Thoracoscopic Findings and Pharmacokinetics of Inhaled Fluorescein in a Pig Model

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Key Words

Sodium fluorescein • Thoracoscopy • Pig model • Fluorescein quantification

Abstract

Background: Fluorescein-enhanced autofluorescence thoracoscopy (FEAT) reveals regions of abnormal fluorescence in patients with primary spontaneous pneumothorax and in normal subjects. Some of these lesions are undetectable by white light thoracoscopy and it has been hypothesized that they represent underlying pleural and/or parenchymal abnormalities. **Objectives:** In order to standardize and evaluate this novel technique, we developed an animal model. **Methods:** Six pigs underwent thoracoscopy after the inhalation of nebulized sodium fluorescein by either volume-controlled mechanical ventilation or spontaneous ventilation. Pleural cavity and lung surface were inspected by white light thoracoscopy and FEAT during a period of 90 min. Fluorescence intensities were quantified in pleura and in blood. Regions of interest were examined postmortem for a histological assessment of the lesions. **Results:** FEAT lesions were observed in all animals, with a maximum intensity of the lesions 20–30

min after the onset of fluorescein administration. The plasma concentrations of sodium fluorescein reached a maximum after approximately 20 min. The microscopic findings suggest that fluorescein accumulates in the subpleural space of better ventilated lung areas. **Conclusions:** This is the first animal model using FEAT. Valuable information has been gathered but further investigations are required to explain the phenomena observed in humans and pigs.

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Introduction

Fluorescein-enhanced autofluorescence thoracoscopy (FEAT) allows the visualization of lung regions with abnormal subpleural fluorescence [1, 2]. In a study conducted by the senior author of the current paper, patients with primary spontaneous pneumothorax inhaled an aerosolized fluorescein solution prior to medical thoracoscopy [2]. During FEAT we found that: (1) macroscopically abnormal lung regions in white light conditions such as blebs and bullae could also be detected in blue light, and (2) normal lung regions in white light often seemed ‘ab-

normal' in blue light conditions. These observations seemed to confirm an association between those 'abnormal' regions and the disease process in pneumothorax, but the finding of abnormal fluorescence at macroscopically normal parts of the lung was intriguing. Moreover, in a group of control subjects undergoing bilateral thorascopic sympathicotomy, FEAT revealed significant regions of abnormal fluorescence in contrast to normal lung inspection in white light. It was hypothesized that lung regions with abnormal fluorescence reflect an underlying pathological process which cannot be detected in white light, but the anatomopathological correlation of these observations remained unclear.

Disodium fluorescein ($C_{20}H_{10}O_5Na_2$) is a relatively low-molecular-weight, highly water-soluble compound which, when exposed to light of wavelengths between 465 and 490 nm (blue), emits light at a wavelength of 520 to 530 nm (yellow-green). The pharmacokinetics of fluorescein have been shown to be linear after intravenous administration. It is rapidly metabolized by the liver with a half-life in plasma of 1.7 min and is excreted in bile and in urine [3]. Only 1 of the 3 metabolites formed in the liver, fluorescein monoglucuronide, is fluorescent. The emission peak is the same as that of fluorescein, but fluorescein glucuronide has been shown to be much more hydrophilic at physiological pH. This means that when using fluorescence intensity as a measurement of fluorescein concentration, fluorescein glucuronide may contribute to the measured intensity [4]. Therefore, plasma concentrations of disodium fluorescein were measured in order to correlate them with the fluorescence intensities observed during thoracoscopy, and to follow its course in time.

Aerosol administration of solid disodium fluorescein has been used in dogs to study the pulmonary and systemic deposition of drug-containing aerosols [5–9]. These studies showed that even a lipophobic compound such as fluorescein is absorbed rapidly via the lung when administered as an inhalation aerosol.

Pleural disease is not uncommon and differential diagnosis sometimes difficult, hence the search for improved technologies to detect pleural abnormalities by thoracoscopy [10, 11]. Autofluorescence bronchoscopy has already been shown to be an interesting and effective tool in the diagnosis of both malignant and nonmalignant lesions [12, 13]. Fluorescein administration by inhalation has been used under white light thorascopic conditions in the past but, thus far, with few clinical applications [14], which stands in sharp contrast to the extensive use of fluorescein by intravenous injection in ophthal-

mology and gastroenterology [15–17]. The aims of this study were: (1) to evaluate and standardize fluorescein administration in an animal thoracoscopy model, and (2) to study the pharmacokinetics of nebulized fluorescein in blood and pleura.

