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Research Report

Assessment of wound perfusion with near-infrared angiography: A prospective feasibility study

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ABSTRACT

Objective: To assess the feasibility of quantitatively measuring skin perfusion before and after suture or staple skin closure of vertical laparotomies using indocyanine green (ICG) uptake with near-infrared angiography.
Methods: This was a prospective, non-randomized feasibility study of patients undergoing surgery with a gynecologic oncology service from 2/2018–8/2019. Feasibility was defined as the ability to quantitatively measure ICG uptake adjacent to the wound at the time of skin closure in $\geq 80\%$ of patients. Patients were assigned suture or staple skin closure in a sequential, non-randomized fashion. Skin perfusion was recorded using a near-infrared imaging system after ICG injection and measured by video analysis at predefined points before and after skin closure. Clinicodemographic, pre- and intraoperative details, and surgical secondary events were recorded.
Results: Of 20 participants, 10 were assigned staple closure and 10 suture closure. Two patients (10%) achieved objective quantification of ICG fluorescence before and after laparotomy closure, failing the predefined feasibility threshold of $\geq 80\%$. Reasons for failed quantification included overexposure (12), insufficient ICG signal uptake (6), and insufficient video quality (2). Near-infrared angiography wound perfusion was subjectively appreciated intraoperatively in 85% (17/20) of patients before and after wound closure.
Conclusions: Objective assessment of laparotomy skin closure with near-infrared angiography–measured perfusion did not meet the pre-specified feasibility threshold. Adjustments to the protocol to minimize overexposure may be warranted. The ability to subjectively appreciate ICG perfusion with near-infrared angiography suggests a possible role for near-infrared angiography in the real-time intraoperative assessment of wound perfusion, particularly in high-risk patients.

1. Introduction

Wound breakdown and infection after laparotomy occur in up to 37% of patients undergoing gynecologic cancer surgery (Nugent et al., 2011; Schiavone et al., 2017; Rivard et al., 2016). The impact of wound complications on morbidity, patient quality of life, and healthcare utilization is marked. Wound complications increase postoperative pain, lead to prolonged recovery times, and cause psychological distress (Brown et al., 2014). A 2012 *meta*-analysis estimated that surgical site infections increase costs on a per-case basis by $20,785 (Zimlichman et al., 2013).

Impaired wound healing can arise from a variety of factors, including infection, compromised perfusion, poor wound substrate, and wound instability (Diegelmann and Evans, 2004). Several randomized studies have assessed interventions for improved healing, including prophylactic vacuum-assisted closure devices, preventative surgical site infection bundles, and altering closure technique or suture type (Leitao et al., 2021; Anthony et al., 2011; Patel et al., 2017;11:CD005661.). With the exception of surgical site infection bundles, these interventions have shown limited benefit.

Gynecologic cancer patients carry a disproportionate risk for wound complications due in part to high rates of pre- and postoperative exposure to agents known to compromise perfusion, including chemotherapy, anti-angiogenic agents, and/or radiation. Interventions that successfully optimize perfusion and decrease complications associated with malperfusion may profoundly impact surgical outcomes and

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patient quality of life. Current approaches for intraoperative assessment of wound perfusion after laparotomy repair are based on unaided visual clinical judgement and subjective surgeon evaluation of features such as tissue color and capillary refill. Currently, there are no available and validated technologies for evaluating wound perfusion at closure.

Indocyanine green is a fluorescent iodide dye that binds intravascularly to plasma proteins and has an excellent safety profile (Hope-Ross et al., 1994; Zammarrelli et al., 2021). Indocyanine green and near-infrared angiography have been used to assess perfusion of skin flaps, wound closures, abdominoplasties, and bowel anastomoses (Diep et al., 2016; Duggal et al., 2014; Mirhaidari et al., 2018; Pruimboom et al., 2020;4:CD013280.; Wyles et al., 2016; Patel et al., 2013; Moukarzel et al., 2020; Wilke et al., 2021; Rinker, 2016; Foster et al., 2019; Shannon et al., 2017). Given its success in similar applications, we sought to investigate the feasibility of using near-infrared angiography to evaluate skin perfusion before and after suture- or staple-assisted laparotomy closure during gynecologic surgery.

