Fluorescence thoracoscopy in the detection of pleural malignancy

Oleg Pikin*, Elena Filonenko, Dmitriy Mironenko, Dmitriy Vursol and Ali Amiraliev

Department of Thoracic Surgery, P.A. Hertsen Moscow Research Institute of Oncology, Moscow, Russian Federation

* Corresponding author. Tel: +7-903-5200529; fax: +7-495-9458020; e-mail: pikin_ov@mail.ru (O. Pikin).

INTRODUCTION

Thoracoscopic examination has become a standard procedure to determine the aetiology of pleural effusion and for staging purposes. In the majority of patients, repeated pleural fluid examination has diagnostic yield of 70.0–80.0% [1]. Cytologic examination of pleural fluid sometimes is inconclusive in differential diagnosis between mesothelioma and pleural adenocarcinoma metastases of different origin. Moreover, in 8–10% of cases with cytologically positive pleural fluid patients, following standard thoracoscopy examination does not find any macroscopic abnormalities of parietal or visceral pleura [2, 3]. Several publications have demonstrated that fluorescence diagnosis (FD) using 5-aminolaevulinic acid (5-ALA) can improve the diagnostic value of laparoscopic and thoracoscopy [4–6]. Only a few reports were dedicated to FD using 5-ALA in humans [7].

5-ALA is the photosensitizer, metabolized in the cell to haem via an intermediate production of protoporphyrin-IX (Pp-IX). It is transformed to haem by a special enzyme, ferrochelatase. The expression of this enzyme is known to be decreased in malignant cells, leading to an accumulation of Pp-IX, which has photodynamic capacities. The differences in concentration and pharmacokinetics between normal and malignant cells facilitate the use of 5-ALA for diagnostic or therapeutic purposes in clinical oncology. The photosensitizer 5-ALA can be administered orally or locally and has very limited side effects. After oral intake, skin photosensitivity lasts for 24–36h and liver enzymes may transiently rise.

Our study was aimed to evaluate the impact of fluorescence thoracoscopy and white-light inspection on diagnosis and staging of pleural malignancies with undiagnosed pleural effusion.

MATERIALS AND METHODS

Patients were eligible when they presented with a pleural effusion with cytologically negative or non-conclusive examination. Patients had to be >18 years of age and fit for general surgery.

METHODS: A total of 23 patients with non-conclusive pleural effusions were enrolled in the prospective single-institution trial. Eligible patients were administered 25 mg/kg of 5-ALA (‘Alasense’, Niopik, Russian Federation) per os 3 h before video-assisted thoracoscopy. After conventional inspection with white light, thorough fluorescence investigation of the visceral and parietal pleura was performed (D-LIGHT Auto Fluorescent System, Karl Storz, Germany). Biopsy specimens of both normal and abnormal sites, as determined from white-light and FD inspection, were obtained for histological examination.

RESULTS: There was no morbidity or mortality due to the procedure. A definitive diagnosis was obtained in all cases: malignant mesothelioma in 13 cases, other malignancies (pleural metastases) in 8 cases and non-specific inflammation in 3 patients. A total of 118 biopsy specimens were available for histological examination. In 20 patients, all pleural deposits (n = 60) detected by white-light thoracoscopy had bright red fluorescence during FD and were proved to be malignant. Upstaging occurred in 12 patients (57.2%) (unsuspected 21 tumour deposits) due to FD examination. Micrometastases of macroscopically normal pleura were detected, only by FD, in one patient. Comparing the results of histological examination of specimens detected by conventional thoracoscopy with that by fluorescence thoracoscopy, we obtained 82 true positive, 10 false-negative, 23 true negative, 3 false-positive results with a specificity of 88.4%, sensitivity of 89.1% and diagnostic accuracy of 88.9%.

CONCLUSIONS: FD using 5-ALA in the pleural cavity is a feasible diagnostic tool when used in addition to white-light thoracoscopy. It improves visualization of additional lesions or even micrometastases in patients with pleural malignancy.

