



ELSEVIER



Flap warming improves intraoperative indocyanine green angiography (ICGA) assessment of perfusion. An experimental study



M.V. Muntean^a, F. Ardelean^b, S. Strilciuc^{c,d,*}, C. Pestean^e,
A.V. Georgescu^b, V. Muntean^f

^a Department of Plastic Surgery, “Prof. Dr. I. Chiricuta” Institute of Oncology, University of Medicine and Pharmacy “Iuliu Hatieganu”, Cluj-Napoca, Romania

^b Department of Plastic Surgery, University of Medicine and Pharmacy “Iuliu Hatieganu”, Cluj-Napoca, Romania

^c Department of Neurosciences, University of Medicine and Pharmacy “Iuliu Hatieganu”, Cluj-Napoca, Romania

^d RoNeuro Institute for Neurological Research and Diagnostic, Cluj-Napoca, Romania

^e Department of Anaesthesiology and Reanimation, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania

^f Department of Surgery, University of Medicine and Pharmacy “Iuliu Hatieganu”, Cluj-Napoca, Romania

Received 17 November 2018; accepted 10 March 2019

KEYWORDS

Flap survival;
Perforator flap;
ICG angiography

Summary *Background:* Indocyanine green angiography (ICGA) is slowly replacing conventional methods of evaluating perfusion during flap surgery. Microcirculatory changes during flap elevation create a marked state of hypoperfusion intraoperatively leading to ICGA underestimation of tissue viability and consequent resection of viable tissue. We propose a novel method of flap warming to induce maximum vasodilation before performing ICGA to increase accuracy in assessing perfusion.

Methods: Submental flaps harvested on a single perforator were created in 8 pigs. ICG angiography was performed in the intraoperative phase (ICGA-C), after inducing maximum vasodilation by warming the flap at 42 °C (ICGA-W) and at 24H postoperative (ICGA-24). By setting a fluorescence threshold of 33% as indicative of necrosis, the flap surface deemed viable by ICGA was measured for ICGAC, ICGAW and ICGA24. The results were then compared to the actual flap survival observed clinically at 7 days.

* Corresponding author.

E-mail address: stefan.strilciuc@snn.ro (S. Strilciuc).

Results: The mean of ICG-C predicted flap survival (FS-C = 49.17%) is 12.97% lower than the mean of actual flap survival on postoperative day 7 (FS = 62.14%). The mean difference between ICG-W and ICG-24 predicted flap survival (FS-W and FS-24) and actual flap survival in the postoperative day 7 (FS) is lower, 3.13% and 2.15%, respectively. Average perfusion recovery over 24 h was 10.83% (FS-24-FS-C).

Conclusions: Conventional intraoperative ICGA underestimated perfusion in all cases. Warming the flap intraoperatively and achieving maximum vasodilation mitigates the effects of vasoconstriction and mimics the microcirculatory environment encountered at 24 h. Performing angiography after induced vasodilation improves ICGA assessment of flap perfusion.

© 2019 British Association of Plastic, Reconstructive and Aesthetic Surgeons. Published by Elsevier Ltd. All rights reserved.

Introduction

Flap design has evolved constantly over the last decades. An in-depth understanding of vascular anatomy and physiology allows modern surgeons to harvest flaps based on a single cutaneous perforator, leading to reduced donor site morbidity, preservation of underlying structures and the ability to tailor any flap to match the exact defect requirements.¹

However, perforator flaps have higher technical demands and their perfusion patterns are less predictable than those of axial or musculocutaneous flaps.² Not being able to accurately determine perfusion leads to serious complications, culminating with the appearance of necrosis, with various implications such as reoperations, increased morbidity and higher overall costs. To this day most surgeons assess flap perfusion empirically by observing temperature, capillary refill and the overall appearance of the flap. These methods are not reliable or reproducible and depend on the clinical experience and intuition of the surgeon.³

Indocyanine green angiography (ICGA) provides dynamic, real-time, intraoperative information about vessel location, perfusion patterns and flap physiology.⁴ ICGA is currently being used successfully to assess flap perfusion and guide resection of necrotic tissue.⁵⁻⁹