Methods

Animals and Preparation

The experiments, approved by the Institutional Animal Care and Use Committee of the Centre Hospitalier Régional Universitaire de Lille, France, were conducted using 6 experimental animals (pigs). The pigs were each 3 months old, of either sex, and weighed 28 kg on average. All animals had been fasted for 48 h prior to the experiments but were allowed free access to water. The external jugular vein was cannulated for blood sampling, and a second peripheral vein was used for the administration of anesthetic drugs and supporting fluids. The pigs were placed in the supine position in the experimental medical thoracoscopy unit. Four pigs were sedated with propofol (0.5 mg/kg/min) and anesthetized with an induction dose of 10 mg/kg of ketamine. These pigs were orotracheally intubated with an endotracheal tube (8 mm inside diameter) and mechanically ventilated with a fixed tidal volume of 8 ml/kg, while positive end-expiratory pressure was set to zero (Cesar II) [19]. Two pigs were spontaneously ventilated during the experiments to demonstrate that the findings during thoracoscopy were independent of mechanical ventilation. The depth of anesthesia was maintained by intermittent propofol injections in all animals. Hemodynamic parameters and oximetry were determined at baseline and thereafter checked every 5 min.

Aerosol Generation

The apparatus used for the nebulization was an Atomisor® MegaHertz ultrasonic nebulizer (Diffusion Technique Française, Saint-Etienne, France) running with a frequency of 2.4 MHz. According to previous studies, the mass median aerodynamic diameter at the outlet of the nebulizer is $4.2 \pm 0.2 \mu m$ (mean $\pm$ SD) [19]. In the mechanically ventilated pigs, the nebulizer was positioned in the inspiratory limb of the ventilatory circuit, 40 cm proximal to the Y-piece, and filled with a 10% sodium fluorescein solution (5 ml; Novartis Pharma, Vilvoorde, Belgium).

Study Design and Thoracoscopy

After the instrumentation and onset of mechanical ventilation, a stabilization period of 5 min was allowed before exploratory thoracoscopy was started. Thoracoscopy was performed through a 1-cm incision on the animal's lateral chest. After blunt dissection of the intercostal space, the parietal pleura was penetrated to create a pneumothorax. A 7-mm-diameter pleural trocar was then inserted to inspect the pleural space and lung using a DAFE autofluorescence endoscopy unit (Richard Wolf, Knittlingen, Germany). After an initial thorough inspection of the pleural cavity, the pigs received an aerosol (10 ml) containing either 500 mg (2 pigs) or 250 mg (4 pigs) of fluorescein in approximately 15 min, maximum 18 min.

Fig. 1. Examples of FEAT lesions (**a, b**) and their corresponding white light images (**c, d**). Hot spots and areas with diffuse accumulation can be seen. **a, c** Discrete subpleural fluorescein accumulation. **b, d** Intense subpleural fluorescein accumulation.

Fluorescein Blood Levels

Venous blood samples were taken at baseline and at 1, 5, 10, 20, 30 and 60 min. The plasma concentrations of sodium fluorescein were determined by spectrofluorometry, and fluorescence intensities were measured as counts per pixel in arbitrary units (AU).

Analysis of Pleural Fluorescence

Video recordings of the visceral pleura were scheduled at regular time intervals using the onset of fluorescein inhalation as reference time point: 0, 2, 5, 10, 15, 30, 45, 60 and 90 min after the onset of nebulization. Video recordings and still images were taken and stored for further image processing. Images were processed using V paint, SigmaScan Pro® (Systat, version 5, trace measurement mode) for fluorescence quantification as described previously [20]. Five fluorescent lesions were analyzed per pig. For each lesion, fluorescence intensities were measured at the center of the lesion (region of interest, ROI) and at surrounding areas with no macroscopic evidence of fluorescence (normal area, NA). Fluorescence intensities were measured as counts per pixel in arbitrary units.

