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Ex vivo use of probe-based confocal laser endomicroscopy in lung cancer visualization: an *ex vivo* pilot study

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Abstract

Lung neoplasia is the main cause of cancer death worldwide, with increasing incidence and an apparent stagnation in the decrease of morbidity. Early lung cancer (LC) diagnosis requires precise pathology and precise surgical intervention at the appropriate time. Current guidelines provide an outline for cancer management, involving multiple imaging methods as well as tissue-collection techniques; however, probe-based confocal laser endomicroscopy (pCLE), although being introduced several years ago, remains an experimental diagnostic method. Based on direct tissue observation that can occur during normal bronchoscopy, with the possibility of combining multiple imaging techniques in a single-step intervention, it however holds great promise. We included 18 consecutive patients diagnosed with LC over a period of six months who underwent curative surgery. After obtaining written consent and ethical approval, we used pCLE on *ex vivo* tissue samples that were later sent to pathology for confirmation. We then compared pCLE findings with the final pathology diagnosis. We included 12 males, median age in the study group being 64 years. We found 10 adenocarcinomas, four small cell lung carcinomas and four squamous cell carcinomas. The pCLE investigation provided key differentiating aspects between normal lung parenchyma, the structure of elastin fiber distribution and the shape and cellular content of alveoli, as opposed to the anarchic organization of the tumoral tissue. In conclusion, we presented here some of the first descriptive findings of lung tissue in pCLE imaging, thus contributing to the standardization of the technique for diagnostic purposes.

Keywords: lung cancer, probe-based confocal laser endomicroscopy, inflammation, lung parenchyma, pathology, diagnosis.

Introduction

Bronchopulmonary neoplasm is one of the most common causes of cancer-associated death in the world [1–3]. The main cause of lung cancer (LC) is considered a combination of exposure to environmental factors and a person's susceptibility to them. It is the only form of cancer in which a significant risk factor has been identified – smoking. In developed countries, the risk of mortality is higher due to the concomitant exposure to multiple risk factors. Difficult and often late diagnosis leads to the identification of the disease at an advanced stage, thus only a small percentage of patients can be treated with curative intent [3, 4]. Detection in the early stages, when the tumor may be resectable, could increase the chances of a patient's life, which proves the need for diagnosis in these stages, as well as detection and rapid treatment of preneoplastic lesions [1–5]. The definite diagnosis of LC is currently supported by histopathological examination. The pathogenesis of LC assumes the existence of many molecular abnormalities, which evolve over a longer period, so the onset of manifestations can occur at up to tens of years [6–8].

Probe-based confocal laser endomicroscopy (pCLE) is a relatively new imaging method that can be used to assist diagnosis during classical endoscopic investigations. Although its applications in the pulmonary sphere are quite limited, the interpretation of the obtained images can be improved by its combination with modern morphometric methods and computer analysis [9–12].

pCLE also has several advantages compared to classical biopsy: lowering the risk of side effects because microscopic analysis of the lesion can be performed by non-invasive method, lung structures can be functionally assessed, and a quick diagnosis can be made with the presence of a pathologist at the time of the intervention.

The interpretation of pCLE images of the lungs must consider several common artifacts and limitations inherent to technology. These factors can sometimes lead to observations that might be misinterpreted or that contribute to the overlap in features seen in healthy and diseased tissue [12–14].

A pCLE system typically comprises a thin, flexible miniprobe, usually ranging from 1 to 1.4 mm in diameter, which contains thousands of optical fibers. These fibers

transmit laser light to the tissue and relay the emitted fluorescence back to a detector. The laser light source commonly operates at a wavelength of 488 nm, which effectively excites the autofluorescence of certain tissue components in the lung, primarily elastin [9].

LC is divided histologically into two major types: non-small cell lung cancer (NSCLC, making up for approximately 85% of all LCs) and small cell lung cancer (SCLC) [15–17].

By using classical histological techniques, Hematoxylin–Eosin (HE) staining and Gömöri silver impregnation for reticulin fibers, we divided as follows changes in lung tissue. NSCLC includes squamous cell carcinomas (SCCs), adenocarcinomas and large cell carcinomas (LCCs).

SCC presents in the form of neoplastic proliferation, which consists of beaches and cords of large, polygonal cells with clear cytoplasm, oval nuclei, polymorphic sometimes with extensive necrosis by keratinization [18–20]. They can be distinguished from other types of NSCLC by the presence of keratinization, pearl formation and intercellular bridge [21]. Adenocarcinoma is defined by cords of cylindrical and cubic cells, which are multilayered and have giant basal nuclei and mucus secretion or gland formation [22, 23]. LCC is characterized by abundant cytoplasm, large cells without squamous or glandular differentiation, with large nuclei and prominent nucleoli, well-represented cell margins [24].

