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Indocyanine Green Angiography Precise Marking for Indeterminate Burn Excision: A Prospective, Multi-centered, Double-blinded Study

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Background: During burn excision, the clinical judgment whether to excise or not excise the area with indeterminate burn depth is difficult. Indocyanine green angiography (ICGA) has been reported to provide high accuracy in diagnosing indeterminate burns. This study aims to evaluate the complete wound closures in both short-term and long-term outcomes after using ICGA precise marking to guide indeterminate burn excision.
Methods: This was a prospective, multi-centered, double-blinded, experimental study. The participants were admitted to the hospital with indeterminate burn wounds. ICGA precise marking was performed. The deep second-degree burn was painted, excised, and subsequently covered with skin grafts and measured on day 5. The superficial burns were measured on day 21. All wounds were followed-up at two months.
Results: Thirty indeterminate burn sites were included in this study. Using ICGA precise marking, the overall rate of short-term complete wound closure, which combined superficial and deep burns, was found to be as high as 96.7% (29/30). The long-term complete wound closures at two months confirmed the short-term result and yielded 100.0% of complete wound closure. The complete wound closures between the short-term and long-term measurements were not significantly different ($P > 0.999$).
Conclusions: Using ICGA precise marking to guide indeterminate burn excision resulted in an excellent rate of complete wound closure and an insignificant difference between short-term and long-term wound outcomes. ICGA is a competent method to aid decision-making in burn surgery of the indeterminate area.
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INTRODUCTION

During burn excision, the clinical judgment whether to excise or not excise the area with indeterminate burn depth is difficult.[1] Indocyanine green angiography (ICGA) has been reported to provide almost 100% accuracy in diagnosing indeterminate burns, compared with 50% by clinical assessment.[2] Although there is plenty of evidence showing the efficacy of ICGA in interpreting burn wounds, the evidence on how to use ICGA in burn surgery is scarce.[3]

A recent study demonstrated how to use ICGA to precisely mark the wounds before burn excision.[4] The superficial and deep burns were significantly different in ICGA, and the junction between the two areas could be marked precisely.[4,5] It was found that the ICGA precise marking provided excellent diagnostic capability with highly accurate long-term outcomes.[2,4,6] However, the current gap of knowledge is the therapeutic outcome after using ICGA precise marking for burn excision. This study aims to

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evaluate the therapeutic outcomes of the ICGA precise marking to guide indeterminate burn excision, including the complete wound closures in both short-term and long-term results.

METHODS

Study Design

This was a prospective, multi-centered, double-blinded, experimental study. The study was investigated and reported in accordance with the Transparent Reporting of Evaluations with Nonrandomized Designs statement.[7] This study was collaborated between Srinagarind hospital, Khon Kaen hospital in Thailand and the University of Wisconsin in USA. This clinical trial was approved by the appropriate ethics committee and was registered in the Thai Clinical Trials Registry, identification number TCTR20200222006. This trial was conducted during February and October 2020.

Participants

Inclusion criteria involved the patients who were admitted to the hospital with indeterminate burn wounds. They were over 18 years old and were hemodynamically stable (mean arterial pressure greater than 65 mm Hg, urine output at least 0.5 mL/kg/h, and adequate consciousness to decide whether to participate in the study). Written or fingerprint informed consent was acquired from all subjects.

Exclusion criteria were a previous history of an allergy to indocyanine green or iodides. The patients with pregnancy, bleeding tendency, and psychiatric disorders were excluded. Patients were also excluded if they had comorbidities that could alter the wound outcomes, including diabetes mellitus, malnutrition, current active infection, immunocompromised host, obesity, advanced age (over 65 years old), and anti-inflammatory drug use. Indeterminate wound areas that are composed of scars, moles, or tattoos were excluded.

Intervention

The study flow diagram is shown in Figure 1 and the study process is shown in the Supplemental Video. (**See Video 1 [online],** which displays the study process of the ICGA precise marking for indeterminate burn excision.) After the injury, the patient was admitted and stabilized. On the same day that the patient was going to have burn excision, the indeterminate burn wound was clinically assessed and ICGA precise marking was performed. A 0.5 mg/kg of indocyanine green (Diagnogreen Injection, Daiichi Sankyo Propharma, Japan) was injected into the burn patient intravenously. The Fluobeam 800 clinical system was used. The machine was approved by the United States Food and Drug Administration, and its depth of penetration was found to be 2.5 cm, which was sufficient to determine the full thickness of the skin.[8,9] The indeterminate area was placed under its viewer.