2. Methods

This was an Institutional Review Board–approved feasibility study investigating the use of near-infrared angiography for the evaluation of wound perfusion before and after laparotomy closure. The primary outcome of the study was assessment of feasibility (ability to quantitatively measure indocyanine green uptake adjacent to the wound before and after skin closure in $\geq$ 80% of patients). The system employed consists of a separate near-infrared light source optimized for indocyanine green detection as well as proprietary software that quantifies near-infrared–captured fluorescence. The secondary outcomes were: a) to quantify mean perfusion impairment, mean dye ingress impairment, and time to maximum dye perfusion before and after skin closure with suture or staples, and b) to determine whether indocyanine green could be subjectively detected adjacent to the wound at the time of skin closure.

Patients undergoing a laparotomy with a vertical midline incision for any indication with the gynecology service from 2/2018–8/2019 were included after informed consent. Exclusion criteria included hepatic dysfunction (elevated transaminases); history of cirrhosis or other chronic liver disease; allergy to iodine; laparoscopic or minimally invasive surgery; laparotomy incisions unable to be closed due to tissue or fascial damage; transverse laparotomy incisions; or laparotomy incisions left open due to a "contaminated" case.

All patients underwent fascial and subcutaneous closure per surgeon preference. For skin closure, patients were assigned to a suture or a staple closure in a sequential, non-randomized fashion. A 25-mg powder bottle of indocyanine green was mixed with 10 mL of sterile water (2.5

mg/mL). After completion of subcutaneous closure and prior to skin closure, 4 mL (10 mg) of indocyanine green was injected intravenously and a video of near-infrared angiography of the incision site was captured for 90 s past the time of initial blush with a default exposure of 30.0 ms. The incision was closed with sutures or staples according to the study's assignment. After closure, an additional 4 mL (10 mg) of indocyanine green was injected. To ensure consistency in the image-captured area, a skin marking ruler was placed parallel to the incision to delineate the site before and after closure in a consistent manner. The camera was placed in the imaging system arm at a height that allowed for the capture of the entire incision and ruler. Indocyanine green fluoroscopy gain was recorded for subsequent quantitative measurements.

To assess the primary outcome, whether perfusion could be quantitatively measured adjacent to the wound, mean perfusion was determined from intraoperative video. Quest medical imaging software was used to measure fluorescence in arbitrary units (a.u.) ranging from 0 to 255 a.u., in 12 areas of skin along the incision before and after wound closure per published protocols (Fig. 1) (Wyles et al., 2016; Verduijin et al., 2019). Successful perfusion measurement was defined as the ability to measure four data points: 1) fluorescence at the start of ingress (defined by a 10% increase in measured fluorescence from baseline); 2) slope of fluorescence intensity; 3) maximum fluorescence intensity; and 4) time to maximum fluorescence (seconds from start of ingress to maximum intensity) (Fig. 2). Success of near-infrared angiography technology was recorded in a binary fashion. Failures included overexposure, insufficient indocyanine green, or insufficient video quality. Failures were classified as overexposure when the maximum value of fluorescence surpassed the imaging software maximum threshold of 255 a.u. Failures were classified as insufficient indocyanine green when measurements were within the software threshold (0–255 a.u.) but there was less than a 10% increase in measured fluorescence from baseline. Insufficient video quality failures included cases where camera movement precluded application of the quantitative imaging software.

