GS GLAND SURGERY
AN OPEN ACCESS JOURNAL DESCRIBING NEW FINDINGS IN GLAND SURGERY

IMPACT FACTOR 2.16

Peer Review File

Article information: https://dx.doi.org/10.21037/gs-22-371

Reviewer A

The authors are to be congratulated for performing the valuable study on ICG-Angiography on the perfusion assessment and perforators in DIEP-flap breast reconstruction. The study is observational and assesses the influence on perforators on the flap perfusion in 67 patients undergoing unilateral DIEP-flap breast reconstruction.

Please find below the review of the papers individual paragraphs

ABSTRACT

The abstract provides the reader with a nice overview of the paper.

Line 30: please clarify if the reconstruction was performed in the delayed or immediate setting

Response 1: Thank you for taking the time to review our article and giving your valuable feedback. We included both immediate and delayed reconstructions in this study. Please refer to the response 2.

INTRODUCTION

Is well-written and justifies the study. The introduction also emphasizes the ever lasting quest of the plastic surgeon i.e. to minimize donor site-morbidity and optimize the reconstructive outcome.

PATIENTS AND METHODS

The patients are selected relevantly to ensure a uniform study population - leaving one question - were all flaps performed in the immediate or delayed setting? This is relevant when also using fat necrosis as an outcome parameter. Fat necrosis might be easier detectable in the delayed setting as the mastectomy skin flaps are not part of the evaluation. Furthermore, how was mastectomy skin flap necrosis handled. I know this is not part of the aim of the paper, but should be mentioned/specified for completeness.

Response 2: We included both immediate and delayed reconstructions in this study. We agree with you that fat necrosis can be more easily detected in the delayed setting. We did not perform statistical analysis on the rate of fat necrosis according to the low incidence of fat necrosis in the study population. It was already included as a limitation of this study in the last sentence of DISCUSSION section. We added "immediate or delayed," to the first paragraph of the PATIENTS AND METHODS section (see Page 5, line 91). Immediate reconstruction was performed in 49 patients and delayed reconstruction in 18. We added this sentence to the first paragraph of the RESULTS section (see Page 9, line 185-186).

In the overall study population, three case of mastectomy skin flap necrosis was

found. We added the definition of mastectomy skin flap necrosis as "Mastectomy skin flap necrosis was defined as any breakdown of skin integrity on the mastectomy site that was treated with surgical intervention." to the PATIENTS AND METHODS section (see Page 9, line 168-170). The result was added to the RESULTS section (see Page 10, line 189)

The patient population is very representative.
Line 106: as the authors refer to the mastectomy specimen weight - we anticipate that the flaps are performed in the immediate setting?

Response 3: We included both immediate and delayed reconstructions in this study. "Estimated mastectomy specimen weight" was calculated preoperatively using preoperative CT angiography. We added "preoperatively" to the second sentence of subheading "Perfusion Assessment Using Indocyanine Green Angiography" of PATIENTS AND METHODS section to clarify the meaning of "estimated mastectomy specimen weight" (see Page 6, line 106).

The perfusion is assessed using the Fluobeam, "quantifying" the relative fluorescence value units.
Line 133-134: please explain this concept to the reader. The reviewer is aquainted with the concept, but many readers may not be.
Is 40% of RVU set at cut-off for sufficient/non-sufficient perfusion?

Response 4: Thank you for pointing out an unnoticed point of this study. We added the definition of RVU and a relevant reference as "RVU was defined as a percentage of fluorescence intensity relative to the surrounding well-perfused tissues designated as 100 percent fluorescent.(21)" to the subheading "Perfusion Assessment Using Indocyanine Green Angiography" of PATIENTS AND METHODS section (see Page 7, line 136-137).

The 40% of RVU is an arbitrary value that was used as the cut-off for sufficient/non-sufficient perfusion in this study. There is still no general consensus on the cut-offs for evaluation of ICG angiography. We discussed this issue in the 3rd paragraph of DISCUSSION section.

