

Mini-Invasive Pleural Probe-Based Confocal Laser Endomicroscopy for Malignant Pleural Effusion Diagnosis

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Keywords

Probe-based confocal laser endomicroscopy · Malignant pleural effusion · Pleural malignancies · Pleural fluid cytology · Pleural biopsies

Abstract

Introduction: Probe-based confocal laser endomicroscopy (pCLE) is an optical imaging tool which allows live imaging tissues at cellular level. pCLE proved to be able to discriminate malignant from benign pleura during medical thoracoscopy and can identify malignant cells in pleural fluids. Since pleural fluid cytology has limited sensitivity for malignant pleural effusion diagnosis, we wanted to determine the feasibility, the safety, and the yield of mini-invasive close-blinded pleural pCLE. **Methods:** We conducted a single-center prospective trial. Every patient referred to medical thoracoscopy because of pleural effusion was eligible. Thoracocentesis for pleural fluid analysis was performed first with a Boutin's needle. Second, the probe was introduced in the pleural space through the Boutin's needle for mini-invasive pleural pCLE. Finally, the medical thoracoscopy and invasive pleural pCLE were performed. Seven pCLE criteria were prospectively rated to assess the sensitivity and specificity of mini-invasive pleural pCLE for malignant

pleural effusion diagnosis. **Results:** Fifty-two patients were enrolled. Mini-invasive pleural pCLE was feasible and safe. Pathological pleural fluid analysis identified malignant effusion with 42% sensitivity and 100% specificity. For the mini-invasive pleural pCLE, five of 7 criteria proved to be significantly discriminant between malignant and benign pleura. Overall, mini-invasive pleural pCLE had 87% sensitivity for malignant pleural effusion identification. The specificity is 52%, and the positive and negative predictive value were 73% both. **Conclusion:** Mini-invasive pleural pCLE was safe and feasible and its sensitivity for malignant pleural infiltration diagnosis was much higher than pleural fluid cytology.

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Published by S. Karger AG, Basel

Introduction

Probe-based confocal laser endomicroscopy (pCLE) is an optical imaging technique allowing live imaging of tissues at a cellular level [1]. The confocal microscope lies in an external column in the endoscopy suit. The tissue of interest is illuminated by a laser beam coming from the column through a probe containing thousands of optical fibers, hence the name “probe based.” Some

molecules of the tissues are fluorescent so that once excited by the laser beam, they produce a fluorescent light which can reach the photodetector in the column, through the same optical fibers of the probe, to give an image which can be analyzed by the investigator. If the tissue is not fluorescent, some fluorophores can be utilized. The acquisition is a video frame with a refreshment frequency of 12 images per second.

Pleural effusion is a common finding in medical practice and often requires thoracentesis and pleural biopsies to determine the underlying disease. However, thoracentesis with pleural fluid cytology has limited sensitivity (up to 60% for repeated thoracentesis) for a malignant pleural effusion diagnosis, one of the most common causes of pleural effusion [2–4]. Therefore, thoracoscopy is often required, allowing direct pleural examination and biopsies with a sensitivity and a specificity of 94% and 100% for malignant diseases [5, 6]. Nonetheless, this procedure is more invasive than a simple thoracentesis. Recently, four studies demonstrated that pleural pCLE could be of value for the diagnosis of malignant pleural effusion. In 2018, Zirlik et al. [7] showed that pCLE with acriflavine or cresyl violet performed in the pleural fluid after thoracentesis was able to identify a malignant pleural effusion with 87% sensitivity and 99% specificity compared pleural fluid cytology. In 2019, Wijmans et al. [8] showed that pCLE was able to guide pleural biopsies when the probe was passed through the biopsy needle during a thoracoscopy, an open thoracotomy or an ultrasound or CT-guided pleural biopsy when a malignant pleural mesothelioma was suspected. In 2021, our team published a prospective trial showing that pCLE during medial thoracoscopy was able to discriminate malignant from benign pleura [9, 10]. The same year, Sawada et al. [11] showed that pCLE performed during surgery for lung cancer was able to identify a visceral pleural involvement with 100% sensitivity and 83% specificity. Considering these results and the low sensitivity of thoracentesis for malignant pleural effusion identification, we conducted this proof-of-concept study to determine if mini-invasive close-blinded pleural pCLE performed through a Boutin's needle was feasible, safe and had a better sensitivity than thoracentesis.

