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Predictive Capability of Near-Infrared Fluorescence Angiography in Submental Perforator Flap Survival

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Abstract

Background—Perforator flaps have become increasingly popular in reconstructive surgery as patients experience less donor-site morbidity than with conventional musculocutaneous flaps. Previously, our laboratory described the intraoperative use of near-infrared (NIR) fluorescence angiography for patient-specific perforator-flap design. This study evaluates the predictive capability of NIR fluorescence angiography for flap survival in submental flap reconstruction.

Methods—NIR angiography was performed using indocyanine green at 0, 0.5, 24, 48, and 72 h post-surgery after flap creation in 12 pigs. A single perforator artery was preserved during flap creation based on location (central or non-central) and dominance (dominant or non-dominant). Venous drainage, arterial perfusion, and perfused area as percentage of total flap were analyzed. Clinical assessments of perfusion were compared with those made using NIR imaging and histology.

Results—Use of NIR fluorescence angiography immediately after flap creation accurately predicted areas of perfusion at 72 h ($p = 0.0013$), compared to the initial clinical assessment ($p = 0.3085$). Identification of necrosis by histology at 72 h correlated with NIR findings of insufficient arterial perfusion immediately after flap creation. No statistically significant differences in perfusion

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John V. Frangioni, M.D., Ph.D.: All intellectual property for the intraoperative near-infrared fluorescence imaging system is owned by Dr. Frangioni's employer, the Beth Israel Deaconess Medical Center (BIDMC), a teaching hospital of Harvard Medical School. None of the technology is licensed at present. As inventor of the technology, Dr. Frangioni may someday receive royalties if a product is developed. Dr. Frangioni has no real or deferred equity interests, whatsoever, in this, or any other technology.

Devices and Drugs Used in This Study: Custom-built intraoperative near-infrared fluorescence imaging system with simultaneous color video and near-infrared fluorescence capabilities; Indocyanine Green (Cardiogreen, Sigma-Aldrich, St. Louis, MO/ IC-GREEN™, Akorn, Decatur, IL), Epinephrine (Hospira, Lake Forest, IL).

metrics were detected based on location or dominance of the preserved perforator; however, flaps containing central perforators had a higher percent perfused area than those with non-central perforators.

Conclusions—The use of NIR angiography immediately after flap creation can predict areas of perfusion at 72 h. This predictive capability may permit intraoperative revision of compromised flaps that have a high likelihood of failure.

Keywords

Perforator flap; near-infrared fluorescence angiography; indocyanine green

INTRODUCTION

Perforator flaps represent an advancement in reconstructive surgery and are an important adjunct to use of traditional muscle and musculocutaneous flaps. The reliability of perforator flaps depends on identification of adequate perforator arteries during flap design in conjunction with a meticulous dissection technique. As reconstructive surgeons continue to refine the indications for perforator flaps, there have been fewer complications, such as flap loss and fat necrosis. The most critical element in reducing the complication rate has been the establishment of adequate flap perfusion. Although the choice of a reliable perforator is important, there are anatomic variations, such as perforator location and dominance, to consider.

Our laboratory previously described the use of the Fluorescence-Assisted Resection and Exploration (FLARE™) system,^{1–3} in conjunction with intravenous injection of indocyanine green (ICG), to perform near-infrared (NIR) fluorescence angiography (reviewed in⁴). Using this technology, we have demonstrated the identification of suitable perforators,⁵ correlated the location and number of perforators identified with NIR imaging to conventional X-ray angiography,⁶ evaluated both abdominal and submental perforator flaps,⁷ and created indices to assess venous drainage and arterial inflow and to produce a graphical representation of perfusion patterns.⁸

Because our previous outcome study evaluated flap perfusion only at 6 h post-surgery,⁸ the predictive capability of NIR imaging beyond this time point is not known. In the present study, we continued to assess the use of NIR angiography to evaluate submental perforator flap perfusion while extending the post-operative observation period to 72 h. The submental flap was chosen for ease of dissection and post-operative care; in addition, the submental site provided minimal pain to the animal. Our goal was to determine the long-term accuracy of our perfusion indices. In addition, we altered perforator location and dominance in order to assess the impact of these parameters on flap perfusion. Finally, we examined flap histology and correlated this data with results of NIR angiography and clinical examination.