The inset-rate is a very nice concept.
Moreover, the time-point of perfusion assessment is set to the relevant post-injection set-off.

STATISTICAL ANALYSES
No comments - sufficient
RESULTS
The results seem to confirm that the perforators located in vertical zone 2 were perferable.
Then the patients are further subdivided - the 33 patients whose DIEPflap was supplied by one dominant and on additional perforator were studied further.

The results nicely show that adding another perforator from a lower zone, given the dominant perforator is located in VZ1 - increases the area of perfusion and thus now leave the reconstructive surgeon with a tool to objectively assess and show this - even though the number of patients are small.

Response 5: Thank you very much for encouraging comments on our manuscript.

DISCUSSION
The discussion covers the relevant topics on ICG-angiography and assesses the different questions/draw-backs on using the technology. The relevant literature is included and discussed. However, the authors should cite one of the papers systematically reviewing and performing meta-analyses on ICG-Angiography in breast reconstructive procedures.

Response 6: We added a reference systematically reviewing and performing meta-analyses on ICG-Angiography in breast reconstructive procedures. Also, some modifications were made in the main text to describe the article.
Changes in the text: "the incidence of fat necrosis has been shown to range from 8.3 percent to 29 percent among patients who have undergone intraoperative ICG angiography to reduce fat necrosis in DIEP flap breast reconstruction." was changed to "the use of ICG angiography in DIEP flap breast reconstruction has been demonstrated to be associated with a reduced risk of fat necrosis,(26) and the incidence of fat necrosis has been shown to range from 8.3 percent to 29 percent among patients who have undergone intraoperative ICG angiography." (see Page 12, line 255-257). A reference "26. Lauritzen E, Damsgaard TE. Use of Indocyanine Green Angiography decreases the risk of complications in autologous- and implant-based breast reconstruction: A systematic review and meta-analysis. J Plast Reconstr Aesthet Surg 2021;74:1703-17." was added.

The issue of race and thereby donor site availablity is covered nicely.
Line 262: Please also add a small comment on the transferablity of studies on mastectomy skin flap necrosis to free or pedicled flaps.

Response 7: We added a sentence to describe the primary result of the study by Gorai et al. (reference #34) to demonstrate the association between perfusion time and clinical outcome.
Changes in the text: "They demonstrated that necrotic skin area showed significantly longer time to reach half of maximal perfusion than viable area." was added to the third paragraph of DISCUSSION section (see Page 13, line 274-275).

Line 272: the authors could add a small paragraph on ICG-angiography and venous drainage as another topic - there is one letter published on this topic...

Response 8: Thank you for letting us know that there is a report that evaluated venous drainage by using ICG angiography. "Although ICG angiography cannot visualize flap venous drainage," was removed from the current manuscript (see Page 14, line 282). We found a letter by Kurita et al. that presented evaluation of venous drainage by using ICG angiography in fingertip replantation. We added a small paragraph to discuss the venous drainage (see Page 14, line 289-294).

35. Kurita M, Shiraishi T, Ozaki M, et al. Usefulness of microscope-based ICG videoangiography for detection of the dominant drainage vein in fingertip replantation. J Plast Reconstr Aesthet Surg 2010;63:2200-1.

CONCLUSION
Is justified and concludes the paper sufficiently
FIGURE 1
Depicts and illustrates the topic nicely - please trim the picture, leaving out the more "bloody" parts. Figure legend relevant and sufficient.

Response 9: Redundant parts were removed from Figure 1B according to your comment (see Figure 1B).

FIGURE 2
Illustrates the concept of vertical zones in conjunction with the CT-angiography.
FIGURE 4
Could be improved by adding a spacing between patient 16 and 17 - and adding vertical-spacing and non - vertical - spacing to the figure

Response 10: Figure 4 was modified accordingly.

FIGURE 5
Very good and illustrative -no comments
TABLE 1
Please specify in the table that Cohort 1 is the vertical zone 1 and likewise for zone 2 - this increases the readability of the table

Response 11: Daggers and relevant descriptions were added to the Table 1.