For the first secondary outcome (3 variables), *mean perfusion impairment* (a.u.) was calculated by taking the difference between maximum dye intensity of the 12 paired areas before and after skin closure; *mean dye ingress impairment* was calculated by taking the difference between maximum slope of the fluorescence curve (a.u. per second) of the 12 paired areas before and after skin closure; and *difference in time to maximum dye intensity* was calculated before and after skin closure (Fig. 2) (Wyles et al., 2016). For the other secondary outcome—to determine whether indocyanine green could be subjectively detected adjacent to the wound at the time of skin closure by the surgeon—members of the gynecologic surgical team reported whether there was any subjective appreciation of indocyanine green fluorescence
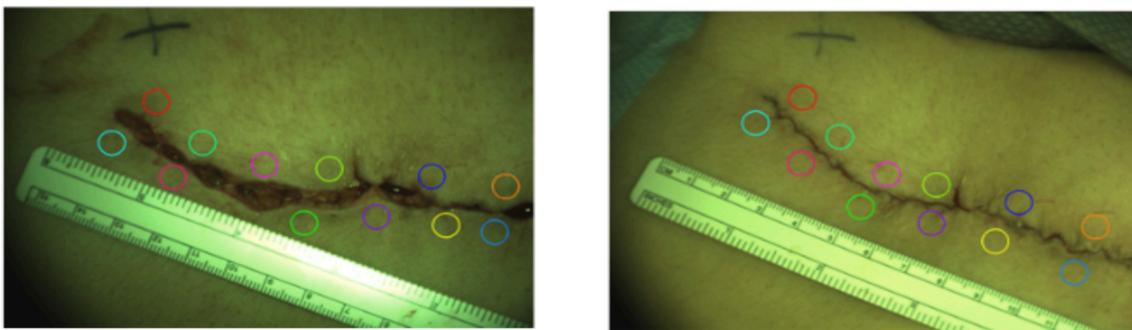

Fig. 1. Schematic of methodology representing the standardized location of perfusion parameter estimations before (left) and after (right) wound closure. Patients were assigned to a suture (as pictured) or a staple skin closure in a sequential, non-randomized fashion. Prior to skin closure, 4 mL (10 mg) of indocyanine green (ICG) was injected intravenously and a video of NIR angiography of the incision site was captured for 90 s past the time of initial blush with the Spectrum NIR imaging system. After skin closure, an additional 4 mL (10 mg) of ICG was injected. To ensure consistency in the image-captured area, a skin marking ruler was placed parallel to the incision to delineate the site before and after closure in a consistent manner. Quantification of perfusion at 12 predetermined, color-designated areas was conducted by Quest medical imaging software.
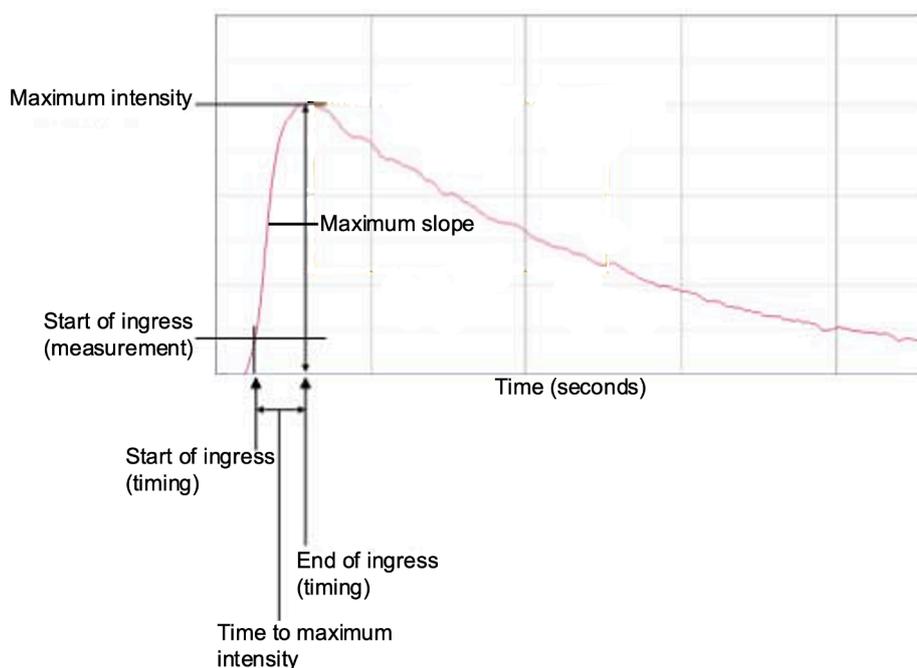
Fig. 2. Schematic of perfusion parameter estimations. Perfusion measurements are plotted as fluorescence intensity (a.u.) over time (seconds) initiated at the time of dye injection. Measurement of time to perfusion intensity is initiated at a threshold of 10% of the maximum.

adjacent to the wound intraoperatively. This outcome was reported in a binary fashion, in the presence of one of two study investigators (BS or RC), who provided consistent guidance on subjective assessment of indocyanine green. The surgical team was blinded to the objective measurements.

