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Feasibility and safety of intraoperative photodynamic diagnosis using 5-aminolevulinic acid in breast cancer surgery

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Abstract

Purpose

Intraoperative margin assessment remains a clinical challenge in breast cancer surgery. We conducted a prospective pilot study to evaluate the feasibility, safety, and diagnostic performance of 5-aminolevulinic acid (5-ALA)–based photodynamic diagnosis (PDD) using a commercially available imaging system in Japan.

Methods

Patients with primary breast cancer scheduled for mastectomy and tumor size > 2 cm received oral 5-ALA (20 mg/kg) 2–4 hours before surgery. Excised specimens were incised immediately after resection and examined under white light and blue-light PDD. Punch biopsies were obtained from areas classified by gross inspection and PDD findings. Pathology served as the reference standard. Primary endpoints were diagnostic odds ratio (DOR) and safety; secondary endpoints included sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

Results

Ten patients received 5-ALA. PDD visualization was feasible in 6 cases when specimens were examined within 2–5 minutes after excision. A total of 33 punch biopsies were analyzed. Compared with gross inspection alone (sensitivity 84.6%, specificity 85.0%, DOR 31.2), the addition of PDD improved sensitivity to 92.3% and increased DOR to 47.9, with specificity of 80.0%. PDD identified several grossly occult tumor foci. No adverse events related to 5-ALA were observed.

Conclusions

In this prospective pilot study, intraoperative 5-ALA–based PDD was feasible and safe in breast cancer specimens. The addition of fluorescence information improved diagnostic performance compared with gross assessment alone. Further studies are warranted to determine its clinical utility in breast-conserving surgery.

Introduction

Breast-conserving surgery (BCS) and mastectomy are the primary surgical options for breast cancer. In recent years, the use of skin-sparing and nipple-sparing mastectomy has increased, often with immediate reconstruction. According to Japan’s national breast cancer registry, 44.8% of patients undergo BCS, 2.4% undergo nipple-sparing mastectomy (NSM), and 1.9% undergo skin-sparing mastectomy (SSM) [1]. In BCS or in NSM/SSM procedures, obtaining negative margins is crucial to

avoiding the need for re-excision or postoperative radiation, since additional treatments impose psychological stress on patients and increase healthcare costs.

In BCS, the reported positive margin rate ranges from 17% to 20% [2–4]. Various techniques have been proposed to improve margin clearance, including wire-guided localization, radio-guided occult lesion localization (ROLL), radioactive seed localization (RSL), and intraoperative ultrasonography [5]. However, in Japan these methods are not widely used, due in part to a shortage of radiologists and regulatory restrictions on the handling of radioactive materials. Instead, a common practice is to perform preoperative ultrasound to identify the lesion and then inject a dye–gel mixture along the planned resection margins. Specifically, blue dye (indocyanine green or patent blue) mixed with sterile lubricating jelly (1.5 mL each) is injected subcutaneously and in the mammary gland at several points along the proposed incision line. At our institution, using this method, the positive margin rate for palpable lesions based on margin evaluation is 21%, and 15% for non-palpable lesions, which is comparable to rates reported internationally. (unpublished data)

5-ALA is a naturally occurring amino acid and precursor of heme and PpIX [6]. Photodynamic diagnosis (PDD) using 5-ALA-induced PpIX fluorescence has been examined for the visualization and detection of various cancers [7–9]. Orally administered 5-ALA is metabolized into PpIX, a photosensitizer that accumulates selectively in tumor cells [10]. Under illumination with blue light, the PpIX emits red fluorescence, highlighting tumor tissue during surgery. PDD using 5-ALA is already approved in Japan for intraoperative guidance in malignant glioma and for endoscopic resection of non-muscle invasive bladder cancer [11, 12]. In 2021, a Canadian group reported the results of a Phase II trial of PDD using 5-ALA for breast cancer. They compared two groups, one receiving 15 mg/kg and the other 30 mg/kg of oral 5-ALA. In both groups, the positive predictive value (PPV) for detecting breast cancer inside and outside the grossly demarcated tumor border was 100.0% and in the 50% range, respectively, confirming the clinical utility of this method. No adverse events were observed [13].