The main limitation of intraoperative ICGA flap assessment is the difference between perfusion patterns observed in perforator flaps intraoperatively and in the early post-operative period.¹⁰ Vasoconstriction, arterial spasm, capillary permeability and A-V shunt flow make it difficult to accurately predict perfused areas during surgery. **Skin perfusion recovers gradually in the post-operative period. This occurs mostly in the first 24 h with maximum blood flow being recorded at 48 h.**¹¹ Intraoperative ICGA tends to underestimate flap perfusion, leading to unnecessary resection of viable tissue.^{12,13}

We hypothesized that intraoperative flap warming to induce vasodilatation would simulate the perfusion patterns recorded in the late post-operative stage. Therefore, intraoperative ICGA performed during flap warming would provide a more precise assessment of flap perfusion and would accurately guide resection of ischemic tissue. To explore this hypothesis, ICGA has been performed three times, twice intraoperatively, immediately after raising the flap (cold angiography, ICGA-C) and after flap warming (warm angiography, ICGA-W), and a third time at 24 h after surgery (ICGA-24). The values for ICGA-C, ICGA-W

and ICGA-24 predicted flap survival (FS-C, FS-W and FS-24 respectively) and actual flap survival in the postoperative day 7 (FS) were compared.

Materials and methods

Animal subjects

The experimental study was developed according to International Guidelines for using Animals in Scientific Procedures and approved by our Institutional Review Board (protocol no. 294-2015). The study was conducted between July-September 2016 at the University of Agricultural Sciences and Veterinary Medicine (UASVM) in Cluj-Napoca. We acquired PIC-FII-337 hybrid pigs ($n=8$) of similar weight (mean \pm SD = 41.2 ± 3.8 kg at study enrolment). The anatomic arrangement of arteries, veins and subcutaneous fat in pigs provide a suitable model for skin flap harvesting.¹⁴ The subjects were isolated into individual spaces and fasted for 6-12 h before being induced using 1-2 mg/kg IV Norofol (Norbrook Laboratories Ltd., Northern Ireland), intubated and maintained under anaesthesia with 2% isoflurane (Baxter Healthcare Corp., Deerfield, IL). Subject temperature was monitored by means of a transoesophageal catheter (38.3-40.2 °C) and kept constant throughout the procedure. Intraoperative submental skin average temperature was 35.4 °C (range \pm 2.9 °C) just before skin incision and 28.3 °C (range \pm 3.1 °C) after flap elevation. Operating room temperature was maintained within 19-20.5 °C.

Surgical procedures

All flaps were harvested by the same surgeon (MM). Identical submental flaps were designed in all cases. Perforators were visualized by means of thermography and Doppler US, and then marked. The submental area was prepared and draped for surgery and a 15 × 12 cm flap was drawn for each subject. Flaps were raised above the fascia and perforators were identified and clipped as dissection proceeded. One eccentric perforator located in the right caudal quadrant of the flap, arising from underneath the submandibular gland was preserved in each case, providing vascularisation to the flap. Perforators of similar calibre were selected. After completing the dissection, meticulous haemostasis was

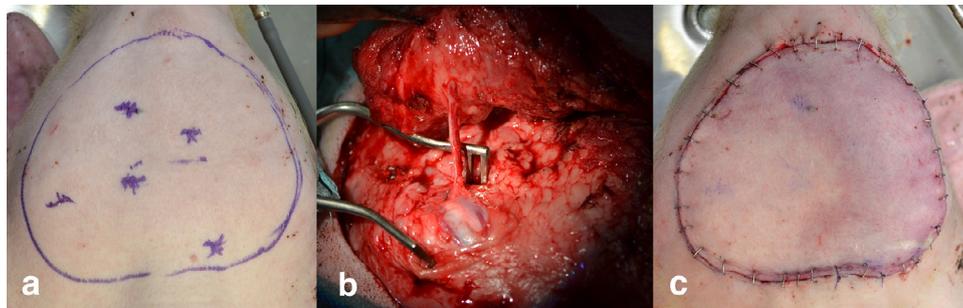


Figure 1 Submental flap design with marked perforators found by thermography and Doppler ultrasound (a), Dissection and isolation of selected perforator (b), Final postoperative appearance after flap inset(c).

achieved and the flaps were placed back in their original location (Figure 1).

