Probe based confocal laser endomicroscopy for pleural malignancies diagnosis.

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Study protocol

This is an ethically approved (clinicaltrial.org NCT03805971) single center cross-sectional study. Every patient ≥ 18 years referred for a medical thoracoscopy (pleural effusion work up, recurrent pneumothorax, talc pleurodesis….) was selected for the present study between 06/2018 and 09/2019. Exclusion criteria were pregnancy and known allergy to fluorescein. The thoracoscopy was performed under sedation (allowing spontaneaous breathing) by two experienced investigators with a systematic procedure. The patient was positioned in lateral decubitus during the intervention. After chest ultrasound examination, a pneumothorax was induced with the Boutin trocar and one entry port was performed though the chest wall. Two different thoracoscopes were used during this study: 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 ®). Five milliliters of fluorescein (10%) were intravenously administrated 5 minutes before image acquisition. After that, the pleural cavity was examined macroscopically. Then the pCLE probe (Alveoflex ®, lateral resolution 3 µm, optical area 1.13 mm^2, depth of focus 0 - 50 µm) (Cellvizio®, Maunakea technologies®, Paris, France) was gently placed on the parietal pleura and videos were recorded. If macroscopic abnormalities were noticed, pCLE was performed on affected zones, if not, three random sites were selected. Finally, biopsies were systematically performed on the same sites for comparison with optical biopsies. During the thoracoscopy, the investigators rated the quality of the pCLE acquisitions (good, acceptable, low).

After the image acquisition, another investigator (not included in those who rated the quality of the acquisition) selected the five best representative images for further analysis and comparison to histological samples. According to our previous experience, 11 criteria were selected to assess the ability of pCLE to distinguish benign from malignant pleura. The abnormal tissular architecture was defined by the identification of tissular/cellular structures not known to belong to the normal pleura (cell clusters, dark clumps, glands, cell cordons, dysmorphic cells, papillar architecture…). The absence of abnormal architecture was defined by the identification of normal pleural cellular and tissular elements as previously reported: the mesothelial monolayer with homogenous and regularly distributed cells; the fibro-adipose connective tissue and its regular and homogeneous vascularization; muscular fibers. The cells homogeneity in size, fluorescence and shape was subjectively assessed by the investigator. The mean cellular sized was assessed on a full field of view (1.13 mm^2). The presence of dysplastic vessels was assessed, as described by Cannizzaro et al., by the identification of vascular leaks (fluorescein leakage outside the vessels), tortuous vessels or giant vessel. For the maximal vascular diameter, we selected the largest vessels among the available pCLE images. For the vascular density, we measured the max number of vessels/image (full field of view) for every patient on the available images. For our study, we selected only the vessels with a length of at least 50 µm. The connective tissue fibers were classified as anarchic or organized. Anarchic connective tissue fibers were defined as irregular in shape or direction, coarse, without a well-defined architecture. Organized connective tissue fibers were defined as regular in shape and direction with a well-defined architecture. The chia seed sign defines the presence on a full field of view of the mesothelial monolayer with well defined, homogeneous and organized mesothelial cells. The criteria were not scored if assessable elements for their interpretation were missing. Every patient included gave informed consent.

Statistical analysis plan

The Fisher's exact test was used to analyze the link between pCLE qualitative variables and the final histological diagnosis ($p < 0.05$ for statistical significance). For quantitative variables, the unpaired T-test (2 sided p-value, $p < 0.05$) was used.