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Diluted Indocyanine Green Angiography: A Novel Approach to Free Flap Perfusion Evaluation in Reconstructive Microsurgery

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Background: Assessing perfusion in free flaps is crucial in clinical practice. Indocyanine green (ICG) angiography offers a more objective and reproducible method, utilizing near-infrared imaging to monitor flap vascularization. This study aims to evaluate the efficacy of diluted ICG as a tool for assessing free flap perfusion.
Methods: This pilot randomized clinical trial compares the fluorescence concentrations of ICG at 3 different dilutions: 5 mg/mL (standard concentration), 2.5 mg/mL (half the standard concentration), and 0.5 mg/mL (one-tenth of the standard concentration). Inclusion criteria required participants to have serum albumin levels above 3 g/dL, hemoglobin levels over 10 g/dL, and no comorbidities. Participants were randomized into 3 groups based on ICG concentration. Fluorescence analysis was performed using ImageJ software to determine mean gray values. Both surgeons and data analysts were blinded to the ICG concentrations administered, ensuring unbiased evaluation.
Results: Forty-five patients undergoing free flap surgery, predominantly male (60%) with a mean age of 37.76 ± 19.79 years and a mean body mass index of 21.23 ± 4.49 kg/m^2, primarily received osteoseptocutaneous fibular free flaps (46.67%), with an average skin flap area of 66.07 ± 46.94 cm^2. The primary etiology was underlying tumors (84.4%), with the head and neck as the most common reconstruction site (82.2%). The superior thyroid artery was the most frequently used recipient vessel (37.78%). Analysis revealed mean gray values of 64.10 ± 8.27 (5 mg/mL), 79.03 ± 2.7 (2.5 mg/mL), and 33.56 ± 3.47 (0.5 mg/mL), with 2.5 mg/mL yielding the highest value ($P < 0.001$).
Conclusions: Findings suggest using 2.5 mg/mL concentration enhances fluorescence emission, offering a dosage alternative in clinical practice. *(Plast Reconstr Surg Glob Open 2024; 12:e6280; doi: 10.1097/GOX.0000000000006280; Published online 5 November 2024.)*

INTRODUCTION

Indocyanine green (ICG) angiography represents a novel approach for the assessment of tissue perfusion, applicable in plastic surgery for both intraoperative appraisal of pedicled flap perfusion and pre- and intraoperative evaluation of free flap perfusion.[1] However, the adoption of intraoperative ICG dosage entails significant considerations, as there is minimal basic scientific

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evidence to establish the correlation between different concentrations and their impact.

The use of ICG angiography is extensive and versatile, allowing for the detection of various conditions, such as aneurysms, and specifically, for the assessment of free flaps.[2,3] Despite its potential to enhance clinical evaluation, the use of ICG remains uncommon in our center, largely due to its rarity in Indonesia. Moreover, ICG has been strongly associated with a reduction in partial free flap loss and the need for re-exploration surgery.[4]

Given the potential benefits offered by diluted ICG, this concept remains hypothetical and unsubstantiated. Therefore, the principal aim of this study is to investigate the efficacy of using a minimal concentration of ICG for assessing free flap perfusion in human subjects.

Takeaways

Question: How to achieve efficacy of employing a minimal concentration of indocyanine green (ICG) angiography for the assessment of free flap perfusion in human subjects?

Findings: The ICG concentration and gray values of patients who underwent free flap surgery showed significant association. The 2.5 mg/mL ICG concentration exhibited a significant difference and yielded the highest mean gray value, demonstrating the most favorable fluorescence emission for evaluating free flaps in humans.

Meaning: The ICG 2.5 mg/mL concentration has potential as a substitute for the standard 5 mg/mL concentration in clinical practice.