Indocyanine green angiography

Flap perfusion was assessed using near-infrared (NIR) fluorescence imaging after i.v. administration of Indocyanine Green (ICG). The Artemis Spectrum (NIRF-800 probe - Quest Medical Imaging BV, Wieringerwerf, the Netherlands) has been thoroughly evaluated and described in previous works.¹⁵ The system has a ring-light source with two imaging sensors: visible RGB ($\lambda = 400\text{-}650\text{nm}$) and NIR ($\lambda = 700/800\text{nm}$). The camera was aligned to the surgical field in a perpendicular plane at a fixed distance of 35 cm using a conventional laser guide, generating a $15 \times 15\text{cm}$ field of view. Colour (70 ms) and NIR (180 ms) frames were captured, juxtaposed and converted to movies in real-time using system firmware. We blinded all sources of light external to the Artemis during NIR imaging to prevent interference.

Angiography (ICGA) was performed by injecting 0.5 mg/kg ICG (Akorn, Delatour, IL) in the right auricular vein, after prior reconstitution with distilled water at 2.5 mg/ml - 3.2 mM). Images were recorded continuously for 10 min after ICG administration.

Three ICGA were performed for each subject. The first angiography was done immediately after flap inset (ICGA-C) by injecting ICG according to the previously mentioned protocol. The second ICGA was carried out 30 min later, (allowing the contrast to clear) after warming the flap at 42 °C to induce maximum vasodilation (ICGA-W). In previous experiments 42 °C has been proven to induce maximal skin vasodilation.¹⁶ An infrared emission lamp, IL 50 (Beurer GmbH, Ulm, Germany), was used to warm the flap to the desired temperature. Flap temperature was monitored using a Testo 890 thermal camera (Testo AG, Lenzkirch, Germany), and maintained between 41.5-42.5 °C. After recovery from anaesthesia, incisions were dressed, and the animals were returned to individual housing. A third angiography (ICGA-24) was performed 24 h after surgery following general anaesthesia.

Flaps were monitored and dressed daily. Colour images were captured using a Nikon D700 (Nikon Inc., Melville, USA) at 7 days postoperative. After the completion of the study the subjects were euthanized using a lethal dose of potassium chloride.

Image analysis

Four images were analyzed for each subject using ImageJ x 64 open source image processing software¹⁷: ICGA-C, ICGA-W, ICGA-24 and a photo of the flap taken on day 7. For ICGA images, raw output was reviewed for each angiography and the frame with peak ICG (grayscale) intensity was selected from the arterial phase of ICGA via batch analysis. Afterwards, selected images were manually cropped to include only flap tissue as regions of interest (ROIs). Resulting images were subsequently quantitatively analysed using a custom-built macro application.

ICGA fluorescence was measured on a grayscale with values between 0-255. Absolute flap fluorescence values were generated automatically by the software to control for background noise. The absolute maximum (ICG-M), the minimum (ICG-m) and specific (ICG-a) pixel group of the flap fluorescence values were automatically generated by the software after rectification for the background noise. To calculate ICGA fluorescence intensity ratio (ICG-r) the pixel groups, clustered on mean values, were divided into a matrix of analysis. The ICG-r for any specific pixel/pixel group of the ROI s was calculated with the formula:

$$\text{ICG} - r = 100 \times \text{ICG} - a / (\text{ICG} - M - \text{ICG} - m).$$

The chosen threshold for ICG-r tissue survival was 33%^{18,19} and used to generate a color overlay on each image (Figure 2). The ICGA predicted flap survival was defined as the percentage of the flap area with a $\text{ICG} - r \geq 33\%$: without warming (FS-C), after warming (FS-W) and 24 h after surgery (FS-24).

Flap survival at postoperative day 7 (FS) was independently assessed by three plastic surgeons, (AG, FA, VM) blinded to the results of ICGA. On the photographs taken in the postoperative day 7 viable and necrotic flap were delineated using a pencil tool, and the consensus line used to measure flap survival (FS) was calculated using the mean \pm SEM of boundaries drawn by 3 independent plastic surgeons blinded to NIR fluorescence results.