TABLE 2 AND 3
Demonstrates and specifies the data supplied in the text. Table legends could be improved by defining the vertical spacing and the non - vertical spacing concept in the heading

Response 12: We agree with you that it is necessary to improve readability of Table 2

and 3. Rather than modifying table legend, we added daggers and descriptions regarding cohort 1 and 2 to maintain consistency with table 1, which was modified according to your previous comment.

TABLE 4
Highlights the findings of the paper. Table legend - as above

Response 13: Daggers and relevant descriptions were added to the Table 4 as above.

Reviewer B

This is a great paper about the perfusion of DIEP free flaps and the supra or infra umbilical location of the perforator, with consistent results. Clinically, it is already known that it is better an infraumbilical the perforator, but this study quantifies the repercussion of the localization and how much the perfusion of the flap changes.
The manuscript is clear, well-referenced and the methodology is impeccable.
In my humble opinion, there is only one mistake: in the text the authors say that they included 67 patients (page 8, paragraph 178) but the figure 3 shows that 70 patients met the inclusion criteria. If there is an explanation for this difference, the authors should justify it in the material and methods section.

Response: Thank you very much for your encouraging comment on our manuscript. We apologize that we included an old version of Figure 3 to the submitted manuscript. 67 patients were included in this study and we changed the old version of Figure 3 to a current version. Thank you for pointing out an important mistake.

Reviewer C

This is an interesting study that presented valuable findings.

The cohort 1 and 2 appeared in the results without description in the method.

Response: Thank you for your praise and encouragement. In the current manuscript, we described regarding definition of cohort 1 and 2 in the subheading "Assignment of Patients Group" in the PATIENTS AND METHODS section as below.
"Patients were categorized into two groups according to the vertical location of the dominant perforator. Patients who had a dominant perforator in vertical zone 1 and 2 were classified into cohorts 1 and 2, respectively." (see Page 8, line 156-157).
Daggers and descriptions regarding definition of cohort 1 and 2 were added to the Table 1 and 2 to enhance readability.

Which cohort do the patients with fat necrosis belong to?

GS GLAND SURGERY
AN OPEN ACCESS JOURNAL DESCRIBING NEW FINDINGS IN GLAND SURGERY
JOURNAL CITATION REPORTS IMPACT FACTOR 2.16

Response: Among the three cases with fat necrosis, two cases belong to cohort 1 and one case belongs to cohort 2. We did not perform statistical analysis to compare the incidence of fat necrosis because the number of total cases is too low to demonstrate the difference between the two cohorts.
Changes in the text: "The incidence of fat necrosis was 4.5 percent (3 of 67 patients)," was changed to "The incidence of fat necrosis was 4.5 percent (3 of 67 patients), including 9.5 percent in cohort 1 (2 of 21 patients) and 2.2 percent in cohort 2 (1 of 46 patients)." (see Page 9, line 187 to Page 10, line 188).

Reviewer D

I enjoyed reading the article. It is well written and deals with an important aspect in DIEP breast reconstruction. Indeed, there has been most focus on the differences between medial and lateral row perforators. The results from this article are there for an important contribution to those working with DIEP breast reconstruction.

I have some comments.

Comment 1
Line 73. I suggest deleting "preferentially" as the donor side of the DIEP flap is the lower abdomen.

Response 1: Thank you for taking the time to review our article and give your valuable feedback. According to your comment, "preferentially" was removed from the current manuscript (see Page 5, line 74).

Comment 2
Line 82. Kelly and Pacifico published in 2013 an article dealing with DIEP flaps based on a para-umbilical perforator and the consequences of designing the DIEP flap on such a perforator. I would include this reference and discussion these findings in the discussion as it supports a part of the authors findings.

