Laparoscopic Fluorescence Detection of Ovarian Carcinoma Metastases Using 5-Aminolevulinic Acid-Induced Protoporphyrin IX

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Supported by Schleswig-Holsteinische Krebsgesellschaft and by Richard Wolf Company, Knittlingen, Germany.

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Received October 14, 2003; revision received January 9, 2004; accepted January 23, 2004.

BACKGROUND. The aim of the current clinical study was to evaluate the in vivo fluorescence detection of ovarian carcinoma metastases in a second-look laparoscopic procedure after intraperitoneally applied 5-aminolevulinic acid (ALA).

METHODS. Five hours before laparoscopic surgery, ALA was applied intraperitoneally via short infusion in a concentration of 30 mg/kg bodyweight in a sterile, 1% solution. Application of ALA resulted in the endogenous production of the fluorescent photosensitizer, protoporphyrin IX (PP IX). The Combilight PDD 5133 system served as a light source, permitting the switch from white light mode to blue light mode to excite the PP IX accumulated in the ovarian tissue specimens. By means of blue light illumination, intraperitoneally located red fluorescent lesions, which were suspected to be metastases, underwent a biopsy. In addition, several biopsy specimens were taken from nonfluorescent areas of the peritoneal cavity.

RESULTS. In 13 of 29 patients, ovarian carcinoma was confirmed histologically or cytologically. In 12 of these patients, metastases were visible by red fluorescence. In total, 123 biopsies were performed. Comparison of histologic assessment of the biopsy specimens with the fluorescence detection showed that strong red fluorescence had a sensitivity of 92% for detecting tumor tissue on specimens. In only 2% of all biopsy specimens was endometriosis observed in benign tissue specimens using fluorescence. In four of 13 patients with ovarian carcinoma, lesions were detected under fluorescence, which were not observed under white light illumination.

CONCLUSIONS. Laparoscopic fluorescence detection of endogenous PP IX after intraperitoneal application of ALA may provide a higher sensitivity of finding peritoneal metastases of epithelian ovarian carcinoma compared with conventional laparoscopy. Direct visualization of in vivo fluorescence after ALA application may improve the early detection of intraperitoneal ovarian carcinoma micrometastases. The high tissue selectivity of PP IX accumulation in tumor tissue specimens also offers the opportunity for therapeutic approaches using photodynamic therapy in the future. ***Cancer* 2004;100:1650–6.**

KEYWORDS: ovarian carcinoma, 5-aminolevulinic acid (ALA), fluorescence detection, second look, laparoscopy.

Ovarian carcinoma is the fourth most frequently diagnosed malignant gynecologic tumor and is responsible for most deaths from gynecologic carcinoma in the U.S. The death rates among with women with ovarian carcinoma exceed the death rates due to cervical and endometrial carcinoma combined.[1] The majority of patients are diagnosed with advanced-stage disease (i.e., FIGO Stage III/IV) be-

DOI 10.1002/cncr.20155
Published online 15 March 2004 in Wiley InterScience (www.interscience.wiley.com).

cause they are asymptomatic during the early stages of the disease. Ovarian carcinoma can spread via the lymphatic system or by hematogenous dissemination. However, the bulk of the tumor is found within the abdominal cavity. The primary treatment modalities are maximal cytoreductive surgical procedures such as adnectomy, hysterectomy, omentectomy, and pelvic lympadenectomy. In $\leq$ 20% of patients with Stage III/IV disease, bowl resection is necessary to achieve optimal tumor reduction at the time of primary surgery. Because of local intraabdominal shedding of tumor cells and tumor growth on peritoneal surfaces, peritoneal debulking is often necessary. Therefore, primary maximal cytoreductive surgery often is necessary for patient survival.[2]

After initial surgery, patients receive platinum-based combination chemotherapy. Overall response and clinical complete response rates have increased over the years. These improvements in ovarian carcinoma therapy are based on radical primary surgery and on improved chemotherapeutic strategies like the introduction of taxanes in the 1990s for adjuvant treatment.[3].