METHODS

Subjects

A randomized clinical trial was conducted on patients who underwent reconstructive microsurgery using free flaps at our institution from July 2022 to July 2023. Subjects were meticulously selected based on specific health parameters as inclusion criteria, and block randomization was used for the subjects' ICG concentration assignment. Eligible individuals included those who had successfully undergone microreconstruction surgery utilizing a viable free flap, had no prior surgical intervention in the area under evaluation, and whose donor area for the free flap had no history of trauma or surgery. Additionally, subjects were required to exhibit serum albumin levels exceeding 3 g/dL and hemoglobin levels surpassing 10 g/dL, and have no comorbidities to be included in the study.

The exclusion criteria for this study encompassed patients with a history of allergy or hypersensitivity to iodine or ICG, those with renal insufficiency or undergoing routine hemodialysis, and individuals regularly consuming sodium bisulfites. Additionally, patients with hepatic dysfunction, those regularly taking anticonvulsant drugs, and those experiencing complications during and postoperation were excluded. Furthermore, patients with psychopathology and/or mental disorders, and those whose transferred free flap had experienced trauma or damage due to external factors during care were deemed unsuitable for inclusion.

This study received approval from the Ethical Committee of the Faculty of Medicine, University of Indonesia/Dr. Cipto Mangunkusumo Hospital, Indonesia, and has been registered as a clinical trial under protocol number NCT06220357, verified on January 23, 2024.

ICG Dilution

This study utilized ICG for injection (Aurogreen; Aurolab, Tamil Nadu, India), with each vial containing 25 mg of lipophilic powder, the active ingredient being ICG. It is packaged with 5 mL of sterile distilled water for dilution, resulting in a 5-mg/mL solution. ICG was further diluted to various concentrations using sterile distilled water. For the standard 5 mg/mL ICG concentration, 1 mL of the ICG solution was drawn from the vial using a 3-mL syringe. To achieve a 2.5-mg/mL ICG concentration, 0.5 mL of the ICG solution was mixed with 0.5 mL of distilled water using a 1-mL syringe. Similarly, for a 0.1 mg/mL ICG concentration, 0.1 mL of the ICG solution was mixed with 0.9 mL of distilled water using a 1-mL syringe. The dilution process was meticulously executed to ensure accuracy.

ICG Concentration Solution and Measurement

The study investigated 3 distinct concentrations of ICG: 5 mg/mL (100% standard concentration), 2.5 mg/mL (50%), and 0.5 mg/mL (10%), which served as the independent variables. The overall subject pool was randomly and evenly distributed into these 3 groups. Dependent variables included the fluorescence intensity emitted from each concentration, visualized by ICG contrast captured by a near-infrared (NIR) camera.

Subsequently, the 1 mL ICG solution was administered as an intravenous bolus, followed by a flush with approximately 3–5 mL of normal saline until no residue remained in the infusion line. This assessment was conducted immediately after the completion of all procedures, including the suturing of the free flap and a clinical evaluation to confirm flap vitality, thereby allowing for the consideration of additional factors that might affect flap fluorescence.

The clinical evaluation of the free flap was independently conducted by clinicians who were not involved in any other part of the research. Free flap viability was determined by comparing these evaluations—which included assessments of color, turgor, temperature, capillary refill time, and bleeding spots on flap edges—with clinical assessments, ensuring a comprehensive understanding of viability. Although the subjects were not blinded, the examination and results were conducted without their involvement, as they were unconscious during the ICG examination.

The fluorescence emitted was quantitatively analyzed using ImageJ software to ascertain mean gray values,

Disclosure statements are at the end of this article, following the correspondence information.