Keywords: Thoracoscopy • Fluorescence diagnosis • Pleural malignancy

Received 26 June 2011; received in revised form 21 July 2011; accepted 1 August 2011
anaesthesia. The chest radiograph, CT scan of the thorax and upper abdomen, bone scintigraphy, chest and abdomen ultrasound, ECG and functional lung tests were routinely performed to all patients. The study was approved by the local Medical Ethical Committee, and written informed consent was obtained from all patients.

5-ALA (‘Alasense’, Niopik, Russian Federation) was administered per os in a dose of 25 mg/kg dissolved in 100 ml of water prior 3 h to thoracoscopy. After intake of ‘Alasense’, patients were kept in the subdued light for at least 24 h.

Fluorescence images were recorded (D-LIGHT Auto Fluorescent System, Karl Storz, Tuttlingen, Germany), which consists of a xenon light source with an integrated filter wheel that enables the selection of white light or blue light (<500 nm for excitation of the Pp-IX). A rigid 10 mm endoscope, integrated with a long-pass filter (cut-off wavelength 470 nm), was used for illumination and observation of the pleura. The HD Storz camera has a white-light mode and a blue-light mode in which the integration time can be increased to correct for the relative low intensity of fluorescent light. An additional low-pass filter (>550 nm) was placed between the endoscope and the camera to increase the contrast of the images. The 23 in. HD Flat Screen monitor was connected by S-Video cable to Karl Storz AIDA system, where all the images were recorded for subsequent evaluation and further analysis.

**Surgical procedure**

All procedures were performed by two experienced surgeons previously skilled in more than 100 thoracoscopic operations. A procedure was performed under general anaesthesia after insertion of a double-lumen tube. A patient was put in a lateral decubitus position and two or three ports were inserted for thorough inspection of the pleural cavity. A single-lung ventilation was started; a standard thoracoscopy was performed and after aspiration of pleural fluid the pleural surface was inspected initially with white light, followed by fluorescence examination (Fig. 1a and b). Biopsy specimens of both normal and abnormal sites, as determined during FD and white-light inspection, were obtained for histological examination. In case of normal pleura during white-light examination, blind multifocal biopsies were performed. In patients with diffuse malignant pleural mesothelioma, pleuroectomy followed by intrapleural photodynamic therapy (PDT) was performed via the thoracotomy approach. In case of metastatic spread, only PDT was performed to prevent the recurrence of the effusion. The port incisions were closed after the procedure, and usually one chest tube was inserted for fluid collection and optimal expansion of the lung.

**RESULTS**

From January 2007 to January 2009, 23 patients (9 men and 14 women) with non-conclusive pleural effusions were enrolled in this study. A right-sided effusion was diagnosed in 15 patients, and a left-sided effusion was diagnosed in 8 patients. A standard thoracoscopy was performed within 3 h after administration of ‘Alasense’. The procedure was uncomplicated in all cases and lasted 35–60 min (median, 42 min). The time required for the additional fluorescence examination varied from 10 to 15 min.

A definitive diagnosis was obtained in all cases: malignant pleural mesothelioma in 13 cases, breast cancer metastases in 4, lung cancer metastases in 4 and non-specific inflammation in

<table>
<thead>
<tr>
<th>Histological examination</th>
<th>Type of the specimen</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V(+)</td>
<td>F(+)</td>
</tr>
<tr>
<td>T(+)</td>
<td>60 (50.9%) (TP)</td>
<td>22 (18.6%) (TP)</td>
</tr>
<tr>
<td>T(-)</td>
<td>3 (2.5%) (FP)</td>
<td>23 (19.5%) (TN)</td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td>45</td>
</tr>
</tbody>
</table>

TP, true positive; TN, true-negative; FP, false-positive; FN, false negative.

*V(+)|F(+) represents deposits visible at white-light and fluorescence mode; V(-)|F(+) denotes deposits visible only by fluorescence examination; V(-)|F(-) denotes specimens obtained from macroscopically normal pleura at white-light and fluorescence modes.
2 patients. A total of 118 biopsy specimens from different pleural sites were available for histological examination.