This PDD approach shows strong potential for application in breast cancer surgery; however, the Canadian study utilized a proprietary camera system (PRODIGI) that is not available in Japan. [14, 15] An alternative PDD imaging system that operates at a similar excitation wavelength is already in clinical use for other cancers in Japan. However, its applicability to breast cancer has not yet been established.

In the present study, we tested whether 5-ALA-based PDD can be implemented for breast cancer using a commercially available endoscopic system. Given differences in tumor localization practices in Japan (e.g., dye-gel marking) and specimen handling protocols from those in Western settings, we also conducted a pilot study to assess the fundamental feasibility of PDD-based tumor visualization in breast cancer, with a focus on establishing technical feasibility and identifying key workflow considerations for future clinical application.

Materials and Methods

Study design

This study was performed as a single-institution prospective trial. The protocol was approved by the hospital's certified ethics review board (approval No. jRCTs041220090), and all patients provided written informed consent. Initially, three patients underwent mastectomy without 5-ALA (serving as fluorescence-negative controls), and their resected specimens were examined under both normal light and PDD illumination to confirm the absence of any background autofluorescence. Subsequently, 10 patients received 5-ALA before surgery as part of the intervention cohort. In these cases, oral 5-ALA (Alaglio®; SBI Pharmaceuticals, Tokyo, Japan) at 20 mg/kg was administered 2–4 hours prior to the operation. After mastectomy, the excised specimens were incised and examined both grossly (with standard overhead lighting) and with PDD (blue-light excitation) in the operating room.

For PDD imaging, we employed a KARL STORZ IMAGE1® S camera head and Telescope System I (Karl Storz SE, Tuttlingen, Germany), a laparoscopy imaging system capable of blue-light excitation and detection of the red fluorescence signal. According to Japanese pathological guidelines, mastectomy specimens are generally sectioned along a plane parallel to a line connecting the nipple and tumor center. In this study, to avoid interfering with the pathologist's final diagnosis, each specimen was sectioned through the tissue along a plane perpendicular to that line to expose a large portion of the tumor and its surrounding margins for PDD observation. From the freshly cut surface, we then collected tissue samples using a 3 mm dermal punch biopsy instrument. Biopsies were taken from representative areas categorized by both modalities: regions that appeared tumor-positive or tumor-negative on gross inspection, and regions that showed fluorescent-positive or -negative findings on PDD (Fig. 1).

Schema of a resected mastectomy specimen. The dotted line indicates the grossly observed tumor margin. The pink shaded area represents the PDD-positive fluorescence region. Biopsy sites were categorized into four groups according to gross inspection and PDD findings: A, grossly tumor-positive area with PDD positivity; B, grossly tumor-negative area with PDD positivity; C, grossly tumor-positive area with PDD negativity; and D, grossly tumor-negative area with PDD negativity. The corresponding classification table is displayed on the right.

Subjects

The study population consisted of adult patients with early-stage primary breast cancer who were scheduled to undergo mastectomy. There were no restrictions on hormone receptor status, HER2 status, or the presence of an in situ component, and both invasive and non-invasive carcinoma cases were eligible. The key inclusion criteria were histologically confirmed breast cancer, patient age 20 years or older, ECOG Performance Status (ECOG PS) of 0–2, scheduled for standard mastectomy for primary breast cancer, disease extension of more than 2 cm on preoperative imaging, and blood test results with appropriate organ function. Key exclusion criteria included significant comorbid conditions that could compromise safety (e.g. poorly controlled hypertension or diabetes), any history of photosensitivity or porphyria, hypotension at the time of enrollment (defined as systolic blood pressure < 100 mmHg or

diastolic < 60 mmHg) or a history of repeated hypotensive episodes, pregnancy or breastfeeding, and current use of medications known to cause photosensitivity (e.g. tetracyclines, sulfonamides, fluoroquinolone antibiotics)