ICGA Objective Interpretation Criteria

Thirty-three percent of maximal perfusion was applied as a cut-point to differentiate between superficial and deep second-degree burns.[10–13] Superficial second-degree burns were defined as the burn areas with a maximal perfusion of >33% that were bright and diffuse, showing patency of small vessels of the subpapillary and dermal plexuses.[10,14] Deep second-degree burns were defined as the burn areas with a maximal perfusion of <33% or the dark area yielding mottled yet diffuse fluorescence demonstrating partial patency of the dermal plexus.[10,14] Thus, the deep second-degree burns were painted with methylene blue to indicate the area to be excised in the operating room.

After ICGA, the painted wounds, which were considered deep burn wounds, were excised and covered with skin grafts. The grafted area was followed-up 5 days after the operation to confirm the complete wound closure. The unmarked areas, which were considered superficial second-degree burns, were applied with a hydrofiber with silver (Aquacel Ag+ Extra; Convatec, UK). The unmarked area was measured on post burn day 21 to determine the complete wound closure, which also confirmed the superficial nature of the wounds. All wounds were followed-up at 2 months after the injury.

Outcomes

The primary outcome was the overall percent of wound closure. Complete wound closure was defined as the wound that achieves 100% re-epithelialization or graft take.[15] The numbers of complete wound closures from both superficial and deep burns were summed and used to calculate the overall percent of wound closures.

The secondary outcome was the percent of wound closure at 2 months after the injury to confirm complete wound closure.[15]

Burn wound surface area was measured by a 3-dimensional wound measurement device (inSight, eKare Inc, Fairfax, Va.), which provided high accuracy and yielded both an inter-rater and intra-rater reliability of >0.99.[16–18] The device was also used in clinical studies as a reliable wound measuring method.[19,20]

Sample Size

The sample size was calculated with an estimating proportion formula.[21] The pilot study was conducted on 10 wounds. The proportion of the overall wound closure from the pilot study was 80%, and the margin of error was set to be 0.15. With an alpha of 0.05, the calculated sample size was 28. With the dropout rate of 10%, the required sample size was 30.

Blinding

The surgeon who assessed complete wound closures was not the surgeon who performed ICGA marking and was blinded to the study process. The participants were blinded to the ICGA outcomes. The statistician who analyzed the data was blinded to the study process.

Statistical Analysis

Data were analyzed using STATA/SE, version 10.1. Data were reported as mean and SD for continuous variables and as number (%) for discrete variables. Binomial probability test was used to compare the complete wound closure to the expected rate of 80%. The McNemar test

