

FIRST REPORT OF PROBE BASED CONFOCAL LASER ENDOMICROSCOPY DURING MEDICAL THORACOSCOPY

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ABSTRACT

Probe based confocal laser endomicroscopy (pCLE) is a new optical endoscopic technique, generating fluorescent light emission from the tissue of interest and allowing *in vivo* live imaging at a cellular level ("optical biopsies").

To the best of our knowledge, this article is the first to present pCLE images during medical thoracoscopy. We present here 3 different patients referred for various health problems. A precise description of pleural cavity pCLE images after intravenous fluorescein injection (a fluorophore) together with corresponding macroscopical and histological studies is performed. This led to the diagnosis of normal pleura in one case, carcinomatous pleuritis in another case and a malignant mesothelioma in the third case.

We believe that optical biopsies could help clinicians to make an early diagnosis, thereby allowing rapid therapeutic intervention (talc pleurodesis for example). Furthermore, it could help to guide biopsies when affected zones are not obvious to macroscopic examination.

In a near future, new fluorophores could be developed to stain some pathophysiological processes, therapeutic targets, or enzymes activities bringing new insights in endoscopic pleural disease work-up.

KEYWORDS: Probe based confocal laser endomicroscopy, pCLE, Pleural carcinomatosis, Malignant mesothelioma, Thoracoscopy, Optical biopsies.

1. Introduction

Probe based confocal laser endomicroscopy (pCLE) is a new optical endoscopic imaging technique which uses a laser as illuminating source, generating fluorescent light emission from the tissue of interest. This technique allows *in vivo* imaging at a cellular level ("optical biopsies") with a video frame of 12 images per second (cellvizio, Mauna Kea technologies®, Paris, France). Although several studies are ongoing, it remains an experimental technique. Domains of interest in pulmonary medicine are numerous [1-7].

To the best of our knowledge, there are no available publications concerning pCLE of the pleura neither in healthy subjects nor in patients with pleural diseases.

In this brief report, we present the results and images of pCLE performed during medical thoracoscopy in 3 different patients referred for various health problems.

2. Methodology

For this case series, we used Alveoflex® CLE probes (lateral resolution of 3 μm , optical area of 1.13 mm^2 , depth of focus of 0-50 μm) which could be easily introduced through the working channel of pleuroscopes after sterilisation. Fluorescein (10%, 5 ml) was intravenously injected 5 min before image acquisition, which, according to our experience, allows the best image quality. pCLE examination did not allow the study of the entire pleural cavity because it would have been time consuming due to the small optical area (1.13 mm^2) of the probe. Consequently, the pleural cavity was first examined with the rigid optic through the pleuroscope for macroscopic evaluation. Thereafter, we performed pCLE on obvious affected zones. If there was no focal abnormality, 4 to 5 elective sites were examined. Biopsies were systematically performed at the sites of pCLE examination. Finally a comparison was made between optical and tissular biopsies. It took approximately 5 min to perform the pCLE study of the pleural cavity. Informed consents were obtained for all patients according to local ethic comity (n: B707201837069).

3. Report

The first patient (Fig. 1) was a 38 years old man referred for talc pleurodesis because of recurrent (third episode) spontaneous pneumothorax (right lung). Macroscopically, the pleura showed no abnormalities: a thin, flat and brilliant membrane coating the pleural cavity (Fig. 1A). Endomicroscopy examination showed a well-organized tissue with cells of similar sizes, shapes and fluorescence intensity (shade of grey) with well delineated intercellular gaps and cells borders (Fig. 1C). Deeper, under the mesothelium, the next pictures showed blood vessels with circulating blood cells (Fig. 1B) and a fibro-adipose connective tissue. Adipocyte are well delimited, large (around 50 μm), round and dark cells surrounded by blood vessels and connective fibres (Fig. 1E). The last pCLE picture (Fig. 1G) showed very large (around 100 μm , dark, long (hundreds of μm)) and parallel cells with a peripheral distribution of their nuclei: striated muscular fibres. Histological analysis confirmed the presence of a normal pleural mesothelium (large flat or cubic cells forming a monolayer epithelium) covering a fibro adipose connective tissue and striated muscular fibres (Fig. 1D, 1F and 1H).

