

# Lymph node metastasis detection of ovarian cancer by porphyrin fluorescence photodetection: case report

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**Abstract** The concept of intraoperative in vivo diagnosis and selective resection of infiltrated lymph nodes in ovarian cancer has not been evaluated despite the increased morbidity associated with pelvic and paraaortic lymph node dissection and its questionable therapeutic value. Fluorescence photodetection is based on the application of a photosensitizer relatively selective for malignant tissue, which after light activation of appropriate wavelength, shows fluorescence. Six hours after oral application of 10 mg/kg body weight 5-aminolevulinic acid, the abdominal cavity of a patient with suspicion of recurrent ovarian cancer was inspected using a laparoscope and blue light at 380–440 nm. Spectral measurements at a wavelength of 635 nm, multiple peritoneal biopsies, and lymph node excisions were performed. White light inspection and porphyrin fluorescence photodetection revealed no intraperitoneal metastases and multiple biopsies were negative. Fluorescence-positive lymph nodes were visible only in the left common iliac region and a specific porphyrin fluorescence peak could be detected. In contrary, no increased

porphyrin fluorescence of intraperitoneal tissues or skin was seen. Fluorescence microscopy showed the characteristic red fluorescence in the infiltrated parts of the lymph node tissue by the papillary ovarian cancer. Histology of the other sites was negative. No systemic or cutaneous side effects were recorded. This data is a proof of the concept that porphyrin fluorescence-guided lymph node metastasis detection is possible in ovarian cancer and should stimulate further research in this field.

**Keywords** Ovarian cancer · Lymph node · Fluorescence diagnosis · ALA · Case report

## Introduction

The therapeutic value of systemic pelvic and paraaortic lymphonodectomy for ovarian cancer is still under debate. According to current protocols, it is indicated in cases where radical debulking surgery has achieved complete macroscopic removal of peritoneal metastases. In early ovarian cancer, 10–20% of lymph nodes are positive and, therefore, complete removal is recommended [1]. A concept of intraoperative in vivo diagnosis and selective resection of infiltrated lymph nodes, however, has not been evaluated so far despite the known increased morbidity associated with pelvic and paraaortic lymph node dissection. In other malignancies such as early breast cancer, similar concepts such as sentinel lymph node detection have meanwhile replaced complete removal of axillary nodes, thereby reducing significantly the rate of patient discomfort [2].

Fluorescence photodetection (PD) is based on the application of a photosensitizer relatively selective for malignant tissue. Light activation of appropriate wave-

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length leads to either fluorescence for diagnostic purposes or to oxidation-mediated tissue destruction for treatment. Exogenous administration of 5-aminolevulinic acid (ALA), the first committed intermediate in the heme biosynthesis pathway, results in a temporary accumulation of fluorescing porphyrin precursors, mainly protoporphyrin IX (PpIX). The negative feedback control is bypassed and ALA induces a preferential accumulation of PpIX in malignant cells of epithelial origin. PpIX can be easily detected as red fluorescence when excited with light of about 405 nm wavelength. Compared to other hematoporphyrin derivatives (HPD), the advantage of ALA-induced PpIX is the decreased skin and organ phototoxicity after topical or systemic application reducing the likelihood of unintended tissue damage. Furthermore, skin phototoxicity lasts only 1 day compared to 6 weeks for HPD [3].

## Materials and methods

### Patient

A 61-year-old patient was hospitalized due to suspicion of recurrent ovarian cancer. The tumor marker CA 125 showed increasing levels and abdominal computed tomography scan revealed retroperitoneal lymph node metastases along the left common iliac artery. Two years before that event, the patient underwent abdominal hysterectomy, bilateral salpingoovarectomy, omentectomy, lymph node sampling, and bowel resection of the sigma at a local hospital with the histologic diagnosis of a papillary–serous ovarian cancer FIGO stage IIIc, pT3c, pN1, G2. After surgery, she received six cycles of polychemotherapy with carboplatin AUC 5 and paclitaxel 175 mg/m<sup>2</sup>.

Before explorative laparotomy, we informed the patient of a feasibility study to detect ovarian cancer metastases by means of fluorescence photodetection and requested her written informed consent according to the protocol approved by the local ethics committee.

