Abstract. Background: Precise staging of gastric cancer is essential when selecting for the appropriate treatment approach. 5-Aminolevulinic acid (5-ALA) is metabolized and accumulated as protoporphyrin IX (PpIX), which is a photosensitizer. In this study, we evaluated the diagnostic usefulness of 5-ALA for demonstrating peritoneal dissemination in patients with gastric cancer. Materials and Methods: Fluorescence laparoscopy (FL) was performed in 13 patients with gastric cancer. All patients received preoperative oral administration of 5-ALA. Fluorescence-light laparoscopies were sequentially performed intraoperatively. Results: In four out of the 13 patients, primary tumors were detected by FL and demonstrated serosal invasion on histological examination. Five out of the 13 patients demonstrated peritoneal metastases, and one patient demonstrated superficial liver micrometastases, by FL. All of these lesions were diagnosed as metastatic lesions by hematoxylin and eosin staining. Conclusion: These findings show that FL diagnosis with 5-ALA is accurate and suitable for the detection of peritoneal metastases and of superficial liver micrometastases in patients with gastric cancer.

Gastric cancer is the second most common cause of cancer-related death worldwide (1). Peritoneal dissemination and liver metastasis are among the most important prognostic factors for patients with gastric cancer and common features of metastasis, associated with advanced gastric cancer (2). Accurate staging of gastric cancer is essential for planning therapeutic strategies and evaluating the prognoses of patients. Enhanced computed tomography (CT) has been commonly used to assess the preoperative staging of gastric cancer (3, 4). However, its diagnostic power is limited to detecting serosal invasion, tumor deposition as peritoneal dissemination, and small superficial liver metastases. Diagnostic laparoscopy is a valuable method used in staging of gastric cancer. By performing accurate diagnostic laparoscopy, it is possible to avoid unnecessary laparotomy and to deliver neoadjuvant chemotherapy. In addition, we can evaluate the effect of neoadjuvant chemotherapy by carrying out diagnostic laparoscopy (5-8).

Photodynamic diagnosis (PDD) is being increasingly used. PDD can discriminate between normal cellular organization and malignant lesions without surgical intervention. 5-Aminolevulinic acid (5-ALA) is an endogenous substance and a natural precursor of the heme pathway. 5-ALA is not a photosensitizer, but protoporphyrin IX (PpIX), to which 5-ALA is metabolized, is. 5-ALA is immediately metabolized to heme in normal cells. On the other hand, since the activity of porphobilinogen deaminase is high in abnormal cells, and the activity of ferrochelatase is low, PpIX accumulates in the mitochondria in cancer cells (9-13). When PpIX is excited by light of 405-nm, a fluorescence spectrum with a 635-nm peak is emitted. Various cancer diagnostic methods using the properties of 5-ALA have been reported (14-20). In addition, we previously demonstrated the usefulness of administration of 5-ALA for the detection of lymph node micrometastases in a mouse model (21).

The purpose of this study was to assess the diagnostic capability of fluorescence laparoscopy using 5-ALA for peritoneal dissemination and small superficial liver metastases that are difficult to identify by CT scanning in patients with advanced gastric cancer.

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Materials and Methods

Patients. This study was performed at the University Hospital of Kyoto Prefectural University of Medicine, Kyoto, Japan, and approved by the Ethics Committee of Kyoto Prefectural University of Medicine, Kyoto, Japan. A total of 13 patients underwent surgery for advanced gastric cancer between April 2010 and February 2012 after written informed consent. Exclusion criteria included the presence of porphyria and pyloric stenosis.

Fluorescence laparoscopic analysis system. The system used for fluorescence laparoscopic analyses is composed of a laparoscopic videoscope (OTV-Y0007; Olympus, Tokyo, Japan) equipped with a video system center (EVIS EXCERAII CV-180; Olympus) and a xenon light source (EVIS EXCERAII CLV-180; Olympus). 5-ALA-induced fluorescence images (>450 nm) (MAJ-Y0048; Olympus) were taken after excitation at 380-430 nm. We performed the observation in white-light mode using the same conditions as for standard laparoscopy, and for the fluorescence observation, we changed to fluorescence mode.