Euthanasia and Histological Assessment

After each experiment, the animals were sacrificed by an injection of potassium chloride, whereas exsanguination was performed through direct cardiac puncture, and the lungs were exposed through a cervicothoracic incision. ROIs and macroscopically normal areas were marked *ex vivo* under blue light inspection with needles placed 0.5 cm away from the periphery of the area. The lungs were preserved in a fixative (4% formaldehyde) and transported to the Pathology Department where the marked regions were examined by a blinded pathologist.

Statistical Analysis

Data are expressed either as median (25th–75th percentile or the interquartile range, IQR) for continuous data, or as n (%) for categorical data. Differences between groups were determined using the nonparametric Wilcoxon signed rank test for paired data and the Mann-Whitney test for unpaired data. SPSS 13® for Windows (SPSS Inc., Chicago, Ill., USA) was used for the analysis, and $p < 0.05$ was accepted as significant.

Results

Clinical Findings

All but one pig had a completely normal lung during white light inspection. There were no blebs/bullae, air leakage or anthracotic lesions present. FEAT revealed different types of lesions: small areas of 1–2 mm diameter with intense fluorescence (hot spots) and larger areas with diffuse fluorescence (table 1). Subpleural accumulation of fluorescein was either discrete (+) or intense (++) as shown in figure 1. Lesions appeared within 5 min after the onset of inhalation, and most lesions were seen at the upper lobe and apex of the pig lungs. Throughout the experiments, SpO₂ remained within the normal range in each animal and no unexpected death occurred.

Fig. 2. Mean plasma concentration of sodium fluorescein after the inhalation of fluorescein solution in pigs. Data are medians and IQR.

Table 1. Thoracoscopic white light thoracoscopy and FEAT findings in 6 pigs receiving fluorescein as aerosol by mechanical or spontaneous ventilation

Pig No.	Ventilation mode	Dose, ml	White light thoracoscopy	FEAT	
				hot spots	diffuse lesions
1	VC	5	normal	++	++
2	VC	5	normal	+	++
3	VC	2.5	normal	++	+
6	VC	2.5	normal	0	+
4	SV	2.5	adhesions	++	+
5	SV	2.5	normal	0	+

Pleural adhesions were seen in one pig.

VC = Volume-controlled mechanical ventilation; SV = spontaneous ventilation; 0 = absence of visible fluorescein accumulation; + = discrete fluorescein accumulation; ++ = intense fluorescein accumulation.

Fig. 3. Fluorescence intensity in pleura before and after the administration of sodium fluorescein in mechanically ventilated pigs. Fluorescein concentration in different lung areas are shown. **a** Mechanical ventilation + 500 mg sodium fluorescein. **b** Mechanical ventilation + 250 mg sodium fluorescein. **c** Pigs spontaneously ventilated + 250 mg sodium fluorescein.

Plasma Concentration of Fluorescein

The median time profile of the fluorescein plasma concentration in AU is presented in figure 2. At 15 min, the end of aerosol administration, the median (25th–75th percentile) plasma concentration reached 220 AU (IQR: 199–253), while 30 and 60 min after the onset of nebulization, the concentrations were 282 AU (IQR: 230–380) and 158 AU (IQR: 133–183), respectively.

Pleural Fluorescence

The time profiles of the pleural fluorescein concentration in pigs before and after fluorescein administration are shown in figure 3. The administration of sodium flu-

orescein by aerosol caused a progressive increase of fluorescence intensity in the visceral pleura (fig. 4). At the end of the administration of fluorescein, the concentration both in the ROI and surrounding areas was significantly higher compared to baseline (Mann-Whitney U test, $p < 0.001$). The overall fluorescein concentration reached 62.2 AU (IQR: 50.4–71.5) at the ROI and 39.5 AU (IQR: 31.3–40.7) at areas with no macroscopic evidence of fluorescein. An example of fluorescence intensity changes observed in time is shown in figure 5. Time profiles for the ROI and NA are presented in figure 6. The highest concentration at the ROI was observed in mechanically ventilated pigs receiving 5 ml of fluorescein solution,

Table 2. Histology of FEAT-positive and FEAT-negative tissue samples

	Inflammation			Atelectasis*	
	+	++	+++	present	absent
Positive areas (n = 26)	22 (85%)	3 (11%)	1 (4%)	6 (23%)	20 (77%)
Negative areas (n = 13)	9 (69%)	2 (15%)	2 (15%)	8 (62%)	5 (38%)
Total	31 (79%)	5 (13%)	3 (8%)	14 (36%)	25 (64%)

Lung inflammation was classified by the pathologist as: + = minimal; ++ = important; +++ = major. Microatelectasis was either absent or present.