Outcomes

The primary endpoints of the study were the diagnostic odds ratios (DORs) for tumor identification based on gross inspection versus PDD observation, and the safety of 5-ALA administration. Secondary endpoints included the diagnostic performance metrics of PDD and standard gross examination, specifically their sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). In practice, the presence of red fluorescence on the specimen surface was assessed qualitatively by visual inspection, since our equipment did not allow for measurement of quantitative fluorescence intensity. All key observations, including both the gross appearance of the specimen and the PDD fluorescence findings, were documented with photographs and video recordings for review. Diagnostic odds ratio (DOR) is defined as the odds of a positive test result in samples with cancer cells divided by the odds of a positive test result in samples without cancer cells, and can be calculated as (sensitivity/(1–sensitivity))÷((1–specificity)/specificity). The value of a DOR ranges from 0 to infinity, with higher values indicating better discriminatory test performance. A value of 1 means that the test does not discriminate between patients with the disorder and those without it, and values lower than 1 point to improper test interpretation [16]. DOR was calculated for both gross and PDD observation. We also closely monitored any adverse events potentially related to 5-ALA, including signs of phototoxic skin reactions, liver function abnormalities, hypotension, or other unexpected reactions to the drug.

Statistical analysis

Biopsy samples collected from cases where PDD observation was feasible were evaluated with regard to PPV, NPV, sensitivity, and specificity based solely on gross observation findings. Results were then compared with those calculated when PDD observations were included. Additionally, DOR were compared for each scenario. All statistical analyses were performed using STATA software version 18.0 (StataCorp, College Station, TX). Given the small sample size and pilot nature of the study, the analysis was primarily descriptive and no formal hypothesis testing was conducted.

Results

Characteristics of patients

Between January and August 2023, three patients underwent mastectomy without 5-ALA (control group) to establish baseline fluorescence observations, and an additional 10 patients underwent mastectomy with preoperative 5-ALA administration (intervention group). Mean age of the 5-ALA group was 64.8 years. Of these 10 cases, 9 had invasive ductal carcinoma and 1 had invasive lobular carcinoma (Table 1). The lobular carcinoma case did not exhibit any fluorescence and was therefore excluded from further

evaluation. In the first three 5-ALA cases, no red fluorescence was detected on the specimens. These three cases had an average interval from specimen removal to PDD observation of approximately 18 minutes. Starting from the fourth case (Fig. 2), we shortened the interval between excision and PDD examination to roughly 2–5 minutes post-excision. Clear tumor fluorescence was observed under these conditions. Overall, 6 of the 10 cases in the 5-ALA group showed visible PDD fluorescence in the tumor bed or margins.

Table 1. Baseline characteristics of the study population and PDD observation results

No.	Age	cT	PDD observation	Luminescence intensity	Time from excision to observation* (min)	No. of punch biopsies	Histological type	pT	pN	subtype
1	56	Tis	fail	(-)	14	0	IDC	1	0	ER+/HER2-
2	49	T2	fail	(-)	19	0	IDC	2	0	ER+/HER2-
3	74	T2	fail	(-)	21	0	IDC	1	0	ER+/HER2-
4	73	T2	success	strong	5	9	IDC	1	0	ER+/HER2-
5	73	T2	success	weak	10	5	IDC	1	2	ER+/HER2-
6	47	T1a	success	weak	unknown	4	IDC	1c	0	ER+/HER2-
7	83	T4a	fail	(-)	2	0	ILC	3	0	ER+/HER2-
8	41	T2	success	strong	3	5	IDC	2	1	ER+/HER2-
9	73	T2	success	strong	2	5	IDC	2	1	ER+/HER2-
10	79	T2	success	strong	unknown	5	IDC	2	2	ER+/HER2-

Abbreviations: IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ER, estrogen receptor; HER2, human epidermal growth factor receptor type 2; PDD, photodynamic diagnosis; SD, standard deviation; N/A, not applicable.