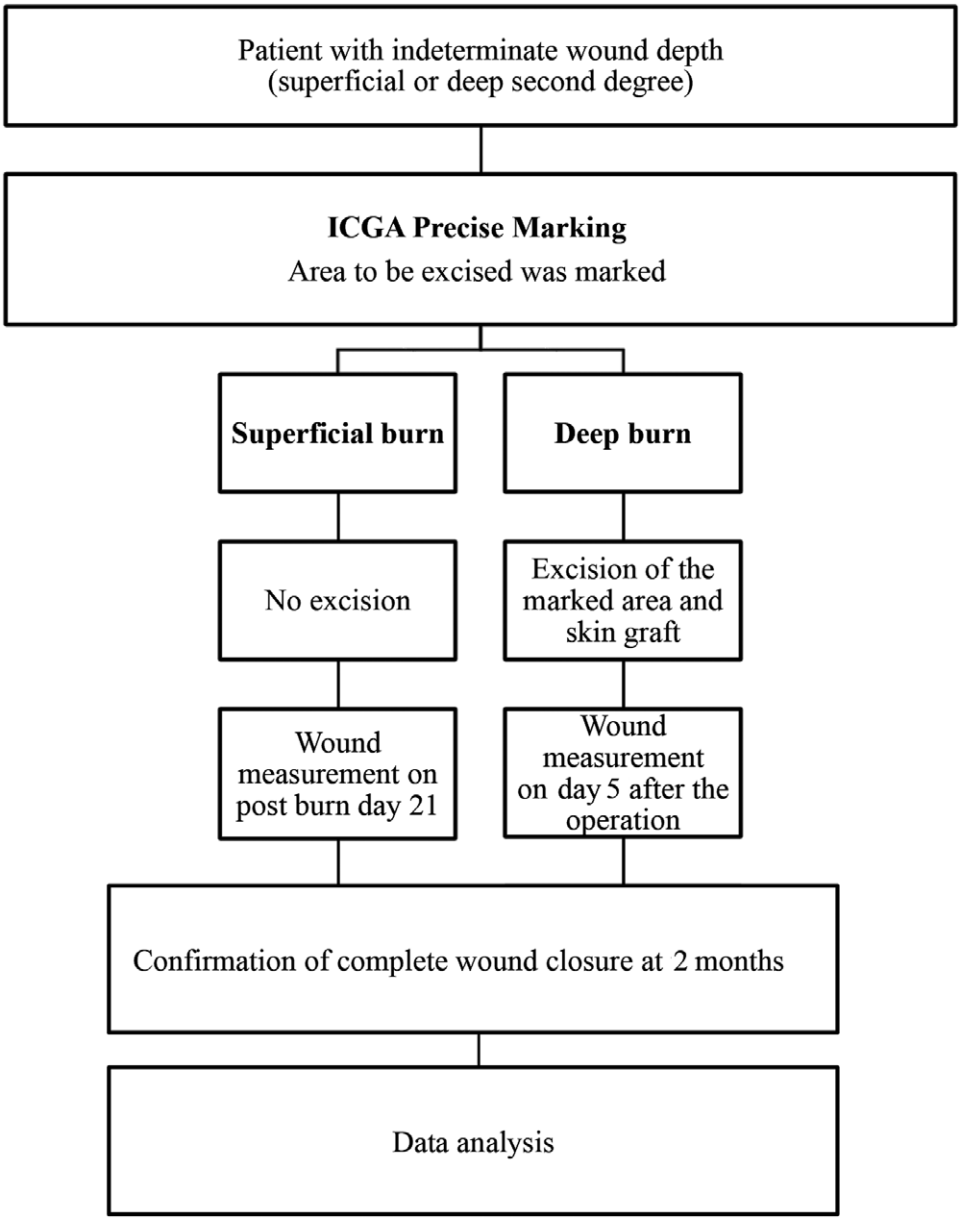

Fig. 1. The study flow diagram.

was used to determine the change in proportion for the dependent variables (short-term and long-term complete wound closure). Post-hoc subgroup analysis was conducted in 2 groups: superficial and deep groups. The aim of the analysis was to determine the wound outcome in the different types of wounds. All test statistics were 2-sided. $P < 0.05$ was considered statistically significant.

RESULTS

Demographic data are shown in Table 1. The illustration of the ICGA assessment and burn wound outcomes was described in Table 2. There were 30 indeterminate burn wounds included in the study. The average wound areas were 65.2 ± 43.8 cm^2. After using ICGA, the burn wounds were 18 (60.0%) superficial burns and 12 (40.0%) deep burns.

Figures 2 and 3 illustrate an example of ICGA precise marking results. The summary of the findings is shown in Table 3. Using ICGA precise marking, the overall rate of short-term complete wound closure, which combined superficial and deep burns, was found to be as high as 96.7% (29/30). This high rate of complete wound closure was significantly greater than the expected rate of 80%, $P = 0.01$. The long-term complete wound closures at 2 months confirmed the short-term result and yielded 100.0% of complete wound closure. The complete wound closures between the short-term and long-term measurements were not significantly different ($P > 0.999$).

Post-hoc analysis was conducted. In the superficial group, the short-term complete wound closure was 94.4% (17/18), and the long-term complete wound closure at two

Table 1. Demographic Data

Demographic Data	N (%) or Mean ± SD
Age (y)	39.1 ± 13.2
Gender	
Men	20 (66.7)
Women	10 (33.3)
BMI (kg/m²)	20.3±2.7
Time of intervention after injury (d)	2.4 ± 0.7
Alcohol use	9 (30.0)
Smoker	8 (26.7)
Hypertension	2 (6.7)
Dyslipidemia	0 (0)
Etiology of burn	
Flame burn	25 (83.3)
Scald burn	5 (16.7)

months was 100.0% (18/18). The difference between short-term and long-term wound closure was not significant ($P > 0.999$). On the other hand, the deep burn group yielded both short-term and long-term complete wound closures of 100% (12/12). The difference between short-term and long-term wound closure was also not significant ($P > 0.999$).