MATERIALS AND METHODS

NIR Fluorescence Imaging System

The FLARE™ imaging system has been described in detail.^{1–3} White (400–650 nm) light and NIR fluorescence excitation (745–779 nm) light are generated by filtered LEDs over a 15-cm field of view. Color video images, showing surgical anatomy, and NIR fluorescence images, showing fluorophore distribution, were obtained simultaneously in real-time. The merged image consists of a grayscale NIR fluorescence image pseudo-colored in a visible color, then overlaid onto the color video image. All images are refreshed up to 15 times per second.

Animal Preparation and 72 h Survival

Animal experiments were in compliance with an approved institutional protocol. Female Yorkshire pigs (E.M. Parsons and Sons, Hadley, MA; body weight, 33.6–41.0 kg; mean, 37.1 kg) were induced with 4.4 mg/kg intramuscular Telazol™ (Fort Dodge Labs, Fort Dodge, IA), intubated, and maintained on 2% isoflurane (Baxter Healthcare Corp., Deerfield, IL). Electrocardiograph, heart rate, oxygen saturation, and body temperature were monitored during the procedure. The neck and submental region were shaved and sterilized with Betadine and a central venous catheter was inserted in the external jugular vein. Preoperative intravenous antibiotic consisting of 5 mg/kg enrofloxacin (Baytril®, 2.27% injectable solution, Bayer HealthCare) was administered prior to incision. Intramuscular injection of 0.01 mg/kg buprenorphine hydrochloride (Hospira, Lake Forest, IL) and application of a 75 µg/h fentanyl patch (Sandoz, Broomfield, CO) were used for post-operative pain relief. Animals were observed for 72 h (3 days) following perforator flap creation.

NIR fluorescence angiography was performed in 12 pigs following injection of 2.5 mg (0.06–0.07 mg/kg) clinical grade ICG (Akorn, Decatur, IL) prior to the incision to identify a single perforator to be preserved.⁸ An 8 × 8-cm round flap was designed at the submental region incorporating the selected perforator and a second injection was given to confirm preservation of the selected artery. Animals were divided into four groups of three animals each based on the location and dominance of the preserved perforator and the groups were designated central/dominant, central/non-dominant, non-central/dominant, and non-central/non-dominant. A perforator artery was identified as dominant if it had higher fluorescence intensity than other perforators in the same flap (Supplemental Fig. 1). This was determined with a pre-operative injection which localized the perforators.

Subsequently, the flap was elevated and a third ICG injection was given to ensure preservation of the appropriate single perforator as well as to determine flap perfusion at 0 h post-operation. The flap was sutured to the original location with a Penrose drain and evaluated by NIR angiography and clinical assessment at 0.5, 24, 48, and 72 h post-operation to monitor flap perfusion. Animals were re-anesthetized at 24 h, 48, and 72 h for both clinical and NIR evaluation. Operative procedures were performed using standard aseptic technique. At 72 h three tissue samples per flap were obtained for histological evaluation; these were from a normally-perfused area adjacent to the perforator artery, an area identified by NIR fluorescence angiography as poorly-perfused at 0.5 h post-operation, and a nearby, non-flap control area.

Quantitative Assessment of Perfusion with NIR Fluorescence Imaging

Quantitative assessment of flap perfusion has been described in detail.⁸ Briefly, fluorescence intensity of the region of interest (ROI) and background fluorescence were quantified and expressed as contrast-to-background-ratio (CBR), defined as (mean fluorescence intensity of the ROI – mean background fluorescence intensity)/mean background fluorescence intensity. Six ROIs were defined: the perforator artery, proximal well-perfused area, distal well-perfused area, poorly-perfused area, non-perfused area, and control area located outside the flap (Fig. 1). Of note, ROIs were defined at a set location for each flap through subsequent injections.