SCLC is distinguished by a proliferation of small cells with poor cytoplasm, poorly defined edges, fine "salt and pepper" chromatin, no nucleoli, and a large number of mitoses [25].

Current pCLE studies have shown significant similarities between *in vivo* imaging and histology, thus being considered an emerging tool for rapid, on-site diagnosis and staging of lung tumors.

Aim

Our aim was to evaluate the importance of pCLE in visualizing lung tissue and differentiate between normal lung parenchyma and malignant tissue specific to LC, as well as attempting to differentiate adenocarcinoma, SCC and SCLC elements.

Patients, Materials and Methods

This research was performed between September 2020 and March 2021 on a group of patients from the Emergency County Clinical Hospital of Craiova (ECCHC), Romania who had curative surgeries for LCs. The study was approved by the Ethical Committee of the University of Medicine and Pharmacy of Craiova (Approval No. 42/17.06.2020) and patients signed the consent forms (ECCHC Ethical Approval No. 26659/08.07.2020).

The histological samples obtained were labeled with Acriflavine and were analyzed using the pCLE (Cellvizio®, Paris, France) used for *ex vivo* studies.

The Cellvizio® pCLE system, developed by Mauna Kea Technologies (Paris, France), integrates advanced optics, fiber-optic technology, and laser scanning to enable real-time microscopic imaging of living tissue. The system comprises a main processing unit connected to a digital display, a laser module, and a flexible miniature probe made up of fiber-optic strands, each only a few hundred micrometers in diameter. This probe can be inserted through an endoscope for direct access to internal tissues. Inside the probe are various optical components, including a scanning mechanism and a single-mode fiber that delivers a low-power laser beam to the target tissue. As the laser interacts with the tissue, fluorescence signals are collected through the same fiber, then processed to produce high-resolution images. Using confocal microscopy principles, the pCLE system captures light from a specific focal plane, filtering out out-of-focus light and providing precise optical sectioning. This allows for real-time visualization of cellular structures, giving clinicians valuable diagnostic insight during endoscopic procedures.

We then processed the same tissue by following standard protocols and stained consecutive sections with HE and Orcein, to confirm the findings obtained by pCLE. An experienced pathologist (DP) analyzed the slides, using a Nikon Eclipse 90i motorized microscope (Nikon Instruments, Europe BV, Amsterdam, The Netherlands) equipped with a Prior OptiScan ES111 motorized stage (Prior Scientific, Cambridge, UK), and a 16-megapixel Nikon DS-Ri2 complementary metal oxide semiconductor (CMOS) cooled camera and Nikon NIS Elements AR image analysis software. Whole-slide scanning was performed, and the same pathologist marked the malignant and benign areas. Final diagnosis of each histological tumor type was obtained from medical records and was based on immunohisto-chemistry, performed as usual medical protocols required at ECCHC.

Results

Statistical data

We included 18 consenting patients that underwent surgery with no complications and were followed for at least four years (up to March 1, 2025). Of these, 12 (66.6%) were males, and the group had an equal distribution between rural and urban provenance within the group. No morbidity associated with surgery was recorded. Median overall age was 64 years (39 years minimum, 78 years maximum age), with no significant differences between males and females (median 64 years for both sexes, 40 and 77 years for males, 39 and 78 years for female participants). Overall, 11 were smokers (seven women and four men). Significant associated conditions included chronic pulmonary obstructive disease (14 cases), type two diabetes (seven cases), hypertension (five cases) and chronic kidney disease (one case).

Based on pathological results, as extracted from medical records, we found 10 (55.5%) adenocarcinomas, four (22.25%) SCLCs and four (22.25%) SCCs. We found eight adenocarcinomas in males (66.6% of males); the remaining two cases accounted for 33.3% of all tumors found in females. We found an equal distribution for both SCLCs and SCCs in both males and females.

pCLE procedure

We performed pCLE on lung tissue before paraffin inclusion and stained the surface with 2% Acriflavine and recorded continuous movies of approximately one minute length (median recording time 66 seconds, minimum 59 and maximum 73 seconds). Recordings were downloaded for analysis by using the dedicated software, Cellvizio®

Viewer (Mauna Kea Technologies, Paris, France). Movies contained both tumor areas as well as surrounding parenchyma that appeared normal upon visual inspection by the pathologist. Orientation was validated by microscopy on slides done from the same areas.