DISCUSSION

Interpretation

This study revealed that using ICGA precise marking to guide indeterminate burn excision resulted in an excellent rate of complete wound closure. The date of the intervention was as early as 2.4 days. This clearly shows the benefit of using ICGA in the indeterminate burns. One of the problems found in burn excision was that the indeterminate burn made surgeons wait for the well-defined depth of the wound, which delayed the excision process.[1] Moreover, the excision of indeterminate burn in the early phase could also result in unnecessary surgery of the viable tissue if the tissue was later found to be a superficial burn.[22] Using ICGA precise marking provides an additional benefit that the removal of indeterminate burns could be minimally invasive and burns could be safely excised early enough, along with the other distinct types of wounds such as deep second degree or third degree burns. After the excision, the area could be closed with a skin graft while the other superficial parts of the wound would heal within 21 days. This adequate and precise surgery not only helps burn patients heal faster, but also helps them start rehabilitation, regain function, and get back to work faster.[23]

Generalizability

Using ICGA is practical and easy to be generalized. The 33% of maximal perfusion cut point used in this study provides objective interpretation, which is easy to reproduce.[2] This study was also among the very few studies[2,3,5,10,24] in which the objective criteria were used in interpreting ICGA. Moreover, extending the usage of an ICGA device that might already be available in several hospitals for other indications, such as flap monitoring or lymphatic assessment, could be cost-effective.[2]

Limitations and Further Studies

Firstly, the wounds included in this clinical trial were only indeterminate burns, which the attending surgeon

Table 2. The Illustration of the ICGA Assessment and Burn Wound Outcomes

Wound Number	Location	Burn Area (cm²)	ICGA Result	Complete Wound Closure*	Confirmation of Complete Wound Closure at 2 Months
1	Right hand	85.4	Deep	Yes	Yes
2	Right chest	61.9	Deep	Yes	Yes
3	Left shoulder	71	Deep	Yes	Yes
4	Left hand	117.9	Superficial	Yes	Yes
5	Right hand	120.4	Superficial	Yes	Yes
6	Left arm	103.0	Deep	Yes	Yes
7	Right hand	21.6	Superficial	Yes	Yes
8	Face	85.8	Superficial	Yes	Yes
9	Left shoulder	23.8	Deep	Yes	Yes
10	Right foot	26.6	Superficial	Yes	Yes
11	Right hand	58	Superficial	Yes	Yes
12	Chin	21.2	Deep	Yes	Yes
13	Right hand	3.1	Deep	Yes	Yes
14	Neck	65.1	Superficial	Yes	Yes
15	Right leg	45.6	Superficial	Yes	Yes
16	Left leg	40.8	Superficial	Yes	Yes
17	Right arm	42.2	Deep	Yes	Yes
18	Right chest	95.3	Superficial	Yes	Yes
19	Right abdomen	90.2	Superficial	Yes	Yes
20	Right arm	85.2	Superficial	Yes	Yes
21	Left leg	72.4	Deep	Yes	Yes
22	Right hand	9.6	Deep	Yes	Yes
23	Right leg	45	Deep	Yes	Yes
24	Left chest	217.3	Deep	Yes	Yes
25	Left hand	87	Superficial	Yes	Yes
26	Right Forearm	73.6	Superficial	Yes	Yes
27	Left hand	87.4	Superficial	No†	Yes
28	Right hand	6.6	Superficial	Yes	Yes
29	Right arm	20.4	Superficial	Yes	Yes
30	Left forearm	72.1	Superficial	Yes	Yes

*Complete wound closure was measured on post burn day 21 for superficial burn and on day 5 after graft placement for deep burn.
†The wound was not healed until post burn day 21 and required skin graft placement.