Variables of interest, including clinicodemographic data, intra-operative details, and surgical outcomes, were prospectively recorded. Comorbidities included history of diabetes mellitus, hyperlipidemia requiring medication, chronic pulmonary disease, hypertension requiring medication, chronic kidney disease, and smoking (current or past if $\geq$ 10 pack years). Postoperative complications specific to wound healing within 30 days of surgery were noted and graded using a pre-viously described and validated institutional grading system (Strong et al., 2015). Descriptive statistics were used to report baseline charac-teristics and intraoperative details. All statistical analyses were per-formed using SPSS version 26.0 (Armonk, NY).

3. Results

Twenty patients were enrolled, of whom 14 underwent laparotomy for an indication of malignancy and 6 for benign disease under the care of one of five surgeons. Median patient age was 59 years (range, 41–78 years), and 70% were white. Nine patients (45%) had a preoperative comorbidity associated with poor wound healing, including diabetes mellitus (n = 2), hyperlipidemia (n = 3), hypertension (n = 5), or a smoking history (n = 4). Three patients (15%) had multiple comorbid-ities. Median body mass index was 26 kg/m^2 (range, 21.5–36 kg/m^2), and median measured thickness of subcutaneous tissue closed before subcuticular closure was 3.0 cm (range, 1.5–5.0 cm). Additional de-mographic, clinical, and intraoperative characteristics are reported in Table 1.

Two (10%) of the 20 patients achieved successful objective quanti-fication of indocyanine green fluorescence both before and after lapa-rotomy closure. This did not meet the predefined feasibility threshold of $\geq$ 80% of patients. Five patients had successful quantification only before closure, 1 after closure, and 13 neither before nor after closure. Reasons for inability to quantify fluorescence included the following: overexposure in 8 patients before and after skin closure, 1 patient before

Table 1
Patient characteristics.

| Variable	All patients n = 20	Staple closure n = 10	Suture closure n = 10
Median age, years (range)	59 (41–78)	60 (42–71)	56.5 (41–78)
Median BMI, kg/m^2 (range)	26 (21.5–36)	27.6 (22–36)	25.8 (21.5–34.2)
Race			
White	14 (70%)	7 (70%)	7 (70%)
Black	2 (10%)	2 (20%)	0 (0%)
Asian	2 (10%)	1 (10%)	1 (10%)
Other	2 (10%)	0 (0%)	2 (20%)
Comorbidities			
Diabetes mellitus	2 (10%)	1 (10%)	1 (10%)
Hyperlipidemia	3 (15%)	2 (20%)	1 (10%)
Hypertension	5 (25%)	2 (20%)	3 (30%)
History of smoking	4 (20%)	2 (20%)	2 (20%)
Surgical indication			
Uterine cancer	6 (30%)	3 (30%)	3 (30%)
Ovary cancer	7 (35%)	4 (40%)	3 (30%)
Other cancer	1 (5%)	1 (10%)	0 (0%)
Benign	6 (30%)	2 (20%)	4 (40%)
History of prior abdominal surgery (total)	13 (65%)	9 (90%)	4 (40%)
Laparotomy	9 (45%)	7 (70%)	2 (20%)
Minimally Invasive	4 (20%)	2 (20%)	2 (20%)
Median preop albumin, g/dL (range)	4.0 (3.1–4.9)	4.4 (3.8–4.9)	4.0 (3.1–4.1)
Median operative time, min (range)	262.5 (142–709)	281 (142–709)	253 (195–475)
Estimated blood loss, mL	300 (50–850)	225 (50–500)	350 (100–850)
Bowel surgery performed	5 (25%)	3 (30%)	2 (20%)
Subcutaneous tissue closed			
Continuous suture	12 (60%)	3 (30%)	9 (90%)
Interrupted suture	8 (40%)	7 (70%)	1 (10%)
Median estimated thickness of subcutaneous tissue, cm (range)	3 (1.5–5)	3.5 (1.5–5)	3 (2–4)