* $p = 0.045$, χ^2 test for atelectasis in FEAT-positive and FEAT-negative areas.

whereas the lowest concentration at the ROI was observed in spontaneously ventilated pigs receiving 2.5 ml of fluorescein.

Histology

Twenty-six abnormal 'FEAT-positive' areas and 13 macroscopically normal 'FEAT-negative' areas were examined microscopically. All tissue samples (n = 39) showed a certain degree of inflammation but a difference was found in microatelectasis (table 2). Significantly more microatelectasis was found in the subpleural space of FEAT-negative samples (χ^2 test, $p = 0.045$).

Discussion

This is the first report of an animal model using FEAT. We compared white light thoracoscopy and FEAT in 6 pigs after the administration of nebulized sodium fluorescein and performed fluorescence quantification. In contrast to a normal lung appearance in white light, FEAT revealed an accumulation of fluorescein in several lung areas in all animals. Fluorescent lesions were visible within 5 min after initiating fluorescein administration and were not associated with blebs, bullae or air leakage. Some of these FEAT lesions were still visible at the time of euthanasia, 90 min after aerosol onset.

FEAT data interpretation depends both on the local concentration of the fluorophore and on the depth of penetration through the lung tissue by the fluorophore [21, 22]. Therefore, FEAT lesions are quantified macroscopically in a semiquantitative fashion as: absence of subpleural fluorescence (0), discrete subpleural glow (+), spots or larger areas with intense fluorescence (++ or +++), and areas with visible fluorophore leakage from the lung (++++), as described previously for humans [2]. In

Fig. 4. Fluorescence intensity changes during FEAT in 6 pigs. At time 0, administration was started. VC = Volume-controlled mechanical ventilation; SV = spontaneous ventilation. SV versus VC: $p = 0.4$, Wilcoxon paired test.

this study, however, FEAT lesions were also analyzed by using a method for the quantification of fluorescence intensity [20]. This method revealed different intensities at the center of FEAT lesions (ROI) and in the surrounding, macroscopically normal areas. It confirms that fluorescein diffuses widely into the visceral pleura, but the achieved concentrations are different within lung regions.

The histological findings of the present study suggest that fluorescein accumulates in the visceral subpleural space. Fluorescein is localized extracellularly since it is mainly a hydrophilic molecule. However, under certain circumstances it can be diffused through cellular membranes, either passively or by active transport [23–25].

Fig. 5. Changes of fluorescence intensity in time observed in the paracardiac region of a right upper lobe during FEAT. From top left to bottom right, images were collected at the 5th, 15th, 30th, 45th, 60th and 90th minute.

Fig. 6. Fluorescein concentration time profiles in the visceral pleura. Center of FEAT lesions: hot spot (**a**), area with diffuse accumulation (**b**). **c** Macroscopically normal surrounding tissue. Fluorescein concentrations at different time points are shown. Data are medians and IQR.

Therefore, an increased accumulation of inhaled fluorescein in subpleural regions may represent an alteration of physiological barriers which could include the alveolar-endothelial membrane and bronchial-epithelial cell-to-cell junctions.

A histological analysis of the pig lungs showed alveolar inflammation of variable severity in all tissue samples. The only difference found in the histological pattern of FEAT-positive and FEAT-negative areas was in the presence of subpleural microatelectasis. Macroscopically normal areas presented with a significantly higher percent-

Fig. 7. Correlation between plasma concentration of sodium fluorescein and pleural fluorescence intensities in FEAT-positive areas.

age of microatelectasis compared to FEAT-positive lesions. This finding suggests that FEAT-negative areas represent lung regions with decreased ventilation due to atelectasis, for example caused by the obstruction of small airways making it impossible for the fluorescein molecules to reach the periphery of the lung.

Hypothetically, FEAT lesions during thoracoscopy may also correspond with an accumulation of fluorescein in areas with inflammation where atelectasis is not present. However, microscopic examination did not reveal a specific relationship between FEAT lesions and inflammation, and thus, further investigation on a cellular level seems necessary.