Methods

We performed a single-center cross-sectional study. Every patient ≥ 18 years referred for a medical thoracoscopy because of a pleural effusion between De-

cember 2020 and November 2023 (pleural effusion work up, talc pleurodesis, etc.) was eligible for the present study. Exclusion criteria were pregnancy, known allergy to fluorescein and lack of pleural effusion to perform a thoracentesis. Before starting the intervention, a chest ultrasound was performed by the investigators to confirm pleural effusion and to select the best puncture area. The intervention was divided into four parts realized by the two same experienced investigators: first, the thoracentesis for pleural fluid sampling and pathological examination; second, the mini-invasive pleural pCLE (Alveoflex[®], lateral resolution 3 μm , optical area 1.13 mm^2 , depth of focus 0–50 μm) (Cellvizio[®], Maunakea technologies[®], Paris, France); third, the invasive pleural pCLE through the thoracoscope; fourth, the traditional medical thoracoscopy with pleural examinations, biopsies, and talc poudrage if required. Two different thoroscopes were used during this study: a Wolf thoracoscope with an outer diameter of 7 mm (Richard Wolf GmbH, Knittlingen, Germany[®]) and a Storz single puncture thoracoscope with an outer diameter of 10 mm (Karl Storz GmbH, Tuttlingen, Germany[®]). For pleural staining, 5 mL of fluorescein 10% were intravenously administrated 5 min before pCLE acquisition. The investigators were aware of the clinical history of the patients during the intervention as it happens in real clinical practice.

For 5 patients, the mini-invasive pleural pCLE was realized under local anesthesia (10 mL xylocaine 2%), without conscious sedation to determine its feasibility and safety. To make the intervention more convenient and faster, the other patients were investigated under conscious sedation and local anesthesia, allowing spontaneous breathing, with the patient lying in lateral decubitus as for the third and fourth part which were performed during medical thoracoscopy. For the mini-invasive pleural pCLE, the probe was blindly introduced in the pleural space through the Boutin's needle (traditionally used in medical thoracoscopy to induce a pneumothorax before insertion of the trocar) until satisfying images were recorded. The Figure 1 shows the Boutin's needle and the Alveoflex probe. At the same time, the two endoscopists rated 7 criteria during the acquisition and provided a general conclusion at the end: Benign or malignant pleura (see the Table 1 for the criteria and their interpretation).

The part three (invasive pleural pCLE) started by the insertion of the trocar allowing to perform the medical thoracoscopy. For this part, the probe was gently applied on the parietal pleura through the working channel of the thoracoscope under vision control. If macroscopic

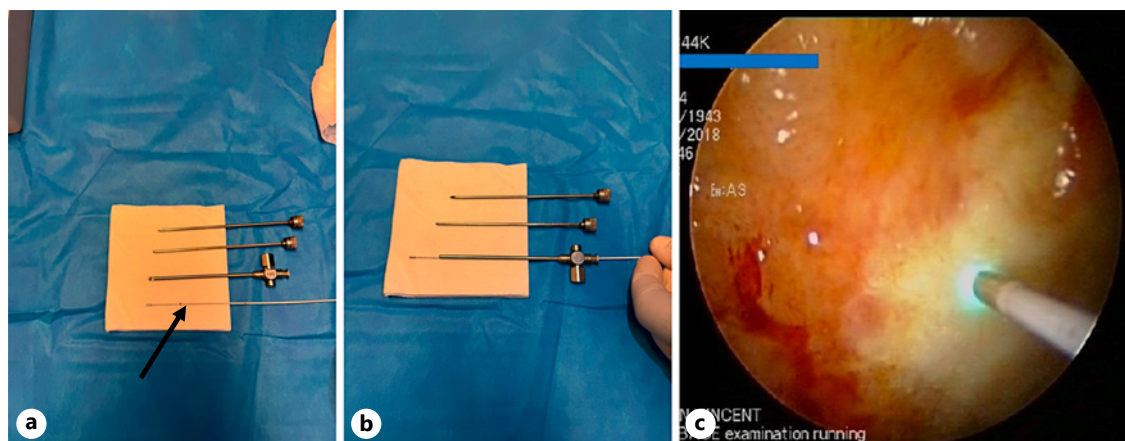


Fig. 1. The Boutin's needle and the confocal Alvéoflex probe. **a** The 2 mm Boutin's needle and its components. The last component (arrow) is the confocal laser endomicroscopy mini probe (Alvéoflex®) (Maunakea technologies, Paris, France). **b** The Alvéoflex probe is introduced through the Boutin's needle, allowing mini-invasive percutaneous pleural assessment. **c** Endoscopic view during a medical thoracoscopy. The Alvéoflex probe is gently applied on the parietal pleura.