### Photodetection procedure

The patient received an oral application of ALA dissolved in mineral water at a concentration of 10 mg/kg body weight. ALA was obtained as a solid (hydrochloride form) from Medac GmbH, Hamburg, Germany. After a time interval of 6 h, midline laparotomy was performed. The open abdominal cavity was inspected using a laparoscope connected to a D-light system (Karl Storz GmbH, Tuttlingen, Germany) with dimmed room light. This light source is a filtered short-arc xenon lamp, which produces either white light for conventional endoscopy or blue light at 380–440 nm to excite sensitized tissue. White light, blue excitation light,

and the reflected tissue fluorescence are detected by the endoscope. A color glass filter (long pass OG 515) was adapted on the ocular piece of the endoscope to transmit the red fluorescence light with an emitted wavelength peak 635 nm and filter out most of the reflected blue excitation light. To prove that the fluorescence originates from PpIX, spectral measurements were performed by imaging a 2-mm diameter tissue area through the endoscope optics via a beam splitter onto a 600- $\mu$ m core quartz fiber connected to a spectrometer (intensified OMA, SI, Gilching, Germany). The spectra were analyzed at a wavelength of 635 $\pm$ 5 nm and normalized to the peak fluorescence intensity of the calibration standard at the wavelength of 600 $\pm$ 50 nm [4]. Fluorescence and white light directed biopsies were taken.

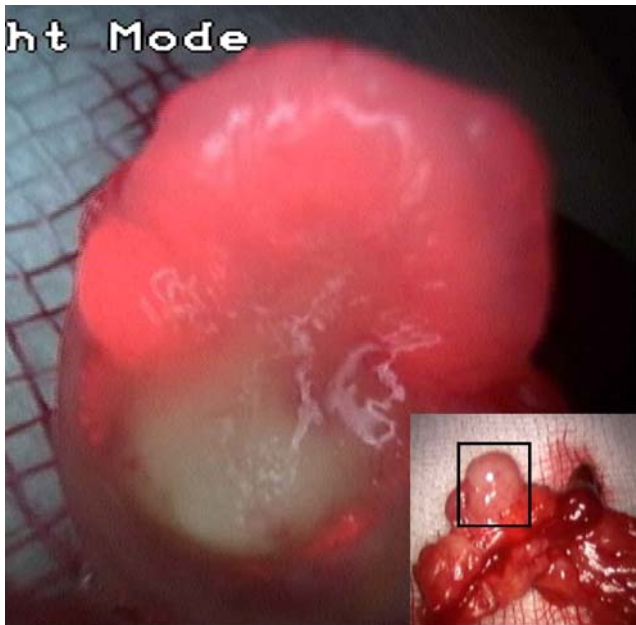
### Fluorescence microscopy

Excised tissues were forwarded for routine diagnostic evaluation, while a segment of the resected specimen was deep-frozen at  $-80^{\circ}\text{C}$  for fluorescence measurements. Samples were cut into serial cryosections for histological evaluation (6  $\mu$ m thickness; staining with hematoxylin and eosin) and for fluorescence microscopy (15  $\mu$ m thickness). All procedures were carried out under reduced ambient light to minimize photodegradation of porphyrins. For qualitative fluorescence microscopy, we used a 100-W mercury light source to excite porphyrins by a wavelength of 355 to 425 nm on a microscope with epifluorescence and transillumination facilities (Orthoplan; Leitz, Wetzlar, Germany).