Normal parenchyma evaluation by standard pCLE imaging

The ability of pCLE to visualize this elastin microstructure in both the airway wall and the alveoli is a key aspect of the technology. Elastin is a major component of the axial backbone of the alveolar ducts and alveolar entries, providing essential structural support to these distal lung units. Upon inspection of the parenchyma recording, we could observe a network-like disposition of elastin fibers, delineating undistorted architecture of the septal walls, with regular structures representing vascular elements and rare diffuse cells of probable inflammatory origin (Figure 1, A–N). This network often appeared as a thin, single-fibered structure with dark alveolar air spaces visible between the fibers (Figure 1, A–L). The elastic structures varied in length, disposition and thickness, ranging from 1 to 3 μm in diameter. Some areas also contained blood vessels and appeared as connective tissue and superposed reticular structures (arrow; Figure 1C). These fibers were typically arranged longitudinally, forming a dense and homogeneous pattern; however, we found characteristic distortion of the alveolar structure, along with increased intricacies of the elastin walls in some areas (Figure 1, D–L). However, when examining normal parenchyma, the structure appeared coherent, with the integrity of the structure maintained and continuous. pCLE also allowed for the visualization of alveolar ducts and extra-alveolar microvessels, with the elastin component being the primary source of contrast for these structures. We observed distinct patterns of elastin fibers depending on the specific location within the bronchial tree. As we observed *ex vivo* tissue samples, we could macroscopically orient the tissue and have anatomical context when interpreting pCLE images obtained from different regions of the respiratory system.

We also identified small, oval or round-shaped cells of various intensities, well-differentiated from surrounding fiber-like structures (star; Figure 1, C, E and F). The cells were of inflammatory nature and could be visualized predominantly near alveoli. We predominantly found these cell infiltrates in healthy lung parenchyma of smokers, since pCLE often can reveal the presence of hyperfluorescent cellular alveolar infiltrates, typically ranging from 15 to 30 μm in size, due to the tobacco tar-induced fluorescence within alveolar macrophages.

At the orifices of the lobar bronchi, a network of tightly compacted, crossing fibers was observed (Figure 1, K and L). Topical administration of Acriflavine resulted in a homogeneous staining pattern with intense fluorescent staining of the nuclei of airway epithelial cells (Figure 1, M and N). This was likely due to the detection of small, light grey, densely packed, and mostly overlapping spots, which correspond to the nuclei of these cells. We have thus demonstrated that pCLE can go beyond the elastin structure, enabling a more comprehensive assessment of the bronchial mucosa at a cellular level.

Tumor evaluation by standard pCLE imaging

The consistent depiction of the delicate and organized elastin network in the alveoli served as a crucial marker of normal lung parenchyma, and any deviations from this pattern, such as thickening or disorganization, was indicative of disease. In contrast to normal lung parenchyma, when observing tumor tissue, irrespective of histological type, we could observe a more irregular structure, with a marked decrease in the number of elastin fibers, increased inflammatory cellularity and anarchic disposition of vascular structures (Figure 2, A–J). Elastic fibers, when present, were torn, incontiguous and did not follow any apparently organized disposition. The consistent observation of a disrupted elastin fiber network, with richer cellular infiltrate, was a constant hallmark of lung tumoral tissue.

The overall image was of a compact field, denoting severe alteration of the normal fiber architecture and dense, irregular disposition of the necrotic tissue, as well as the tumor-altered tissue and underlying cellular elements. Neoplastic lesions often exhibited heterogeneous architecture with areas of poor uptake of Acriflavine. We could identify darker cells of neoplastic origin. They appeared to have large nuclei of irregular shape and larger in size. These nuclei showed weaker Acriflavine reaction and had an irregular arrangement when compared with the overlapping and homogeneous distribution of cell nuclei seen in normal mucosa. We could also note larger dark areas of necrotic origin, with inconsistent fibrillary surrounding structures. Cellular elements were irregular in shape, more present in conjunction with a lack of background fibers, cellular detritus being a constant feature of all analyzed images (Figure 2, A–J). Vascular structures also appeared with thick walls and associated multiple cellular elements, round or oval, of various sizes (Figure 2D). Rich associated inflammatory infiltrate was seen in most cases.

In adenocarcinoma cases, pCLE revealed an amorphous masses filling the alveolar space and causing destruction of the alveolar walls (Figure 2, A and B). This mass-like structure sometime exhibited alternating areas of high fluorescence, due to misshapen aggregates of elastin, and dark cavities, representing large blocks of tumor cells that lack autofluorescence (Figure 2, C and D). The stromal component of adenocarcinoma consistently appeared as a highly fluorescent field penetrated by dark hollows. Furthermore, we found pCLE changes such as mottled elastin, septal studding, and disorganization or fragmentation of tissue, correlated with the degree of differentiation of adenocarcinoma cells.

