PROBE-BASED CONFOCAL LASER ENDOMicroScopy FOR Diagnosis of Nasopharyngeal Carcinoma In Vivo

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OBJECTIVES/HYPOTHESIS: Probe-based confocal laser endomicroscopy (pCLE) is a novel technique allowing real-time evaluation of the histological features of tissues in vivo at the cellular level. This study aimed to evaluate the feasibility of using pCLE in the diagnosis of nasopharyngeal carcinoma (NPC).

STUDY DESIGN: Feasibility study.

METHODS: In this study, the pCLE images of the lesions, as well as the surrounding or contralateral normal mucosa of the lesions, were acquired in vivo from each patient after intravenous injecting of 2.5 mL fluorescein. Biopsy specimens were collected at the imaged sites followed by a histopathological diagnosis by the pathologists, which was used as the gold standard. The pCLE images were compared to histopathological diagnosis of visualized sites by using sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV).

RESULTS: Diagnoses based on pCLE images correlated well with the gold standard diagnoses based on tissue histology. The overall sensitivity, specificity, PPV, and NPV for diagnosis of carcinoma versus nondysplasia were 93.8% (67.7%-99.7%), 90.5% (68.2%-98.3%), 88.2% (62.3%-97.9%), and 95.0% (73.1%-99.7%), respectively, and the four indices for pCLE diagnosis of dysplasia versus nondysplasia were 60.0% (17.0%-92.7%), 80.9% (57.4%-93.7%), 42.9% (11.8%-79.8%), and 89.5% (65.5%-98.2%), respectively. The overall sensitivity for diagnosis of carcinoma versus dysplasia was 93.8% (67.7%-99.7%), specificity was 40% (7.3%-83.0%), PPV was 83.3% (57.7%-95.6%), and the NPV was 66.7% (12.5%-98.2%).

CONCLUSIONS: CLE is a suitable and valid method for otolaryngologists to diagnose of NPC in vivo.

KEY WORDS: Probe-based confocal laser endomicroscopy, nasopharyngeal carcinoma, optical biopsy.

LEVEL OF EVIDENCE: NA

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INTRODUCTION

Nasopharyngeal carcinoma (NPC) is an rare malignancy in most parts of the world, but it is rather prevalent in South China, Southeast Asia, and North Africa.1 Globally, there are approximately 80,000 new cases annually and approximately 50,000 patients die of NPC.1,2 This neoplasm is a malignancy of the epidermoid in the nasopharyngeal mucosa with unique histological, epidemiological, and biological characteristics.3 Even though there has been substantial improvement in NPC therapy over the last decades, such as intensity-modulated radiotherapy and chemotherapy, one of the most crucial factors to ensure patient survival and quality of life still remains early detection and accurate diagnosis of NPC.4

Nowadays, various diagnostic techniques can be used to evaluate the nasopharynx in patients suspected of having NPC, particularly if high-risk clinical symptoms are present. Head and neck contrast-enhanced computed tomography (CT) and magnetic resonance imaging (MRI) have been used to delineate the tumor extent for staging and evaluation.5,6 White light nasendoscopy, with an invasive biopsy for pathological diagnosis, is routine in the diagnosis of NPC, but is time consuming and dependent on the quality and quantity of the biopsy samples.1,2

However, probe-based confocal laser endomicroscopy (pCLE) was recently introduced as a novel technique providing real-time, in vivo, histological visualization of mucosal tissue during the endoscopy examination7–9 and is described as an optical biopsy. It produces magnified images for the architectural comparison of normal cellular structure with neoplastic tissue after injected contrast agents, such as fluorescein, that stain the extracellular matrix of the mucosal tissues.10,11 To date, numerous studies have shown the

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applications of pCLE in vivo diagnosis of esophageal squamous cell carcinoma,12 biliary tract malignancies,13 as well as its advantages such as eliminating unnecessary biopsies, detecting tumor margins following resection, and allowing for immediate decision making.14 Furthermore, pCLE also has been used in the diagnostic differentiation between non-neoplastic, precancerous, and cancerous lesions of the head and neck.15–18

The aim of our study was to depict the applicability of pCLE examination for diagnosing NPC in comparison to the gold standard of histopathological examination based on the morphology and architecture of the pCLE images.