## Results

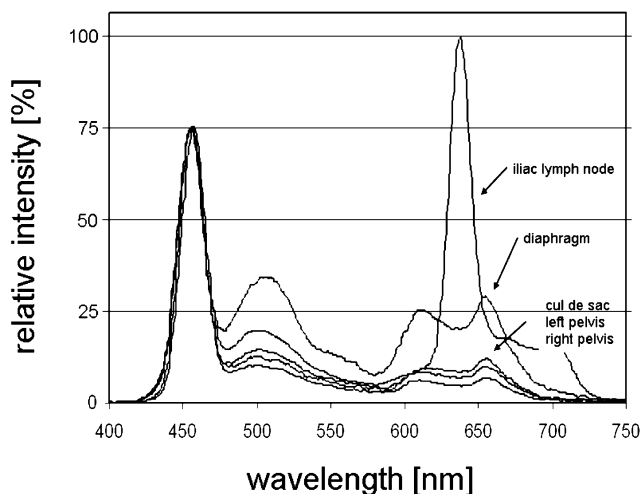
White light inspection and fluorescence photodetection of the abdominal cavity revealed no intraperitoneal metastases. Multiple biopsies taken from various peritoneal sites were negative. However, fluorescence-positive lymph nodes were visible in the left common iliac region under fluorescent imaging (Fig. 1). In the paraaortic region, lymph nodes were not enlarged and were fluorescence-negative. Spectroscopic measurements were performed from the left common iliac lymph node, cul de sac, left and right pelvis, and from the peritoneal surface of the diaphragm (Fig. 2). Fluorescence spectral analysis via endoscope did not reveal any protoporphyrin IX fluorescence of intraperitoneal tissues or skin. A specific fluorescence peak of the 5-aminolevulinic acid (ALA) induced protoporphyrin at 635 nm wavelength could be detected in the enlarged left common iliac lymph nodes reaching 5600 arbitrary units.

A systemic pelvic and paraaortic re-lymphonodectomy was performed and multiple biopsies from the abdominal



**Fig. 1** Red porphyrin fluorescence of the enlarged left common iliac lymph node after excitation at 380–440 nm using the D-Light system (K. Storz GmbH, Tuttlingen, Germany). The hilus region of the lymph node presents only the greenish autofluorescence. The *inset* shows the excised chain of lymph nodes along the left common iliac vessels in conventional white light

cavity were taken. Microscopic analysis confirmed lymph node infiltration of the left common iliac nodes by an epithelial ovarian cancer of papillary differentiation. Fluorescence microscopy showed the characteristic red fluorescence in the infiltrated parts of the lymph node tissue by the papillary ovarian cancer. The lymph node hilus presented



**Fig. 2** Spectroscopic measurements of the protoporphyrin IX fluorescence observed at various intra-abdominal locations 6 h after oral administration of 5-aminolevulinic acid at a dose of 10 mg/kg and excitation with blue light at 380–440 nm. Only the left common iliac lymph node displayed a highly increased porphyrin fluorescence level at 635 nm wavelength. Fluorescence level adjusted to highest level measured (=100%)

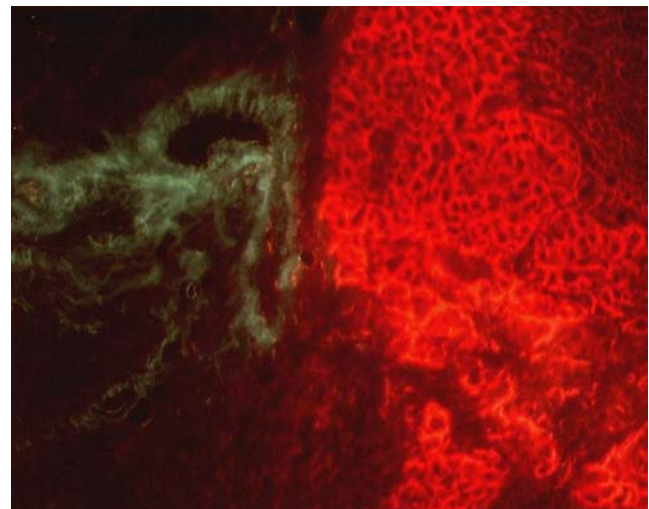
only with green autofluorescence of the connective tissue (Fig. 3). Histology of the other sites was negative.

The oral administration of ALA using a concentration of 10 mg/kg body weight was well-tolerated. No systemic effects such as nausea, vomiting, or elevation of liver enzymes were recorded. There were no symptoms of cutaneous photosensitization as exposure to intense white light had to be avoided for 24 h after oral administration of ALA.

## Discussion

The manifestations of metastatic lymph nodes in ovarian cancer are very diverse and range from clearly palpable enlarged nodes to only microscopic detectable nodes. The lymphatic drainage of ovarian cancer is into the pelvic and paraaortic regions. Therefore, these lymph nodes have to be removed by extensive surgery along the iliac, aortal, and inferior caval vessels causing an increase in the perioperative and postoperative complication rate. Since the therapeutic value of lymphonectomy is still under debate, an easier detection of potentially positive nodes would be advantageous. In early stages of ovarian cancer, 10 to 20% of lymph nodes are metastatic. This rate gradually increases to 50% in advanced ovarian cancer [1].