In SCCs, pCLE also revealed a somewhat amorphous mass, with less fluorescent, thicker, and more numerous branches, which represent the stroma of the tumor (Figure 2, E and F). Darker layers could be observed, corresponding to the cellular component of the tumor. The stromal component of SCC appeared as highly fluorescent fibers, with increased cellular densities, irregular stratified patterns, and capillary neovascularization.

Small cell carcinoma, in contrast to adenocarcinoma and SCC, has shown a much less prominent stromal component under our *ex vivo* pCLE investigation. Instead, the cellular component was dominant, with an observed light scattering pattern and characteristic dark cells (Figure 2, G–J).

These distinct features observed with pCLE in different histological types of LC suggest that this technology may

have a role in aiding in their differentiation. We could conclude that Acriflavine significantly aided the identification of various tumor patterns by confocal imaging and may allow the mapping of tumor cells within the neoplastic areas of the lung.

Discussions

Diagnostic applications of pCLE in lung cancer

pCLE has shown promise in the detection of malignant lesions within the central airways. Studies have described characteristic pCLE patterns indicative of malignancy, including disorganization of the normally structured elastin fiber network, increased cellular density, irregular stratification of epithelial cells, and the presence of capillary neovascularization [26]. These features, visualized in real-time, can aid in identifying suspicious areas during bronchoscopy. In our present work, we found similar aspects to those described in literature. Furthermore, we could differentiate different types of malignant lesions.