Jamie A. Kelly, Marc D. Pacifico. Lateralising paraumbilical medial row perforators: Dangers and pitfalls in DIEP FLAP planning A systematic review of 1116 DIEP flaps. Journal of Plastic, Reconstructive & Aesthetic Surgery (2014) 67, 383e388.
They concluded "The perforasome concept has improved our understanding of perfusion from perforators in DIEP flaps. However, when the umbilicus presents a physical barrier to blood vessel passage resulting in lateralizing paraumbilical medial row perforators it appears an exception to the "perforasome" rule. Our experience suggests that when a paraumbilical perforator is harvested, a hemi flap is safe but caution should be exercised when further

volume is needed from the contralateral side".

Response 2: We feel grateful for informing us an important preceding study. Suggested reference was added to the INTRODUCTION section as No. 17 reference (see Page 5, line 81). "Kelly et al. demonstrated that umbilicus act as a physical barrier to paraumbilical perforators of DIEP flap and contralateral perfusion could be compromised when paraumbilical perforators were harvested" was added to the 4th paragraph of the DISCUSSION section (see Page 14, line 299-301).

Comment 3
Line 115. I would suggest defining "inset rate" more clearly even when table 1 makes is clear for the reader. Instead of "inset rate" one could use percentage of total flap weight used for DIEP flap breast reconstruction.

Response 3: We agree with you that it is necessary to describe the definition of "inset rate". We added "The flap inset rate was defined as the proportion of inset flap to harvested flap weight." To the subheading "Perfusion Assessment Using Indocyanine Green Angiography" of the PATIENTS AND METHODS section (see Page 6, line 104-105).

Comment 4
Line 121-123. The authors write:"The first stage ICG angiography was performed after completion of intramuscular dissection of targeted perforators."

Does this mean that there is no continuity in the source DIEA vessel, meaning is the cranial (distal) end of the DIEA occluded? Such will have an impact on perfusion through the dominant perforator.

Response 4: We performed the first stage ICG angiography after dominant perforator was completely separated from the rectus abdominis muscle to minimize the potential effect of intramuscular dissection to perfusion of the flap. During the dissection, cranial end of the DIEA was ligated.
Changes in the text: "The first stage ICG angiography was performed after completion of intramuscular dissection of targeted perforators." was changed to "The first stage ICG angiography was performed after completion of intramuscular dissection of dominant perforator and cranial end (superior continuation) of the deep inferior epigastric vessels were ligated." (see Page 7, line 123-124).

Comment 5
Line 135-137. The authors write: "After that, the microvascular clamps were removed, and complete pedicle dissection was performed. The second stage perfusion assessment was performed before pedicle division to evaluate effect of additional perforators on the perfusion

zone.

Like comment to line 121-123. By occluding the distal end of the DIEA, the flow through the remaining perforators will increase as it can not be bypassed. This must be made clear to the readers, so they know that measurements were made under the same conditions. If complete pedicle dissection means that the distal end of the DIEA is occluded than perfusion pressure through the DIEA changes and therewith through the perforators.

Response 5: We performed the first and second stage of perfusion assessment in the same condition that the distal end of DIEA was ligated. Please refer to the response 4.

Comment 6

In the introduction (line 122-126 the authors write, “At the time of measurement, mean intra-arterial blood pressure was regulated between 70 and 90 mmHg and body temperature between 36°C and 37°C.

Both the blood pressure and body temperature have an impact on the perfusion area and influence the results from stage 1 and stage 2 if it occurs. A 20 mm Hg difference of / and 1℃ will most definitely influence the perfusion area of perforators and might be mentioned in the discussion as a limitation.

Response 6: We agree with you that the differences of blood pressure and body temperature could affect perfusion of flaps. We added “Lastly, mean intra-arterial blood pressure was regulated between 70 and 90 mmHg and body temperature between 36°C and 37°C in this study, but the differences of 20 mmHg and 1°C still have potential to affect the flap perfusion.” to the last paragraph of DISCUSSION section (see Page 16, line 340-343).

Comment 7
Line 284-286 The authors write, “lower margin of umbilical stalk as the threshold of vertical perforator location because we assumed that this line can divide perforators into two groups that have quite different characteristics in terms of flap perfusion.”

