A Comparison of Three Methods for the Analysis of Skin Flap Viability: Reliability and Validity

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Objective: Technological advances have provided new alternatives to the analysis of skin flap viability in animal models; however, the interrater validity and reliability of these techniques have yet to be analyzed. The present study aimed to evaluate the interrater validity and reliability of three different methods: weight of paper template (WPT), paper template area (PTA), and photographic analysis.

Approach: Sixteen male Wistar rats had their cranially based dorsal skin flap elevated. On the seventh postoperative day, the viable tissue area and the necrotic area of the skin flap were recorded using the paper template method and photo image. The evaluation of the percentage of viable tissue was performed using three methods, simultaneously and independently by two raters. The analysis of interrater reliability and viability was performed using the intraclass correlation coefficient and Bland Altman Plot Analysis was used to visualize the presence or absence of systematic bias in the evaluations of data validity.

Results: The results showed that interrater reliability for WPT, measurement of PTA, and photographic analysis were 0.995, 0.990, and 0.982, respectively. For data validity, a correlation >0.90 was observed for all comparisons made between the three methods. In addition, Bland Altman Plot Analysis showed agreement between the comparisons of the methods and the presence of systematic bias was not observed.

Innovation: Digital methods are an excellent choice for assessing skin flap viability; moreover, they make data use and storage easier.

Conclusion: Independently from the method used, the interrater reliability and validity proved to be excellent for the analysis of skin flaps' viability.

Keywords: test validity, reproducibility of results, surgical flaps and tissue survival

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INTRODUCTION

GRAFTS AND FLAPS are transplanted tissues often used in aesthetic and reconstructive plastic surgeries^{1,2} as important resources in skin loss due

to trauma or surgical procedures. Although grafts and flaps have been widely used, important complications can lead to tissue necrosis.^{3,4} Consequently, other interventions become necessary to repair it, and therefore, prolong the duration of hospital stays

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and delay return to daily activities of the patient.⁵ Based on this, a considerable amount of research has been done with the aim to increase tissue viability.^{6–12}

The evaluation of the results of interventions performed in experimental models of skin grafts and flaps include some methods, such as gene expression analysis,^{10,13} protein expression analysis,^{11,12,14–16} assessment of histological changes,^{11,12,15} and the percentage of viable tissue.^{6,7,17,18} The latter is widely used for being fast, inexpensive, and efficient. Three methods can be used to evaluate the percentage of viable tissue: weight of paper template (WPT),^{17,18} paper template area (PTA),¹⁹ and photographic area (PA).^{11,14,16}

The paper template method was first described by Sasaki and Pang in 1980²⁰ and since then it has been used to evaluate the percentage of flap necrosis on the seventh postoperative day. This technique takes into consideration the weight of the template paper where the flap has been drawn to calculate its viability. Technological advances have provided some new alternatives to evaluate skin flap viability; among these are the digitization of the paper template and the measurement of area through software. Another even more recent alternative is the photographic analysis of the skin flaps. Despite the wide use of these assessment tools, no reports on the comparison of interrater validity and reliability of these methods have been found in literature. It is well known that reliability and validity are two most important technical properties that indicate the quality and usefulness of an assessment test.²¹ Therefore, the purpose of the present study was to evaluate the interrater validity and reliability of the three different methods (WPT, PTA, and PA) used to assess the viability of skin flaps.

CLINICAL PROBLEM ADRESSED

Flap necrosis is an undesirable postoperative outcome; therefore, further studies are necessary to reduce complications. Novel techniques for the assessment of skin tissue viability have emerged, however, the feasibility of these innovations has yet to be evaluated, otherwise, the interpretation of results may be inaccurate and would directly affect the study results, and indirectly, the use of the methods studied in clinical practice.

MATERIALS AND METHODS

Animals

Sixteen male adult Wistar rats (*Rattus norvegicus*: var. *albinus*, *Rodentia Mammalia*) (259.35 ± 21.85 g

body weight) from the Vivarium of the Universidade Federal de São Carlos (UFSCar) were used in the study. Postoperatively, the animals were kept in individual cages. The temperature was maintained at $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ with a 12-h light–12-h dark cycle. The rats were given *ad libitum* access to food and water during the experimental protocol.

This study was approved by the Research Ethics Committee of the institution (protocol N. 6224290515).

Surgical procedure

The animals were anesthetized with intraperitoneal injection of ketamine (95 mg/kg) and xylazine (12 mg/kg; Syntec®, Santana de Parnaíba–SP, Brazil). The rats were positioned over a flat surface, with extended limbs, their backs were shaved and a random pattern, cranially based dorsal skin flap, measuring 10 cm long and 4 cm wide,²² was planned. The flap was then incised with scalpel and elevated from the deep muscular fascia, including the superficial fascia, panniculus carnosus, and skin. After flap elevation, a plastic barrier (Film F1), cut with the same dimensions, was placed between the flap and its donor bed to prevent flap revascularization by new blood vessel growth from the recipient bed. Flaps were then sutured with 4-0 nylon stitches.