abnormalities were noticed, pCLE was performed on the affected zones. In the absence of macroscopic abnormality, three random sites were selected. The same criteria were rated by the same two investigators. Finally, biopsies were systematically performed on the same sites for pathological diagnosis.

The assessed visual criteria were selected in accordance with our previous publications and experience [9, 10]. Patients were followed up according to clinical practice standards and depending up on clinical condition. Medical records and follow-up data were checked until the redaction of the manuscript discretion during the study and thereafter, until redaction of the manuscript.

Statistical Analysis

The Fisher's exact test was used to analyze the link between pCLE qualitative variables and the final histological diagnosis.

Results

Patients' Characteristics, Feasibility, and Safety

Sixty-seven patients were screened and 52 were included in the analysis (28 men and 24 women). The mean age was 67 years. Fifteen patients were excluded for multiples reasons, 8 patients did not consent (see Table 2). The Table 3 refers to baseline characteristics for included patients. Twenty-one patients had benign

pleural diseases while 31 had malignant pleural infiltration in the pleural biopsies.

Concerning the feasibility and safety, mini-invasive pleural pCLE investigation was feasible and allowed images recording when the Boutin's needle could be introduced in the pleural space, which was not possible for 3 patients because of pleural adhesions. The pCLE acquisition took approximately 5 min. The quality of the acquisition was variable according to the patient as it was already shown in our first prospective trial about pleural pCLE [9]. The pCLE probe was damaged by the Boutin's needle during one procedure precluding any image acquisition. For all included patients, the invasive pleural pCLE was recorded immediately after the mini-invasive phase without any complication occurring during thoracoscopy.

For the 5 patients investigated under local anesthesia for the mini-invasive phase through the Boutin's needle, moderate pain and discomfort were reported without requiring further analgesia. Images were recorded for every patient without difficulties.

Pathological Pleural Fluid Analysis

After thoracoscopy and pleural biopsies, it turned out that a malignant infiltration was observed in 31 patients out of the 52 patients investigated. Pathological pleural fluid analysis identified malignant effusion with a sensitivity of 42%, a specificity of 100%, a positive predictive value (PPV) of 100% and a negative predictive value (NPV) of 54%.

Table 1. Criteria for pCLE pleural evaluation

Criteria	Details
Abnormal tissular architecture	- No: correct identification of the previously described normal pleura characteristics [9, 10] - Mesothelial monolayer with a regular distribution of the cells - Fibro-adipose connective tissue with regular and homogenous vascularization - Muscular fibers - Yes: identification of cellular/tissular structures which are not known to correspond to normal pleura (cellular clusters or dark clumps, glands, cells cordons, dysmorphic cells, papillar organization. ...) - Not assessable: cellular structures are not visible
Cellular homogeneity in size, shape and fluorescence	Subjective description by the investigator with reference to the normal pleura [9, 10] - Yes - No - Not assessable if cellular structures are not visible
Dysplastic vessels [12]	- Yes: - Presence of tortuous vessels - Vascular leak - Giant vessel - No: no criteria for dysplastic vessel - Not assessable: absence of vessels assessable
Organized connective fibers	- Anarchic: coarse fibers, irregular in shape, without well-defined architecture - Organized: regular in shape and direction, well-defined architecture - Not assessable: no connective fibers identified
Chia seed sign on a full field of view	- Yes: full field of view with chia seed sign - No: absence or presence on only one portion of the field of view - Not assessable: no cellular structure assessable
General physician conclusion	- Synthetic conclusion of the entire pCLE acquisition - Malignant: presence of malignant pleural pCLE features - Benign: absence of malignant pleural pCLE features or presence of full chia seed sign

pCLE, probe-based confocal laser endomicroscopy.