Statistical analysis

The values for ICGA predicted flap survival (FS-C, FS-W, FS-24) and actual flap survival in the postoperative day 7 (FS) were introduced as continuous variables (percentages) in

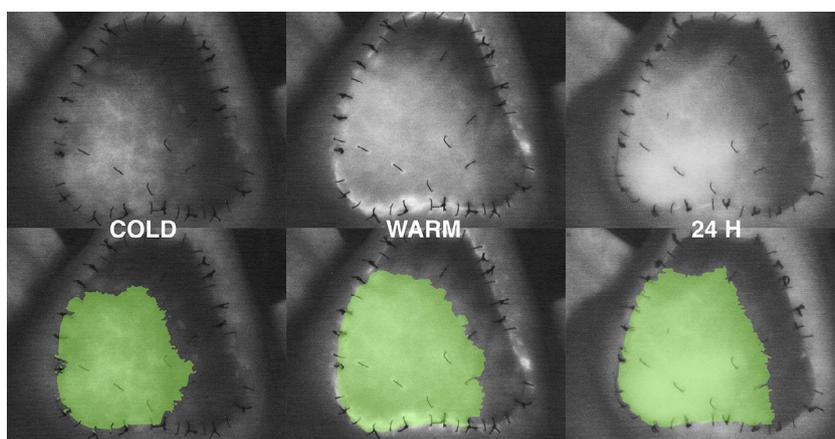


Figure 2 The ICGA images analysed with the post-processing application to apply the ICG-r 33% threshold overlays.

a study database, using R 3.4.1 programming language (R Foundation for Statistical Computing, Vienna, Austria). The small size of the experimental sample required bootstrapping in order to increase the statistical significance of the results. The values for flap survival and necrosis were extracted and used to generate new observations in the bootstrap model, as described by Efron.²⁰ The process was repeated 1000 times and perfusion value differences were compared using a 95% confidence interval (CI).²¹ Boxplots were used to visualize resulting output.

Results

Image processing of raw ICGA grayscale perfusion values produced 24 independent observations for the study sample ($n=8$). The mean of ICG-C predicted flap survival (FS-C = 49.17%) is 12.97% lower than the mean of actual flap survival in the postoperative day 7 (FS = 62.14%). The mean difference between ICG-W and ICG-24 predicted flap survival (FS-W and FS-24) and actual flap survival in the postoperative day 7 (FS) is lower, 3.13% and 2.15%, respectively.

Boxplots of ICGA predicted flap survival after bootstrapping (FS-C, FS-W and FS-24) and flap survival in the postoperative day 7 (FS) (Figure 3) confirm initial descriptive analysis. There is a statistically significant underestimation of flap survival with ICG-C when compared to ICG-W and ICG-24.

Figure 4 illustrates the overlays of ICGA predicted flap survival on the same image at postoperative day 7. Intraoperative warming of the flap highly improves ICGA prediction of flap survival.

Discussion

NIR angiography after intravascular administration or ICG is a valuable method for assessing perfusion intraoperatively,⁸ but for ICGA to accurately determine perfusion in certain areas of the flap, the contrast has to be able to penetrate its microcirculation.

In our study, conventional intraoperative ICGA (FS-C) underestimated flap perfusion in all cases, the mean predicted flap survival (FS-C = 49.17%) being 12.97% lower than the mean final flap survival in the postoperative day 7

(FS = 62.14%). This underestimation of flap viability is not an inherent flaw of the method but merely a consequence of the microcirculatory changes induced by surgical trauma.

Flap blood flow is laminar,²² directly proportional to the radius of the vessel and inversely proportional to blood viscosity. Any change in pressure, radius, viscosity or vessel length will affect blood flow.

Blood flow at the arteriolar level is mainly dependent on systemic pressure, but flap perfusion is determined by the state of its microcirculation.²³ When compared to the late postoperative phase, there is a marked reduction in flap perfusion intraoperatively and in early postoperative phase as a consequence of multiple factors that act synergistically and potentate each other. Pressure is significantly decreased by preserving a single source vessel for the flap, the perforator.²⁴ The predominant hyperadrenergic state caused by the sectioning of sympathetic nerve fibers during flap raising,²⁵ causes vasoconstriction and reduces vessel radius. Most importantly the decrease in flap temperature during raising causes a number of changes in rheology, all contributing to hypoperfusion. Temperature in modern operating rooms ranges between 18–20 °C.²⁶ Low temperature inhibits NO secretion, and causes overexpression of α_2c adrenergic receptors,²⁷ thus amplifying the vasoconstrictor effects of circulating catecholamines. The temperature drop also causes an increase in viscosity and a decrease in the cellular deformability.²⁸ Between 38 °C and 22 °C, the decrease in blood flow is linear and can be as high as 3.41% per degree Celsius.