It is also conceivable that the ventilation/perfusion abnormalities in pig lungs could make it possible to evaluate FEAT as it might appear in diseases of the pleura, where the pleura may be very thin or the underlying lungs have emphysematous bullae. Thus, pooling of the fluorescein in these areas might make it possible to evaluate the permeability of the lung tissue, and not only of the pleura.

We found that nebulized fluorescein is rapidly absorbed in the bloodstream reaching a peak concentration approximately 20 min after initiating its administration, which is comparable to data from previous pharmacokinetic studies in experimental animals [9]. Fluorescein accumulation in visceral pleura was promptly visible after its administration, and its concentration followed a pattern similar to that in the blood, as assessed by fluorometric analysis (fig. 7). Despite the differences found between

the absolute values of fluorescein concentration in different types of FEAT lesions, no significant differences were observed in the elimination time of fluorescein between hot spots and areas with diffuse accumulation of fluorescein. At 60 min after inhalation, the fluorescein concentration for both was comparable (fig. 6). From a practical perspective this could be useful information, considering that this time interval (15–60 min) after fluorescein administration might be the optimal time period for assessing pleural abnormalities when using inhaled sodium fluorescein during thoracoscopy.

The merit of the current study lies in evaluating a technology that might turn out to have clinical applications, not only pertaining to primary spontaneous pneumothorax but also in the assessment of other visceral pleural diseases such as malignant pleuritis [26]. Furthermore, the fluorescence intensity quantification of ROI and NA by digital image analysis is an improvement compared to the semiquantitative scoring system previously used for FEAT data interpretation. Its negativity, regarding specific underlying pathology, does not diminish its importance in the systematic evaluation of this novel technique called FEAT. After all, from the published human studies it is not clear if all regions that fluoresce represent a clinically significant abnormality, hence the need for an animal study.

Limitations of the current paper include the presence of substantial, widespread evidence of chronic lung inflammation, a common finding in otherwise healthy laboratory pigs that certainly weakens the validity of our model. The pig was chosen as a model for this project because the anatomy and physiology of its lungs can be compared to human lungs. Our study lacks a systematic examination of mechanism, for example by pathological verification of the depth of deposition. Indeed, deeper deposition may scatter the fluorescent light explaining the more diffuse areas. Despite these limitations, our experimental study shows that the pig is a valid animal model for the study of FEAT, and the observed time course profile of inhaled fluorescein can be used in further human studies.