Table 2. Screened patients excluded from the analysis: $n = 15$

Unwilling to participate	8
Not possible to insert the Boutin's needle	3
Lack of probe or other technical problem	4

Mini-Invasive Pleural pCLE

For the mini-invasive pleural investigation, 5 among 7 imaging criteria were significantly discriminant between malignant and benign pleura (Table 4). The abnormal architecture had high sensitivity (87%) for malignant effusion, but the specificity was only 43% with a PPV and NPV of 69% for both of them. Conversely, the full chia seed sign had low sensitivity (29%) for benign pleura but was very specific (94%) yielding a PPV of 75% for benignity. The cell ho-

mogeneity in size, color and shape was also significantly associated with benign pleura but with lower specificity than the full chia seed sign. The general conclusion of the physician at the end of the examination had 87% sensitivity and 52% specificity for malignant pleuritis with a PPV and a NPV of 73%. See Figure 2 for illustrative images.

Invasive Pleural pCLE

With reference to our previous experience, the invasive pCLE phase was performed as a quality comparator to the mini-invasive acquisition. The results between the two phases are highly concordant (Table 4).

Patients Follow-Up

The entire patient cohort was followed up for a mean duration of 17.31 ± 15.2 months. In our cohort, 18 patients showed benign pleuritis. They were followed up for 20

Table 3. Baseline patients' characteristics and histological diagnosis: *n* = 52

Sex	28 m/24 w
Mean age	67
Histological diagnosis (malignant, <i>n</i> = 31/benign, <i>n</i> = 21)	Chronic lymphocytic pleuritis, <i>n</i> = 14 Epithelioid mesothelioma, <i>n</i> = 7 NSCLC, <i>n</i> = 9 Ovarian adenocarcinoma, <i>n</i> = 4 Subacute pleuritis, <i>n</i> = 4 Granulomatous pleuritis, <i>n</i> = 3 Breast cancer, <i>n</i> = 2 Lymphoma, <i>n</i> = 2 Biphasic mesothelioma, <i>n</i> = 2 Other cancers: SCLC, prostate, colon, esophagus, undifferentiated
NSCLC, non-small cell lung carcinoma; SCLC, small cell lung carcinoma.	

Table 4. Mini-invasive pleural pCLE criteria

<i>n</i> = 52 (31 malignant/21 benign)	Abnormal tissular architecture	Homogeneous cell size	Homogeneous cell shape	Homogeneous cell fluorescence	Blood vessel dysplasia	Organized conjunctive fibers	Full chia seed sign	General physician conclusion
TP	27	15	15	11	20	19	6	27
FP	12	10	10	7	9	23	2	10
TN	9	21	21	24	12	8	29	11
FN	4	6	6	10	11	2	15	4
<i>p</i> value	0.023	0.01	0.01	0.013	0.16	0.17	0.049	0.0041
Sensitivity, %	87	71	71	52	/	/	29	87
Specificity, %	43	68	68	77	/	/	94	52
PPV, %	69	60	60	61	/	/	75	73
NPV, %	69	78	78	71	/	/	66	73
Concordance with invasive assessment, %	94	96	96	96	/	/	100	100

FN, false negative; FP, false positive; PPV, positive predictive value; NPV, negative predictive value; TN, true negative; TP, true positive.

months $\pm$ 15.56 months (mean $\pm$ sd) (until November 2023, end of the study). From November 2023 to April 2025, no more patients died in the benign pleuritis cohort so that surviving patients (*n* = 12) were followed up for 40 months $\pm$ 10 months without evidence of pleural malignancies. Among the deceased patients (*n* = 6), the mean follow-up was 12.8 $\pm$ 11.0 months. Causes of death included non-small cell lung cancer in 5 patients, small cell lung cancer in 1 patient, and suicide in 1 patient. Importantly, none of these patients exhibited clinical or pathological evidence of pleural malignancy prior to death.

Discussion

Our study is the first to investigate mini-invasive pleural pCLE for pleural effusion. In this proof-of-concept study, we showed that the technique is feasible and safe. It allowed live imaging of pleural tissue at a cellular level during a mini-invasive procedure, and we provided evidence that the imaging criteria we prospectively assessed may discriminate malignant from benign effusion with a greater sensitivity than thoracocentesis.