* Observation time was defined as the interval from specimen excision to the start of gross and/or PDD inspection.

In this pilot study, patients who had undergone neoadjuvant chemotherapy or had more advanced disease were not included, resulting in all tumors in the 5-ALA group being ER+ and HER2–. Interval from specimen excision to PDD observation was shortened after the initial three 5-ALA cases to capture fluorescence before it faded.

Primary and secondary outcomes

In the six cases with successful PDD visualization, a total of 33 punch biopsy samples were collected from the incised specimen surfaces for pathological analysis. The distribution of sampled sites and their diagnostic category by method is summarized in Table 2. Among samples, 13 (39%) were from areas that appeared positive for tumor by both gross inspection and PDD, whereas 4 (12%) were from areas where the PDD finding and gross inspection were discrepant (e.g. PDD-positive but grossly inconspicuous, or vice versa). The comparative diagnostic performance of gross examination alone and PDD observation is presented in Table 3. Using pathology as the gold standard, gross inspection alone

yielded a sensitivity of 84.6%, specificity of 85.0%, PPV of 78.6%, and NPV of 89.5%. This corresponded to a DOR of 31.2 for gross examination. When PDD findings were added to the assessment, sensitivity improved to 92.3%, with a specificity of 80.0%, PPV of 75.0% and NPV of 94.1%. The inclusion of PDD increased the DOR to 47.9. No adverse events attributable to 5-ALA were observed.

Table 2. Correlation between gross inspection and PDD observation samples

		PDD		
		Positive	Nagative	
Gross	Positive	13	1	
	Negative	3	near*	9
			distant**	7

* Near indicates samples collected from areas adjacent to the gross tumor margin

**Distant indicates samples collected from locations remote to the gross tumor margin

Table 3: Diagnostic performance of gross and PDD observation

	Gross		PDD	
	Positive	Negative	Positive	Negative
Pathological diagnosis				
Cancer cells present (+)	11	2	12	1
Cancer cells absent (-)	3	17	4	16
PPV	78.6%		75.0%	
%(95%CI)	(49.2-95.3)		(47.6-92.7)	
NPV	89.5%		94.1%	
%(95%CI)	(66.9-98.7)		(71.3-99.9)	
Sensitivity	84.6%		92.3%	
%(95%CI)	(54.6-98.1)		(64.0-99.8)	
Specificity	85.0%		80.0%	
%(95%CI)	(62.1-96.8)		(56.3-94.3)	
DOR	31.216		47.948	

Abbreviations: PPV, positive predictive value; NPV, negative predictive value; DOR, diagnostic odds ratio; PDD, photodynamic diagnosis.

All indices were calculated using biopsy-confirmed pathology as the reference standard.

Assessment of gross and PDD findings and pathological findings of individual samples (Table 2)

The largest number of samples were collected in both the PDD- and grossly negative area, with 16 samples. Of these, 8 were collected close to outside of the gross tumor margin and 8 were collected from the area near the specimen margin. One sample taken close to outside the gross tumor margin showed invasive carcinoma with lobular features on pathological examination (Supplementary Fig. 1) while the others were unremarkable.

The second largest number of samples were collected in the area where both the PDD and gross findings were positive, with 13 samples collected. Of these, 11 samples were positive for invasive carcinoma, one showed inflammatory changes due to preoperative biopsy, and one was unremarkable.

Of the three samples taken from PDD-positive but grossly negative areas, pathology showed invasive cancer in one case (Fig. 3) and benign findings in two cases. Specifically, one case demonstrated inflammation (Supplementary Fig. 2A), and another case was diagnosed as mastopathy with ductal dilatation (Supplementary Fig. 2B). The sample in which invasive carcinoma was identified was obtained from a PDD-positive area located just outside the gross tumor margin.