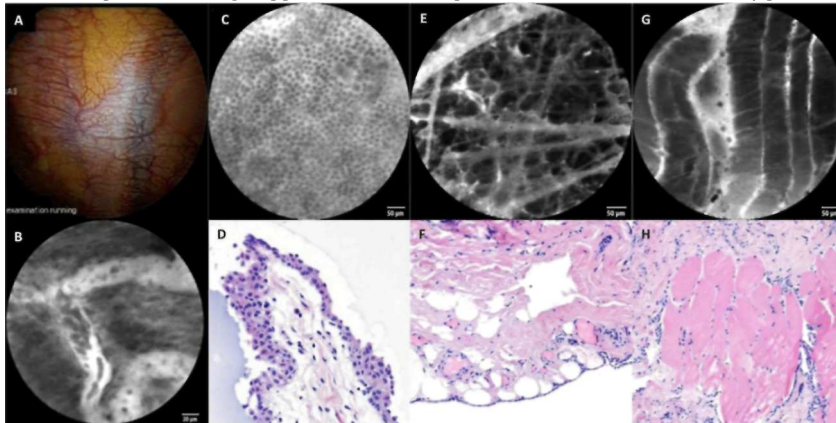
The second patient (Fig. 2), a 79 years old woman, was referred for a talc pleurodesis because of recurrent exudative pleural effusion in the context of a non-small cell lung carcinoma confirmed by pleural fluid cytology. Pleural cavity examination showed macro nodular pleural infiltration suggesting a carcinologic invasion (Fig. 2A). Endomicroscopy exploration showed a pleural infiltration with cells of different sizes, shapes and fluorescence intensity (shades of grey) with distorted intercellular gaps and indistinct cell borders. Those cells are forming multiple cellular clusters of different sizes. In some places, they are organized around blood vessels and connective tissues with a papillary architecture. Normal mesothelium is no more visible (Fig. 2B). Histological analysis showed a pleural infiltration by a non-small cell lung carcinoma with a papillary and nodular architecture (Fig. 2C).

The third patient (Fig. 3) was referred for investigation of an exudative pleural effusion of unknown origin. Pleural cavity examination showed a diffuse, irregular, white macular infiltrate of the pleura becoming either micro or macro nodular (Fig. 3A). Endomicroscopy showed a pleural infiltration with large cells of different sizes and shapes with distorted intercellular gaps and indistinct borders. In some places, cells can take a glandular or a papillar architecture (Fig. 3B). Histological analysis (Fig. 3C) confirmed those observations and concluded to a malignant mesothelioma after immuno-histological studies.

Fig. 1. Normal pleura.

A. Normal pleura endoscopic view.

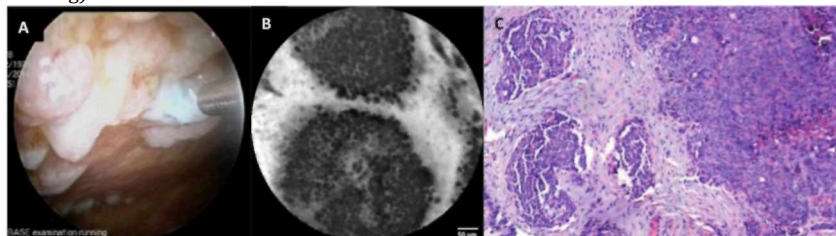
- B. pCLE examination showing a blood vessel in the normal pleura.
- C. pCLE examination showing normal mesothelium: well-organized tissue with cells of similar sizes, shapes and fluorescence intensity (shade of grey) with well delineated intercellular gaps and cells borders.
- D. Normal pleura histologic appearance (optical microscope, hematoxylin and eosin staining).
- E. pCLE examination of Sub-pleural connective tissue showing well delineated, large (around 50 μm), round and dark cells (adipocytes) surrounded by blood vessels and connective fibers.
- F. Normal pleura histologic appearance with sub pleural fibro adipose connective tissue (optical microscope, hematoxylin and eosin staining).
- G. pCLE examination of sub-pleural tissue showing very large, dark, long and parallel cells with a peripheral distribution of their nuclei, under the cytoplasmic membrane: striated muscular fibers.
- H. Normal pleura histologic appearance with sub pleural striated muscular fibers (optical microscope, hematoxylin eosin staining).



With informed patient consent
pCLE: probe based confocal laser endomicroscopy.

Fig. 2. Non small cell lung carcinoma pleural infiltration.

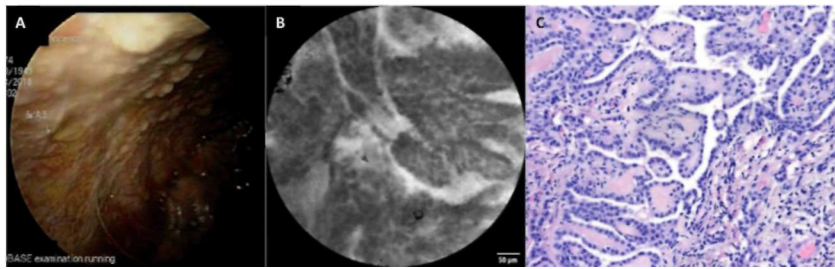
- A. Endoscopic view showing macro nodular pleural infiltration
- B. pCLE examination showing pleural infiltration with cells of different sizes, shapes and fluorescence intensity (shades of grey) with distorted intercellular gaps and indistinct cells borders. Those cells are forming multiple cellular clusters of different sizes.
- C. Histological appearance showing pleural infiltration by a non-small cell lung carcinoma (optical microscope, hematoxylin eosin staining).