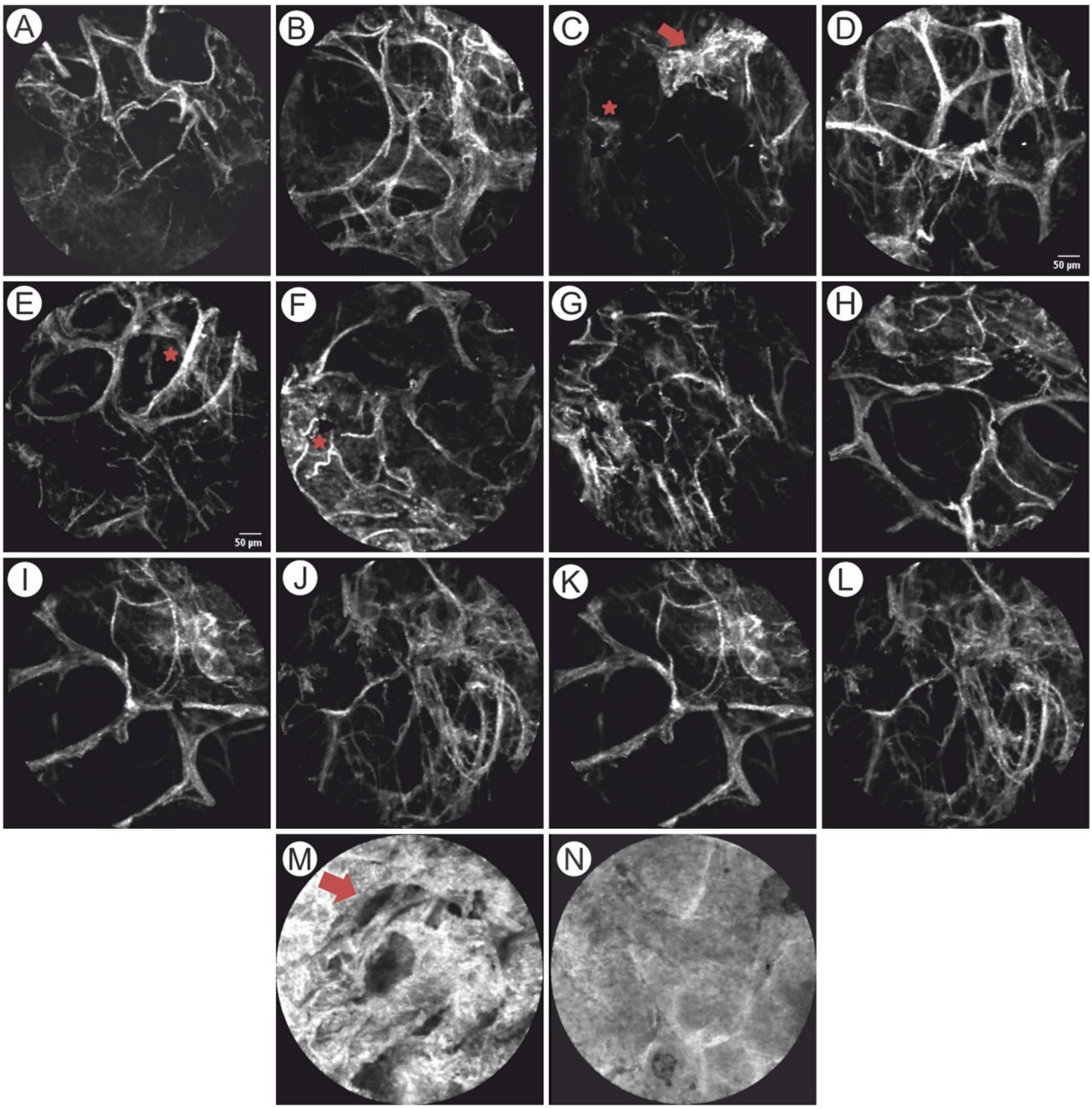

Figure 1 – ***Representative aspects of normal lung parenchyma inspected by pCLE: (A) Elastin fibers with a regular disposition, clear delimitation of alveolar structures; (B) Elastin fibers of various diameters, contiguous, blood vessels and supporting fiber structure; (C) Dense connective tissue and reticular structures with included blood vessels (arrow) and possible inflammatory cells, oval-round in shape and of various shapes and intensities (star); (D) Blood vessels included in elastin structures of various lengths and disposition; (E) Well-delimitated oval structures of airwaves and alveoli, with accompanying cells of various intensities, oval in shape (star); (F) Similar aspect with the presence of rare inflammatory cells of low intensity (star); (G–J) Reticular fibers, elastin structures of various thickness and disposition; (K) Healthy bronchial mucosa, with longitudinally arranged elastic fibers and opening of bronchial gland (arrow); (L) Healthy lung tissue with increased cellularity and glandular structures; (M) Bronchial gland (arrow) in a compact elastin network, specific for the distal tract; (N) Multiple nuclei of constant sizes, thick elastin fibers and gland-like structure within normal parenchyma.***