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References

- Noppen M, Stratakos G, Verbanck S, D'Haese J, Meysman M, Vincken W: Fluorescein-enhanced autofluorescence thoracoscopy in primary spontaneous pneumothorax. *Am J Respir Crit Care Med* 2004;170:680–682.
- Noppen M, De Keukeleire T, Hanon S, Stratakos G, Amjadi K, Madsen P, Meysman M, D'Haese J, Vincken W: Fluorescein-enhanced autofluorescence thoracoscopy in patients with primary spontaneous pneumothorax and normal subjects. *Am J Respir Crit Care Med* 2006;174:26–30.
- Desmettre T, Devoisselle JM, Mordon S: Propriétés de fluorescence et particularités métaboliques de la fluorescéine. *J Fr Ophtalmol* 2000;23:821–834.
- Grotte D, Mallox V, Brubaker RF: Fluorescein glucuronide: can this metabolite interfere with fluorescein tracer studies? *Trans Am Ophthalmol Soc* 1983;81:125–129.
- Blair NP, Evans MA, Lesar TS, Zeimer RC: Fluorescein and fluorescein glucuronide pharmacokinetics after intravenous injection. *Invest Ophthalmol Vis Sci* 1986;27:1107–1114.
- Byron PR, Clark AR: Drug absorption from inhalation aerosols administered by positive-pressure ventilation. I. Administration of a characterized, solid disodium fluorescein aerosol under a controlled respiratory regime to the beagle dog. *J Pharm Sci* 1985;74:934–938.
- Byron PR, Clark AR: Drug absorption from inhalation aerosols administered by positive-pressure ventilation. II. Effect of disodium fluorescein aerosol particle size on fluorescein absorption kinetics in the beagle dog. *J Pharm Sci* 1985;74:939–942.
- Robertson HT, Glenny RW, Stanford D, McInnes LM, Luchtel DL, Covert D: High-resolution maps of regional ventilation utilizing inhaled fluorescent microspheres. *J Appl Physiol* 1997;82:943–953.
- Pillai RS, Yeates DB, Miller IF, Hickey AJ: Controlled dissolution from wax-coated aerosol particles in canine lungs. *J Appl Physiol* 1998;84:717–725.
- Jantz MA, Antony VB: Pathophysiology of the pleura. *Respiration* 2008;75:121–133.
- Ishida A, Ishikawa F, Nakamura M, Miyazu YM, Mineshita M, Kurimoto N, Koike J, Nishisaka T, Miyazawa T, Astoul P: Narrow band imaging applied to pleuroscopy for the assessment of vascular patterns of the pleura. *Respiration* 2009;78:432–439.
- Häussinger K, Becker H, Stanzel F, Kreuzer A, Schmidt B, Strausz J, Cavaliere S, Herth F, Kohlhäufel M, Müller KM, Huber RM, Pichlmeier U, Bolliger CT: Autofluorescence bronchoscopy with white light bronchoscopy compared with white light bronchoscopy alone for the detection of precancerous lesions: a European randomised controlled multicentre trial. *Thorax* 2005;60:496–503.
- De Keukeleire T, Hanon S, Dieriks B, Vanmaele L, Noppen M: Tracheobronchial amyloidosis: a bright sight with autofluorescence light. *Respiration* 2009;77:454.
- Boutin C, Viallat JR, Aelony Y: Practical Thoracoscopy. Berlin, Springer, 1991, pp 76–78.
- Norton EW, Gutman F: Diabetic retinopathy studied by fluorescein angiography. *Trans Am Ophthalmol Soc* 1965;63:108–128.
- Larsen M: Ocular fluorometry methodological improvements and clinical studies – with special reference to the blood-retina barrier permeability to fluorescein and fluorescein glucuronide. *Acta Ophthalmol Suppl* 1993;211:1–52.
- Polglase AL, McLaren WJ, Skinner SA, Kiesslich R, Neurath MF, Delaney PM: A fluorescence confocal endomicroscope for in vivo microscopy of the upper- and the lower-GI tract. *Gastrointest Endosc* 2005;62:686–695.
- Maunoury V, Mordon S, Geboes K, Klein O, Debaert A, Cortot A, Desreumaux P, Colombel JF: Early vascular changes in Crohn's disease: an endoscopic fluorescence study. *Endoscopy* 2000;32:700–705.
- Goldstein I, Wallet F, Nicolas-Robin A, Ferrari F, Marquette CH, Rouby JJ: Lung deposition and efficiency of nebulized amikacin during *Escherichia coli* pneumonia in ventilated piglets. *Am J Respir Crit Care Med* 2002;166:1375–1381.
- Collinet P, Sabban F, Cosson M, Farine MO, Villet R, Vinatier D, Mordon S: Laparoscopic photodynamic diagnosis of ovarian cancer peritoneal micro metastasis: an experimental study. *Photochem Photobiol* 2007;83:647–651.
- Schuster DP, Kovacs A, Garbow J, Piwnicka-Worms D: Recent advances in imaging the lungs in intact small animals. *Am J Respir Cell Mol Biol* 2004;30:129–138.
- Goujon D, Zellweger M, Radu A, Grosjean P, Weber BC, van den Bergh H, Monnier P, Wagnières G: In vivo autofluorescence imaging of early cancers in the human tracheobronchial tree with a spectrally optimized system. *J Biomed Opt* 2003;8:17–25.
- Rockey JH, Li W: Binding of fluorescein and carboxyfluorescein by normal and glycosylated human serum proteins. *Ophthalmic Res* 1982;14:416–427.
- Romanchuk KG: Fluorescein: physiochemical factors affecting its fluorescence. *Surv Ophthalmol* 1982;26:269–283.
- Moldow B, Larsen M, Sander B, Lund-Andersen H: Passive permeability and outward active transport of fluorescein across the blood-retinal barrier in early ARM. *Br J Ophthalmol* 2001;85:592–597.
- Chrysanthidis MG, Janssen JP: Autofluorescence videothoracoscopy in exudative pleural effusions: preliminary results. *Eur Respir J* 2005;26:989–992.