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Fig. 3. Pleural mesothelioma.

- A. Endoscopic exploration of the pleural cavity showing a diffuse, irregular, white macular infiltrate of the pleura becoming either micro or macro nodular.
- B. pCLE examination showing pleural infiltration by large cells of different sizes and shapes with distorted intercellular gaps and indistinct cells borders. In some places, they can take a glandular or a papillar architecture.
- C. Histological appearance of a malignant mesothelioma (optical microscope hematoxylin eosin staining).



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4. Discussion

With this brief report, we show that pleural cavity pCLE examination after fluorescein injection is possible and valuable. Our pictures demonstrate a clear distinction between the normal pleura and a carcinomatous pleuritis. To our knowledge, this is the first report of pleural cavity examination through pCLE. It is important to keep in mind that most of pleural carcinomatosis are metastatic, resulting in large histological variability, depending on the origin of the primary tumor [8].

Thoracoscopy is required for pleural effusion work-up and pleurodesis in case of recurrent pneumothorax or carcinologic pleural effusion [9]. During the intervention, if abnormalities are noticed, it is difficult to make a diagnosis on macroscopic appearance and biopsies are necessary. Direct *in vivo* cell imaging with optical biopsies can be interesting in order to help clinicians making the right diagnosis early during the procedure thereby allowing rapid therapeutic intervention. Furthermore, affected pleural zones are sometimes not easy to identify on macroscopic appearance. *In vivo* cell imaging can help to target biopsies and consequently could increase thoracoscopy sensitivity [10,11]. Biopsies and histopathological examination remain currently the gold standard for pleural carcinomatosis diagnosis. Large longitudinal studies with comparison to this gold standard will be necessary to establish pCLE diagnostic criteria and their accuracy. If high negative predictive value for pleural carcinomatosis or if specific criteria for non-malignant pleural effusions can be shown, then minimally invasive pleural pCLE examination could be developed (needle based for example). This could be of great value for patient quality of life. Recently, Zirlik et al. showed a sensitivity and specificity of 87% and 99% respectively to diagnose malignant pleural effusion by CLE *ex-vivo* analysis [7].

Pleural cavity pCLE examination requires a fluorophore administration which was fluorescein in our cases. This hydrophilic fluorophore mainly stains the blood vessels, the connective tissue and the cellular cytoplasm. This therefore provides a negative cellular nucleus staining (as eosin in standard histology) [12]. This agent was chosen because it is the safest and best known fluorophore [12]. In our experience, there were no adverse events to mention. In the future, different fluorophores with other tissular distributions and diagnostic properties could be tested. Fluorophores with specific biological properties could be used to explore some pathophysiological processes or stain specific targets (receptors, enzymes activities, vascularisation, capillary leaks, microorganisms ...) not visible during standard histology, bringing new insights in endoscopic pleural disease work-up and diagnosis [12,13]. In fact, pleural pCLE live imaging allows real time examination of live tissues, which is a great difference compared to standard histology. The interest of live tissular imaging was clearly demonstrated in gastroenterology for pancreas cystic disease work-up [14].

5. Limitations

In this brief report, only three cases are presented but those are the first worldwide well documented pCLE examinations of the pleura.

Additional cases are currently investigated in our centre to extend our experience in that field.

Even if pCLE differences between normal pleura and pleural carcinomatosis seem obvious in our present report, it is important to keep in mind the large histological variability of pleural metastatic carcinomatosis or malignant mesothelioma [8]. Furthermore, inflammatory pleural diseases accompanying infectious or autoimmune processes need to be considered as well. Consequently, further studies are necessary for a precise differential diagnosis between those entities.

6. Conclusion

This report is the first publication of pCLE imaging during medical thoracoscopy and shows the feasibility of the technique. Our study indicates a clear difference between normal pleura and carcinologic pleuritis, which highlights the great potential of the technique. In the future, fluorophores could be tested to stain some pathophysiological processes or therapeutic/diagnostic targets. Larger cross sectional and longitudinal studies will be needed before defining the place of pCLE in clinical investigation of pleural diseases.

DECLARATION OF INTEREST

None with the present work.

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