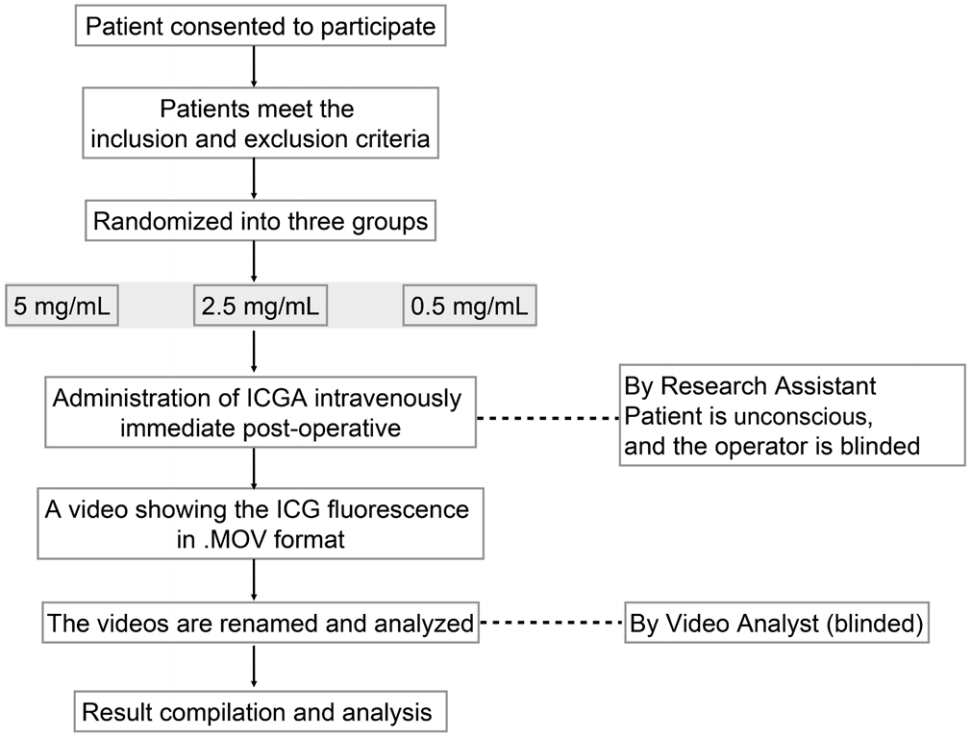

Fig. 1. Research workflow.

providing a robust measure of ICG concentration. To maintain study integrity, both surgeons performing the procedures and data analysts interpreting the results were blinded to the specific concentrations administered to each subject.

Flap Perfusion Fluorescence Evaluation

Flap perfusion fluorescence evaluation was conducted by an assessor who examined postoperative NIR camera imaging results relative to ICG fluorescence in the flap area. The assessor was blinded to which concentration was used for every image sequence analyzed. Imaging was captured using an NIR camera (Fluoro 4000XL, Tohaoptics, Japan) positioned 15–20 cm perpendicular to the flap. The outcomes were image sequences converted from video format (.MOV extension) at 4 frames per second. The mean gray values from each image were calculated using ImageJ.

To calculate the mean gray values, the software first determines the gray value of each pixel, which represents the intensity or level of grayness in a particular pixel, measured on a scale from white (0) to black (255) in an 8-bit image. The mean gray value is then calculated by summing all the gray values within the selected area and dividing this sum by the number of pixels in that area. The measured area is adjusted according to the flap's skin paddle. The mean gray value is unitless.

The mean gray values create a sequence of data, starting from the lowest (before ICG enters the flap), peaking when fluorescence is at its maximum, and then diminishing as ICG passes through the flap. The highest mean gray value (the peak) is selected for analysis, as it represents the maximum fluorescence capability of the given concentration. This process is illustrated in Figure 1.

Statistical Analysis

Categorical data were presented as frequency (percentage), whereas numerical data were presented as mean ± SD or median (minimum – maximum), depending on the data distribution. The normality of numerical data was assessed using the Kolmogorov–Smirnov test. The distribution of patient characteristics between concentration groups was evaluated using χ^2 and one-way analysis of variance (ANOVA) tests. Mean differences among groups were assessed using the one-way ANOVA test. The association between patient characteristics and concentration groups was determined using the χ^2 test. When the criteria for the χ^2 test were not met, the Fisher exact test was utilized as an alternative. A *P* value of less than 0.05 was considered indicative of statistical significance. All statistical analyses were performed using IBM SPSS Statistics version 26 (SPSS, Chicago, Ill.).