BMI, body mass index.
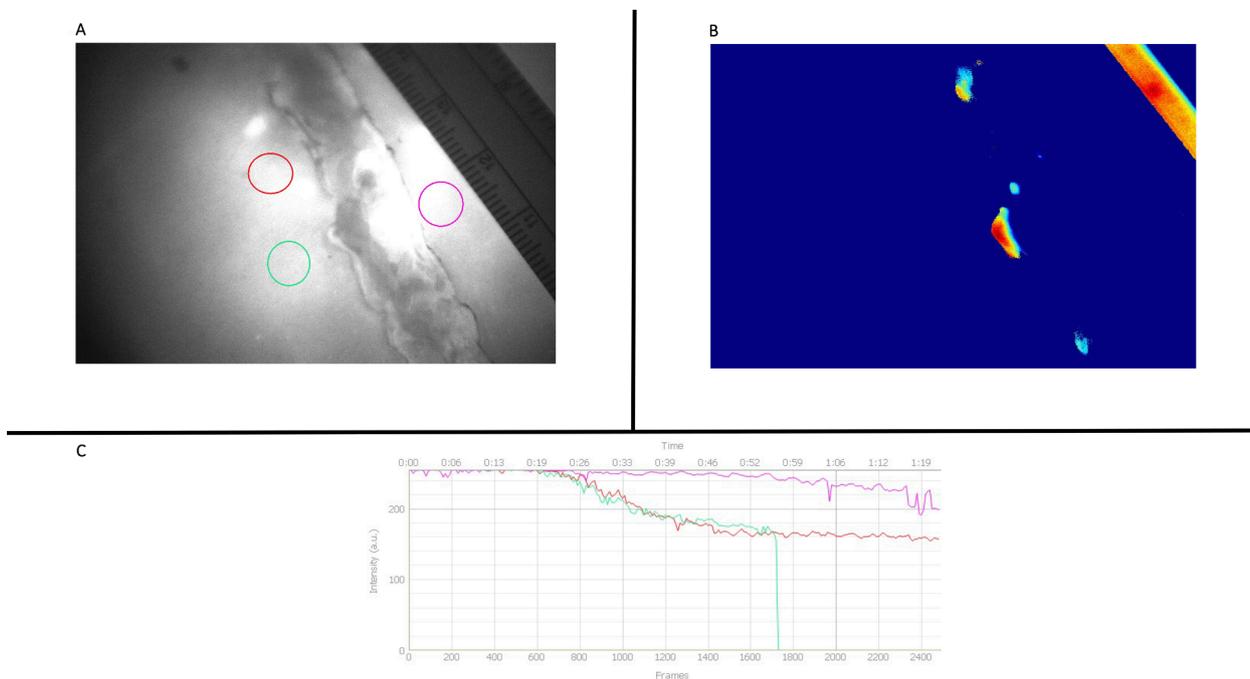
Fig. 3. A. Representation of overexposure. Example of overexposure precluding accurate fluorescence quantification. Pictured here is pre-closure fluorescence assessment, NIR channel. **B. Representation of overexposure.** Example of overexposure (peak intensity) precluding accurate fluorescence quantification. Pictured here is pre-closure fluorescence assessment, maximum value mapping. **C. Fluorescence intensity measurements in overexposed imaging.** Example of fluorescence measurements obtained in cases of overexposure. Pictured here are measurements from pre-closure fluorescence assessment as pictured in Fig. 3A and 3B.

skin closure, and 3 patients after skin closure (Fig. 3A-C); lack of or insufficient indocyanine green signal uptake in 2 patients before and after skin closure, 1 patient before skin closure, and 3 patients after skin closure (Fig. 4A-C); and insufficient video quality due to movement in 1 patient before closure and 1 patient after closure. There were no adverse reactions attributable to indocyanine green.