However, to our knowledge, the overall survival rate is still low in patients with advanced-stage disease. Many of these patients will experience disease recurrence.Less than 17% of patients with Stage IV disease and $<$ 30% of patients with Stage III ovarian carcinoma will survive $>$ 5 years.[4]

The presence of residual tumor and metastases, which can be established by second-look procedures, demonstrate the unsuccessful results of initial therapy. Although second-look procedures cannot ensure positive effects on patient survival, they are the only way to assess residual disease or disease progression in patients with histologically proven disease.[5,6] A second-look operation (SLO) is an increasingly common procedure in clinical trials. A negative SLO may be an important end point and an early surrogate for disease-free survival. Laparoscopic assessment in a second-look evaluation is equivalent to a laparotomy and has a low incidence of complications.[7]

The principle of photodynamic therapy (PDT) and fluorescence diagnosis is based on a predominant accumulation of a nontoxic photosensitizer that is selectively accumulated or retained by malignant tissue specimens. By exposure to light of an appropriate wavelength in the visible range, this excitation can lead to selective destruction of malignant tissue specimens. One disadvantage of many photosensitizers is a general photosensitization, especially of the skin, which may last several days or weeks. The prolonged photosensitivity requires the patient to avoid sunlight to prevent severe sunburn. The administration of 5-aminolevulinic acid (ALA) with subsequent accumulation of endogenous protoporphyrin IX (PP IX) reduces the duration of photosensitization to 1 or 2 days.[8] If cell and tissue specimens photosensitized with ALA-induced PP IX are exposed to blue light (wavelength of 400–450 nanometers), red fluorescence can be detected.[9]

TABLE 1
Indication for Reassessment Procedures

Reassessment procedures	No. of patients
Second look	22
Surveillance (third, fourth look)	4
Increasing CA 125 level (>35 U/mL)	4

ALA-mediated PP IX fluorescence detection of malignomas, such as carcinoma of the bladder and glioblastoma (as detailed below), has been reported to have improved tumor diagnostic decisively in several medical disciplines. In contrast to conventional white light conditions, fluorescence diagnosis allows significant improvements in tumor detection of very small lesions as well as occult lesions. Since the first data on fluorescence detection of early bladder carcinoma were published by Kriegmair et al. in 1996,[10] the use of this diagnostic tool is now becoming routine in cystoscopy.

The better intraoperative differentiation between malignant and healthy tissue specimens in patients with glioblastoma when using ALA appears to improve surgical results and overall survival rates.[11] The potential of ALA-guided fluorescence diagnosis is also being evaluated in the specialties of otolarynology, pulmonology, dermatology, and gastroenterolgy.[12–16]

After showing the feasibility of detecting metastases of ovarian carcinoma in animal studies,[17,18] we evaluated the practicability of ALA-induced PP IX-based fluorescence diagnosis in vivo during diagnostic laparoscopy after the intraperitoneal application of ALA in patients with ovarian carcinoma.

MATERIALS AND METHODS

Patient Characteristics

Thirty patients were included in the current study. The indications for reassessment procedures are listed in Table 1. The current study was approved by the local ethics committee of the Medical University of Lübeck (Lübeck, Germany). Informed written consent was obtained from all women before they were enrolled in the study.

The mean age of the subjects was 58 years (range, 24–72 years). Twenty-five women had epithelial ovarian carcinoma, 2 had tumors of low malignant poten-

TABLE 2
Patient Characteristics

Characteristics	No. of patients
Mean age (yrs) (range)	58 (range 24–72 yrs)
Tumor type	
Epithelial	25
Epithelial-LMP (borderline lesion)	2
Germ cell	1
Fallopian tube carcinoma	2
FIGO Stage at initial surgery	
I	6
II	4
III	17
IV	3
Primary adjuvant therapy	
Surgery and platinum-based chemotherapy	26
Surgery alone	3
Surgery and radiotherapy	1

LMP: low malignant potential; FIGO: International Federation of Obstetrics and Gynecology.

tial (borderline tumor of the ovary), 1 had a Sertoli–Leydig carcinoma, and 2 had fallopian tube carcinoma. Twenty-six patients had received previous surgical therapy and platinum-based chemotherapy, 3 had received surgical therapy alone (2 with borderline lesions and 1 with Sertoli–Leydig carcinoma), and 1 had received surgery and adjuvant radiotherapy.

The clinical characteristics of the patients (age, tumor classification and stage, primary therapy, and histology) are shown in Table 2. For all the patients enrolled in the current study, the concentration of the tumor marker CA 125 was determined in serum samples.