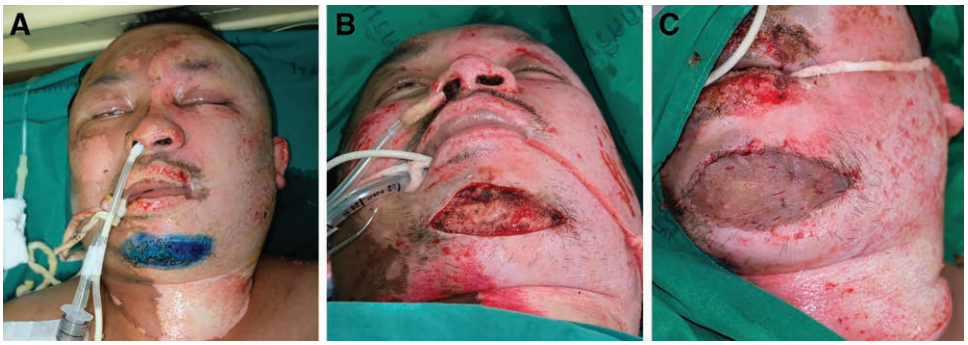

Fig. 2. An example of ICGA precise marking results. A, ICGA precise marking. B, Excision along the marking. C, Skin graft placement.

Fig. 3. An example of ICGA precise marking results (cont.). A, Five days after the operation shown in Figure 2 to confirm the complete wound closure. B, The unmarked area was measured on post burn day 21 to determine the complete wound closure. C, The patient's wound was followed-up at 2 months after the injury.

Table 3. Summary of Results

Wounds	Wound Closure on Day 5 or Day 21	Wound Closure at 2 months	*P*
Superficial group	17	18	>0.999
Deep group	12	12	>0.999
Overall	29	30	>0.999

could not decide whether the wound was either superficial or deep. The wound that was easy to classify was excluded. If other distinct types of wounds, such as first, second, or third-degree burns were included in the study, the rate of complete wound closure could be higher. Secondly, ICGA requires the injection of indocyanine green. Using ICGA precise marking in patients with allergy to iodide is limited. Thirdly, although the ICGA is used, minimal uncertainty in the diagnosis of indeterminate burn still remains. This is reflected in the wound that was assessed by the ICGA to be a superficial burn, but did not heal within 21 days. The explanation could be the cut-point used in interpreting ICGA. It was found that there was a gray zone in the diagnosis of ICGA in indeterminate areas ranging from 25% to 45% of maximal perfusion.[10] The use of 33% cut-point was proposed to solve this problem[10] and was chosen to use in this study. The 33% cut-point was found to provide as high as 88% positive predictive value of excising nonviable tissue and as low as 16% negative predictive value of excising viable tissue.[10] However, the overall rate of short-term complete wound closure of 96.7% in this study reveals that this cut-point provides excellent results, but is not totally perfect. Further study should explore more on the diagnostic criteria used in interpreting ICGA in burns to perfect this method. Fourthly, this study uses the ICGA *diagnostic* definition as superficial and deep burn, which was supported by a previous diagnostic study that compared ICGA results with pathological results. The purpose here is to explain the rationale of the interventions between the 2 groups (debridement or no debridement) better because ICGA precise marking for excision is newly described in this study. However, further research could benefit from using ICGA *therapeutic* definition as debridement and no debridement because the main therapeutic purpose of assessing the indeterminate burn is to know whether the wound could heal within 21 days to prevent the hypertrophic scar.[25] Fifthly, a further study with randomization on

the use of ICGA versus clinical judgment for indeterminate wounds will help clarify its utility, and the long-term results of >2 months are recommended to evaluate true efficacy since scar maturation could take up to 1 year for full wound quiescence.[26] If the long-term wound closure is significantly different between using ICGA and clinical judgment, this could thoroughly add ICGA to the new outstanding method of treating burns. Lastly, the marking requires a short learning curve of 2–3 cases because of the hand-eye coordination between the real marking and the ICGA monitor. Nevertheless, the marking is simple, and the marking's outcomes are worth the price of the practice.

CONCLUSIONS

Using ICGA precise marking to guide indeterminate burn excision resulted in an excellent rate of complete wound closure and an insignificant difference between short-term and long-term wound outcomes. Therefore, ICGA is a competent method to aid decision-making in burn surgeries of the indeterminate areas.

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PATIENT CONSENT

The patients provided written consent for the use of their images.

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