Among the 2 patients who achieved successful objective measurement of indocyanine green fluorescence before and after laparotomy closure, 1 underwent staple-assisted closure and 1 suture-assisted closure. For the former patient, the mean difference between incision
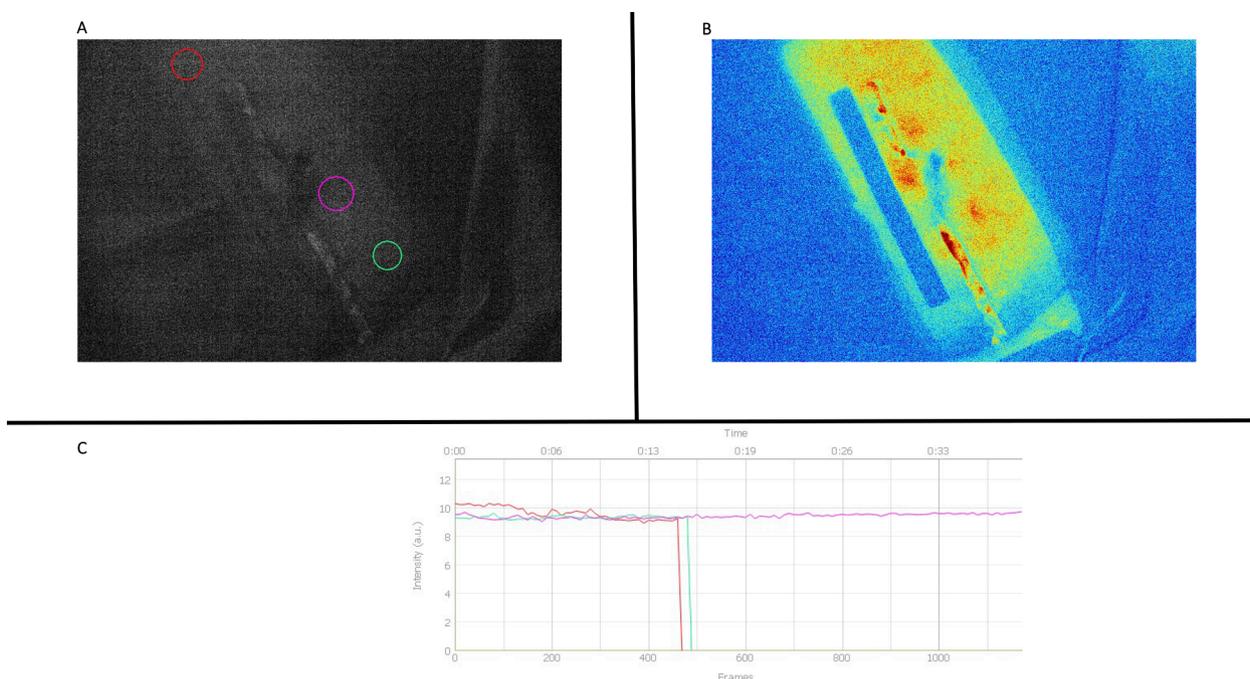

Fig. 4. A. Representation of indocyanine green (ICG) failure. Example of overexposure precluding accurate fluorescence quantification. Pictured here is post-closure fluorescence assessment, NIR channel ingress. **B. Representation of ICG failure.** Example of ICG failure (peak intensity) precluding accurate fluorescence quantification. Pictured here is post-closure fluorescence assessment, maximum value mapping. **C. Fluorescence intensity measurements in cases of ICG failure.** Example of fluorescence measurements obtained in cases of ICG failure. Pictured here are measurements from post-closure fluorescence assessment as pictured in Fig. 4A and 4B.

Table 2
Difference in perfusion pre- versus post-closure perfusion.

Closure method	Perfusion parameter	Before closure (SD)	After closure (SD)	Change
Staple-assisted closure	Maximum slope of fluorescence (a.u./s)	21.1 (6.8)	25.3 (10.0)	+4.2
	Maximum dye intensity (a.u.)	107.5 (34.1)	105.1 (36.0)	−2.4
	Time to maximum dye intensity (s)	8.4 (0.5)	6.6 (1.4)	−1.8
Suture-assisted closure	Maximum slope of fluorescence (a.u./s)	5.4 (1.1)	8.8 (1.1)	+3.4
	Maximum dye intensity (a.u.)	37.6 (8.8)	64.8 (9.1)	+27.2
	Time to maximum dye intensity (s)	11.8 (2.5)	10.4 (0.6)	−1.4

perfusion before closure versus after closure in fluorescent units was + 13.0 a.u. (42.2 a.u. [SD 15.7 a.u.] before closure and 55.2 a.u. [SD 15.9 a.u.] after closure). For the latter patient, the mean difference between incision perfusion before closure versus after closure was + 14.0 a.u. (27.0 a.u. [SD 6.6 a.u.] before closure and 41.0 a.u. [SD 6.2 a.u.] after closure). Other perfusion parameters, including maximum slope of fluorescence, maximum dye intensity, and time to maximum dye intensity were similar before and after skin closure for both patients (Table 2).