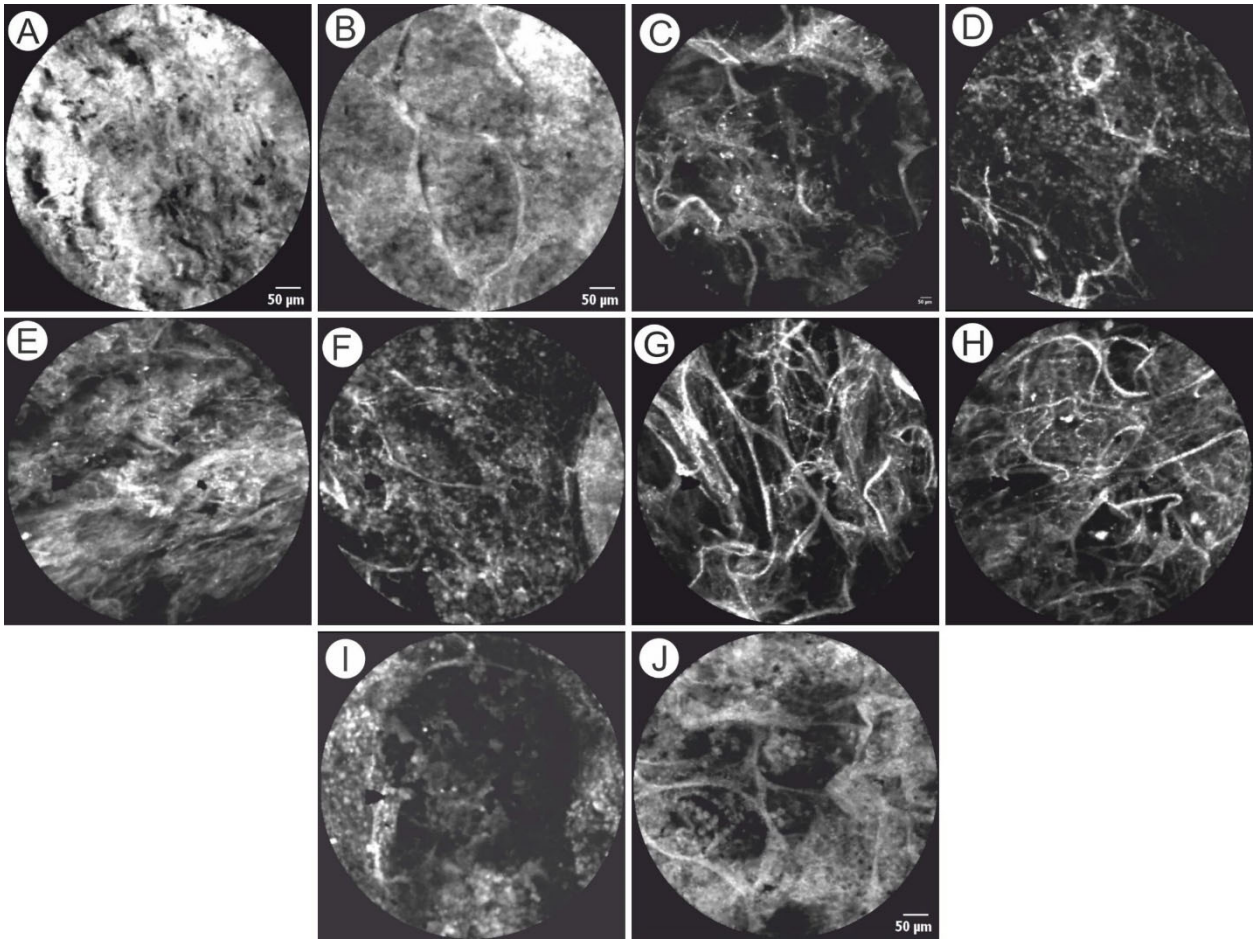

Figure 2 – ***Representative aspects of lung cancer inspected by pCLE: (A) Amorphous mass with no apparent structure, thick elastin fibers of irregular shapes and disposition; (B) Important cellular infiltrate of various nuclear intensities, shapes and disposition; (C) Inflammatory infiltrate with irregular elastin disposition, specific of tumor environment; (D) Blood vessel with increased wall density, inflammatory infiltrate and lower elastin fiber count; (E) Thick branches of an amorphous mass, typical for squamous cell carcinomas; (F) Similar aspect with the presence of multiple inflammatory infiltrates; (G–J) Multiple cellular infiltrates, light-scattering pattern and multiple dark cells and accompanying areas (I), specific for small cell carcinoma.***

For the diagnosis of peripheral LC, needle-based confocal laser endomicroscopy (nCLE) has emerged as a valuable tool. Research has demonstrated that bronchoscopic nCLE imaging of peripheral lung lesions suspected of malignancy is feasible, safe, and boasts a high degree of accuracy, reportedly up to 95%, in distinguishing between cancerous tissue and normal airway or lung parenchyma [26, 27]. This capability is particularly significant given the challenges associated with diagnosing peripheral lung lesions using conventional bronchoscopic techniques, which often suffer from a high rate of near misses during sampling [28]. The criteria for malignancy observed with nCLE include enlarged pleomorphic cells, dark clumps, and directional streaming of cells [28].

Furthermore, pCLE has been investigated for its ability to discriminate between malignant and benign peripheral pulmonary nodules. Studies have identified specific pCLE patterns associated with malignancy, such as a compact alveoli pattern, destroyed alveoli, and the presence of auto-fluorescent cells [29]. While initial experiences with pCLE for LC diagnosis showed indirect abnormal patterns like dark hollows, elastin disorganization, and cellular infiltrates, the technology initially faced limitations in directly visualizing individual tumor cells despite the use of Fluorescein [27, 28]. This was attributed to the limited penetration depth of pCLE and the thickness of the image plane [28]. However, advancements, particularly with nCLE, have allowed for imaging within the tumor, overcoming these earlier limitations [28].

Comparisons between pCLE and traditional bronchoscopic techniques, such as white light bronchoscopy and autofluorescence bronchoscopy, have been conducted to assess their relative efficacy in detecting premalignant and malignant bronchial lesions [26]. These studies suggest that pCLE, especially when used with contrast agents like Acriflavine, can enhance the detection of subtle mucosal abnormalities that might be missed by conventional bronchoscopy [26, 27]. Moreover, pCLE has the potential to guide biopsies of suspicious areas identified during bronchoscopy. By providing real-time microscopic information, pCLE can help clinicians target the most likely sites of malignancy, potentially increasing the diagnostic yield of biopsies and reducing the number of samples required [26]. This capability of providing real-time microscopic assessment, often referred to as "optical biopsy" underscores pCLE's role as a synergistic tool that can enhance the information obtained from traditional tissue biopsies [29].

pCLE and lung cancer staging

While pCLE is not a primary modality for LC staging based on the tumor, node, metastasis (TNM) system, it has the potential to contribute to more accurate staging in several ways [30, 31]. The TNM system, which is the cornerstone of LC staging, primarily relies on assessing the size and extent of the primary tumor (T), the involvement of regional lymph nodes (N), and the presence of distant metastasis (M) [30]. These assessments are largely based on macroscopic imaging techniques such as computed tomography (CT) and positron emission tomography (PET)–CT scans [30–32].