Se comment on Line 82. Kelly and Pacifico had the same idea. I would suggest mentioning their article in the discussion as it supports the authors assumption.

Comment line 290.294 The authors write "We consistently found that most of the contralateral paraumbilical area was not included in the rapid perfusion area in the first stage of perfusion assessment (for single dominant perforators in vertical zone 1), as depicted in Figure 5.”

As mentioned earlier, if the distal end of the DIEA was not occluded in stage 1 , but was in stage 2 this could contribute to an increased perfusion area. If in both stages the distal end of DIEA was occluded, the results are easier compared. According to me this has to be clarified or discussed.

Response 7: After responding to the comment 2 and 4, the issues have been resolved. Please refer to the response 2 and 4.

Comment 8
Line 466. Fig 3. Of the 101 patients, 70 met the Inclusion criteria.

The authors mention in the manuscript 67 patients. Have I misunderstood something?

Response 8: We apologize that we included an old version of Figure 3 to the submitted manuscript. 67 patients were included in this study and we changed the old version of Figure 3 to a current version. Thank you for pointing out an important mistake (see Figure 3).

Comment 9

Line 499 Table 3

The vertical spacing group had a larger % of patients with a pfannenstiel incision This form of previous surgery has been reported to have a form of ischemic preconditoning effect. Could this have contributed to the increased perfusion area and mafe te differences between the cohorts larger?

As the authors write (line 100) “Patients who had midline vertical abdominal scars were also excluded, as these scars can affect flap perfusion contralaterally to the pedicle”. As the article by Mahajan et al showed, the pfannestiel incision also influences flap perfusion. A the authors did not exclude the pfannestiel incision due to previous surgery it might be correct to mention in the discussion the findings by Mahajan et al and mention that this could possible influence the results.

Reference Ajay L. Mahajan, M.Med.Sc., M.D. Assaf Zeltzer, M.D. Karel E. Y. Claes, M.D. Koenraad Van Landuyt, M.D., Ph.D. Moustapha Hamdi, M.D., Ph.D.. Are Pfannenstiel Scars a Boon or a Curse for DIEP Flap Breast Reconstructions? Plast. Reconstr. Surg 2012129: 797-805

Response 9: We did not exclude patients who had Pfannenstiel incision scars because the difference of proportion of each scar did not reach statistical significance. However, as

you commented, there were more patients who had Pfannenstiel incision scars in the vertical-spacing group and the difference might affect the study results. We investigated further on this issue. Among the 16 patients in the vertical-spacing group, 9 patients had Pfannenstiel incision scars and 7 patients did not. To evaluate the potential effect of Pfannenstiel scar on the perfusion-related outcomes, we compared perfusion-related variables between the patients with Pfannenstiel incision scars and other scars. Below is the result.

Variable	Pfannenstiel incision scar	Other scars	*p*
Increment of perfused area, cm^2	41.1 ± 15.2	46.4 ± 12.3	0.298
Increment of perfused proportion	0.15 ± 0.04	0.20 ± 0.05	0.278
Increment of maximal distance of midline cross, cm	2.2 ± 1.3	2.3 ± 1.6	0.340

We could not observe significant differences of perfusion-related outcomes between the two groups. On the other hand, quantitative evaluation of the potential effect of Pfannenstiel incision scars on the perfusion-related outcomes is still a good candidate for further research, we added this issue as a limitation of this study.

Changes in the text: "Similarly, the potential effect of previous Pfannenstiel incision on the perfusion-related outcome was not evaluated in this study.(38) Further quantitative analysis of the effect of the Pfannenstiel incision on the perfusion-related outcome would be warranted." was added to the last paragraph of the DISCUSSION section (see Page 16, line 336-338).

Reviewer E

The authors described the concept of vertical Zone classification in this manuscript. I considered the multiple perforator DIEP and MS-TRAM were superior than the single perforator DIEP at the point of flap circulation. This concept of vertical Zone was very interesting and well described. Therefore, I think this manuscript is worth for publication.

Response: Thank you very much for your supporting comment on our manuscript.