Subjectively, near-infrared angiography wound perfusion was appreciated in 85% (17/20) of patients both before and after wound closure. In 1 (5%) of 20 patients, near-infrared angiography could not be detected pre-closure. In 2 (10%) of 20, near-infrared angiography failed both pre- and post-closure, as no dye ingress was observed intraoperatively. The patient with failed pre-closure subjective perfusion assessment was a 60-year-old woman of unknown race with a body mass index of 25.7 kg/m^2 and no medical comorbidities who also failed objective pre-closure fluorescence measurement. One patient with failed subjective perfusion assessment pre- and post-closure was a 42-year-old black woman with a body mass index of 36 kg/m^2 and no medical comorbidities who also failed objective pre- and post-closure fluorescence measurement. The second patient with failed subjective perfusion assessment pre- and post-closure was a 65-year-old white woman with a body mass index of 32 kg/m^2 and a history of hypertension who also failed objective pre- and post-closure fluorescence measurement. None of the patients with failed wound perfusion on subjective assessment experienced any postoperative complications. Among the remaining 17 patients with good subjective wound perfusion, only 1 patient experienced a wound complication, which was a grade 1 wound separation after a staple-assisted subcuticular closure. This patient was a 45-year-old white woman with a body mass index of 22.6 kg/m^2 and no medical comorbidities. Near-infrared angiography perfusion could not be objectively measured before or after closure in this patient due to overexposure. On intraoperative subjective assessment, indocyanine green perfusion was observed both pre- and post-closure.

4. Discussion

Optimization of wound perfusion is crucial to successful wound healing (Sen, 2009). Several studies have investigated the association of different skin closure techniques with wound complications; however, there are limited data on how perfusion can be assessed. In the orthopedic literature, Wyles et al. performed a randomized trial evaluating skin perfusion using laser-assisted indocyanine green angiography following stapled closure, subcuticular suture closure, or vertical mattress sutured closure of skin following total knee arthroplasty (Wyles et al., 2016). The authors reported that a running subcuticular suture was associated with the greatest perfusion (p < 0.001). To our knowledge, no studies have evaluated wound perfusion using near-infrared angiography following laparotomy.

In gynecologic surgery, near-infrared angiography is widely used in the detection of sentinel lymph nodes during endometrial and cervical cancer surgeries. Furthermore, emergent data suggest near-infrared angiography may improve outcomes after bowel anastomoses; a recent study demonstrated a 1.5% rate of anastomotic leaks with near-infrared angiography, compared to 4.7% without it (Moukarzel et al., 2020). The established use of near-infrared angiography in gynecologic cancer surgery suggests it could be readily applied elsewhere, such as assessment of wound closure.

In this study, we sought to investigate whether near-infrared angiography could be used to quantify perfusion before and after skin closure following laparotomy for gynecologic indications, and whether this technology could be applied intraoperatively. We found near-infrared angiography did not meet the pre-specified feasibility threshold, as only 2 (10%) of 20 patients had successful perfusion measurement before and after wound closure. Under this protocol, it is unlikely that near-infrared angiography can be used to quantify perfusion and compare closure techniques, or to evaluate other interventions to optimize laparotomy perfusion. Overexposure both before and after wound closure was responsible for the failed objective assessment of 12 (67%) of 18 cases, suggesting that protocol modifications should focus on methods to reduce overexposure and standardize the capture of intraoperative imaging. Possible strategies to minimize overexposure include ensuring a standardized interval of at least 15 min to allow for complete washout before post-closure indocyanine green injection (Reinhart et al., 2016). Although indocyanine green has a short plasma half-life of ~ 3–5 min, and repeated evaluations during the same surgical procedure are common, rapid skin closure or disruption in skin integrity from transection, retractors, and other surgical manipulation possibly resulted in the cumulation of indocyanine green and subsequent overexposure (Gurtner et al., 2013). Introducing a mandatory delay of 15–20 min between indocyanine green injections possibly could decrease rates of overexposure after wound closure. An additional strategy to enhance clearance between evaluations could be to reduce the dose of indocyanine green from 10 mg, although the 10-mg indocyanine green dosage was selected based on the manufacturer's instructions and prior reports and standardized protocols across multiple anatomic sites, including the breast, groin, and pelvis, which successfully used doses ranging from 7.5 to 50 mg (Mirhaidari et al., 2018; Capozzi et al., 2019; Gentileschi et al., 2019; Phillips et al., 2012; Elite System Spy). Other factors that could be standardized to reduce overexposure may include: altering sensor settings to reduce the gain or lower exposure time, ensuring ambient lighting is turned off, ensuring proper camera positioning (perpendicular to the body surface), and introducing a standardized distance for the camera arm from the skin (e. g., 30 cm) (Gurtner et al., 2013).