However, pCLE, particularly nCLE, shows promise in improving the assessment of mediastinal lymph node involvement, a critical component of the N stage in LC [26]. The ability of nCLE to visualize individual malignant cells and the microstructure of lymph nodes allows for a more detailed evaluation compared to standard bronchoscopic techniques [26]. By providing real-time microscopic feedback during fine-needle aspiration of mediastinal lymph nodes, nCLE can potentially increase the accuracy of detecting metastatic spread to these nodes, thus contributing to a more precise N staging [28].

Furthermore, pCLE can indirectly aid LC staging by enhancing the diagnostic yield of biopsies taken from suspicious areas within the lung parenchyma or lymph nodes [26]. By guiding the biopsy forceps or needle to the most likely sites of malignancy based on real-time microscopic visualization, pCLE can increase the chances of obtaining representative tissue samples for pathological analysis. This improved diagnostic accuracy can lead to a more informed assessment of the T stage (tumor extent) and the N stage (nodal involvement). Additionally, pCLE has been used to identify malignant cells in pleural effusion [26]. The presence of malignant cells in pleural fluid signifies advanced-stage disease (M1a in the TNM system), and pCLE's ability to detect these cells can contribute to the accurate determination of the M stage [26]. While pCLE does not directly measure tumor size or detect distant metastases in the same way as macroscopic imaging, its high-resolution, real-time capabilities can significantly improve the accuracy of tissue sampling and the assessment of regional lymph nodes, both of which are crucial for determining the pathological stage of LC.

Comparison of pCLE with traditional diagnostic modalities

pCLE offers several advantages over conventional bronchoscopy. Standard bronchoscopy provides a macroscopic view of the airways, whereas pCLE enables real-time *in vivo* visualization at the cellular level, allowing for the assessment of distal airways and alveolar structures that are beyond the reach of conventional bronchoscopes [6]. Furthermore, pCLE has the potential to guide biopsies to specific areas of interest identified through microscopic imaging, potentially increasing the diagnostic yield of bronchoscopic procedures [26]. However, pCLE also has limitations compared to conventional bronchoscopy, including a small field of view, which makes systematic examination of the entire airways impractical [26–29]. Additionally, the interpretation of pCLE images requires specific training and expertise [29].

When compared to biopsy techniques, pCLE can be considered a less invasive "optical biopsy" that provides real-time histological information of the surface epithelium [26]. This can potentially reduce the need for more invasive procedures like surgical lung biopsy and their associated complications such as pneumothorax and hemorrhage [33]. Moreover, pCLE can guide transbronchial biopsies and cryobiopsies to specific areas with abnormal microscopic features, potentially improving diagnostic accuracy and reducing the risk of complications by avoiding sampling of vessels or pleura [26]. Nevertheless, pCLE may not entirely replace traditional biopsies, especially when large tissue samples are required for comprehensive histological and molecular analysis, which are essential for the diagnosis and subtyping of certain LCs and interstitial lung diseases (ILDs) [26, 27].

In comparison to imaging modalities like CT, high-resolution CT (HRCT), and PET–CT, pCLE offers a significant advantage in terms of resolution, providing microscopic views of the lung tissue *vs.* the macroscopic images obtained with these radiological techniques [33]. pCLE can complement the findings from CT and HRCT by providing real-time cellular-level information in areas that appear suspicious on these scans [6]. For instance, while a CT scan might reveal a pulmonary nodule, pCLE can help determine whether the nodule exhibits microscopic features suggestive of malignancy [29]. However, pCLE does not provide the same level of anatomical overview and staging information as comprehensive CT and PET–CT scans, which are crucial for assessing the overall extent of disease and detecting distant metastases in LC [31, 32]. Therefore, pCLE is best viewed as a complementary tool that can enhance the diagnostic capabilities of traditional methods by providing unique microscopic insights during bronchoscopy.

Advantages and limitations of pCLE in clinical practice

The integration of pCLE into clinical practice for lung disease diagnosis and staging offers several notable advantages. One of the primary benefits is the capability for real-time, *in vivo* microscopic visualization of lung tissue [9]. This allows clinicians to assess tissue architecture and cellular morphology at a level of detail not achievable with standard bronchoscopy or macroscopic imaging. In certain cases, pCLE has the potential to facilitate non-invasive or minimally invasive diagnosis, potentially reducing the need for more invasive surgical biopsies and their associated risks [33]. By providing real-time microscopic guidance during bronchoscopy, pCLE can improve the diagnostic yield of biopsies by allowing clinicians to target specific areas of interest that exhibit abnormal features at the cellular level [26]. Furthermore, pCLE's ability to visualize elastin and cellular structures is particularly valuable in the diagnosis of a wide range of lung diseases, including ILDs and LC, where characteristic microscopic patterns can be identified. There is also evidence suggesting a potential role for pCLE in monitoring treatment response and disease progression over time [29].