All the events generated by surgical trauma, act together to create a profound state of vasoconstriction intraoperatively. However, these changes are temporary, the hyperadrenergic state subsides and perfusion gradually recovers over the next 24h. In our study, the average perfusion recovery over 24h was 10.83% (FS-24 - FS-C).

ICGA is only useful if it can be used during surgery to identify and aid resection of nonviable tissue. Most of the current research is focused on identifying a fluorescence intensity value capable of predicting necrosis. Giunta et al. suggested that a fluorescence perfusion index of less than 25% indicates skin necrosis while viable tissue has values exceeding 40%.²⁹ Monahan et al. published similar data, reporting a “grey zone” between 25% and 57% in which ICGA is unable to accurately determine perfusion,³⁰ while

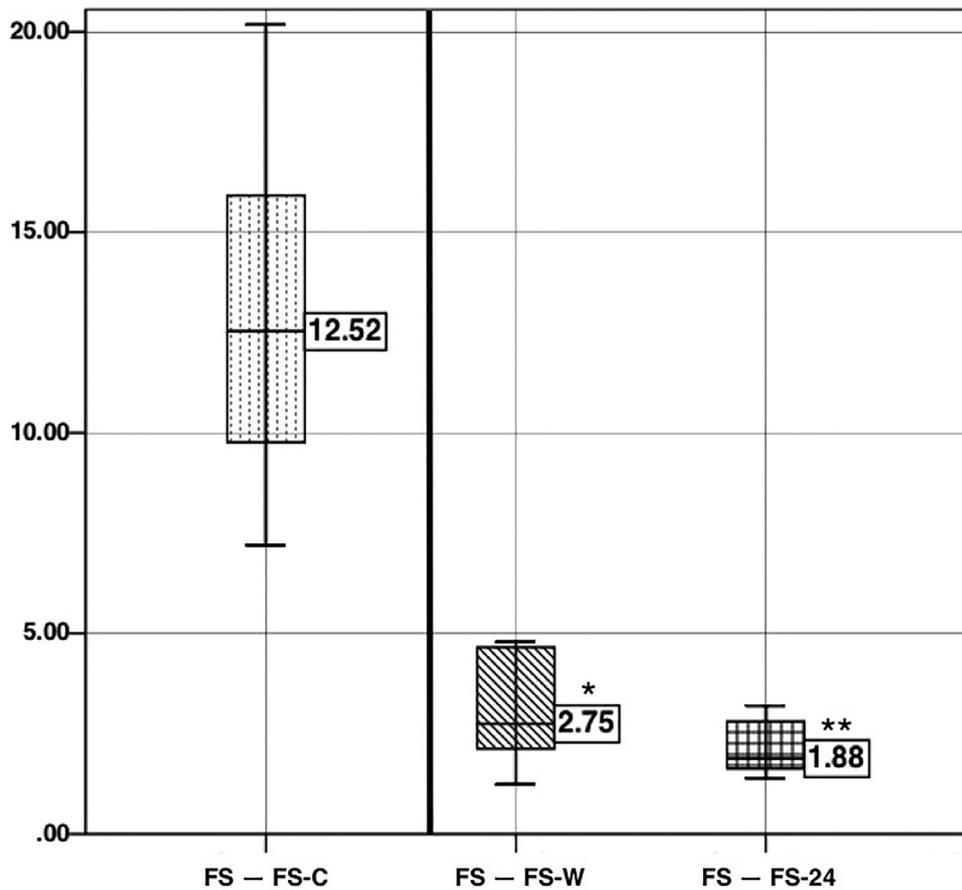


Figure 3 Boxplots of ICGA predicted flap survival after bootstrapping. The difference between flap survival in the postoperative day 7 and ICGA predicted flap survival: (FS - FS-C) the difference between postoperative day 7 flap survival and estimated flap survival with ICG-C; (FS - FS-W) the difference between postoperative day 7 flap survival and estimated flap survival with ICG-W; (FS - FS-24) the difference between postoperative day 7 flap survival and estimated flap survival with ICG-24. A means comparison analysis shows statistically significant differences between (FS ? FS-C) versus (FS - FS-W) (* = $p < 0.005$) and between (FS ? FS-C) versus (FS - FS-24) (** = $p < 0.001$). Boxes illustrate the interquartile range; horizontal lines indicate median values of measurement differences (% of total flap surface); whiskers indicate minimum and maximum values.



Figure 4 ICGA predicted flap survival areas on the same image at postoperative day 7: black = overlay of necrosis in the postoperative day 7 (FN); yellow = overlay of flap survival in the postoperative day 7 (FS); blue = overlay of predicted ICG-C flap survival (FS-C); red = overlay of predicted ICG-W flap survival (FS-W); orange = overlay of predicted ICG-24 flap survival (FS-24). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Wada et al.³¹ use a fluorescence threshold between 40 and 50% to determine viable tissue in rats. Gorai et al. suggest resecting mastectomy flaps at a fluorescence value below 34%.³² The difference in anatomy, perfusion patterns and physiology between rats, pigs and humans, together with interindividual differences render establishing an exact and applicable threshold value to predict necrosis very difficult.

In our study we used a fluorescence threshold of 33% to delineate perfused and nonperfused areas. In our study, the 33% threshold provided the best correlation of predicted ICG-24 flap survival (FS-24) with actual flap survival in the postoperative day 7.