Fluorescence laparoscopy using 5-ALA. Patients received a dose of 10 to 15 mg per kg body weight of 5-ALA (Cosmo Bio Co., Ltd., Tokyo, Japan) dissolved in 20 ml of 50% glucose solution 3 h prior to surgery. All patients were protected from direct light for 24 h after administration of 5-ALA, to avoid sunburn.

The patient was placed in the supine position under general anesthesia. A small subumbilical incision was made and a 12-mm disposable trocar was inserted for the camera, and two or three 5-mm disposable trocars for retraction or dissecting organs were inserted into the upper abdominal wall. Under 10-mmHg abdominal air pressure, we performed a careful examination of the abdominal cavity, liver surface, and omentum. Laparoscopic examination was performed under alternate white light mode and fluorescence mode.

Suspicious lesions detected under the fluorescence mode were sampled. In patients requiring gastrectomy, the procedure was performed under laparotomy.

Fluorescence images and fluorescence spectrum. The system used for stereomicroscopic analyses of fluorescence and the spectral analytic system were as previously described (21). We applied the excitation at 405±20 nm and observed the fluorescence at wavelengths of >430 nm. We compared the fluorescence spectra of peritoneal disseminated lesions with that of pure PpIX (Sigma-Aldrich, St. Louis, MO, USA) dissolved in dimethyl sulfoxide (DMSO).

Fluorescence analysis of primary tumors. The resected primary lesions were subjected to formalin fixation overnight and were processed for pathological examination. The specimens were observed using fluorescence stereomicroscopy.

Histopathological examination. Formalin-fixed, paraffin-embedded specimens were prepared from surgically-removed lesions for histopathological diagnosis.

Results

Diagnosis using fluorescence laparoscopy. Details of the patients’ characteristics are summarized in Table I. The mean age was 68 years (range 51 to 81 years), and the male/female ratio was 2.3. Two patients underwent distal gastrectomy, three patients total gastrectomy, one patient gastrojejunostomy, and two patients explorative laparotomy. In six out of the 13 patients, the lesions of serosal invasion were observed as primary tumors and exhibited a specific speckled pattern of PpIX fluorescence signal by fluorescence laparoscopy. Tumor in four patients demonstrated serosal invasion on histological examination.

<table>
<thead>
<tr>
<th>Case</th>
<th>Gender</th>
<th>Age (years)</th>
<th>Depth of invasion</th>
<th>Peritoneal dissemination</th>
<th>Liver metastasis</th>
<th>Histology</th>
<th>Operation</th>
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<td>Total gastrectomy</td>
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(Figure 1A); two patients were not diagnosed histologically. Five out of the 13 patients demonstrated peritoneal disseminations, and one patient demonstrated superficial liver micrometastases as red signals of PpIX on fluorescence laparoscopy (Figure 1B and C). All of these lesions were diagnosed as metastatic lesions by hematoxylin and eosin staining (data not shown). Table II shows the comparative diagnostic accuracy of fluorescence laparoscopic imaging and white light imaging for the lesions of...
peritoneal metastasis after 5-ALA administration. The white light imaging diagnosis of a white nodule was peritoneal dissemination. On the other hand, we diagnosed a nodule with red fluorescence as peritoneal metastasis by using fluorescence laparoscopy. The accuracy of the fluorescence imaging was greater than that of the white light imaging. The side-effects of elevation of liver enzyme levels, nasal swelling, edema, and photosensitivity reaction are known to occur following ALA administration at 60 mg per kg of body weight. We used a concentration of 10 to 15 mg per kg of body weight. None of the patients developed notable side-effects. The results showed that laparoscopic fluorescence imaging by administration of 5-ALA, detected more metastatic lesions compared to white light laparoscopy and can provide qualitative diagnoses.

Visualization of primary tumor in excised specimens by 5-ALA administration. We analyzed excised specimens after overnight formalin fixation but before paraffin embedding. We could not distinguish cancer lesions from normal tissue under white light (Figure 2, left), but detected red fluorescence by fluorescence microscopy observation (Figure 2, center). Furthermore, the portion with red fluorescence by fluorescence observation was confirmed by hematoxylin and eosin (H&E) staining (Figure 2, right) to be cancerous tissue. The fluorescence spectra of both the resected peritoneal dissemination and the pure PpIX were determined by using a spectrophotometer. The result of each measurement is shown in Figure 3. It was confirmed that the result for the emission spectrum of peritoneal dissemination was in accordance with that for the pure PpIX spectrum, and it was confirmed that PpIX had accumulated at the cancer site.