The specimens were classified into white-light positive (V+) or white-light negative (V−) and fluorescence-positive (F+) or fluorescence-negative (F−). Specimens proved to be malignant at histological examination were classified into tumour-positive (T+), benign and tumour-negative (T−). The results are presented in Table 1.

According to this analysis, a positive fluorescence image was considered to be correct if histological examination found tumour tissue in the specimen. When white-light and fluorescence thoracoscopy were both negative in the detection of pleural malignancy, but pathological examination of multiple-blinded biopsies proved malignancy, these cases were considered as false-negative, while the biopsies with no tumour were considered to be true negative. The specimens obtained from positive fluorescence spots and negative at histological examination were considered as false negative.

In 20 patients, all pleural deposits (n = 60) detected by white-light thoracoscopy had bright red fluorescence during FD and were proved to be malignant (Fig. 2a and b). Additional lesions not visible by white-light mode were found in 12 patients (57.2%) (unsuspected 21 tumour deposits) due to FD examination. In three patients with macroscopically normal pleura by white-light mode, micrometastases were detected by FD only in one patient, while in two patients with normal pleura no any fluorescence were seen (Fig. 3a and b). Histological examination of obtained specimens proved lung adenocarcinoma metastases of fluorescence spots in the first case and did not find any malignancy (non-specific inflammation) in the last two cases.

Comparing the results of histological examination of the specimens detected by conventional and fluorescence thoracoscopy, we obtained 82 true positive, 10 false negative, 23 true negative, 3 false positive results with specificity of 88.4%, sensitivity of 89.1% and diagnostic accuracy of 88.9%.

**DISCUSSION**

Fluorescence methods have been intensively studied since 1990 with the aim of enhancing the optical contrast of malignant tissue over normal surrounding tissue. Both autofluorescence and fluorescence methods with 5-ALA are being used in clinical practice for diagnostic purpose. Chrysanthidis et al. [8] used autofluorescence thoracoscopy in patients with pleural effusion for diagnosis of pleural spread and differential diagnosis between malignant and benign disease. The first experimental report by Prost et al. [6] proved the advantage of thoracoscopic FD with 5-ALA in rats to the standard white-light inspection. 5-ALA was used locally by intrapleural instillation of different doses that could be challenging in humans [6]. We preferred oral intake of fluorescence agent prior 3 h to thoracoscopy exploration in doses determined by the weight of the patient and previously published in the literature [6, 7]. We consider 5-ALA to be an ideal sensitizer for FD due to its short-time skin phototoxicity and very few side effects.

![Figure 2: Thoracoscopic fluorescence diagnosis with 'Alasense'. Bright red fluorescence of tumor deposits (a and b).](image)

![Figure 3: Thoracoscopic fluorescence diagnosis with 'Alasense'. Macroscopically normal pleura by white-light inspection (a); solitary tumour deposit (red) in fluorescence mode, not visible by standard examination (b).](image)
Generally, we obtained satisfactory view of both small and large lesions presented as bright red spots on the monitor with good contrast to normal pleura. To our experience, FD was especially effective in the detection of small lesions (<3 mm) on the parietal and visceral pleura not visible by white-light examination. We support Baas et al. [7] who consider fluorescence thoracoscopy to be very useful in diagnosis of small lesions on the parietal pleura and lesions on the rim of the partly collapsed lung or diffuse spots on the visceral pleura in patients with pleural malignancies. Time is needed to get accustomed to the handling of the fluorescent equipment and to interpret the fluorescent images. One should remember that the time of fluorescent inspection is restricted to 10–15 min that more than enough for thorough investigation of the pleural cavity and to perform multiple biopsies.