Evaluation of viability

Viability was evaluated using three different methods: WPT, PTA, and photographic analysis (PA). The three methods were applied independently and simultaneously on the seventh postoperative day by two blinded raters. Before the viability evaluation of the skin flap, the animals were anesthetized by intramuscular injection of ketamine and xylazine for analysis.

Weight of paper template

The percentage of skin flap necrosis area was calculated on the seventh postoperative day through the WPT method described by Sasaki and Pang.²⁰ The animals were positioned over a flat surface, with extended limbs. The limit between viable tissue (soft, rosy, warm, and with hair skin) and necrotic tissue (dark, cool, and without hair skin) was demarcated in the animals. A mold of entire flap was then drawn and cut out in a writable transparency paper (tracing paper), being checked in a precision balance (± 0.0001 g error). It was cut out from this fragment, just the correspondent area to flap necrosis, which was also checked (Fig. 1A).

After that, the following formula [1] was used:

$$\% \text{ of necrosis area of the flap} = \frac{\text{weight of paper template of flap necrosis}}{\text{weight of paper template of total area of flap}} = X \ 100 \quad [1]$$

Figure 1. WPT (A) drawing the skin flap and its necrotic area in a transparent (vegetal) paper; (B) mold drawn; (C) mold cut; (D) checking the weight of the mold of entire flap in a precision balance; (E) splitting the viable and necrotic areas; (F) checking the weight of the mold representing the necrotic area in a precision balance; PTA (G) drawing the skin flap and its necrotic area in a transparent (vegetal) paper; (H) mold drawn; (I) measurement of the entire flap area; (J) measurement of the necrotic area; PA (K) standardized photo of the entire skin flap; (L) measurement of the entire skin flap's area; (M) measurement of the necrotic area. WPT, weight of paper template; PTA, paper template area; PA, photographic area.

Paper template area

For the analysis of PTA, the molds designed in tracing paper were scanned (LaserJet100 color MFP M175a) with a 300 dpi resolution and the flap survival area was calculated using image analysis software ImageJ.* The total flap area was demarcated and then only the necrosis area was delineated. The values obtained were expressed in pixels (Fig. 1B). The following formula [2] was then applied:

Photographic analysis

After positioning the animals, a photograph was taken at a distance of 20 cm using a digital camera. Following, these photographs were analyzed using ImageJ software. The same procedure was performed where the total flap area was demarcated and only then the necrosis area was delineated. The values obtained were expressed in pixels (Fig. 1C). The following formula [3] was then applied:

$$\% \text{ of necrosis area of the flap} = \frac{\text{number of pixels of necrosis area of the flap}}{\text{number of pixels of the total area}} = X \ 100 \quad [2]$$

*<https://imagej.nih.gov/ij/download/>

$$\% \text{ of necrosis area of the flap} = \frac{\text{number of pixels of necrosis area of the flap}}{\text{number of pixels of the total area}} \times 100 \quad [3]$$

Euthanasia of animals

The animals were euthanized individually by carbon dioxide asphyxia on the seventh day after surgery.

Statistical analysis

The intraclass correlation coefficient (ICC) was used to assess the validity between methods (WPT, PTA, and PA) and also the interrater reliability. The interpretation of the strength of the linear relationship between the variables was performed according to the one proposed by Koo and Li.²² Based on the 95% confident interval of the ICC estimate, values less than 0.5, between 0.5 and 0.75, between 0.75 and 0.90, and greater than 0.90 are indicative of poor, moderate, good, and excellent reliability, respectively.

The Bland Altman Plot Analysis was used to visualize the presence or absence of systematic bias in the evaluations of validity.²³ Data were expressed as mean $\pm$ standard deviation. The SPSS (20) statistical software package was used.

RESULTS

Interrater reliability

The observed interrater reliability values for all the tests were excellent (Table 1). Interestingly, it was noted that the photographic analysis of the flap area showed greater difference between the percentage of necrosis between raters A and B (2.28%).

Validity

The ICC analysis between the different evaluation methods showed excellent correlation, as well

as the 95% confidence intervals for the different comparisons, as shown in Table 2. With the Bland Altman Plot Analysis, there was also agreement between the comparisons of the methods used: WPT $\times$ PTA, WPT $\times$ PA, and PTA $\times$ PA, as shown in Figures 2–4.

