area adjacent to the perforator and out toward the periphery, whereas the ICG seems to reach the central parts of the flap rather fast but beyond a certain distance from the perforator, the ICG will fill the tissue considerably slower. This will give rise to a poorer correlation in the peripheral parts of the flap (Fig. 2). An additional condition that could affect the correlation between the two methods are the different shapes of the curves that can be seen in the ICG-FA assessment. This probably has a larger effect on the spread of the values of the maximum slope than on the area under the curves, which explains the poorer correlation when maximum slope is compared with the LSCI measurements (Fig. 4).

CONCLUSIONS

The results of this study indicate that there is a correlation between perfusion measurements done on DIEP free flaps with ICG-FA (when data are presented as AUC) and LSCI. However, there is no correlation when maximum slope is calculated from the same ICG-FA measurement and compared with LSCI data.

A limitation of this study is, of course, the small number of flaps. It is hard to draw any definite conclusions from five flaps, and this work therefore has to be considered more of a preliminary study. We believe that further studies should focus on end points such as flap necrosis to see if the two methods are comparable in predicting future ischemic areas.

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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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