Using a high threshold to delineate necrosis is safe, but in many instances may lead to over-resection of viable

tissue. The perfusion “gray zone” is actually the distal portion of the flap, which, partially recovers in the postoperative period.

Other authors have tried waiting a period of time before doing the angiography to benefit from perfusion recovery. Matsui et al. suggest that a single ICGA recording carried out 30 min post-operative is sufficient in predicting necrosis at 72 h in submental flaps in pigs.¹³ Wu et al. suggest performing ICGA 20 min after mastectomy and the using a 25% cutoff value to guide resection of nonviable tissue.³³ However, this prolongs the operating time while not benefiting from maximal perfusion recovery which can take up to 24 h.

We propose a novel approach to solve these problems. Because temperature is the main regulator of skin vascular supply,³⁴ warming the flap intraoperatively counteracts the delirious effects of hypothermia, vasoconstriction and increased blood viscosity. Inducing maximum vasodilation and achieving total relaxation of the vascular smooth muscle, independent of changes in vascular tonus, requires warming the flap to 42 °C. This temperature provides maximum tissue vasodilation, in a biphasic pattern, with the first peak on increased perfusion recordable in the first 3-5 min after maximum temperature is reached.³⁵ This allows each individual flap to reach its maximum perfusion recovery potential. To benefit from this effect, ICGA was done in the first five minutes after reaching the desired temperature.

In our study, conventional intraoperative ICGA (ICG-C) had a mean underestimation of flap survival (FS - FS-C) of 12,97%. The mean perfusion recovery by warming at 42 °C (FS-W - FS-C) was 9.84%. Induced vasodilation improves flap perfusion and more accurately predicts flap viability, mimicking microcirculatory conditions encountered at 24 h post-operative (FS-24 - FS-W = 0.98%).

Indocyanine green angiography offers real time information on flap perfusion. It is reproducible, easy to perform, radiation free and with no toxicity. Conventional ICG performed during surgery underestimates perfusion and leads to over-resection of viable tissue. Inducing vasodilation before performing ICGA, by warming the flap, counteracts the circulatory changes induced by surgical trauma, allowing the surgeon to make an accurate assessment of flap viability. This leads to a precise resection of ischemic tissue, with better outcomes, reduced morbidity, and a lower reintervention rate.

Warming the flap before angiography is safe, fast and easy to perform. It adds a couple of minutes of additional time to the procedure and in return improves the ability of ICGA to assess perfusion. The limited sample size in our study is a limitation that requires attention in further research. Results need confirmation in larger experimental studies, and on patients undergoing flap reconstructions.