Before application of ALA, porphyria was excluded in all patients anamnestically and by the Hoesch test.[19] Five hours before laparoscopic surgery was performed, the patients received a sterile, 1% ALA solution (pH 6.5) (Medac, Wedel, Germany), intraperitoneally. The solution (given at a concentration of 30 mg/kg body weight) was administered via short infusion by a needle placed subcostally on the left side of the body. . After ALA application, the patients were shielded from direct light exposure for 36 hours to avoid phototoxic reactions.

Laparoscopic Procedure

Five hours after ALA sensitization, laparoscopy was performed (Fig. 1). To minimize intraabdominal injuries, a Veress needle (Richard Wolf GmbH, Knittlingen, Germany) was placed subcostally on the left side of the body and a pneumoperitoneum was built up. After the retraction of the Veress needle, a 2-mm minilaparoscope was placed subcostally on the left side of the body. Then, a 10-mm 0 ° Combilight PDD 5133 laparoscope (Richard Wolf GmbH) was placed in view subumbilically. The laparoscope served as the light source, which permitted switching from white light mode to blue light mode (wavelength of 350–440 nanometers). The blue light mode efficiently yields PP IX fluorescence. In the white light mode, a special filter restricted the wavelength range to 455–700 nanometers to prevent photobleaching or phototoxic effects.

After taking an intraabdominal round view, two accessory 5-mm ports were placed suprapubicly on the right and left side in view. All laparoscopies were video-documented. By means of blue light illumination of the light source, 50 small biopsy specimens were excised from intraperitoneally located red fluorescent lesions, suspected to be metastases. In addition, several biopsy specimens were excised from nonfluorescent areas of the peritoneal cavity ($n = 73$). They also were suspicious for metastases. All patients underwent peritoneal washing cytology.

RESULTS

All patients received the intraabdominal ALA application via short infusion without complications. In one patient who had received ALA, the laparoscopy was not performed because of a technical defect of the equipment needed for the visualisation of PP IX fluorescence. The laparoscopy was performed without complications for 29 patients.

In 13 of the 29 patients, ovarian carcinoma was confirmed histologically or cytologically. For 12 of these patients, metastases were visible as strong red fluorescence. In four of these patients, metastases were extremely small and were not detected with conventional white light imaging. In addition, four of these patients had negative results after washing cytology. In only one of the tumor-positive patients was no red light fluorescence emitted by the metastatic lesions, which included metastases > 3 cm in dimension.

Altogether, 123 biopsy specimens were excised. For 46 biopsy specimens obtained from red fluorescing areas, tumor cells were confirmed by histology, whereas 70 biopsy specimens from nonfluorescent areas were negative for tumor cells. Four biopsy specimens from one patient in whom endometriosis was detected histologically yielded false-positive results. Three biopsy specimens from one patient with large nonfluorescent metastases yielded false-negative results. From these data, a sensitivity of 92% and a specificity of 95% were calculated (Fig. 2).

Tumors were confirmed histologically in 5 patients with levels of CA 125 $>$ 35 U/ml. In 8 patients

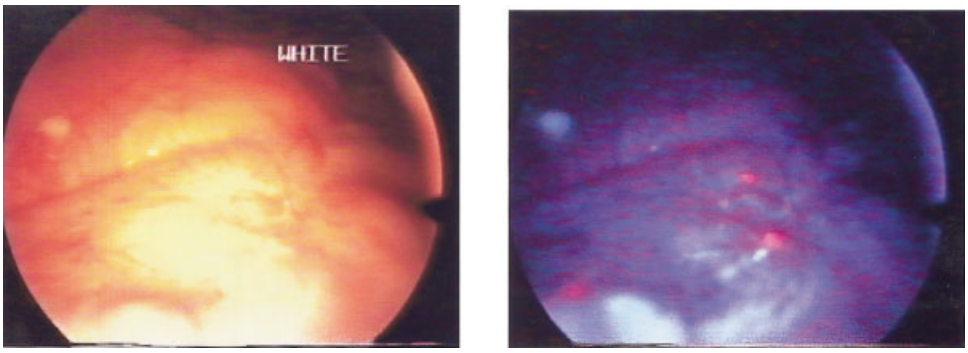

FIGURE 1. Laparoscopic image of a 5-aminolevulinic-sensitized tissue specimen showing metastatic ovarian carcinoma lesions under white and blue light illumination. Tumor was detected on tissue specimens by strong red fluorescence, even in lesions measuring < 0.5 mm. The peritoneum, bowel, and liver showed no fluorescence.

