Probe-based Confocal Laser Endomicroscopy (pCLE) in the Diagnosis of Diffuse Parenchymal Lung Diseases: Two Cases

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Introduction

The working principle of confocal endomicroscopy was first described in 1957 and it is based on the narrow-point illumination of the light source focused on a single point in the sample and the return of the reflected light through a small opening or a pinhole in the probe pathway [1-3]. This procedure allows for the exclusion of the information from outside, under, and above the focused area, and to obtain high quality images of a very thin focusing field. It is called “confocal” due to having both illumination and detection systems in the same focal plane. In this way, since there is no longer a need to obtain a biopsy from the targeted area or at least due to the biopsies that can be taken from the exactly targeted area and from the spot, a diagnosis can be achieved.

CellVizio (Mauna Kea Tech, Paris, France) confocal endomicroscopy system employs a proximal scanning system. Confocal microscopy allows in vivo imaging of cells and tissues by fragmentation through obtaining lateral and axial resolutions.

When stimulated by a laser light of 488 nm, various extracellular and cellular fluorophores containing intracellular fluorines resulting from the epithelial cells and the cross links between the collagen and elastin in the subepithelial area are resolved at different concentrations.

The microspectrometer studies demonstrated that the main fluorescence signal is spread by the elastin component of human bronchial and alveolar system at this wavelength. It can be easily performed during a routine fiber optic bronchoscopy applied under local anesthesia [4,5]. The in vivo bronchial pCLE imaging technique is simple: a mini-probe is placed through the 2-mm operating channel of the bronchoscope and the probe tip is applied over the bronchial mucosa under visual inspection. With a 50 µm under contact surface for the depth of the focus, the system may likely provide imaging of the basal lamina densa and lamina reticularis, the first layers of the bronchial subepithelial connective tissue. Elastin is found in the acinus, axial backbone of the alveolar ducts, and alveolar entries, and also in the outer sheath of the extra alveolar microvessels. Alveolar fluorescent imaging in active smokers severely differs from the imaging performed in non-smokers. The alveolar areas of the smokers are generally full of high fluorescent cells corresponding to the alveolar macrophages [4,6].

In the present article, the potentials of the system were discussed, accompanied by the optical biopsy images of a case with Pneumocystis jirovecii pneumonia (PCP) and a case with early pulmonary fibrosis, of which the imaging of distal airways was performed via probe-based, fibered confocal laser endomicroscopy (pCLE) by using CellVizio (Mauna Kea Technologies, Paris, France).

Cases

Case 1

A 32-year-old male patient who admitted to our polyclinic with shortness of breath, cough, sputum, and fever did not have any known medical history and he had shortness of breath, fever, cough, and sputum for two months. He was charged under the pre-diagnosis of pneumonia. The patient was taken into the intensive care unit (ICU) upon the worsening of respiratory failure. The patient was monitored in the ICU via the support of non-invasive mechanical ventilation (NIVM) and the HIV serology was found positive. Since the radiological and clinical appearances were consistent, P. jirovecii pneumonia was considered. Figures 1 & 2 present the chest X-rays and the computed tomography sections of thorax of the patient.
The patient had a history of smoking 30 packs/year and he had six months of regular treatment due to tuberculosis in 1992.

Despite the coarse rales heard in the entire bilateral lung areas in the physical examination, tachypnea and distinct oxygen support, no significant characteristics were found other than the oxygen saturation being 85%.

Proper antibiotics, prophylactic treatment, and ventilatory support were administered to the patient. In clinical terms, PCP was considered and a clinical response was achieved to the treatment administered. Before referring for antiretroviral treatment, alveoloscopy was performed for the imaging of the alveolar area and the fluorescent cellular structures within this area (Figure 3). The consolidation and the rich cellular content observed in the patient’s alveolar area was consistent with pneumonia. Consent was obtained to use the procedure findings for scientific purposes.

Case 2

A 63-year-old male patient admitted with cough, shortness of breath, low back pain, and fatigue was diagnosed COAH and initiated with bronchodilator therapy for four months. However, despite regular treatment, his cough did not recover. There were no background and family history. The patient had a history of smoking 20 packages/year and he had not smoked for 20 years.

Other than mild desaturation (spO2 93%) observed in room air during the physical examination, end-inspiratory rales were heard in the bilateral basal lung; nothing was found in other system examinations.

Nothing was identified in the routine blood tests. The chest X-rays and thorax CT sections of the patient, who was considered to have early pulmonary fibrosis with the radiological appearance, are presented in Figures 4 & 5. The result of transbronchial biopsy was consistent with the usual interstitial pneumonia (Figure 6). The imaging of the alveolar structure was performed via confocal endomicroscopy (Figure 7). It was established that the architecture of the elastin structure comprising the alveolar wall was lost and disintegrated, and the alveolar area was significantly poor in cells. Consent was obtained to use the procedure findings for scientific purposes.