RESULTS

Baseline Characteristics

We enrolled 45 patients who underwent free flap surgery, achieving an overall flap viability of 100%. The numerical data were normally distributed and are presented as mean ± SD. The majority of patients were male (60%), with an average age of 37.76 ± 19.79 years and an average body mass index of 21.23 ± 4.49 kg/m^2. The procedure predominantly involved osteoseptocutaneous fibular

Table 1. Baseline Characteristics of Patients and Flap

Patient Characteristics		N (%)	Mean ± SD
Sex	Male	27 (60)	
	Female	18 (40)	
Age (y)			37.76 ± 19.79
Body mass index (kg/m²)			21.23 ± 4.49
Flap characteristics			
Free flap type	ALT	13 (28.89)	
	FFF	21 (46.67)	
	RFFF	9 (20)	
	DIEP	2 (4.44)	
Etiology	Tumor	38 (84.44)	
	Trauma	4 (8.89)	
	Infection	3 (6.67)	
Reconstruction site	Head and neck	38 (84.44)	
	Trunk and breast	3 (6.68)	
	Upper extremity	1 (2.22)	
	Groin	1 (2.22)	
	Lower extremity	2 (4.44)	
Recipient vessel used in			
Head and neck	External carotid	1 (2.22)	
	Superior thyroid	17 (37.78)	
	Lingual	2 (4.44)	
	Facial	16 (35.56)	
	Temporal superficial	2 (4.44)	
Trunk and breast	Internal mammary	3 (6.68)	
Upper extremity	Deep brachial	1 (2.22)	
Groin	Diep inferior epigastric	1 (2.22)	
Lower extremity	Dorsalis pedis	2 (4.44)	
Skin flap area (cm²)			66.07 ± 46.94

ALT, anterolateral thigh free flap; DIEP: deep inferior epigastric perforator free flap; FFF, fibular free flap; RFFF, radial forearm free flap.

Table 2. Characteristics Distribution of Patient and Flap between Concentration Groups

		ICG Concentrations			
Patient and Flap Characteristics		**5 mg/mL**	**2.5 mg/mL**	**0.5 mg/mL**	***P***
Sex	Male	10 (66.67%)	11 (73.33%)	7 (46.67%)	0.293*
	Female	5 (33.33%)	4 (26.67%)	8 (53.33%)	
Age (y), mean ± SD		39.86 ± 22.0	34.33 ± 20.08	39.06 ± 18.02	0.719†
Weight (kg), mean ± SD		57.16 ± 18.8	54.70 ± 12.4	51.06 ± 13.3	0.554†
Body mass index (kg/m²), mean ± SD		20.40 ± 5.87	20.83 ± 4.07	20.45 ± 3.18	0.461†
Free flap type	Osteoseptocutaneous	7 (46.67%)	5 (33.33%)	9 (60%)	0.343*
	Fasciocutaneous	8 (53.33%)	10 (66.67%)	6 (40%)	
Skin flap area (cm²) median (minimum–maximum)		78.75 (22.5–209.0)	43.75 (15.0–189.0)	74.50 (17.50–120)	0.173†

*χ^2 test.
†One-way ANOVA analysis.

free flaps (46.67%), with an average skin flap area of 66.07 ± 46.94 cm². Underlying tumors constituted the primary etiology (84.4%), with the primary reconstruction site being the head and neck (82.2%), where the most commonly used recipient vessel was the superior thyroid artery (37.78%), as shown in Table 1.

The subjects were randomly distributed into 3 groups, each consisting of 15 subjects, based on different ICG concentrations (5, 2.5, and 0.5 mg/mL). Upon comparing demographic variables such as sex, weight, age, types of free flaps, and skin flap area, which could potentially affect ICG fluorescence among the different concentration groups, no statistically significant differences were observed between the groups ($P > 0.05$), as demonstrated in Table 2.