The fluorescence pattern for each injection was recorded graphically and analyzed. Maximum fluorescence ($I_{\max}$), time to $I_{\max}$ ($T_{\max}$), and fluorescence intensity at 120 seconds post-injection (I_{120}) were obtained for individual injections. The $T_{\max}$ ratio and drainage ratio were determined to quantify arterial inflow and venous drainage, respectively. For arterial perfusion we have previously shown that a $T_{\max}$ ratio less than 200% had a 75% sensitivity and 100% specificity with 87.5% accuracy, whereas for venous compromise a Drainage ratio less than 86% had a 100% sensitivity and 98.9% specificity with 98.9% accuracy.⁸ Drainage ratio = $I_{120}/I_{\max} \times 100$ (%). $T_{\max}$ ratio = $T_{\max}'/T_{\max}^* \times 100$, where $T_{\max}'$ and $T_{\max}^*$ are defined as

the time of maximal fluorescence intensity at preoperative and postoperative injections, respectively. Using Vision Assistant (National Instruments) software for analysis of the NIR fluorescence images, the perfused area was quantified as a percentage of total flap area. This analysis was performed for all conditions at the time point where maximum perfusion was achieved ($P_{\max}$). A staff plastic surgeon (BTL) clinically assessed the viable areas of the flap at each injection; the surgeon was blinded to the results of NIR fluorescence angiography during this evaluation. Clinically-assessed areas of perfusion were compared to areas assessed by NIR fluorescence angiography. Clinical assessment of perfusion was based on skin color and capillary refill and a line of demarcation was drawn to determine perfusion as a percent of total area.

Statistical Analysis

The Wilcoxon matched-pairs signed rank test or Mann-Whitney test was employed to determine statistical differences between two matched or unmatched groups, respectively. Correlation was expressed by Spearman's rank correlation coefficient. All values were calculated using Prism 4 (Graphpad Software, La Jolla, CA). $P < 0.05$ was considered statistically significant. Values represent mean $\pm$ SEM.

RESULTS

CBR Patterns, Perfusion Indices, and Recovery of Perfusion in Perforator Flaps

The typical CBR patterns seen within flaps have been described.⁸ All preoperative injections at the control ROI showed an "arterial" pattern of perfusion (Supplemental Fig. 2A), which was also frequently seen within the perforator artery ROI. We also observed a "capillary" pattern that was characterized by a gradual increase in the CBR in the absence of the inflow peak. This capillary pattern identified areas within the flap that were under-perfused by primary arterial flow, but were perfused by a distant artery via the capillary bed. The arterial pattern became less prominent and the capillary pattern more prominent with increasing distance from the perforator artery (Supplemental Fig. 2B). The "arterial-insufficient" or capillary patterns were frequently observed immediately after flap creation and were gradually replaced by an arterial pattern over time. This trend was observed in the peripheral areas of the flap (Supplemental Fig. 2B). The arterial pattern represented normal perfusion, while the arterial insufficient and capillary patterns corresponded to areas of compromised perfusion; these latter two patterns gradually shifted to the arterial pattern over 72 h. Drainage ratios evaluated at the perforator artery and the proximal and distal well-perfused areas demonstrated continuous recovery of the venous drainage of flaps (Supplemental Fig. 2C). The drainage ratio at 0 h post-operation was $68.5 \pm 6.2\%$ at the perforator artery and $83.8 \pm 7.0\%$ in the proximal and distal well-perfused areas. These ratios were significantly compromised compared to the preoperative ratios of $38.0 \pm 2.1\%$ at the perforator artery ($p = 0.001$) and $39.3 \pm 1.9\%$ in the proximal and distal well-perfused areas ($p = 0.002$). At 0.5, 24, 48, and 72 h post-operation the drainage ratio at the perforator artery was 68.5 ± 6.2 , 52.4 ± 4.3 , 51.5 ± 6.6 , and 48.9 ± 6.3 , respectively; significant recovery was only observed during the first 24 h (Wilcoxon signed rank test, $p = 0.042$).