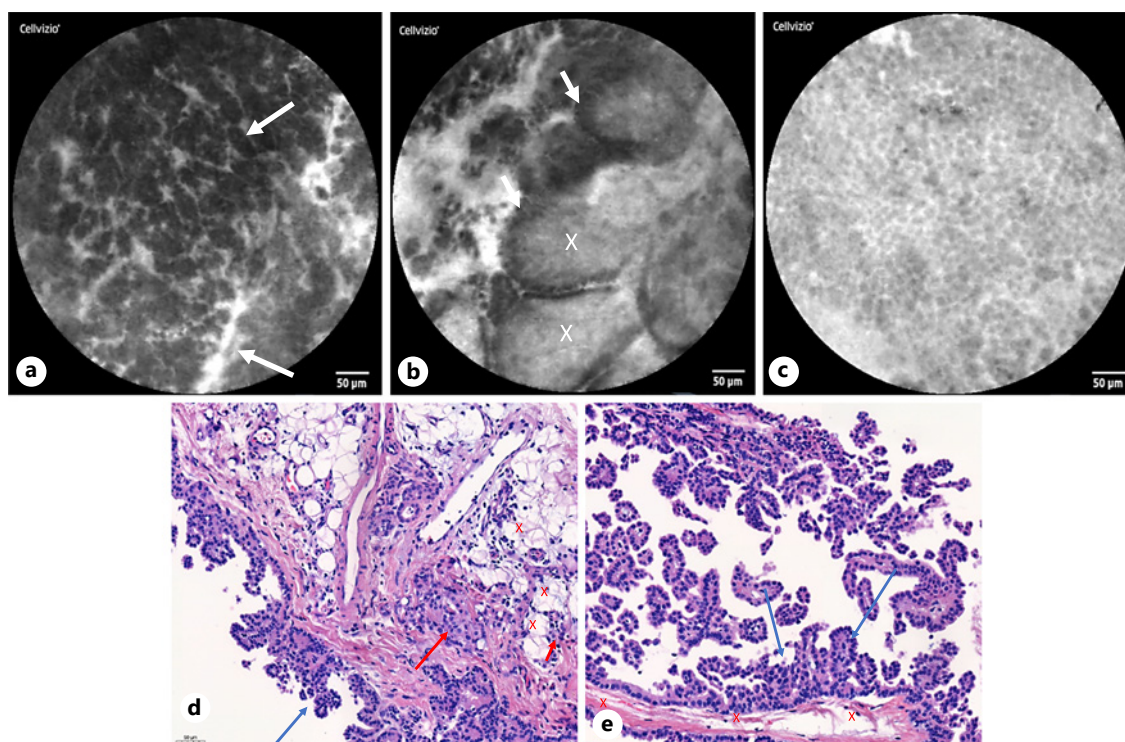


Fig. 2. Illustrations of mini-invasive pleural probe-based confocal laser endomicroscopy (pCLE) images. **a** Abnormal tissular architecture, the normal pleural structures are not visible. Cells are organized in clusters, cords and in some places in papils as pointed by the upper arrow. The lower arrow points to a vessel with fluorescein leaks. **b** Another pCLE image of the same patient as in **a** showing big papils. The crosses are pointing to the fibrovascular structural axes of the papils while the arrows point to the epithelial lining. **c** Illustration of full chia seed sign with a

patchy and regular distribution of the mesothelial cells. **d** Histological section of pleural biopsies from the same patient as in **a** and **b** and showing a malignant mesothelioma with a papillar architecture. The blue arrow points to the pleural surface showing malignant cells taking a papillar architecture. These malignant cells are infiltrating the pleural stroma and the adipose tissue (crosses) as pointed by the red arrow. **e** A giant papil is shown, the fibrovascular axis is pointed by the crosses while the epithelial lining is pointed by the blue arrow.

We found that some of the prospectively assessed criteria such as abnormal tissular architecture, full chia seed sign and homogeneity of the cells in size, shape, and fluorescence intensity were discriminant between malignant and benign pleura. Furthermore, the mini-invasive procedure was highly concordant with the invasive one, which was reassuring on the quality and the representativeness of the mini-invasive pleural acquisition. Moreover, in 5 patients the procedure was performed under local anesthesia only, as for a thoracocentesis without requiring further analgesia opening the way to easy going routine procedure to explore pleural effusion in the future.