MATERIALS AND METHODS

Patients

The Ethical Committee of the Eye and ENT Hospital of Fudan University approved the protocol. From January 2017 to June 2017, in the Eye and ENT Hospital of Fudan University, 21 patients with suspected NPC lesions who required nasopharyngeal biopsy were enrolled from 450 patients with nose bleed, stuffy ear, or otitis media with effusion according to the results of nasendoscopy, CT, or MRI. Inclusion criteria were defined as follows: 1) age ≥ 18 years, 2) requiring nasopharyngeal biopsy for patients with suspected NPC lesions, and 3) willing and able to comply with study procedures and provide written informed consent to participate in the study. Exclusion criteria were defined as follows: 1) age < 18 years, 2) allergies to fluorescein, 3) current pregnancy/lactation period, 4) renal insufficiency at any stage. All included patients provided written informed consent. This protocol was registered at ClinicalTrials.gov (identifier NCT03202173).

pCLE Imaging Protocol

The target lesions were first identified endoscopically. Because normal tissue background fluorescence is not able to generate a sufficient signal-to-noise ratio, intravenous fluorescent dye was applied (10%). The pCLE images were obtained within 20 minutes after intravenous injection of 2.5 mL 10% fluorescein (Alcon Research, Ltd., Fort Worth, TX) using the Cellvizio system (Mauna Kea Technologies, Paris, France) through a pCLE probe (GastroFlex, CellVizio; Mauna Kea Technologies) while the patients were awake. This pCLE probe, with a diameter of 2.6 mm, could provide a depth of examination from 55 to 65 μm, a 240 × 240 μm² field of view at a resolution of 1 μm, and a magnification of 1,000 at 12 frames per second.

The probe tip was first placed on the background normal mucosa (contralateral to the lesion) to obtain a control, nonneoplastic image. Then, the targeted lesion sites and adjacent surrounding mucosa were scanned with pCLE to obtain diagnostic images. After images were obtained, patients underwent the biopsy of the scanned lesions. All specimens were sent to pathology for routine histologic diagnosis by two professional head and neck oncology pathologists according to the histologic diagnosis criteria of NPC in Pathology And Genetics of Head and Neck Tumours, World Health Organization Classification of Tumours.19

Classification of pCLE Images

Two principal investigators with prior pCLE experience performed all video and image quality control for this study. Images were rejected if the field of view was out of focus or showed evidence of a motion artifact. Forty-two high-quality pCLE images with correlating pathological diagnoses were selected based on the presence of interpretable epithelial images as determined by the principal investigator. Then, these images were sent to three otolaryngologists with pCLE experience. They were asked to review and categorize the pCLE images into three categories based on the cellular and vascular criteria: nondysplasia, dysplasia, or carcinoma. For cellular criteria, normal nasopharyngeal epithelial cells were uniform, with a regular structure and clearly visible squamous cell boundaries. The pCLE images of dysplasia showed a less regular cellular shapes, with dark areas as well as more irregular intercellular spaces when compared to normal nasopharyngeal epithelial cells. In contrast, malignant epithelial cells are not uniform, and squamous cell structures are irregular. In most cases, these cells do not show clearly visible boundaries between cells. For vascular criteria, normal capillaries in the tissues appeared routinely without fluorescein leakage, and usually appeared as more noticeable blood vessels in the cases of dysplasia, whereas malignant capillaries were irregular, distorted, dilated, and elongated. Occasionally, fluorescein leakage could also be observed in malignant tumors.

All reviewers were blinded to the patient’s medical history and pathological diagnoses.

Outcome Measures

The corresponding pathological diagnoses were used as the gold standard in assessing the diagnostic accuracy of pCLE imaging through pooled sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

RESULTS

Patient Characteristics

Table I summarizes the demographics and clinical presentation of patients in this study and associated lesion characteristics. A total of 21 patients (male = 17, female = 4) with nasopharyngeal lesions were enrolled in the study. The mean age ± standard deviation of enrolled patients was 48.8 ± 15.2 years (range, 21–75 years). On final pathological diagnoses, there were eight dysplastic diagnoses and 13 nasopharyngeal carcinoma in the 21 targeted lesion of the patients.

pCLE Image

Homogeneous configuration of the flat and polygonal epithelial cells with relatively uniform cell gap were noted in pCLE images of normal nasopharyngeal mucosa (Fig. 1). The pCLE images of the dysplasia mucosa showed an unorganized architecture of tissue with slightly intensified fluorescein leakage and irregular cells (difference of cell shape and size) (Fig. 2). Figure 3 showed the pathology and pCLE images of nasopharyngeal carcinoma, which was associated with crowded, unorganized tissue architecture (a dark-gray background without identification of mucosal structures), irregular cell clusters, amplified fluorescein leakage, and irregular vessel changes.