In the single sample taken from a PDD-negative and grossly positive area, pathology was unremarkable.

Discussion

In this pilot trial, we demonstrated that intraoperative photodynamic diagnosis using 5-ALA is feasible during breast cancer surgery and can be performed safely. The addition of PDD fluorescence information improved the detection of tumor-involved margins compared to gross inspection alone, as evidenced by an increase in the diagnostic odds ratio from 31.2 to 47.9. Clinically, this suggests that PDD could serve as a useful adjunct to standard surgical margin assessment, potentially helping surgeons identify residual cancer more effectively. Importantly, no adverse events were observed with the use of 5-ALA in our patients, confirming the safety of the 20 mg/kg oral dose in this context. Our findings indicated the importance of timing: tumor fluorescence was only reliably observed when the surgical specimen was evaluated within a few minutes after resection. Delays longer than this allowed the fluorescence signal to fade, resulting in the initially missed detections in our first few cases. Accordingly, for PDD to be effective, the workflow must incorporate very prompt imaging of the fresh specimen. Furthermore, although these fluorescence-negative specimens had not yet been sectioned, they had been exposed to ambient room light for an extended period, which may have contributed to the failure of fluorescence detection by PDD. Previous basic research has documented the photobleaching of 5-ALA-induced PpIX fluorescence upon light exposure [17].

Our main result, the diagnostic performance of gross and PDD observation (Table 3) demonstrated that the PPV, NPV, and sensitivity of PDD were comparable to or better than those of gross observation alone, whereas specificity was slightly lower (gross 85%, PDD 80%). This reduction in specificity was attributed to several PDD false-positive samples (Fig. 3 and Supplementary Fig. 2). These samples did not represent completely normal tissue; rather, they showed benign histological changes such as inflammation or mastopathy with ductal dilatation. This finding is consistent with previous reports in the neurosurgical field, where PDD positivity was observed in association with edema or inflammation [18, 19], as well as with prior phase II breast studies which reported false positivity in benign proliferative lesions such as atypical ductal hyperplasia and sclerosing adenosis [13]. The use of PDD may enable safer surgery by ensuring an adequate but minimal resection margin, and thereby contribute to an improved cosmetic outcome. However, a relatively frequent occurrence of false-positive fluorescence would lead to concerns about unnecessary enlargement of the resection area. On the other hand, since benign proliferative breast lesions are known to confer an increased risk of breast cancer development, resection of such areas might not necessarily be disadvantageous. Further studies are warranted to clarify the mechanisms underlying PDD false positivity in benign lesions and to establish optimal criteria for determining the appropriate surgical margins when using PDD.

We observed some notable differences between our findings and those of the previous Phase II study by the Canadian group [13]. In that study, PDD was successful even in cases of invasive lobular carcinoma, whereas in our series the single lobular carcinoma case did not exhibit any detectable fluorescence. Additionally, the Canadian trial reported that even very small tumor foci could be detected with their PDD

system, whereas our study struggled to identify scattered microscopic invasive foci. One likely explanation for these discrepancies is the difference in imaging equipment and technique. The Canadian team used a dedicated handheld fluorescence imaging device (PRODIGI) optimized for breast tissue, which included the use of filters to block out blue excitation light and enhance contrast [13, 15]. In contrast, while our present system operates at the appropriate excitation wavelength for PpIX (around 405 nm) and is capable of detecting the emitted red fluorescence, its sensitivity and image clarity are probably inferior to those of a purpose-built device. The lack of a specialized long-pass filter in our setup may have also reduced the signal-to-noise ratio, making faint fluorescence harder to visualize. These technical factors could explain why some small cancerous areas (especially the diffuse pattern of lobular carcinoma) were not identified by our PDD method.

Limitations of this study include the small number of cases and the small number of cases in which PDD was observable. In addition, only ER-positive, HER2-negative cases were enrolled, owing to our avoidance of cases after preoperative chemotherapy, and the relationship between differences in biology and the results of PDD observation could not be observed. DCIS cases were also not enrolled. These issues await future study.

