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Indocyanine green dye angiography as an adjunct to assess indeterminate burn wounds: A prospective, multicentered, triple-blinded study

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BACKGROUND:	Clinical assessment of indeterminate burn wounds has been reported to yield poor accuracy, even when performed by burn experts. Indocyanine green (ICG) dye angiography has been found to be highly accurate in assessing burn depth, but there is still limited evidence of its use in indeterminate burn wounds. This study aims to compare the accuracy of ICG angiography to that of clinical assessment in assessing indeterminate burn wounds.
METHODS:	This is a prospective, multicentered, triple-blinded, experimental study. Participants were stable patients, admitted to the hospital with burn wounds of indeterminate depth. The burn wounds were clinically assessed by an attending plastic surgeon. ICG angiography was performed and evaluated by another surgeon. Tissue biopsies were obtained and sent for histological study to be assessed as the gold standard.
RESULTS:	In the 30 burn sites that were assessed, the accuracy of ICG angiography was 100.0%, compared with 50.0% for clinical assessment ($p < 0.001$). Clinical assessment yielded a sensitivity of 33.3% and specificity of 66.7%, while ICG angiography yielded both a sensitivity and specificity of 100.0%. Therefore, the number needed to treat for using ICG angiography in indeterminate burn wounds was two.
CONCLUSION:	Indocyanine green angiography yields a significantly higher accuracy than clinical assessment in indeterminate burn wounds. This intervention can, thus, be a useful tool to aid clinical judgment.
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KEY WORDS:	Indocyanine green dye angiography; burn; indeterminate burn wound; burn assessment.

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Burn assessment in indeterminate burn wounds can be problematic.¹ Indeterminate burn wounds are defined as second-degree burn wounds in which the differentiation between superficial and deep dermal involvement cannot be made by observation alone.

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The accuracy in assessment has been reported to be 50% to 75%, even when performed by burn experts.^{1–4} A standard treatment for deep dermal burn wounds is early excision and grafting. In some centers, these wounds are excised early to reduce morbidity, infection, length of hospital stay, and cost.⁵ However, this approach leads to some patients being subjected to unnecessary surgery. Thus, a method to more accurately evaluate indeterminate burn wounds would help alleviate this issue.

Indocyanine green (ICG) dye angiography has been reported to be good in assessing burn depth (with an accuracy of almost 100%)² and predicting long-term wound outcomes. In addition, images can be obtained using this process in a short amount of time.^{6,7} In these ways, it is superior to Laser Doppler Imaging, which is another good option for evaluating burn wounds.^{6,8} The drawbacks of ICG angiography, on the other hand, are that it is an invasive procedure and that it requires the patient to be injected intravenously with ICG. However, it is considered to be safe, as it has been used for other indications for more than 40 years.⁹ Despite this, there is little information in the available literature on using ICG primarily to assess indeterminate burn wounds.⁹ This study aims to compare the accuracy of ICG angiography to that of clinical assessment in assessing indeterminate burn wounds.

METHODS

Study Design

This is a prospective, multicentered, triple-blinded, experimental study. The study was conducted and data were analyzed in accordance with the Transparent Reporting of Evaluations with Nonrandomized Designs statement.¹⁰ This trial was registered in the Thai Clinical Trials Registry, number TCTR20170821001.

Participants

Inclusion criteria were that patients were stable, older than 18 years, and were admitted to the hospital with burn wounds of indeterminate depth on any part of the body. The stable patients were defined as patient with hemodynamically stable (mean arterial pressure ≥ 65 mm Hg), urine output of 0.5 mL/kg per hour to 1 mL/kg per hour, and adequate conscious to understand the study protocol and could make decision whether to participate to the study or not. Written informed consent or fingerprint consent form was obtained from all participants. Exclusion criteria were allergy to ICG and iodides, pregnancy, and bleeding tendency. Indeterminate wound areas that contained tattoos, moles, or scars were also excluded. This study took place in Srinagarind Hospital, Khon Kaen Hospital, and Chulabhorn Hospital in Thailand.

Intervention

The study flow diagram is shown in Figure 1. Burn wounds with indeterminate depth were clinically assessed to be either

superficial or deep second degree by the attending plastic surgeon. Indocyanine green angiography was then performed by another surgeon. A single 0.5-mg/kg dose of ICG (Diagnogreen Injection; Daiichi Sankyo Propharma, Japan), similar to the amount used in ICG angiography for other indications, was injected intravenously to the patient. The Fluobeam 800 clinical system was used. It was approved by United States Food and Drug Administration and its depth of penetration was 2.5 cm, which was sufficient to evaluate full thickness of skin.^{11,12} The Fluobeam 800 machine was then activated and the area to be assessed was placed under its viewer. Digital photographs and video recordings of the area were captured until 5 min after ICG injection. Quantification of perfusion was then performed on the saved images.