Discussion

Despite improvements in therapeutic resection techniques, 60-70% patients with gastric cancer who have undergone a radical curative resection, experience recurrence within two years after surgery (22). Moreover, most cases of death are associated with recurrence. It is thought that this is due to micrometastases that could not be diagnosed at the time of surgery. Therefore development of diagnostic technology that provides accurate staging diagnosis is urgently needed.

Although significant improvements in preoperative tumor staging have been developed due to diagnostic modalities such as CT scanning, Magnetic Resonance Imaging (MRI), and positron emission tomography-CT, peritoneal dissemination and small superficial liver metastases are only detected during surgery in many cases (23, 24). Several reports have indicated that preoperative staging of peritoneal dissemination and superficial metastasis aids in avoiding unnecessary laparotomy (5-8). However, standard staging laparoscopy cannot provide an accurate diagnosis without biopsy. Many patients with advanced gastric cancer have peritoneal dissemination, and peritoneal dissemination is a significant prognostic factor. Effective therapy has been reported (25, 26), and it is thought that discovery at an earlier stage is important for a positive prognosis. Furthermore, accurate prognostic judgment and planning of postoperative chemotherapy are strongly associated with peritoneal dissemination (27). The most common route of peritoneal dissemination is tumor invasion as far as the
gastric serosal layer (28). Conventional cytology has low sensitivity (29). Therefore, a means of diagnosis of serosal invasion is necessary.

Various fluorescent probes which are cancer-specific have been developed and applied (30-33). The advantages of the fluorescence diagnosis are that it is a minimally invasive procedure, the diagnostic device is compact, and it makes real-time diagnosis possible. However, only a few fluorescent probes can be applied in a clinical setting, for reasons that include safety considerations. Fluorescence diagnosis using 5-ALA has been applied for diagnosis of such conditions as bladder cancer and brain tumor in a clinical setting (17-20). Because 5-ALA is an endogenous substance and is included in many food types, there is no report of any serious side-effect associated with administering it (14, 33). As for our results, it was confirmed that fluorescence laparoscopic diagnosis using 5-ALA had sufficient sensitivity for detecting peritoneal disseminations and superficial liver micrometastases. This technique can accurately diagnose metastases without the need for biopsy. No acute or major complications were observed, although patients should avoid direct sun exposure for 24 h after 5-ALA administration.

Our study indicates that PDD using fluorescence laparoscopy after 5-ALA administration offers the potential for the detection and accurate diagnosis of peritoneal dissemination and superficial liver micrometastases in patients with advanced gastric cancer. Fluorescence diagnosis seems not only to make it easy to look for metastatic nodules, but also to allow diagnosis of whether they are metastases without H&E staining. Because neoadjuvant chemotherapy has been developed for peritoneal dissemination (25, 26), a more accurate diagnosis of peritoneal metastasis is necessary. In addition, it is not possible to change chemotherapy regimens upon shifting to surgery when an accurate diagnosis is not possible at second staging laparoscopy after neoadjuvant chemotherapy has been initiated. Therefore, a fluorescence laparoscopic method using 5-ALA will be useful. However, there is a limitation of this study in terms of the object of clinical application. A weak point of this technique is that deep observations are not possible because the penetration of light is low. This method can be used to diagnose large lymph node metastases without excision, but the micrometastases are difficult to detect without resection by observation in the abdominal cavity (data not shown). If deep observation were possible, diagnosis of lymph node metastasis would be possible and it could be possible to select individual lymph nodes for extraction. Therefore a cancer-specific probe, sensitive to near-infrared light excitation should be developed. Because this study was a pilot study, it includes only a few patients and was a comparison between sampled metastatic lesions and pathological examination results. However, the number of cases will be increased in the future, and it is necessary to further examine the diagnostic capability. In addition, it is

Figure 3. Typical normalized fluorescence spectrum of peritoneal dissemination with 5-aminolevulinic acid (5-ALA) administration in advanced gastric cancer patients (left) and typical normalized fluorescence spectrum of protoporphyrin IX (PpIX) dissolved in dimethyl sulfoxide (DMSO) (right).
necessary to consider diagnosis of small metastatic foci. This technique is worth further investigation and development for intraoperative diagnosis using fluorescence laparoscopy.

**Competing Interests**

None.

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**References**


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