Conclusions

Conventional intraoperative ICGA underestimates flap survival. Surgical trauma coupled with a drop-in flap temperature induce a marked state of vasoconstriction, increased blood viscosity and decreased perfusion. Warming the flap intraoperatively and achieving maximum vasodilation mitigates the effects of vasoconstriction and mimics the microcirculatory environment encountered at 24 h.

Performing ICGA after induced vasodilation permits a more accurate prediction of flap survival and minimizes resection of viable tissue.

Conflict of interest

Authors state no conflict of interest.

Funding

This study was funded from own resources part of doctoral studies of first author.

Financial disclosure statement

The authors have nothing to disclose.

References

1. Saint-Cyr M, Schaverien MV, Rohrich RJ. Perforator flaps: history, controversies, physiology, anatomy, and use in reconstruction. *Plastic Reconstr Surg* 2009;123(4):132e-145e.
2. Narushima M, Yamasoba T, Iida T, et al. Pure skin perforator flaps: the anatomical vascularity of the superthin flap. *Plastic Reconstr Surg* 2018;142(3):351e-360e.
3. Chubb D, Rozen WM, Whitaker IS, Acosta R, Grinsell D, Ashton MW. The efficacy of clinical assessment in the postoperative monitoring of free flaps: a review of 1140 consecutive cases. *Plastic Reconstr Surg* 2010;125(4):1157-66.
4. Gurtner GC, Jones GE, Neligan PC, et al. Intraoperative laser angiography using the SPY system: review of the literature and recommendations for use. *Annals Surg Innov Res* 2013;7(1):1.
5. Komorowska-Timek E, Gurtner GC. Intraoperative perfusion mapping with laser-assisted indocyanine green imaging can predict and prevent complications in immediate breast reconstruction. *Plastic Reconstr Surg* 2010;125(4):1065-73.
6. Newman MI, Samson MC. The application of laser-assisted indocyanine green fluorescent dye angiography in microsurgical breast reconstruction. *J Reconstr Microsurg* 2009;25(1):21-6.
7. Holm C, Tegeler J, Mayr M, Becker A, Pfeiffer UJ, Muhlbauer W. Monitoring free flaps using laser-induced fluorescence of indocyanine green: a preliminary experience. *Microsurgery* 2002;22(7):278-87.
8. Alstrup T, Christensen BO, Damsgaard TE. ICG angiography in immediate and delayed autologous breast reconstructions: perioperative evaluation and postoperative outcomes. *J Plastic Surg Hand Surg* 2018;52(5):307-11.
9. Rinker B. A comparison of methods to assess mastectomy flap viability in skin-sparing mastectomy and immediate reconstruction: a prospective cohort study. *Plastic Reconstr Surg* 2016;137(2):395-401.
10. Matsui A, Lee BT, Winer JH, Laurence RG, Frangioni JV. Submental perforator flap design with a near-infrared fluorescence imaging system: the relationship among number of perforators, flap perfusion, and venous drainage. *Plastic Reconstr Surg* 2009;124(4):1098-104.
11. Chubb DP, Taylor GI, Ashton MW. True and 'choke' anastomoses between perforator angiosomes: part II. Dynamic thermographic identification. *Plastic Reconstr Surg* 2013;132(6):1457-64.