Impact of Location and Dominance of the Preserved Perforator

Preoperative NIR fluorescence angiography permitted selection of a single perforator artery; the location and dominance of this perforator could be altered. Drainage ratio, $P_{\max}$, and percent perfused area were also evaluated between groups. No statistical differences in these parameters were observed at 72 h post-operation (Fig. 2 and Supplemental Fig. 3) although the percent-perfused area in the central-perforator flaps tended to be significantly greater than that of the non-central-perforator flaps at 0, 24 h, and 48 h.

Predictive Factors for Flap Survival and Histology

Table 1 shows the histological properties of flap specimens. All controls showed normal histology (data not shown). All specimens with normal histology had an arterial perfusion pattern at the time of surgery. Four specimens shown to be necrotic by histological analysis had no clinical signs of discoloration; these included three cases of fat necrosis and one of full-thickness necrosis. The data in Table 1 also suggest that serious tissue damage could persist even as the perfusion patterns improved over time. It is noteworthy that the arterial-insufficient pattern was identified in poorly- and non-perfused areas of flaps (Table 2). Non-perfused areas always abutted or overlapped with poorly-perfused areas, suggesting that a centrally located perforator artery is critical to flap perfusion.

Comparison of Clinical Assessment and Results of NIR Fluorescence Angiography

Many differences were observed between perfusion areas identified by clinical assessment and by NIR fluorescence angiography. Clinical assessment was based on the demarcation between normal and discolored skin. The clinical assessment done immediately after flap creation (0 – 0.5 h) often failed to accurately predict areas that would develop necrosis by 72 h (Fig. 3A). Using clinical assessment alone, no correlation was found in individual groups or overall between the perfused area at 0.5 h and 72 h post-surgery (Table 3). The difference between the clinical assessment and NIR fluorescence angiography in predicting overall flap perfusion was statistically significant (Fig. 3B). Clinical assessment was also unable to predict areas in which perfusion would be subsequently restored. Clinical assessment and the results of NIR fluorescence angiography were also compared based on perforator artery location and dominance (Supplemental Fig. 4). A statistical correlation existed between the two techniques in the case of dominant and non-dominant perforator arteries; a weaker correlation was seen with central and non-central perforators (Table 3). Finally, the percent-perfused area determined based on NIR results at 0.5 h was very strongly correlated with clinical assessment at 72 h in non-dominant and non-central perforator arteries and overall (Fig. 3B, Supplemental Fig. 4, and Table 3).

DISCUSSION

Accurate prediction of flap perfusion status can be a challenge at the time of surgery and clinical findings of inadequate perfusion may be delayed. In cases of partial flap loss or compromised perfusion it can take up to 72 h to identify areas of demarcation between normal and discolored skin that are indicative of compromised perfusion. This is a common clinical observation, e.g., in the case of mastectomy skin-flap loss. The goal of this study was to determine if NIR angiography can accurately predict areas of normal and compromised perfusion. We characterized areas of perfusion using NIR angiography immediately after flap creation (0 – 0.5 h) and used this data to accurately predict the perfusion status of a flap at 72 h.

These assessments were in agreement with clinical assessments carried out at 72 h. We have presented quantitative assessment of flap perfusion using previously described metrics, including the drainage ratio and the $T_{\max}$ ratio.⁸ Here, we validated the application of these metrics in the immediate post-operative period to predict flap survival and demonstrated the correlation of our predictions with clinical outcome. We observed flap perfusion during the initial 72 h after flap creation, a standard time frame for post-operative monitoring in plastic surgery.⁹ The CBR patterns observed during this time indicated that the (1) arterial pattern represents normal perfusion; (2) compromised perfusion patterns arterial-insufficient and capillary occur more frequently with increasing distance from the perforator artery; and (3) these compromised patterns improve to normal arterial patterns over time. From these observations we conclude that perfusion is a function of distance from the perforator vessel.