Discussion

The previous studies have demonstrated that 50% of the...
peripheral lung support tissues consist of elastin [4]. At the level of
the alveoli, elastin comprises the basic structure of alveolar sac and
sac entries. Furthermore, the external sheaths of the extra-alveolar
microvessels are made of elastin. This basic information allows for the
explanation of intra acinar pCLE images. The first in vivo
studies with pCLE demonstrated that the procedure was safe and well tolerated
in awake individuals under local anesthesia and spontaneous
ventilation. Additionally, contrary to the frequently observed during
the transbronchial biopsy, acinar imaging does not cause significant
bleeding in the proximal airways. This may be explained by the
change to the low pressure in the alveolar capillaries while advancing
the probe. Moreover, the flat surface design of the probe tip allows
the probe to move without damaging the extra-alveolar vessels during
shifting. None of the studies conducted to date have reported pleural
complications [1,2].

With the pCLE probe safely advanced until the alveolar cavity, it has been possible to obtain information on many parenchymal
diseases. Salaün et al. [7] published an alveolar proteinosis case, for
which they had an image with pCLE. In the said case, there were diffuse
alveolar and interstitial opacities in the lung graphies, and confocal
endomicroscopy enabled the imaging of globular lipoproteinaceous
material. This finding was confirmed also with the examination of
bronchoalveolar lavage.

On the other hand, Finler et al. [8] analyzed the images in different
pathological conditions and investigated the repeatability in technical
terms. In the said study, the researchers highlighted the fact that the
images from pCLE might be reliably evaluated through the thickness
and brightness of the fiber, which varied between the different
observers, in the different examinations of the same observer and also
based on the anatomical location during the re-evaluation of the
same patient. They stated that their findings supported the use in the
diseases involving respiratory bronchiole. The authors suggested that
the normal and pathologically-proven diseased tissues and malignant
lesions might be differentiated via this approach. However, they also
reported that they had difficulties in differentiating the inflamed
lung tissue and malignant lesions via this imaging. They stated that
establishing an image library, and analyzing and recording of these
images would be a guide for future studies.

Newton et al. [9] analyzed 116 bronchopulmonary segments
from 38 patients and four healthy subjects in their study and they
demonstrated that the pCLE images were correlated with the results
of the high-resolution computed tomography (HRCT) of the
thorax and the biopsy. The primary objective of their study was to
demonstrate the image differences upon the imaging of the structural
modifications occurred in various parenchymal lung diseases via
confocal endomicroscopy and to establish the role of pCLE in such
diseases. The elastin-rich structure of the lung, as distinct from the
gastrointestinal system, requires the use of exogenous fluorescent
contrast agent, such as intravenous fluorescein, or topical acriflavine
in order to clearly exhibit the boundaries of the cellular architecture
and to display the epithelium-embedded gland structures and
dynamic in vivo blood flow. The authors reported the secondary
objective of the said study was to identify the additional advantages
provided (such as differentiating the vessel structures clearly) via in
vivo pCLE with the use of fluorescein in the healthy and diseased
tissues. Finally, the safety of using pCLE in patients with parenchymal
lung diseases was described as the tertiary objective. At the end of
the study, the authors stated that they observed some advantages of
pCLE compared to the conventional histological examination. They
observed both dynamic cells and cyclic movements due to respiration
within the acinus. They suggested that the advantages provided by
transbronchial biopsy during the surveillance bronchoscopy in the hand, another purpose for pCLE imaging is to remove the need for particles upon the development of staining techniques. On the other instance, it will be possible over time to observe asbestos fiber directly within the alveolar structure or to analyze and identify various toxic agents in the alveolar cavity, which was poor in cellularity and autofluorescence in the alveolar and acinar area were observed. Although there are no similar published cases yet, a limited number of cases included in the ongoing “Optical Lung Atlas” studies exhibit similar findings. It has not yet been possible to identify the disease-specific patterns. On the other hand, the images obtained in the second case are consistent with the study by Newton et al. [9], which is the most extensive study published so far. The deterioration of the alveolar structure and the unaccompanied cellular movement, many data could not be seen and this was a significant disadvantage compared to the conventional histological examination. The mentioned study is the first published study in this field to date, and the optical biopsy image patterns specific to the distinct diseases have not yet been identified. All studies performed using pCLE summarized in Table 1.