In our study, pleural fluid cytology had 42% sensitivity for malignant effusion identification with 100% specificity, which is in line with previous studies [13]. When based on general physician conclusion, mini-

invasive pleural pCLE was clearly more sensitive with 87% sensitivity but had limited specificity (53%). Among our cohort, 16 patients with negative pleural fluid cytology had in fact true malignant effusion according to the mini-invasive pleural pCLE assessment reaching a PPV of 73%. Otherwise, only 4 patients had false negative mini-invasive pleural assessment. Consequently, mini-invasive pleural pCLE can improve the poor sensitivity of pleural fluid cytology which can sometimes delay the diagnosis and patient management [14]. In fact, if the Light's criteria show an exudative effusion, a negative pleural fluid cytology does not exclude a malignancy, as the sensitivity is poor and further investigations are required. However, medical thoracoscopy or percutaneous ultrasound or CT-guided pleural biopsies are not always readily available or suitable, considering clinical background and comorbidities. In this context, we

believe that pCLE (given its high sensitivity) could play a valuable role in expediting patient management or facilitating timely referral to specialized centers for definitive diagnosis and care.

Otherwise, specificity could be improved with other fluorophores (see below). Although being highly sensitive (87%) for malignant effusion it turned out that abnormal architecture was this time much less specific for malignancies compared to our previous study on invasive pleural pCLE during medical thoracoscopy: 43% and 72%, respectively [9]. In fact, in our first trial, some patients were referred for talc poudrage because of recurrent pneumothorax (8/62 patients). These patients had the typical pCLE features of normal pleura with full chia seed sign and no abnormal tissular architecture. The cells were rigorously homogeneous in size shape and fluorescence. Therefore, these patients were easily identifiable as benign pleura which increased the sensitivity and the specificity of benign and malignant criteria, respectively. Furthermore, patients with nonmalignant pleuritis can show tissular alterations with reactive and hyperplastic mesothelial cells which can be difficult to distinguish from malignant infiltrations, especially for mesothelioma. This is also true for standard histological assessment and immunohistochemistry is mandatory for an accurate differential diagnosis [15]. This can explain the disappointing specificity of mini-invasive pleural pCLE.

In the present study, only 60% of the included patients had a malignant pleuritis as a final diagnosis, which is in line with other series of medical thoracoscopy for pleural effusion [16, 17]. This means that a lot of patients are referred for medical thoracoscopy while suffering from benign diseases without these biopsies results implying necessarily a modification in clinical management. This highlights the clinical need for a higher sensitivity and specificity for malignancies in the field of mini-invasive assessment of pleural effusions before referring the patient for a medical thoracoscopy. The low specificity of pleural pCLE for detecting malignancies could potentially be improved through the use of new fluorophores. For instance, Zirlik et al. [7] demonstrated that pCLE applied to centrifuged pleural fluid and enhanced with intracellular fluorophores such as acriflavine or cresyl violet achieved a sensitivity of 87% and a specificity of 99% for identifying malignant cells, compared to conventional pleural fluid cytology. Acriflavine and cresyl violet are distributed intracellularly and intranuclearly, allowing for direct

visualization of cells and their nuclei. In contrast, fluorescein is hydrophilic and primarily stains the extracellular matrix and vascular compartments. (Malignant) cells thus appear as negative images within the highlighted extracellular matrix, which hinders detailed cellular analysis and likely contributes to lower specificity.

In our study, we did not use acriflavine or cresyl violet due to their unsuitability for systemic administration. Additionally, most previous investigations in thoracic oncology have relied on fluorescein. Future studies should investigate intracellular fluorophore with systemic administration. Otherwise current development of new specific staining agent by the association of a fluorophore with a specific target tracer opens the field for endoscopic molecular imaging. This promising technique combined with pCLE could increase the specificity and the yield of pleural pCLE assessment as already shown for single pulmonary nodules [18].