DISCUSSION

The analysis of skin flap and skin graft viability after a surgical procedure is a method widely used to compare and demonstrate the effectiveness of treatments. With the latest research findings, more accurate and up-to-date information are necessary to evaluate this complication, considering that the paper template method proposed by Sasaki and Pang was first described in 1980, when there was restricted access to digital devices. In this method, a transparent (vegetal) paper is placed on the area to be evaluated and then the drawing of the whole skin flap area is delimited, as well as the necrotic and viable areas. Afterward, this paper containing the total area and necrotic area of the skin flap has its weight measured on a precision scale. A disadvantage of this technique is that it cannot be redone after separation of the viable and necrotic area. Therefore, an alternative method is to scan the paper template to later carry out the measurements of the areas, total, necrotic, and viable, using a computer software. In this way, the template is stored digitally and whenever necessary it can be reassessed several times. However, this method still had the limitation that only the first evaluator who performed the paper template identified the necrotic and viable areas. With this concern in mind, the method of photographic analysis of skin flap area was developed, since it allows the digital registration and storage of the skin flap area and it can be evaluated and reevaluated by different evaluators and in different places.

Table 1. Values for mean and standard deviation of each test and each rater

Instruments	Rater	<i>M</i> $\pm$ <i>SD</i>	ICC	95% Confidence Interval		F-test with True Value 0			
				Lower	Upper	Value	df1	df2	Sig
WPT	A	49.70 $\pm$ 19.53	0.995	0.985	0.998	384.20	15	15	0.000
	B	50.62 $\pm$ 19.61							
PTA	A	49.95 $\pm$ 19.69	0.990	0.972	0.997	205.08	15	15	0.000
	B	50.57 $\pm$ 19.73							
PA	A	49.99 $\pm$ 19.68	0.982	0.950	0.994	111.678	15	15	0.000
	B	52.27 $\pm$ 20.58							

Instruments: WPT, weight of paper template; PTA, paper template area; PA, photographic area; ICC, intraclass correlation coefficient.

Table 2. Values for the validity tests

Comparisons	ICC	95% Confidence Interval		F-test with True Value 0			
		Lower	Upper	Value	df1	df2	Sig
WPT $\times$ PTA	0.999	0.997	1	1905.85	15	15	0.00
WPT $\times$ PA	0.986	0.959	0.995	137.64	15	15	0.000
PA $\times$ PTA	0.988	0.982	0.996	162.67	15	15	0.000

Figure 2. Differences between WPT and PTA.

Many authors have been using alternatives to evaluate tissue viability, as described previously. However, only a few have analyzed the validity of the tests proposed,^{24,25} and so far, none of them has evaluated interrater reliability specially in the skin flap model. das Neves *et al.*²⁴ tested the reliability of the paper template method using the semiautomatic image analysis (Minimop). The ICC obtained (0.64) with a confidence interval of 95% indicated a substantial level of concordance between the methods.

The results of the present study found a validity value higher than 0.98 for all comparisons, which showed that, regardless of the method used, an excellent relationship was observed between the methods. Therefore, it is possible to compare the studies that have investigated the effectiveness of

Figure 3. Differences between WPT and PA.

Figure 4. Differences between PTA and PA.

some treatments to increase the skin flap viability, but using different methods. Sano *et al.*²⁵ have used rat transverse rectus abdominis musculocutaneous flaps to compare WPT with digital planimetry to measure the necrosis area. Although no significant differences were found between both methods, the latter is less used.

Nowadays, validity of an assessment tool can be determined not only by analyzing the relationship between variables, but also by using the Bland Altman Plot Analysis, a graphical method to compare two measurement techniques.²⁶ In the present study, the results of both assessment tools showed that the three different methods for the analysis of skin flap viability can be applied due to their high degree of correlation and little disagreement. The interrater reliability is another alternative method to be considered. It provides a way of quantifying the degree of agreement between two or more raters in the measurement procedures for the acquisition of data.²¹ In our study, we evaluated the interrater reliability for the three methods studied. When evaluating the interrater reliability for each method, it was observed that the photographic analysis showed the greatest difference in the percentage of necrosis between evaluator A and B (2.28%). This finding may be related to the greater difficulty in confirming two characteristics that define the necrotic area, the cold and hard skin, which through a photograph cannot be properly evaluated. However, this small interrater difference does not compromise the validity or reliability of the measurement methods used in this study, since they presented a correlation between raters greater than 0.98 for the three methods.

INNOVATION

Our study proved to be effective in using three assessment methods by different raters, to assess the viability of skin flaps in rats. The photographic analysis can be used to evaluate the viable area and tissue necrosis by multiple raters, in different places. Images can be stored and reviewed to solve doubts. WTP does not allow reassessment after use. When WPT is the only choice, the ImageJ software should be used to measure PTA, thus, the scanned images can be evaluated and reviewed by different raters.

CONCLUSION

In summary, this study showed the validity of three different methods (WPT, paper template area, and photographic analysis) to evaluate the viability of the skin flap. Moreover, the interrater reliability for all methods was excellent. Thus, we can suggest that the three methods are valid and reliable for the analysis of the viability of the skin flap.

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AUTHOR DISCLOSURE AND GHOSTWRITING

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Abbreviations and Acronyms

ICC = intraclass correlation coefficient
PA = photographic area
PTA = paper template area
WPT = weight of paper template