12. Pascone C, Agostini T, Lazzeri D, Pascone M. Refinements in postoperative free flap monitoring. *Plastic Reconstr Surg* 2011;127(6):2512 author reply.
13. Matsui A, Lee BT, Winer JH, Laurence RG, Frangioni JV. Predictive capability of near-infrared fluorescence angiography in submental perforator flap survival. *Plastic Reconstr Surg* 2010;126(5):1518-27.
14. Vargas CR, Nguyen JT, Ashitate Y, et al. Near-infrared imaging for the assessment of anastomotic patency, thrombosis, and reperfusion in microsurgery: a pilot study in a porcine model. *Microsurgery* 2015;35(4):309-14.
15. van Driel PBAA, van de Giessen M, Boonstra MC, et al. Characterization and evaluation of the Artemis camera for fluorescence-guided cancer surgery. *Mol Imaging Biol* 2015;17(3):413-23.
16. Kellogg DL Jr, Johnson JM, Kenney WL, Pergola PE, Kosiba WA. Mechanisms of control of skin blood flow during prolonged exercise in humans. *Am J Physiol* 1993;265(2 Pt 2):H562-8.
17. Schindelin J, Rueden CT, Hiner MC, Eliceiri KW. The ImageJ ecosystem: an open platform for biomedical image analysis. *Mol Reprod Dev* 2015;82(7-8):518-29.
18. Newman MI, Jack MC, Samson MC. SPY-Q analysis toolkit values potentially predict mastectomy flap necrosis. *Ann Plastic Surg* 2013;70(5):595-8.
19. Moyer HR, Losken A. Predicting mastectomy skin flap necrosis with indocyanine green angiography: the gray area defined. *Plastic Reconstr Surg* 2012;129(5):1043-8.
20. Singh K. On the asymptotic accuracy of Efron's Bootstrap. *Ann Stat* 1981;9(6):1187-95.
21. Mancin M, Toson M, Grimaldi M, Barco L, Trevisan R, Carnieletto P, et al. Application of bootstrap method to evaluate bimodal data: an example of food microbiology proficiency test for sulfite-reducing anaerobes. *Accred Qual Assur* 2015;20:255.
22. Sigurdsson GH, Thomson D. Anaesthesia and microvascular surgery: clinical practice and research. *Eur J Anaesthesiol* 1995;12(2):101-22.
23. Pereira CM, Figueiredo ME, Carvalho R, Catre D, Assuncao JP. Anesthesia and surgical microvascular flaps. *Rev Bras Anesthesiol* 2012;62(4):563-79.
24. Cutting C, Ballantyne D, Shaw W, Converse JM. Critical closing pressure, local perfusion pressure, and the failing skin flap. *Ann Plastic Surg* 1982;8(6):504-9.
25. Pearl RM. A unifying theory of the delay phenomenon-recovery from the hyperadrenergic state. *Ann Plastic Surg* 1981;7(2):102-12.
26. Hakim M, Walia H, Dellinger HL, et al. The effect of operating room temperature on the performance of clinical and cognitive tasks. *Pediatr Qual Saf* 2018;3(2):e069.
27. Jeyaraj SC, Chotani MA, Mitra S, Gregg HE, Flavahan NA, Morrison KJ. Cooling evokes redistribution of alpha2C-adrenoceptors from Golgi to plasma membrane in transfected human embryonic kidney 293 cells. *Mol Pharmacol* 2001;60(6):1195-200.
28. Tsai TM, Jupiter JB, Serratori F, Seki T, Okubo K. The effect of hypothermia and tissue perfusion on extended myocutaneous flap viability. *Plastic Reconstr Surg* 1982;70(4):444-54.
29. Giunta RE, Holzbach T, Taskov C, et al. Prediction of flap necrosis with laser induced indocyanine green fluorescence in a rat model. *Br J Plastic Surg* 2005;58(5):695-701.
30. Monahan J, Hwang BH, Kennedy JM, et al. Determination of a perfusion threshold in experimental perforator flap surgery using indocyanine green angiography. *Ann Plastic Surg* 2014;73(5):602-6.
31. Wada H, Vargas CR, Angelo J, et al. Accurate prediction of tissue viability at postoperative day 7 using only two intraoperative subsecond near-infrared fluorescence images. *Plastic Reconstr Surg* 2017;139(2):354-63.
32. Gorai K, Inoue K, Saegusa N, et al. Prediction of skin necrosis after mastectomy for breast cancer using indocyanine green angiography imaging. *Plastic Reconstr Surg Global Open* 2017;5(4):e1321.
33. Wu C, Kim S, Halvorson EG. Laser-assisted indocyanine green angiography: a critical appraisal. *Ann Plastic Surg* 2013;70(5):613-19.
34. Kellogg DL Jr, Pergola PE, Piest KL, et al. Cutaneous active vasodilation in humans is mediated by cholinergic nerve cotransmission. *Circ Res* 1995;77(6):1222-8.
35. Charkoudian N, Eisenach JH, Atkinson JL, Fealey RD, Joyner MJ. Effects of chronic sympathectomy on locally mediated cutaneous vasodilation in humans. *J Appl Physiol* (1985) 2002;92(2):685-90.