After obtaining the ICG images, a punch biopsy was performed. The central part of the indeterminate wound area was infiltrated with 1 mL of 2% lidocaine with 1:100,000 epinephrine (Xylocaine; AstraZeneca BV, Zoetermeer, the Netherlands). A sample of the full thickness of the dermis was obtained using 5-mm punch biopsy. A single interrupted suture of 4-0 nylon (Ethilon; Ethicon, Inc., Sint-Stevens-Woluwe, Belgium) was placed to close the wound. The tissue was then sent for histological study.

Histological specimens were judged by using pathological criteria of assessing burn depth.¹³ There were five parameters: collagen discoloration, intercollagen basophilic material, endothelial cell necrosis, epithelial cell necrosis, and mesenchymal

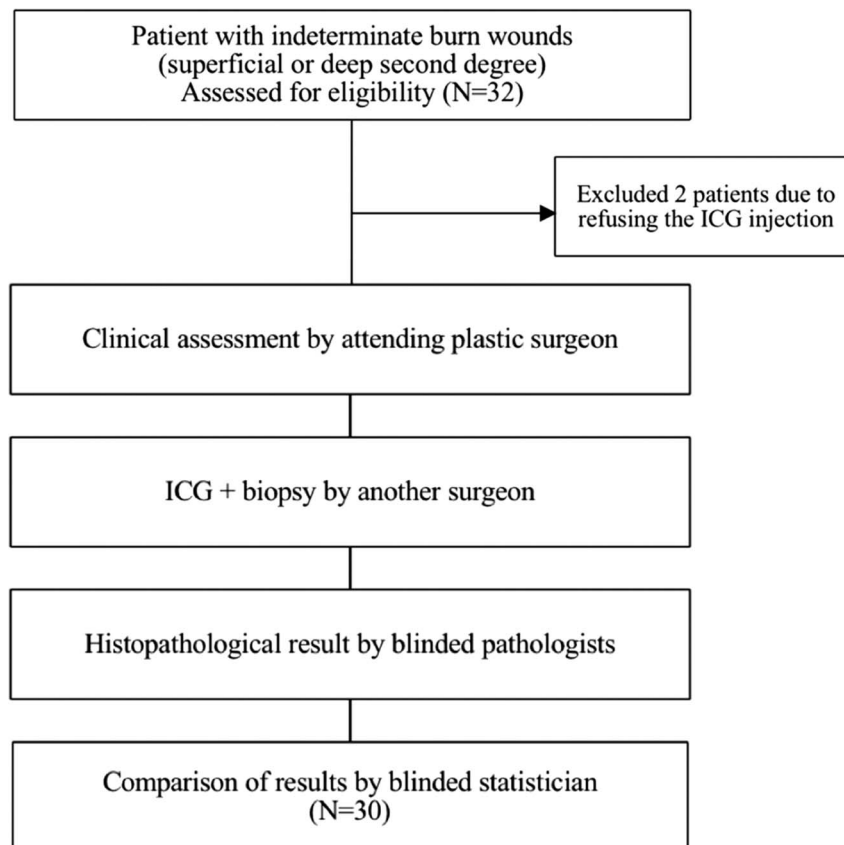


Figure 1. Study flow diagram.

cell necrosis.¹³ If these parameters were damaged greater than midpoint of the dermis or deep skin adnexal structures were totally necrosis, this specimen was considered deep burn wound.^{13–16} On the other hand, if these parameters were damaged not greater than midpoint of the dermis or there was lymphocytic infiltration in dermis without totally damaged deep adnexal structures, this specimen was considered superficial burn wound.^{13–16}

All interventions took place at bedside on the same day and occurred within 30 minutes. They were illustrated in Figure 2 and Video, Supplemental Digital Content 1 (<http://links.lww.com/TA/B265>). Indocyanine green angiography results were available immediately after finishing the intervention, while the histological results were available within 1 week. This method of intervention does not affect the participant's routine treatment decision and does not delay the treatment process.

ICG Angiography Interpretation

Percent of maximal perfusion was used to distinguish between superficial and deep second-degree burns.^{17–20} A value of less than 33% has been found to provide a good predictive value for nonviable tissue.¹⁷ Thus, superficial second-degree burns were defined as burns with maximal perfusion of more than 33% (Fig. 2B) while deep second-degree burns were defined as burns with maximal perfusion of less than 33%.^{2,17} Video,

Supplemental Digital Content 1, <http://links.lww.com/TA/B265> demonstrates how to interpret the results with examples.

Outcomes

The primary outcome is the accuracy of clinical assessment and ICG angiography. The gold standard used in this study was the histological result, which was reported to correlate with healing outcome.¹⁶ Secondary outcomes are the sensitivity, specificity, and number needed to treat for each method.