Associations between the ICG Concentration and Gray Values of Patients Who Underwent Free Flap Surgery

The one-way ANOVA test revealed a significant association between ICG concentration and mean gray values ($P < 0.001$). Post hoc analysis indicated that the 2.5 mg/mL ICG concentration exhibited a significant difference and yielded the highest mean gray value, as depicted in Table 3. The fluorescence emission differences are depicted in Figure 2. From this figure, it is apparent that there is a discernible difference in fluorescence emission between the skin flap and normal skin. Moreover, the fluorescence emission shown in this figure cannot be directly correlated to the superiority of any concentration, as various free flaps exhibit different thicknesses and other factors that might influence the ICG fluorescence.

Table 3. Association between ICG Concentration and Gray Values of Patients Underwent Free Flap Surgery in Our Institution

ICG Concentration	n (%)	Mean Gray Value (Mean ± SD)	*P*	*P* between Groups
5 mg/mL	15	59.7 ± 5	<0.001*	Ref
2.5 mg/mL	15	75.9 ± 2.9		0.014*
0.1 mg/mL	15	32.7 ± 3		<0.001*

*$P < 0.05$.
Ref, reference.

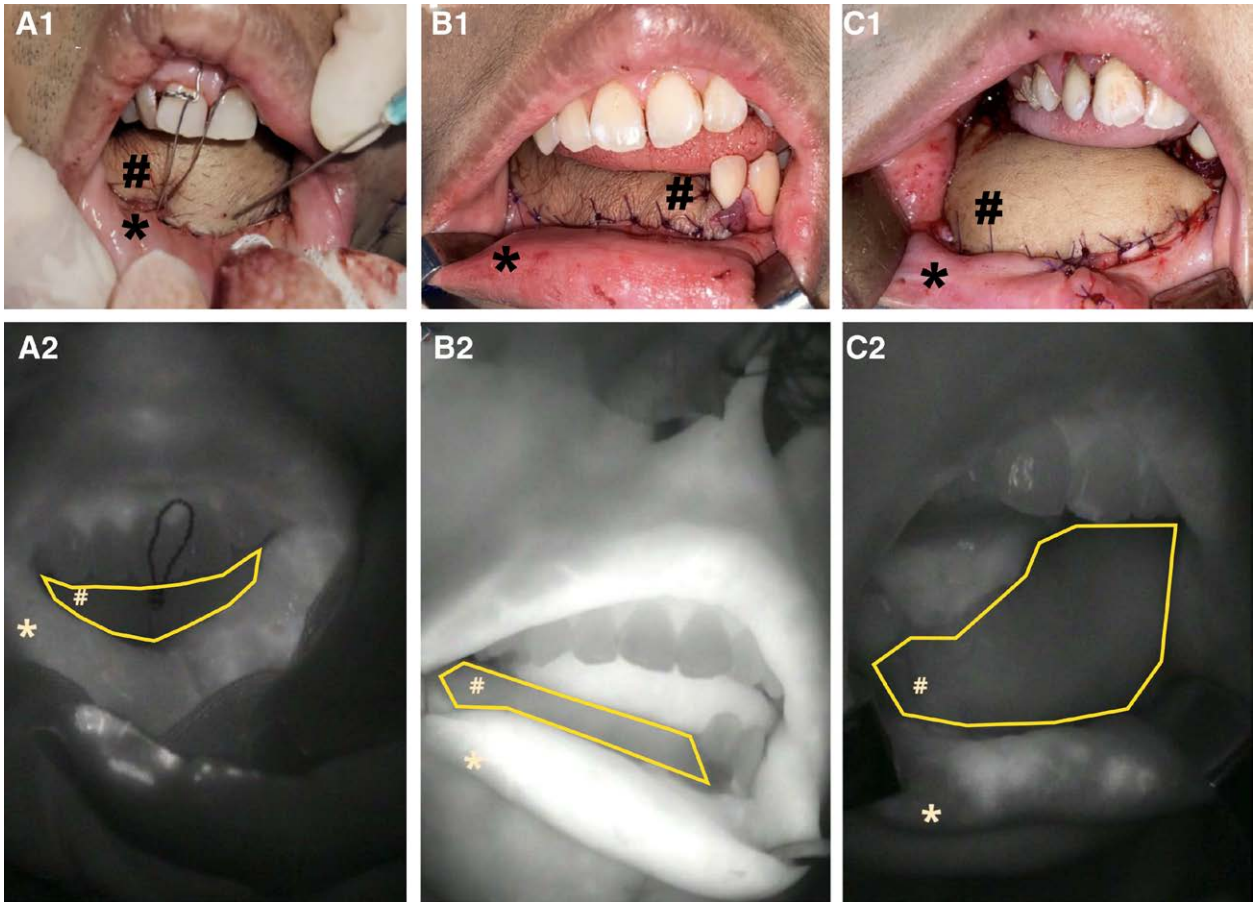

Fig. 2. Free flap imaging (normal and gray scale) at different concentrations. A1, Normal image of an intraoral flap, and (A2) the same image in grayscale, evaluated with the standard concentration of 5 mg/mL ICG. B1, Normal image of an intraoral flap, and (B2) the same image in grayscale, evaluated with a concentration of 2.5 mg/mL ICG. C1, Normal image of an intraoral flap, and (C2) the same image in grayscale, evaluated with a concentration of 0.5 mg/mL ICG. (*) indicates area of normal skin, (#) indicates area of skin flap, and the yellow line represents the skin flap area evaluated for mean gray values.

DISCUSSION

Patient Characteristics and ICG's Influence

Although ICG serves as a diagnostic tool, it shares similarities with conventional drugs in its absorption, distribution, metabolism, and excretion mechanisms. Therefore, factors that affect the drug's pharmacokinetics could potentially influence its functionality. To mitigate this bias, we ensured an even distribution of these factors among the ICG concentration groups, which is shown in Table 2.