A follow-up prospective study has already been initiated at our institution, incorporating a wider range of breast cancer subtypes, including DCIS and breast-conserving surgery cases, and using an improved fluorescence imaging system and optimized workflow. This ongoing study is designed to clarify the clinical utility of the PDD approach and determine whether it can be integrated into routine surgical practice.

Declarations

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Conflict of Interest

The authors declare that they have no conflict of interest.

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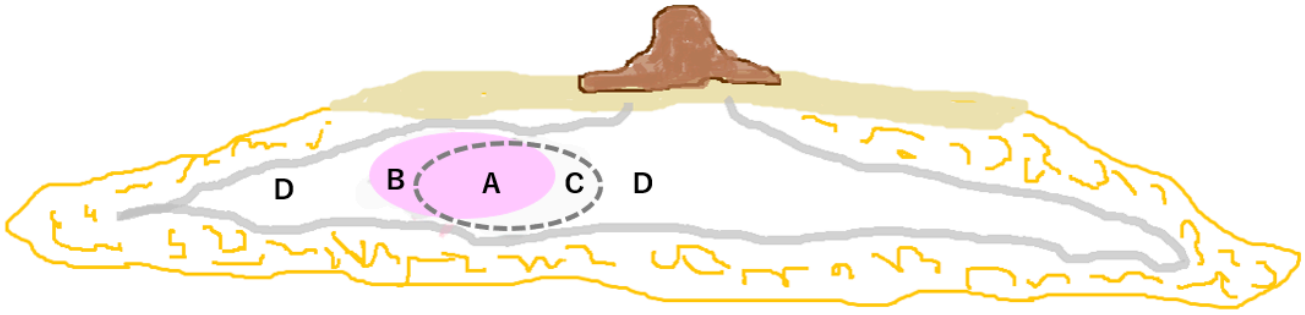
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Figures



Grossly observation margin

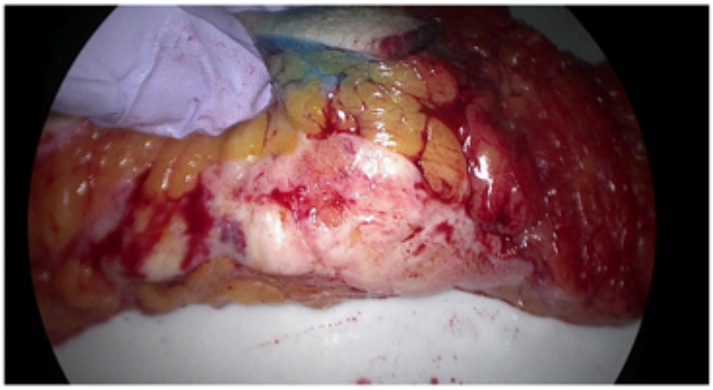
PDD observation positive area

		PDD	
		Positive	Negative
Gross	tumor	A	C
	non-tumor	B	D

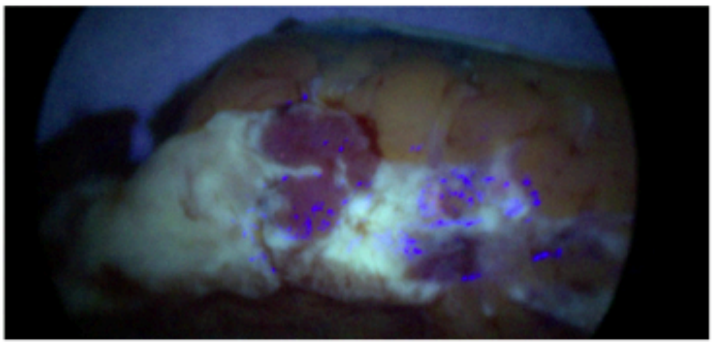
Figure 1

Schematic illustration of gross- and photodynamic diagnosis (PDD)-based tissue sampling