Sample Size

The accuracy of clinical assessment in indeterminate burn wounds has been reported to be 50% to 75% (approximately 62.5%), while the accuracy of ICG angiography has been found to be almost 100%.^{1,2} Comparing accuracy in paired sample with expected probability of disagreement between the two methods was $100 - 62.5 = 37.5\%$.^{1–4} With power of 0.8% and 95% confidential interval, the calculated sample size was 19.²¹

Blinding

First, the attending plastic surgeon who provided clinical assessment result and the surgeon who performed ICG angiography and tissue biopsy were not the same person and were each blinded to the other's processes. Pathological results were reported by two standardized pathologists who were also blinded



Figure 2. Illustration of study interventions. (A) Clinical assessment was made at the blue mark located on the left hand. The attending surgeon's answer was deep burn wound. (B) However, ICG angiography was then performed and revealed that the wound was superficial burn because the percentage of maximal perfusion was greater than 33%. (A scale of percent of maximal perfusion was shown on the right side of the picture) (C) A punch biopsy was performed at the blue mark. The histological result confirmed ICG angiography result that the wound was actually superficial burn. (D) Apart from the study, the wound was followed on day 3 and day 7, which also confirmed that it was superficial burn.

TABLE 1. Demographic Data (N = 30)

Demographic Data	n (%) or Mean \pm SD
Age, y	44.8 \pm 11.6
Sex	
Male	15 (50.0)
Female	15 (50.0)
BMI, kg/m ²	21.7 \pm 2.8
Time of intervention after injury, d	3.5 \pm 1.5
Total body surface area	21.5 \pm 10.1
Alcohol use	0
Smoker	0
Diabetes	3 (10.0)
Hypertension	3 (10.0)
Dyslipidemia	0
Wound location	
Trunk	14 (46.7)
Extremities	16 (53.3)
Etiology of burn	
Flame burn	14 (46.7)
Scald burn	8 (26.7)
Contact burn	4 (13.3)
Electrical burn	4 (13.3)

to the patient wounds and ICG results. Second, participants were blinded to the results. Third, data were analyzed and reported by a blinded statistician.

Statistical Analysis

Data were analyzed on an intention-to-treat basis. STATA/SE version 12 was used for the analyses. Data are reported as mean and standard deviation for all continuous variables and as number (percentage) for discrete variables. The test of equality of ROC areas was used to determine the difference in terms of accuracy between clinical assessment and ICG angiography. All test statistics were two-sided, and a *p* value of less than 0.05 was considered statistically significant.

RESULTS

Participants and Recruitment

Patients were enrolled from September 2017 to January 2018. There were 32 burn sites that met the inclusion criteria. Two burn sites were excluded due to patients refusing the ICG injection. Thus, a total of 30 burn sites were included in the final analysis. The participants were 44.8 \pm 11.6 years old. They were 50.0% male and had an average BMI of 21.7 \pm 2.8 kg/m². Demographic data is shown in Table 1.

Outcomes

Summary of results is shown in Table 2. The accuracy of ICG angiography was 100.0%, compared with 50.0% for clinical assessment (*p* < 0.001). Clinical assessment yielded a sensitivity of 33.3% and specificity of 66.7%, while ICG angiography yielded both sensitivity and specificity of 100.0%. Therefore, number needed to treat using ICG angiography in indeterminate burn wounds was two. Table 3 demonstrated clinical data of all wounds.

DISCUSSION

Interpretation

Clinical assessment yields mediocre accuracy in indeterminate burn wounds. It must be emphasized that this study included only indeterminate burn wounds. The clinicians had to be uncertain as to whether the wounds were superficial or deep prior to making his or her final diagnosis of the wounds, leading to just a 50–50 chance of correct assessment, odds that are no better than a coin toss. Our study yielded a result of 50.0% which is also correlated with those of previous studies.^{1–4} This result strongly suggests that an additional intervention should be used to improve the chances of correct diagnosis.

Indocyanine green accuracy was almost 100%, as assessed through comparison to pathological results. The fact that ICG angiography detects the presence and strength of blood flow in the wound area (one of the criteria used in making burn pathological diagnosis) could contribute to the accuracy of the method.^{14,19} Nevertheless, there are some circumstances in which ICG results could be misinterpreted. In cases of unburned skin with intact melanin, it has been reported that melanin could absorb the wavelength used in ICG, making the area appear darker, which could be misinterpreted as indicating a deep burn wound.^{2,7,20} Therefore, knowing whether the skin in the area being tested is burned or unburned is necessary prior to interpreting ICG angiography results. Because of this, ICG angiography should be used adjunctive to clinical assessment, rather than alone.