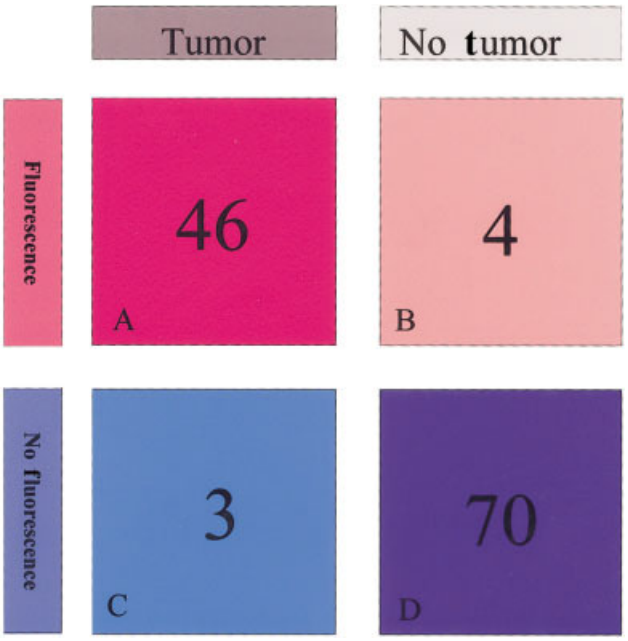

FIGURE 2. Comparison of macroscopic fluorescence with the histologic evaluation of the biopsy specimens.

TABLE 3
Tumor Marker CA 125 and Intraabdominal Metastases

CA 125 (U/mL)	No. of histologically proved tumors	No detected tumor residuals
>35	5	0
<35	8	17

with normal levels of CA 125 (< 35 U/mL), metastases were proven histologically (Table 3).

DISCUSSION

To our knowledge, the current study is the first report to evaluate the use of fluorescence detection to diagnose ovarian carcinoma metastases in humans. We used an intraperitoneal application of ALA because it offers the advantage of local and systemic sensitization of malignant peritoneal tissue specimens. Because ALA as a small molecule is readily absorbed by the blood stream via the peritoneum, this leads, in addition to the local sensitization, to systemic distribution of ALA at a rate that is comparable to that found in published studies in which an oral or intravenous application of ALA was used.[20–24] Therefore, no initial biodistribution study, which would introduce additional risks without benefit for the patients, was conducted.

Because of the few patients in the current study and our reliance on the results on the examiner, we cannot prove statistically that fluorescence diagnosis is superior to white light laparoscopy. However, based on the current study results, it is very likely that very early metastases can be detected more sensitively with fluorescence than with conventional laparoscopy. First, no side effects or complications (in addition to photosensitization of the skin) were observed. In rats, phototoxic effects on intraperitoneal tissue samples were observed after intraperitoneal application.[25,26] In the current study, care was taken to minimize the applied light dose by using a special filter in the white light illumination. No clinical side effects due to phototoxic reaction were observed. However, further studies are needed to exclude side effects when very high ALA or light doses, especially during open surgery, are used. ALA and PP IX are part of the cellular metabolism with high clearance rates. Therefore, chronic toxicity is not expected from a few ALA applications. Second, the strong fluorescence contrast that was observed between lesions and the peritoneum allows for the easy identification of suspicious lesions. Third, we observed only one patient with false-negative biopsy results and one patient with false-positive results due to endometriosis. In addition, peritoneal metastatic lesions measuring < 0.5 mm were detected in 4 patients using scattered fluorescence. However, these lesions were not observed using standard white light conditions.

Fluorescence diagnosis was also more sensitive than washing cytology, which was only found to be positive in 4 of 13 patients. This high false-negative rate of washing cytology is in accordance with a recently published study.[27] Direct visualization of in vivo fluorescence after ALA application therefore may improve the very early diagnosis of intraperitoneal ovarian carcinoma micrometastases, even before elevated CA 125 levels are detected.

We believe that laparoscopic fluorescence detection of PP IX after intraperitoneal application of ALA as an addition to conventional laparoscopy will provide a higher sensitivity and specificity in the detection of intraperitoneal metastases of epithelial ovarian carcinoma compared with the use of conventional laparoscopy alone. When substantiated in Phase III studies, fluorescence-guided biopsies after ALA application might help to take directed tissue samples from tumor-suspicious areas, thereby avoiding random biopsies.