A): Macroscopic appearance



B): PDD observation



C): Invasive carcinomas identified in a sample collected in both a PDD- and a grossly positive area (H&E staining, original magnification x10)

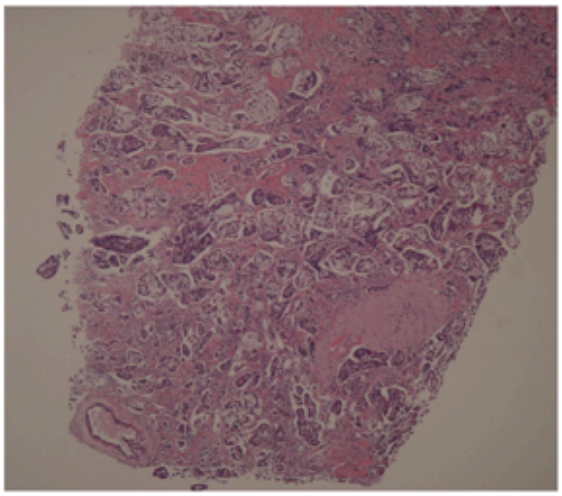
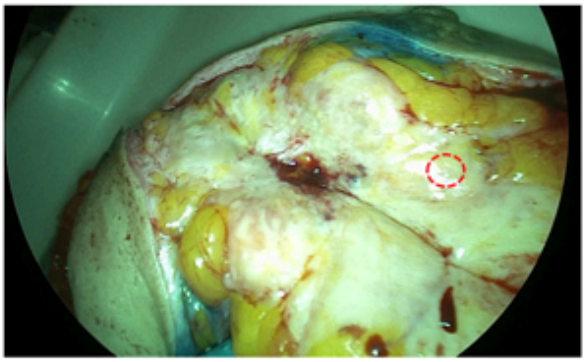


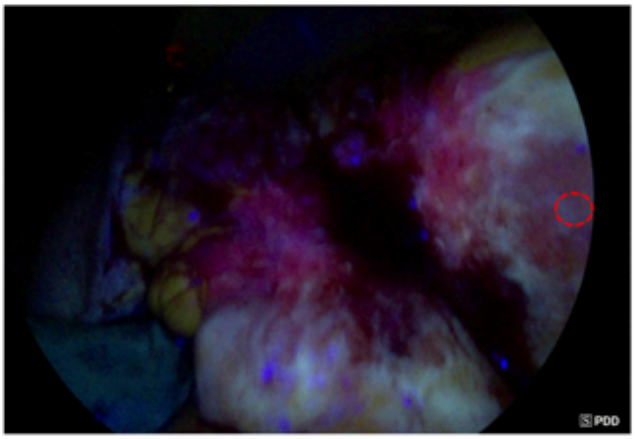
Figure 2

Representative case demonstrating PDD fluorescence (Case 4)

A) Gross observation (sample collected from the circled area)



B) PDD observation (sample collected from the circled area)



C) Pathological examination revealed invasive carcinoma (H&E staining, original magnification x10)

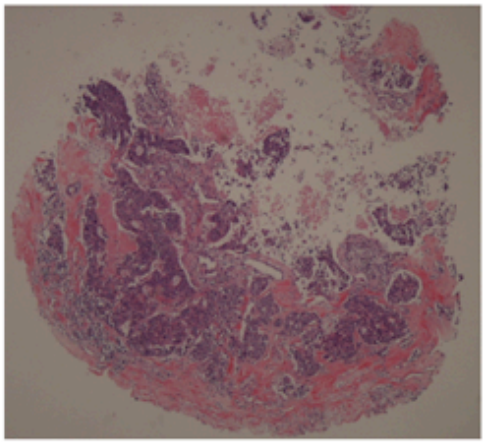


Figure 3

In Case N08, a PDD-positive area was identified just outside the gross tumor margin (grossly negative area).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfigure1.docx