The accuracy of ICG angiography is significantly higher than clinical assessment in indeterminate wounds. Thus, ICG angiography can be a good alternative adjuvant in cases of indeterminate wounds, especially in critical areas such as face, palm, or sole of the foot (Fig. 2). Due to its wide “applications” in various departments (ophthalmology, neurosurgery, cardiac surgery, general surgery, and plastic surgery), equipment for conducting ICG angiography may already be available in many hospitals. Thus, expanding its use to burns could be both beneficial and cost-effective.⁹

Generalizability

The multicentered design in this study enhances generalizability. ICG angiography can be performed at the bedside, and obtaining results using this process can take as little as 1 minute. Indocyanine green is also safe and has only a 0.4% rate of adverse reactions.²²

Limitations

This study involved only indeterminate wounds in order to reflect the fact that surgeons would only require an adjuvant procedure when they think that the wounds are indeterminate. Thus,

TABLE 2. Summary of Results (N = 30)

	Clinical Assessment	ICG Dye Angiography
Sensitivity	33.3 (11.8–61.6)	100.0 (78.2–100.0)
Specificity	66.7 (38.4–88.2)	100.0 (78.2–100.0)
Accuracy	50.0 (32.5–67.5)	100.0 (100.0–100.0)

Values of sensitivities, specificities, and accuracies are % (95% CI).

TABLE 3. Clinical Data of All Wounds

Number	Location	Days Postburn	Burn Diagnosis		
			Clinical	ICG	Biopsy
1	Right flank	2	Superficial	Superficial	Superficial
2	Left arm	5	Superficial	Superficial	Superficial
3	Left flank	4	Deep	Deep	Deep
4	Abdomen	1	Deep	Superficial	Superficial
5	Upper chest	4	Superficial	Superficial	Superficial
6	Right leg	5	Deep	Deep	Deep
7	Left distal leg	5	Superficial	Deep	Deep
8	Upper back	2	Deep	Superficial	Superficial
9	Left hand	4	Deep	Superficial	Superficial
10	Abdomen	1	Deep	Deep	Deep
11	Right foot	5	Superficial	Deep	Deep
12	Right distal thigh	4	Deep	Superficial	Superficial
13	Abdomen	4	Superficial	Deep	Deep
14	Chest	1	Deep	Deep	Deep
15	Right proximal leg	5	Deep	Deep	Deep
16	Left flank	4	Deep	Superficial	Superficial
17	Left proximal leg	5	Deep	Deep	Deep
18	Chest	1	Superficial	Superficial	Superficial
19	Right distal leg	4	Deep	Deep	Deep
20	Left foot	5	Superficial	Deep	Deep
21	Right back	2	Deep	Superficial	Superficial
22	Right forearm	4	Deep	Superficial	Superficial
23	Left forearm	1	Superficial	Deep	Deep
24	Left leg	4	Deep	Deep	Deep
25	Upper chest	5	Deep	Superficial	Superficial
26	Right forearm	4	Deep	Superficial	Superficial
27	Right proximal leg	5	Deep	Deep	Deep
28	Right flank	1	Deep	Superficial	Superficial
29	Chest	4	Superficial	Superficial	Superficial
30	Right distal leg	5	Deep	Deep	Deep

the accuracy of the study findings was lower than in other studies that included general burn wounds (first-, second-, and third-degree burns), which might be easier to distinguish and could result in higher accuracy.

Apart from ICG angiography, there are several methods to assess indeterminate burn wounds.²³ Pathological study is a gold standard, but its drawbacks are the invasive nature of tissue biopsy, inability to assess entire area of suspected wound and waiting time required for tissue fixation and pathological report.²³ Thermography measures burn wound temperature to indicate its depth by using a notion that deeper wounds are colder than superficial wounds.^{23,24} Although this method is easy to use, its accuracy can be confounded by evaporative heat loss and a formation of granulation tissue.^{23,25} Laser Doppler Imaging is another good method for evaluating burn wounds because it is not invasive and provides good accuracy.^{8,26} It detects moving red blood cells in cutaneous microcirculation to generate images.²⁷ It has some drawbacks that it requires immobilization of the scanned part, long scanning time, and limited depth of penetration.²⁷ Indocyanine green angiography can cope with these drawbacks. It provides real-time images, which the scanned part can be mobilized, and its depth of penetration is 2.5 cm, which is adequate for full skin thickness scan.¹¹

Although ICG angiography is good for burn assessment, it still has some drawbacks that it requires ICG intravenous injection and the images remain 5 minutes to 10 minutes after injection. Anyway, the allergic rate of ICG is low²² and 5 minutes to 10 minutes is sufficient to take images of desired burn areas which can also be saved and used later.

CONCLUSION

The ICG angiography yields a significantly higher accuracy than clinical assessment in indeterminate burn wounds. This intervention can, thus, be a useful tool to aid clinical judgment.

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DISCLOSURE

The authors declare no conflicts of interest.

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