Because all flaps were designed as single-perforator flaps we were able to control the location and dominance of the selected perforator artery to evaluate the impact of these parameters on flap survival. Although our study failed to show a statistically significant difference in perfusion based on perforator location or dominance, we did demonstrate that a central perforator had a statistically significantly higher percent-perfused area than a non-central perforator at 0, 24 h, and 48 h post-operation. These findings suggest that, in larger flaps, a central perforator may be a crucial component of flap design. Although flaps with dominant perforators also had higher percent-perfused areas than those with non-dominant perforators, the difference was not statistically significant. Note that the percentage of perfused area showed continuous recovery in all flaps (with the exception of one flap that developed total flap failure), which was consistent with our previous study.⁷

The compromised arterial-insufficient and capillary perfusion patterns observed immediately after flap creation could be risk factors for the development of tissue damage. Although these areas often showed signs of recovery based on perfusion metrics, especially in the flap periphery (PP and NP, Supplemental Fig. 2B), histological changes were already apparent at 72 h. The arterial-insufficient pattern was observed at the time of surgery in 15 areas; nine of these were sampled for histological analysis at 72 h and showed evidence of fat necrosis (Tables 1 and 2). Of the areas sampled that showed fat necrosis, all areas had elements of patterns of compromise by NIR at 0.5 h (capillary, arterial insufficiency, or arterial/delayed spasm patterns). Some of these areas showed recovery by NIR at 72 h, suggesting that the NIR assessment at 0.5 h more accurately predicts fat necrosis determined by histology. The identification of an arterial-insufficient pattern at the time of surgery could potentially aid in the identification of areas in the flap that will eventually undergo necrosis. In contrast, all specimens sampled from areas with arterial perfusion patterns at the time of surgery were histologically normal at 72 h. Because only a limited number of histology specimens were taken from sites with a capillary pattern, we were not able to draw conclusions about the relation between the presence of this pattern of perfusion at the time of surgery and tissue histology at 72 h. However, three histological samples with necrosis were obtained from areas that showed a capillary pattern, suggesting that presence of the capillary pattern soon after surgery could predict areas of necrosis as well.

Although total flap loss is well-described in free-flap reconstruction,¹⁰ partial flap loss and fat necrosis are more likely indicators of poor perfusion and tissue ischemia.^{11,12} In our study, five of six histologically-demonstrated sites of fat necrosis were associated with the compromised arterial perfusion patterns arterial-insufficient and capillary at the time of surgery. One area of fat necrosis developed at an area with the compromised perfusion pattern “delayed drainage pattern with spasm” as well (Table 1). Venous congestion can also eventually result in ischemia.¹³ We previously proposed that a drainage ratio greater than 85% represents the threshold of venous congestion.⁸ Drainage ratios in the areas of fat necrosis were greater than 96%, suggesting the existence of venous congestion. We also evaluated drainage ratio at the perforator artery, in the well-perfused/proximal/contralateral flap regions, and in control areas to determine the timing and extent of recovery in venous drainage. We found that improvements in venous outflow continued for up to 24 h after flap creation (Supplemental Fig. 2C).

Finally, we observed that clinical assessment of perfusion was inaccurate in the immediate post-operative period (0–0.5 h) in terms of identifying areas that would be necrotic at 72 h and in fact, early post-operative clinical assessment over-estimated vascularity. However, NIR fluorescence angiography performed immediately after flap creation was able to identify areas of demarcation seen clinically at 72 h. Because discoloration is a result of severe congestion or ischemia, there is always a lag period between the appearance of discoloration and onset of compromised perfusion. And, since NIR fluorescence angiography reflects blood flow, it

appears capable of predicting perfusion in the flap. Interestingly, NIR imaging at 72 h showed a slightly larger percent-perfused area than was indicated by clinical assessment at the same time. It is possible that perfusion in the periphery of the flap may continue to recover beyond 72 h (Fig. 3A). In addition, it should be noted that four of 13 specimens showing abnormal histology presented in areas with normal skin color (Table 1).