The present article discussed the imaging of the fluorescent cellular structures within the alveoli, which were full of diffuse exudate in a patient with PCP and also the imaging of the deterioration in the alveolar structure and the alveolar cavity, which was poor in cell in a patient with early IPF. In the first case, a distinct exudation and cellular structures with apparent autofluorescence in the alveolar and acinar area were observed. Although there are no similar published cases yet, a limited number of cases included in the ongoing “Optical Lung Atlas” studies exhibit similar findings. It has not yet been possible to identify the disease-specific patterns. On the other hand, the images obtained in the second case are consistent with the study by Newton et al. [9], which is the most extensive study published so far. The deterioration of the alveolar structure and the unaccompanied cellular increase and infiltration are consistent with the images from the optical biopsies of other patients with IPF. Since the purpose of this procedure was the imaging of the specific parenchymal status via pCLE in both cases, the procedure did not result in any changes to the treatment approach. Although the findings specific to the diseases is still at the level of identification, it may be estimated that some various approaches may be produced in the diagnosis and etiology of interstitial lung diseases with the confocal endomicroscopy. For instance, it will be possible over time to observe asbestos fiber directly within the alveolar structure or to analyze and identify various toxic particles upon the development of staining techniques. On the other hand, another purpose for pCLE imaging is to remove the need for transbronchial biopsy during the surveillance bronchoscopy in the lung transplantation cases. The identification of findings specific to the disease may aid in the diagnosis without the necessity of more advanced invasive procedures in many cases with in vivo optical biopsy.

Consequently, the probe-based confocal endomicroscopy may be a leading method for the imaging of peripheral lung nodules, interstitial pathologies, bronchial, and bronchial structure modifications. However, the limitations such as the restricted number of images due to the ability of imaging only the elastin fibers and the lack of taking concurrent tissue samples should be overcome.


In conclusion, although the findings with pCLE were helpful in suggesting the diagnosis, it did not show that it could alleviate the need for the tissue biopsy, and the histopathological examination to confirm the diagnosis. We still believe that these cases are worth sharing with the medical community, as publishing this data could stimulate further advancement, and development of the in vivo microscopic cellular exam, hopefully leading to reducing the need for performing transbronchial, and open lung biopsies, and the potential risks involved.

The challenge remains, the selection of the alveolar units to be examined in a patchy heterogeneous disease like IPF.

Further description and correlation of the pCLE findings with histopathological examination of lung biopsies may prove helpful in developing pathognomonic criteria for various lung pathologies, which, when combined with radiographic findings, may suffice for making a diagnosis, reducing the need for invasive biopsy techniques.

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Table 1: All studies performed using pCLE summarized.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Country</th>
<th>n</th>
<th>Study design</th>
<th>Disease</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
<th>Conclusion</th>
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<tr>
<td>Sorokina et al</td>
<td>2014</td>
<td>Russia</td>
<td>18</td>
<td>Ex vivo</td>
<td>Lung cancer</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>Successful to identify carcinoma</td>
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<td>Yserbyt et al</td>
<td>2014</td>
<td>Belgium</td>
<td>105</td>
<td>In vivo</td>
<td>AR in lung Tpx</td>
<td>0.93</td>
<td>0.83</td>
<td>ns</td>
<td>Existence of specific pCLE characteristics in AR</td>
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<td>2013</td>
<td>Belgium</td>
<td>26</td>
<td>In vivo</td>
<td>Lung Tpx</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>Test-retest reliability is good for cellularity and autofluorescence quantification</td>
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<tr>
<td>Yserbyt et al</td>
<td>2013</td>
<td>Belgium</td>
<td>2</td>
<td>In vivo</td>
<td>PAM</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>Describing the endoscopic findings of calcium-phosphate microliths accumulate</td>
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<td>2013</td>
<td>Belgium</td>
<td>5</td>
<td>In vivo</td>
<td>Miscellaneous</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>Needs standardisation of measurement techniques and evaluation of regional differences in diffuse lung diseases</td>
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<tr>
<td>Morisse et al.</td>
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<td>France</td>
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<td>Animal</td>
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<td>ns</td>
<td>ns</td>
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<tr>
<td>Salaün et al</td>
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<td>France</td>
<td>36</td>
<td>In vivo</td>
<td>Amiodarone related pneumonia</td>
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<td>0.88</td>
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<td>Successful to identify amiodarone related pneumonia</td>
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<tr>
<td>Fuchs et al</td>
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<td>Lung cancer</td>
<td>0.96</td>
<td>0.87</td>
<td>0.91</td>
<td>May enable the rapid diagnosis of neoplasia during endoscopy</td>
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<td>Newton et al</td>
<td>2012</td>
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<td>42</td>
<td>In vivo</td>
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<td>ns</td>
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<td>A basis for future work to harness pCLE</td>
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References