Sex, encompassing both male and female genders, may influence drug pharmacokinetics, particularly regarding distribution and metabolic pathways. Gender-based differences in body composition—men typically have greater weight and water composition, whereas women have higher fat composition—could potentially affect ICG distribution. However, ICG distribution primarily relies on albumin, which is minimally influenced by sex, though this lacks empirical validation.[5]

Age and body weight significantly influence ICG pharmacokinetics, as dosing regimens may vary between pediatric and adult populations to achieve comparable outcomes. Previous studies have used either weight-adjusted or fixed doses of ICG. Moreover, flap characteristics could also impact the emitted fluorescence of ICG. ICG fluorescence penetration typically ranges from 10 to 20 mm. Therefore, we ensured an equitable distribution of flap types—osteoseptocutaneous and fasciocutaneous—across

groups. Additionally, the uniform distribution of skin flaps helped mitigate potential factors that could influence fluorescence emission among groups.[6]

Despite potential differences in subject characteristics, as noted above, fluorescence intensity may still be influenced. ICG serves as a global diagnostic tool to differentiate between vital and nonvital areas, aiding in free flap evaluation. Further investigation reveals that fluorescence angiography, using iterative injections of ICG at minimal dosages, is a safe and effective technique for free flap monitoring.[2]

The Optimal Concentration

This study demonstrates that a concentration of 2.5 mg/mL of ICG yields a significantly higher mean gray value compared with the standard concentration. Our findings represent a novel contribution to the field by establishing the efficacy of a diluted ICG concentration for angiography in free flap surgery. This result aligns with our previous study, which found no significant differences in rat models between the standard concentration (5 mg/mL) and the diluted concentrations of 3.75, 2.5, 1.25, and 0.5 mg/mL. However, the 2.5-mg/mL ICG concentration group received the highest scores from the assessors, indicating that its fluorescence emission was the most favored.[7]

