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Comparing Probe-Based Confocal Laser Endomicroscopy With Histology. Are We Looking at the Same Picture?

Comparando endomicroscopía confocal con histología. ¿Estamos mirando la misma imagen?

Dear Editor,

Probe-based confocal laser endomicroscopy (pCLE) provides real-time vision of respiratory tissues at the cellular level through a flexible bronchoscope. This technique might guide sampling of pulmonary nodules,[1–4] lymph nodes[5] or pleural biopsies.[6] First studies of the respiratory tract were performed with a 488 nm wavelength probe that allows elastin visualization without adding fluorophores in the tissue. Because cells are not visualized, lung cancer pattern descriptions were limited to changes on the stromal component.[1,7–9] Later studies used fluorophores like methylene blue or fluorescein that could be excited at a 488 or a 660 nm wavelength, respectively, to visualize cell nuclei.[2,4,10] In these studies, different imaging patterns were described. In particular, healthy tissue was described as having homogeneous architecture with bright, partially overlapping nuclei. Inflammation was considered when heterogeneous tissue architecture without overlapping nuclei but expanded cytoplasms were observed, and neoplasia was reported as a chaotic distribution of dark cells with heterogeneous nuclei.[2,8,9,11,12] Although pictures of these patterns were frequently presented together with pictures of histology samples, none of the studies correlated the measurements performed in pCLE images to those of histology samples. In this pilot study, we aimed to explore the feasibility of correlating pCLE and optical microscopy features of normal and pathological airway samples.

Under general anesthesia and orotracheal intubation with a rigid bronchoscope (Efer-Dumon type, La Ciotat, France) pCLE was performed after applying a drop of 1% MB in nine regions of normal mucosa and in six tumors, as determined with white-light bronchoscopy. The AlveoFlex® probe and a laser scanning unit equipped with 660 nm laser wavelength (Cellvizio®, Mauna Kea Technologies, Paris, France) were used. After pCLE image registration, biopsies were taken and histology was studied with optical microscopy following haematoxylin-eosin (H&E) staining. A total of 15 patients were studied. The biopsies revealed normal epithelium in 6 cases, inflammatory infiltrate in 3 cases and thoracic tumors in 6 cases, which included B cell lymphoma, adenocarcinoma, squamous cell carcinoma, small cell lung cancer, and non-small cell lung cancer. The study was approved by the local ethics review board (Clinical Research Ethics Committee of Bellvitge University Hospital – Act 08/13) and written informed consent was obtained from all participants.

One representative image from the pCLE registration was selected and compared to one image of the H&E-stained sample. Nuclei were segmented in all images using ImageJ software (National Institutes of Health, Bethesda, MD, USA).[13] In the pCLE images, we accounted for the number and mean size of nuclei, the relative area occupied by nuclei and the intensity of fluorescence. In the H&E-stained histological sample images, we accounted for the number and mean size of nuclei and the relative area occupied by nuclei. We chose these features because these structures can be identified both in the optical and the pCLE images.

We made two different comparisons. First, we compared the patterns of pCLE alone. A logistic regression model was fitted to predict the probability of pathological tissue (inflammation or malignancy) based on every feature. We observed that changes in these features were associated with variations in the probability of disease. In particular, we observed that the odds of disease increased by a factor of 1.03 (95% CI: 0.99–1.07) for every one unit increase in the mean size of nuclei, by a factor of 1 (95% CI: 1–1) for every one unit increase in the intensity of fluorescence, and by a factor of 1.088 (95% CI: 1.01–1.224) for every one unit increase in the relative area occupied by nuclei. Next, we registered the measurements performed in the optical microscopy and pCLE images (see Table 1) and compared their distributions. After a Wilcoxon rank sum exact test we observed that the distributions of the mean size and relative area occupied by nuclei were different in the images of optical compared to pCLE microscopy. Although this small analysis is not adequately powered to achieve statistical significance, our results of the pCLE alone patterns are in line with the previously mentioned studies showing that it is possible to discern between normal and pathological tissue patterns, while the measurements performed in the optical microscopy and pCLE images cannot be correlated to the tissue structures observed in the biopsy specimens.