From practical point, the detection of microscopic pleural metastasis could change the treatment strategy in patients with pleural malignancies. In our study, the additional lesions were found in 12 (57.2%) patients that is better than the results by Baas et al. In their series, the fluorescence thoracoscopy improved the visualization of the additional nodules on the visceral pleura only in 4 (26.7%) of 15 patients with mesothelioma [7]. On the one hand, the detection of additional lesions in patients with macroscopic pleural spread does not influence the treatment policy in the majority of cases. On the other hand, the detection of pleural micrometastases especially in patients with peripheral lung cancer and visceral pleural invasion could change preoperative plan dramatically. We found lung cancer pleural micrometastasis by FD only in one patient with macroscopic normal parietal and visceral pleura.

One should be surprised by a relatively high rate of false-negative results in our study. We have explained these phenomena by diffuse tumour growths mimicking a view of normal pleura both at white-light and fluorescence modes.

The limitation of our study is the small number of patients enrolled. Further investigations are needed to determine the role of FD in clinical practice. To our opinion, in the future, FD might be routinely used in patients with peripheral lung cancer and visceral pleural invasion to detect pleural micrometastases. It could also be useful in patients with non-conclusive pleural effusion to facilitate biopsy.

CONCLUSIONS

FD using 5-ALA in the pleural cavity is a feasible diagnostic tool when used in addition to white-light thoracoscopy. It improves visualization of additional lesions or even micrometastases in patients with pleural malignancies.

Conflict of interest: none declared.

REFERENCES


APPENDIX. CONFERENCE DISCUSSION

Dr K. Papagiannopoulos (Leeds, UK): in pleural malignancies, especially when dealing with mesothelioma, this will most probably aid in the diagnosis. Do you consider that in patients with lung cancer with small pleural effusions, you might start using this routinely, because we know that the diagnostic yield of the aspirations is extremely low, isn’t it?

Dr Probst: This was our first experience, and we enrolled patients with non-conclusive pleural effusion. I think that the main field for employing this method, fluorescence thoracoscopy, will be, first, in peripheral lung cancer, especially with microscopic pleural invasion undetected by white light thoracoscopy, where we can detect micrometastases by fluorescence diagnosis; it should change the treatment strategy of such patients greatly. It can be used routinely, too, for upstaging of patients with peripheral lung cancer and visceral pleural invasion.

Dr A. Toker (Istanbul, Turkey): As they have shown here, we have a 10% false negative rate, and this is the major problem in our series, also, the false negative patients.

Dr Pikin: Of course. The limitation of this study is that the number of patients is too small, 23 patients. I think that when we collect a larger series, maybe the false positive results will be less.

Dr H. Eid (Dubai, UAE): I would like to just ask whether you really need fluorescence to identify pleural lesions to take a biopsy from – do you need such a light to take it? Usually all pleural lesions are evident. Whenever you place the thoracoscope with the light, giving illumination and magnification, you can see any pleural lesion. What I saw in the video you showed is pleura with a smooth surface, and no lesion to take. If there is a lesion, you will take it.

This fluorescence is useful for a peripheral lung lesion when you cannot differentiate between normal tissue and the lung lesion which is mainly subpleural with visceral pleural invasion. But on the parietal pleura, I think any lesion will be evident. If there is a lesion, or pathology, it will be evident to the naked eye and you can take a biopsy without the need for injecting either a dye or using the fluorescence.

Dr Pikin: The fluorescence thoracoscopy was done on these patients to confirm the specific sensitivity and diagnostic accuracy of this method. We still perform a biopsy for microscopically normal and microscopically abnormal pleura to compare each with histological examination. And, of course, the main aim of the thoracoscopic fluorescence diagnosis is to determine the micrometastases that couldn’t be detected by white light thoracoscopy.

Dr A. Toker (Istanbul, Turkey): Certainly there are some lesions that could not be seen with video thoracoscopy, they may be micrometastases. Sometimes we could experience that you take samples, especially in lung cancer patients, as Kostas indicated, and you see that they develop pleural recurrences within six or eight months, or within the first year after resection. So I think this technique may be useful, especially for these patients whose pleura seems clear.