Because neither study examined chemical changes, we can theoretically postulate that an equilibrium is attained between the dosage of ICG and the accessible plasma protein at a concentration of 2.5 mg/mL. This equilibrium facilitates favorable binding between the monomeric configuration of ICG and plasma proteins, leading to a decrease in the extent of aggregation and polymerization, and resulting in enhanced fluorescence emission. Furthermore, high concentrations of ICG are known to cause quenching effects. The precise optimal concentration in humans, however, remains unknown.[7]

This discovery addresses a notable gap in the literature regarding the optimal dosage of ICG in human subjects, presenting a potential paradigm shift in clinical practice. Previous studies have reported a wide range of dosages for ICG administration, varying from 2.5 to 25 mg for fixed doses and 0.025 to 0.5 mg/kg for weight-adjusted doses.[8–12] Notably, the most commonly observed fixed dose was 10 mg, whereas the predominant weight-adjusted dose was 0.5 mg/kg. However, there remains a lack of consensus regarding the ideal dosage of ICG for flap surgery, as highlighted by the previous study by Li et al. This absence of agreement underscores the complexity and individuality inherent in medical practice.[13]

Our results align with a recent systematic review and meta-analysis by Smit et al,[14] which concluded that ICG angiography is one of the most suitable methods for assessing intraoperative free flap tissue perfusion, leading to improved free flap survival. The findings of this study not only enrich the existing body of knowledge but also significantly impact reconstructive microsurgery by offering a more efficient alternative dosage for assessing free flap perfusion.[14]

The utilization of ICG facilitates the precise evaluation of flap perfusion by medical practitioners. The assessment is characterized by the discernment of fluorescence intensity following ICG administration, thereby delineating perfusion from the central to distal regions of the flap. This capability enables the identification of any impediments to vascularization. Such attributes underscore ICG as an effective and straightforward modality for distinguishing tissue perfusion adequacy. Nevertheless, the findings of this study indicate a 100% viability rate of free flaps, indicative of the absence of flap failure, whether partial or total.

Although our study offers valuable insights, several limitations should be acknowledged. First, the small sample size necessitates further investigation with a larger cohort to validate and extend our findings. Second, despite blinding our surgeons and data analysts to the concentration administered, the patients were not blinded, potentially introducing bias. Third, we did not assess how varying ICG concentrations affected venous outflow evaluation or fluorescence emission in nonvital or partially necrotic flaps. Finally, our study lacked patient follow-up to evaluate the long-term implications of different ICG concentrations. These limitations underscore the need for caution when interpreting our results.

Although our findings are promising, they remain preliminary. Further research is imperative to validate the efficacy of diluted ICG and strengthen its rationale for clinical application. Future studies should also aim to address the limitations of our research, such as the small sample size and absence of long-term follow-up. We posit that our findings pave the way for future investigations in this realm and hold considerable promise for advancing patient outcomes in reconstructive microsurgery.

CONCLUSIONS

The results of our study demonstrate that a diluted concentration of 2.5 mg/mL for ICG exhibits the most favorable fluorescence emission for evaluating free flaps in human subjects. This finding suggests its potential as a substitute for the standard concentration in clinical practice. Additionally, our study reinforces the fundamental scientific principles underlying the preference for lower concentrations, as adopted by several medical centers.

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DISCLOSURES

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ETHICS APPROVAL

This study was approved by the institutional review board of the Faculty of Medicine, University of Indonesia/Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

DECLARATION OF HELSINKI

The research was performed following the Declaration of Helsinki.

Written informed consent was obtained from all parents who participated in this study.

All the data generated or analyzed during this study are included in the submission. The raw data are available from the corresponding author upon reasonable request.

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