Perfusion was subjectively observed in 17 (85%) of 20 patients. This finding is meaningful, as several prior studies on the incorporation of indocyanine green perfusion assessment in intraoperative management relied on the subjective detection of fluorescence, with improved outcomes tied to successful subjective perfusion (Pruimboom et al., 2020; Moukarzel et al., 2020). Given the higher success rate of subjective perfusion assessment, it is possible that this subjective strategy may be useful in higher-risk settings in which surgeons may intervene in cases of compromised perfusion. For example, a clinical trial could assess whether the use of near-infrared angiography improves wound outcomes in patients undergoing flap-based closure following pelvic exenteration or if perfusion assessment could be used before laparotomy incision to demarcate well-perfused tissue in patients with extensive surgical histories. Complementing subjective perfusion assessment with

refined objective assessment may optimize the implementation of this technology in settings such as the evaluation of bowel anastomoses.

The strengths of this study are its prospective design, with intentional inclusion of both staple- and suture-based skin closure, and its incorporation of both objective and subjective measures of perfusion. Furthermore, this study prospectively collected factors of potential relevance to wound healing, including subcutaneous closure technique and median measured thickness of subcutaneous tissue. This study included a diverse group of patients, spanning a wide range of ages (range, 41–78 years), body mass indexes (range, 21.5–36 kg/m^2), and medical histories. This study also examined the feasibility of this technique among five surgeons to examine broader applicability.

The limitations of this study are primarily related to small sample size and failure to adequately quantify indocyanine green ingress intraoperatively mostly due to high rates of overexposure. Further, this study had a low rate of postoperative complications (5%). As this was not a randomized trial, it was not powered to discern whether near-infrared angiography enhances intraoperative perfusion assessment, alters intraoperative decision making, or improves postoperative outcomes. These findings suggest that future studies on the impact of near-infrared angiography on intraoperative assessment, surgical decision making, or postoperative outcomes should focus on cases with higher risk of wound complications, such as in surgical fields previously exposed to radiation, patients who have received chemotherapy/bevacizumab, or in patients with a history of multiple prior surgeries.

This prospective feasibility study demonstrated that objectively measuring abdominal incision perfusion with near-infrared angiography is not feasible under the current protocol and could not be used to compare perfusion between staple- and suture-based skin closures. Adjustments to the protocol to minimize overexposure may allow for comparisons of these closure methods in the future. Further, differences in subjective indocyanine green perfusion appreciated with near-infrared angiography suggest there may be a role for it in the real-time intraoperative assessment of wound perfusion in high-risk patients.

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CRediT authorship contribution statement

Beryl L. Manning-Geist: Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Renee A. Cowan:** Data curation, Formal analysis, Writing – review & editing. **Brooke Schlappe:** Conceptualization, Data curation, Writing – review & editing. **Kenya Braxton:** Data curation, Writing – review & editing. **Yukio Sonoda:** Writing – review & editing. **Kara Long Roche:** Writing – review & editing. **Mario M. Leitao Jr:** Writing – review & editing. **Dennis S. Chi:** . **Oliver Zivanovic:** Writing – review & editing. **Nadeem R. Abu-Rustum:** Conceptualization, Writing – review & editing. **Jennifer J. Mueller:** Data curation, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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