It should be emphasized that the conclusion of this study is that a single intravenous injection of ICG at 30 min post-op, followed by a 2-min measurement period, is all that is required to predict flap outcome at 72 h. Moreover, all of the ROI measurements can be fully automated, thus reducing error and simplifying analysis.

However, our study also has limitations. The time frame for observation here was 72 h and it is possible that areas showing poor perfusion at 72 h can continue to recover at later time points. As noted above, the NIR assessment at 72 h showed a higher percent-perfused area than was indicated by clinical assessment at the same time. It is certainly possible that a clinical assessment beyond this time frame may correlate with the NIR findings; however, the areas with compromised perfusion may be unreliable indicators of clinical outcome. The evaluation of perforator artery location and dominance required classifying the flaps in smaller groups; it is possible that a larger sample size may be needed to observe statistically significant differences.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Perforator Selection and Establishment of ROIs using NIR Fluorescence Angiography
ROI selection. On a major axis of the flap that includes the selected perforator artery, a point 1 cm from the flap edge was defined as a poorly-perfused area (PP). In the groups with centrally-located perforators identified using NIR fluorescence angiography, the line between the perforator artery and poorly-perfused area was divided in half and the midpoint was defined as the ipsilateral, well-perfused area; the area designated contralateral well-perfused was located on the same line on the opposite side of and equidistant from the perforator (right). In the groups with non-centrally-located perforators, the line connecting the perforator artery and poorly-perfused area was divided into three equal segments. Points proximal and distal to the perforator marking the ends of these segments were designated proximal and distal well-perfused areas, respectively (right). Areas showing the weakest or no fluorescence at 0.5 h post-operation were designated non-perfused areas. Shown are the color video (left), NIR fluorescence (middle), and pseudo-colored (green) merged image (right) of a central (top) and non-central (bottom) PA recorded after flap elevation.

Figure 2. Effect of Location and Dominance of the Perforator Artery

Perfused area as a percentage of total flap was evaluated at the PA. Comparison of parameters between central and non-central PAs are shown on the left and between dominant and non-dominant PAs on the right. Asterisk indicates statistically significance difference. Values represent mean $\pm$ SEM. X-axis indicates the time before or after flap elevation.

Figure 3. Perfused Area as a Percentage of Total Flap Determined by NIR Fluorescence Angiography and Clinical Assessment Over Time

A. Visual assessment of perfused area as a percentage of total flap determined by clinical assessment (top) and NIR fluorescence angiography (bottom) at the indicated times. Black dashed line indicates the border between viable and non-viable areas based on clinical assessment and white dashed line indicates the border between perfused and non-perfused areas assessed using NIR fluorescence angiography.

B. Quantitation and comparison of viable area determined by clinical assessment and perfused area determined by NIR fluorescence as a percentage of total flap. X-axis indicates time before or after flap elevation. Asterisk indicates a statistically significant difference. Values represent mean $\pm$ SEM.

Table 1

Histological Evaluation and Perfusion Patterns

Histology	Flap Type	ROI	Color	Edema	Post-Operative Perfusion Pattern			
					0.5 h	24 h	48 h	72 h
NS	C/D	PA	-	+	A	A	A	A
NS	C/D	PA	-	-	A	A	A	A
NS	C/D	PA	-	+	A	A	A	A
NS	C/ND	PA	-	+	A	A	AD	AD
NS	C/ND	PA	-	+	A	A	A	A
NS	C/ND	PA	-	+	A	A	A	A
NS	NC/D	PA	-	+	A	A	A	A
NS	NC/D	PA	-	+	A	A	A	A
NS	NC/D	PA	-	+	A	A	A	A
NS	NC/ND	PA	-	+	A	A	AD (SP)	A
NS	NC/ND	PA	-	+	A	A	A	A
Fat necrosis	C/D	PP	-	+	Cap	A (SP)	AD (SP)	A (SP)
Fat necrosis	C/D	PP	-	+	AD (SP)	AD	AD	AD
Fat necrosis	C/D	NP	-	+	AI	Cap	Cap	AD (SP)
Fat necrosis	C/ND	NP	+	+	AI	Cap	Cap	A
Fat necrosis	NC/D	NP	+	+	AI	AI	AI	A (SP)
Fat necrosis	NC/ND	PP	+	+	AI	AI	AD (SP)	Cap
EG necrosis	C/D	NP	+	+	AI	Cap	Cap	AD (SP)
EG necrosis	C/ND	NP	+	+	AI	Cap	Cap	A
EG necrosis	NC/D	NP	+	+	AI	AI	AI	A (SP)
EG necrosis	NC/D	NP	+	+	AI	AI	AI	A (SP)
EG necrosis	NC/ND	NP	+	+	Cap	AD	AD (SP)	A (SP)
FT necrosis	C/ND	NP	-	+	Cap	AD (SP)	A (SP)	AD (SP)
FT necrosis	NC/ND	PA	+	n/a	AI	Cap	AI	AI