The hypothesis of fluorescence diagnosis being a diagnostic tool for infiltrated lymph node detection represents a very attractive approach. The potential of selective accumulation of a photosensitizing drug may be especially useful for the detection of positive nodes in ovarian cancer. In breast cancer or even cervical cancer, the sentinel



**Fig. 3** Fluorescence microscopy of the left common iliac lymph node shows the characteristic red porphyrin fluorescence after excitation at 380–440 nm. The hilus region of the lymph node (*left half* of the picture) presents only the greenish autofluorescence. Hematoxylin & eosin staining (not shown) revealed infiltration of the lymph node by an ovarian carcinoma of papillary differentiation

technique employs a preoperative and perilesional injection of blue dye and/or radioactively labeled colloids. However, this is hardly possible in the case of ovarian cancer where a preoperative intra-abdominal injection of the bilaterally localized organs would be necessary. This is even more difficult in patients with cystic adnexal masses because this may lead to rupture, which is associated with a worse prognosis.

In this patient, we could show that PD of lymph node metastases in ovarian cancer by means of systemic administration of ALA is feasible and well-supported. Spectroscopy of these lymph nodes showed high a peak around the PpIX-related wavelength in contrast to the other regions tested by spectroscopy and controlled by histology (Fig. 2). These results from a first case are promising and may be a significant step toward selective resection of only infiltrated lymph nodes with a decrease in the patient's discomfort.

Recent studies have shown the potential use of PD for the detection of peritoneal metastases in ovarian cancer. Major et al. [5] topically applied sterile swabs with 1% ALA solution to the rectum and peritoneum of the abdominal wall for 1 h. All visible metastases showed a red porphyrin-like fluorescence. Some of the small cancerous lesions were only observed in the fluorescence mode and not under white light inspection, which was proven by fluorescent-guided biopsy. The false negative rate of conventional staging surgery may be high and even the most thorough reexploration with cytology or biopsies does not always result in sensitive diagnosis of residual disease. By taking more than 100 biopsies during second-look procedure from the peritoneal surfaces, the false negative rate was reduced from 55–80 to 28%, which can impact positively on survival [6, 7]. Since patient survival is dependent on the size of the largest tumor lesion after performing cytoreductive surgery, a minimally invasive diagnostic procedure with the potential to assist in the detection of residual microscopic disease would be of great value. However, the intraoperative topical application with sterile swabs onto the abdominal wall for 1 h is time-consuming and is restricted to accessible areas only. Löning et al. [8] presented data comparing the *in vivo* fluorescence detection of ovarian carcinoma metastases in a second-look laparoscopic procedure after intraperitoneally applied 30 mg/kg bodyweight 5-ALA with white light inspection in 29 patients. 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. In 4 of 13 patients with ovarian carcinoma, lesions were detected under fluorescence, which were not observed under white light illumination.

Porphyrin fluorescence diagnosis of metastatic lymph nodes relies on a completely different approach compared

to the classical sentinel concept. The classical concept tries to identify the first lymph nodes draining a solid and localized malignant tumor. The porphyrin fluorescence concept aims to identify only the metastatic infiltrated lymph nodes irrespective of the tumor origin or localization. The latter concept has the charm of being linked only to positive nodes, being independent of tumor spread such as most ovarian cancers and ease of drug application. Furthermore, the use of porphyrin fluorescence may improve the detection of minimal residual disease not otherwise visible by the naked eye in patients with ovarian cancer, which can even be combined with intraperitoneal photodynamic therapy to treat the residual disease [9, 10]. Further systematic exploration of lymph node metastasis detection with the porphyrin fluorescence concept has to focus on critical details such as whether lymph node with partial infiltration will stain sufficiently, whether retroperitoneal detection is feasible, and finally, whether restriction of excising only positive nodes are advantageous in the combined surgical and chemotherapy treatment for ovarian cancer. However, this unique case shows proof of the concept that porphyrin fluorescence-guided infiltrated lymph node detection is possible in ovarian cancer and should stimulate further research in this field.

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