The full chia seed sign had comparable sensitivity and specificity to our previous study (29% and 94%, respectively). However, the PPV was lower for benignity (75% vs. 100%). Two patients had false positive in the present study. We think that a patchy distribution of carcinomatous nodules could not explain these results because the invasive pleural pCLE assessment performed under the control of sight gave the same results. For these patients, the malignant cells were located deep in the pleura so that it is possible that they were missed by the pCLE assessment which is quite superficial: the confocal plane stands between 0 and 50 μm . The pleural biopsies which were large and deep could reach them. Consequently, the PPV of 75% and 73% for benignity (for full chia seed sign and general physician conclusion) are not high enough to exempt patient for a further and invasive pleural assessment when malignancy is suspected.

The dysplastic vessels criterion was not significant in the present study in contrast to what we found previously. In fact, tortuous vessels and vascular leaks identified in pCLE were described in the field of gastroenterology as a criterion for colorectal or gastric cancer characterization [12]. However, in our experience, these vascular abnormalities are also identified in inflammatory involvement of the pleura which is known to be part of the pleural inflammatory pathophysiological process [19, 20]. These vascular alterations seemed not to be easily distinguishable from malignant one with pCLE. Furthermore, the probes

used in the field of gastroenterology have a better lateral resolution than our Alvéoflex probe, allowing for a more precise assessment of vascular abnormalities [21]. Another drawback linked to the probes we used is that we had to deplore some probes damages after sterilization yet performed according to the manufacturer prescription.

Limitations

Apart the fact of being monocentric, our study has some other limitations. First, in some patients, adequate visualization of the pleural structures and the mesothelial layer may have been impaired by the presence of a thick fibrinous pleuritis with a suboptimal fluorescein staining.

Second, the investigators who performed the pCLE acquisition and interpretation were aware of the patients' clinical histories and pretest probabilities of malignancy, which may have influenced their interpretation of the criteria. However, this study design was intentionally chosen to reflect real-world clinical practice. Indeed, if pCLE is implemented in routine care, the acquisition will be interpreted in real time by the endoscopist, who is inevitably informed of the patient's clinical background and pretest probability of malignancy.

Third, since the thoracoscopy was performed immediately after the mini-invasive pCLE, we could not assess the real incidence of pneumothorax that could have occurred during the mini-invasive phase. Fourth, as the invasive criteria were assessed a few minutes after the mini-invasive phase, this could have led to an overestimation of the concordance between the mini-invasive and the invasive assessment. Finally, the current management of (pleural) malignancies requires a thorough molecular assessment to precisely determine the underlying histology and to identify specific molecular target for patient management. So far, this is impossible to perform with pleural pCLE.

Conclusion

Our study is the first to investigate the potential of mini-invasive pCLE for pleural effusion work up. We showed that this technique is feasible, safe and has a much higher sensitivity for malignant effusion identification than pleural fluid cytology, which could

help clinicians to drive patients to medical thoracoscopy faster. However, the pleural pCLE specificity and the PPV for benign pleura are not high enough to exempt patients from additional investigations and thoracoscopy if malignancies are suspected. Nowadays, in case of malignant pleural infiltration, biopsies for a thorough molecular assessment are mandatory.

In the future, the yield of pCLE could be further increased with specific fluorophores and molecular imaging. Whether the information provided through this new but expensive technique will lead to a change in clinical management of pleural effusion or not, needs further investigations.

Statement of Ethics

Our study was approved by the CHU Liege Ethics Committee (B707201837069). Every patient provided written informed consent.

Conflict of Interest Statement

The authors declare no conflict of interest with the present work.

Funding Sources

This work was supported by the CHU of Liège (university hospital).

Author Contributions

Olivier Bonhomme: conceptualization-lead, formal analysis-lead, methodology-lead, project administration-lead, writing – original draft-lead, writing – review and editing-lead. Vincent Heinen: conceptualization-equal, data curation-lead, formal analysis-equal, investigation-lead, project administration-equal, validation-equal. Renaud Louis: investigation-equal, methodology-equal, supervision-equal, writing – review and editing-lead. Nancy Detrembleur: supervision-equal and validation-equal. Jean-Louis Corhay: conceptualization-equal, methodology-equal, supervision-equal. Bernard Duysinx: conceptualization-lead, data curation-lead, investigation-lead, methodology-lead, project administration-lead, supervision-lead, and validation-lead.

Data Availability Statement

Research data are not publicly available but are available from the corresponding author upon request.

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