ROI, Region of interest; NS, No significant abnormality; EG, Eccrine gland; FT, Full-thickness; C, Central; NC, Non-central; D, Dominant; ND, Non-dominant; PA, Perforator artery; PP, Poorly-perfused area; NP, Non-perfused area; AI, Arterial-insufficient; Cap, Capillary; A, Arterial; AD, Arterial/delayed drainage pattern; SP, Arterial spasm; n/a, Not applicable.

Table 2

Perfusion Over Time of ROIs Showing the Arterial-Insufficient Pattern at the Time of Surgery.

Flap Type	ROI	Post-Operative Perfusion Pattern			
		0.5 h	24 h	48 h	72 h
C/D	NP	AI	Cap	Cap	AD (SP)
C/ND	PP	AI	Cap	Cap	A (SP)
C/ND	NP	AI	AI	Cap	Cap
NC/D	PP	AI	AI	AI	A (SP)
NC/D	PP	AI	Cap	A	A
NC/D	NP	AI	AI	AI	Cap
NC/D	NP	AI	Cap	AI	AI
NC/D	NP	AI	Cap	AI	AI
NC/ND	PA	AI	Cap	AI	AI
NC/ND	WPp	AI	Cap	AI	AI
NC/ND	WPd	AI	AI	AI	AI
NC/ND	PP	AI	AI	AD (SP)	Cap
NC/ND	PP	AI	AI	AI	AI
NC/ND	NP	AI	AI	AI	AI
NC/ND	NP	AI	AI	AI	AI

ROI, Region of interest; C, Central; NC, Non-central; D, Dominant; ND, Non-dominant; PA, Perforator artery; WPp, Well-perfused area proximal to PA; WPd, Well-perfused area distal to PA; PP, Poorly-perfused area; NP, Non-perfused area; AI, Arterial-insufficient; Cap, Capillary; A, Arterial; AD, Arterial/delayed; SP, Arterial spasm.

Table 3

Statistical Correlations for Perfused Area.

Correlation between	Perforator	Number of pairs	Spearman r	p
Clinical	Dominant	30	0.4231	0.0198*
Clinical	Central	30	0.3369	0.0687
Clinical	Non-dominant	30	0.4461	0.0135*
Clinical	Non-central	30	0.3511	0.0571
Clinical	Overall	60	0.4527	0.0003*
Clinical 0.5 h	Dominant	6	0.3592	0.4972
Clinical 0.5 h	Central	6	-0.1437	0.8028
Clinical 0.5 h	Non-dominant	6	0.2794	0.5639
Clinical 0.5 h	Non-central	6	0.5768	0.2417
Clinical 0.5 h	Overall	12	0.3173	0.3085
NIR 0.5 h	Dominant	6	0.5852	0.2417
NIR 0.5 h	Central	6	0.2572	0.6583
NIR 0.5 h	Non-dominant	6	0.9276	0.0167*
NIR 0.5 h	Non-central	6	0.9429	0.0167*
NIR 0.5 h	Overall	12	0.8325	0.0013*

* Wilcoxon signed rank test, $P < 0.05$.