In primary surgery of suspicious adnexal masses, fluorescence diagnosis could be helpful in patients with Stage I ovarian carcinoma to discriminate between Stage IA tumors and higher-grade tumors because very small tumor outspread might be differentiated on the ovarian surface or an occult peritoneal outspread. However, any risk of extensive phototoxic damage during the surgical procedure should be excluded.

Different methods exist for the follow-up of patients with ovarian carcinoma. The sensitivity of computed tomography (CT) scan is reduced in patients with tumor implants of $\leq$ 1 cm. A further limitation of this imaging procedure is low interobserver agreement.[28]

The combination of positron emission tomography (PET) and CT scan may be effective in identifying patients with recurrent ovarian carcinoma.[29] However, all patients investigated in the current study also showed elevated CA 125 levels. Currently, the only tumor marker shown to have a well defined and validated role in the management of epithelial ovarian carcinoma is CA 125. Changes in the level of CA 125 can be used as a reliable indication of response or disease progression.[30,31] In the current study, we confirmed the diagnosis by detection of fluorescing tumor in all patients exhibiting elevated CA 125 levels, but we also identified eight patients with provable tumor having normal CA 125 levels. Other diagnostic instruments appear to be less sensitive for early detection of tumor recurrence.

The SLO is defined as the surgical reassessment of asymptomatic patients without clinical and instrumental signs of disease after completed primary surgery and chemotherapy. The aim of a second-look procedure is to verify the treatment response to obtain prognostic and therapeutic indications. The SLO remains the most reliable method for assessing disease status in patients after they have received initial therapy. Tumor stage, grade, and residual tumor measuring $>$ 2 cm after initial surgery are significant predictors of disease recurrence.[32] However, $\leq$ 50% of the patients will experience disease recurrence after a negative second-look evaluation.[33]

This high rate of disease recurrence, despite a negative second-look evaluation, may be the result of residual micrometastases. However, it cannot be explained by the finding that retroperitoneal lymphogene recurrences were found in 12.3% of negative second look procedures as reported by Vaccarello et al.[34]

Husain et al.[35] recently assessed second-look laparoscopy as a safe and accurate method with a low incidence of complications in patients with ovarian carcinoma who had undergone previous extensive abdominal surgery. Their rate of negative evaluations and their rate of recurrences in patients with negative second look procedures were equivalent to those described in former studies of second-look assessment by laparotomy. Therefore, laparoscopy in second-look evaluation appears to be as predictive as evaluation by laparotomy with the advantage of lower morbidity and costs.[5]

However, second-look procedures are not used as an integral part of tumor management, which could change in the future. General strategies in oncology are to detect and halt disease recurrences at an early stage. In reference to an older study, there are suggestions that patients with serum levels of CA 125 $\leq$ 35 U/mL at the time of disease recurrence had a better prognosis than patients with higher values.[36] To our knowledge, published randomized trials do not evaluate whether serum CA 125 levels $>$ 35 U/mL have prognostic significance for the early detection of disease recurrence when initiating an early second-line therapeutic approach.

Most women with progressive ovarian carcinoma experience disease recurrence and are candidates for further therapy. The progress in the development of highly effective second-line chemotherapy in ovarian carcinoma and the increase of the sensitivity of tumor detection by fluorescence diagnosis are expected to increase the usefulness of second-look surgical interventions, which have been controversial until now.[37]

The development of new and more promising second-line therapies may create a resurgence in the use of second-look procedures. Innovative strategies in cancer treatment are aimed at immunotherapy.[38] Im-

munotherapeutic approaches for the treatment of ovarian carcinoma include locoregional and systemic cytokine application, prophylactic and therapeutic vaccines, and adoptive immunotherapy strategies. The efficacy and success of new strategies in cancer treatment may only be high if the tumor burden is small. Patients with platinum-resistant ovarian carcinoma with a poor prognosis might benefit from strategies using a fluorescence staining procedure as a very sensitive method for detecting minimal tumor residuals.

The capability of ovarian carcinoma cells to metabolize ALA to PP IX holds the possibility for photodynamic therapy. The evaluation of the effectiveness of this therapeutic approach in patients with peritoneal carcinomatosis has been reported previously.[39]

The strong red fluorescence of ALA-induced PP IX offers a distinct optical differentiation between healthy and malignant tissue for selective biopsies via laparoscopy. This principle of precise detection of malignancy may further open new avenues to diagnose peritoneal metastases of other malignant diseases, such as pancreatic and colon carcinoma.

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