Despite these advantages, the clinical application of pCLE also presents several limitations. The small field of view of the probe makes it impractical for systematic

examination of the entire tracheobronchial tree or lung parenchyma [29]. The limited penetration depth, typically up to 50 μm, restricts visualization to superficial tissue layers, which may not be sufficient for diagnosing conditions that primarily affect deeper structures [33]. In many applications, especially for visualizing cellular details crucial for cancer diagnosis, the use of exogenous contrast agents is necessary [26]. The interpretation of pCLE images can be subjective and requires specialized training and experience to accurately identify pathological patterns [29]. The cost and availability of pCLE systems and probes may also be a barrier to widespread adoption in all clinical settings [26]. Additionally, the procedure can be susceptible to artifacts caused by tissue movement during breathing or probe manipulation [29]. Finally, the pCLE processor requires an initialization process before each bronchoscopy, which adds a short amount of time to the overall procedure [29]. Addressing the subjectivity in image interpretation through the development of standardized criteria and potentially automated analysis tools is crucial for facilitating broader clinical use [33].

Current consensus and guidelines regarding the use of pCLE in lung diseases

Currently, there are no widespread, standardized guidelines for the routine use of pCLE in the diagnosis and staging of most lung diseases [26]. However, the growing body of research has led to the emergence of expert opinions, consensus statements, and preliminary diagnostic criteria for specific conditions. For instance, studies have proposed semi-quantitative criteria for interpreting pCLE images in the context of ILDs, focusing on features like alveolar distortion, density of structures, consolidations, and the presence of loaded alveolar macrophages [6]. These criteria are often used in research settings and in centers with expertise in pCLE imaging. The common aspects encountered during the evaluation of histology slides with chronic or acute inflammatory infiltrate, macrophage [34, 35] or lymphocytes [36–38] is a promising field of application for pCLE, as our study has shown from the various aspects of cellular infiltrates found in both the parenchyma of smokers, as well as the adjacent tumor tissue. Inflammation remains an associated condition of cancer [39–41], and the role pCLE will further play in its evaluation remains of high importance.

In the diagnosis of LC, while pCLE is not yet part of standard staging guidelines, research has identified characteristic pCLE patterns for both central and peripheral malignancies, and nCLE has shown high diagnostic accuracy for peripheral lesions [26]. These findings may eventually lead to the incorporation of pCLE into diagnostic algorithms for specific clinical scenarios.

Given the complexity of diagnosing many lung diseases, particularly ILDs, a multidisciplinary approach is often emphasized. In this context, pCLE findings can be integrated with clinical information, radiological imaging (such as HRCT patterns), and pathological data from biopsies to reach a more accurate and confident diagnosis [9]. The importance of multidisciplinary discussion is highlighted in the diagnostic guidelines for idiopathic pulmonary fibrosis, where the interpretation of HRCT patterns and, when available, histopathology findings are considered in conjunction with clinical context [42]. While these guidelines do not specifically recommend for or against pCLE, the integration of any novel diagnostic modality like pCLE would likely occur within such a multidisciplinary framework.

Recognizing the need for consistency and reproducibility in pCLE imaging, there are ongoing efforts to standardize imaging protocols and interpretation criteria [26, 28]. The development of image libraries and automated software for image analysis are also being explored to reduce inter-observer and intra-observer variability in the interpretation of pCLE images [26]. As the evidence base for pCLE in pulmonology continues to grow, and as standardization efforts progress, it is anticipated that more specific guidelines regarding its clinical use for diagnosing and staging various lung diseases will emerge.

Conclusions

pCLE represents a promising advancement in the field of pulmonology, offering real-time, high-resolution microscopic visualization of lung tissue *in vivo*. While pCLE offers numerous advantages, it also has limitations such as a small field of view, limited penetration depth, and the need for specialized expertise in image interpretation. The development of standardized imaging protocols and interpretation criteria is crucial for ensuring the widespread and reliable adoption of this technology. While challenges remain, pCLE has the potential to significantly improve the diagnosis and management of a wide spectrum of lung diseases in the years to come.

Conflict of interests

The authors declare that they have no conflict of interests.

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