Our pilot study supports the use of pCLE to identify patterns of normal and pathological airway tissue but discourages comparisons between pCLE and histology images. These findings might reflect that the final histology diagnosis is based on a set of information not obtainable from a sequence of nuclei as it is observed in pCLE images. To empower pCLE as a useful tool for diagnosis of lung cancer, future studies should focus on identifying further discriminative features[14] or specific tumor markers. Otherwise, this technique will be limited to the identification of pathological areas for biopsy guidance.

Table 1
Measurements Performed in the Histological Samples (Optical Microscopy) and pCLE Images.

	Final Diagnosis	Optical Microscopy (H&E Stain, 40× Magnification)	Probe-based Confocal Laser Endomicrosocopy	*P*-Value
Number of nuclei	Normal ($n = 6$)	28.8 (5.78)	35.3 (4.13)	.1025
Mean (SD)	Pathological ($n = 9$)	33.8 (16.2)	27.8 (16.6)	.8946
Mean size of nuclei (μm^2)	Normal ($n = 6$)	103 [90.8;129]	36.5 [31.7;40.9]	<.05
Median [Q1;Q3]	Pathological ($n = 9$)	127 [114;155]	29.5 [27.9;59.0]	<.05
Relative area occupied by nuclei (μm^2)	Normal ($n = 6$)	60.6 (18.2)	26.5 (10.8)	<.05
Mean (SD)	Pathological ($n = 9$)	80.1 (13.4)	38.9 (14.3)	<.05

Pathological refers to inflammation or malignancy.
H&E: haematoxylin–eosin, SD: standard deviation.

Authors' Contributions

M.D-F, B.T. and A.R. generated the hypothesis; M.D-F., B.T. and N.B. designed the study; M.D-F., B.T, N.B, N.C, R.L., J.D and A.R contributed to data acquisition. C.T. performed the statistical analysis, and M.D-F. wrote the manuscript. All members of the study critically reviewed the submitted article for important intellectual content, provided final approval of the version to be published, and agreed to be accountable for all aspects of the work and to ensure that any questions related to the accuracy or integrity of any part of the work will be appropriately investigated and resolved.

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Conflict of Interest

All authors of this manuscript report that no potential conflicts of interest exist with any companies/organizations whose products or services may be discussed in this article.

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Ascending necrotizing mediastinitis. An exceptional case☆

Mediastinitis ascendente necrosante. Un caso excepcional

To the Editor:

Necrotizing mediastinitis (NM) is defined as the progression of a distant acute infection towards the mediastinum[1], involving its structure[2,3] and causing necrosis of the tissues involved. This is a rare but potentially lethal complication with a mortality ranging from 10% to 40%, depending on the series[4]. This high morbidity is derived from the severity of the process[4] and the need for prolonged admission in most cases[2].

The most frequently associated foci are otolaryngological infections[1], usually from a dental or oropharyngeal abscess[5], and less frequently from cervical spine infections[4,6]. The infection spreads via deep retropharyngeal[6], pretracheal, or vascular[7] fascia, facilitated by intrathoracic negative pressure, severity, and breathing[1,4,7]. For this reason, it is normally called descending NM. Dissemination from a source other than the head and neck is exceptional, although other foci, such as abdominal, chest wall, lung, lymph nodes, and even hematogenic spread, have been described[1].

We report the case of an 83-year-old patient with a history of hypertension and type 2 diabetes mellitus, permanent atrial fibrillation treated with anticoagulation, complete atrioventricular block with pacemaker implantation, and obstructive sleep apnea syndrome. The patient had undergone endoscopic retrograde cholangiopancreatography to perform a sphincterotomy with plastic stent placement to treat repeated biliary colic complicated with acute pancreatitis of biliary origin and cholangitis. As endoscopic revisions were required due to large calculi obstructing the stent, we decided to perform a cholecystectomy.

He was scheduled to be admitted from home but reported vomiting and diarrhea in the days prior to admission. On the morning of the intervention, he presented hypotension 50/30 mmHg with generalized deterioration of his condition and metabolic acidosis with hyperlactacidemia (19 mmol/l) and pH 7.22, so the procedure was suspended and the patient was admitted to the intensive care unit. During admission, his hemodynamic status was highly unstable and he developed acute renal failure (creatinine 2.42 mg/dl), elevated acute phase reactants (mild leukocytosis of 13.43×10^9/l, C-reactive protein 150 mg/l, and procalcitonin 48.59 ng/dl), elevated liver enzymes, and hyperbilirubinemia.

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