The sensitivity, specificity, PPV, and NPV for pCLE images of the suspected lesions were compared to the gold standard histologic diagnosis. The overall specificity,
sensitivity, PPV, and NPV for pCLE diagnosis of carcinoma versus nondysplasia mucosa were 93.8% (67.7%-99.7%), 90.5% (68.2%-98.3%), 88.2% (62.3%-97.9%), and 95.0% (73.1%-99.7%) (Table II). The overall sensitivity for diagnosis of dysplasia versus nondysplasia mucosa was 60.0% (17.0%-92.7%), specificity was 80.9% (57.4%-93.7%), and PPV was 42.9% (11.8%-79.8%), and the NPV was 89.5% (65.5%-98.2%) (Table II). The overall sensitivity for diagnosis of carcinoma versus dysplasia mucosa was 93.8% (67.7%-99.7%), specificity was 40% (7.3%-83.0%), PPV was 83.3% (57.7%-95.6%), and NPV was 66.7% (12.5%-98.2%) (Table II). Meanwhile, there was good interobserver agreement for the detection of mucosal carcinoma ($\kappa = 0.75$) between the otolaryngologists.

DISCUSSION

The pCLE is an advanced technology that provides real-time, in vivo microscopic images of individual cells and tissue architecture of the mucosa including gastroenterology, urology, pulmology, and dermatology with good outcome. Recent studies showed promising results using this imaging method in the area of the head and neck, with a sufficient differentiation of pathological lesions to physiological tissue in the upper gastrointestinal tract such as the pharynx, larynx, and esophagus. Furthermore, preliminary results regarding CLE examinations in the oral cavity show potential for detecting cancerous lesions even in their early forms, which have generated interest in applying this technology to the diagnosis of nasopharyngeal lesions.

In the normal population, an average area of 60% (±10%) of the whole epithelial surface (approximately 50 cm² in the adult) of the nasopharynx is covered by squamous epithelium. In this study, flat and relatively
uniform polygonal epithelial cells were found in pCLE images of normal nasopharyngeal mucosa, which was consistent with the hematoxylin and eosin staining. Furthermore, well-formed blood vessels were also found within the nasopharyngeal mucosa that were situated close to the surface between the stratified layers of the epithelium. Although pathologic review is an indispensable tool in the diagnosis of nasopharyngeal lesions, the
pCLE images obtained were highly comparable with the histology of the nasopharyngeal mucosa, suggesting that optical biopsy using pCLE may be another good alternative in the diagnosis of NPC.

Prior studies regarding pCLE imaging of malignant tumor focused on specific tissue patterns and cellular components that are characteristic for malignancy. Liu identified dark epithelial cells with irregular arrangement as distinctive for squamous-cell carcinoma of the esophagus. These characteristic changes in malignant mucosa were in accordance with the pCLE images of NPC as we found in this study, which implied that the tissue architecture and cell morphology are the main criteria in pCLE imaging for diagnosing NPC.

Additionally, Nathan and Cannizzaro described a disorganized epithelium with fluorescence leakage in pCLE images of squamous cell carcinoma. Our findings regarding increased fluorescence leakage in nasopharyngeal carcinoma were in accordance to those findings. Therefore, fluorescence leakage can also be a main criterion for the diagnosis of NPC. This pilot study demonstrated that the diagnoses of NPC based on pCLE imaging were highly consistent with the histopathologic findings in the nasopharynx, which were consistent with the diagnostic yield of pCLE imaging in other anatomic sites. Furthermore, another distinctly superior advantages of in vivo pCLE imaging is the ability to scan and evaluate the entire lesion at the cellular level, which can identify superficial mucosal lesions that may be missed by standard white-light imaging endoscopy and further guide invasive biopsy. This method can avoid sampling errors associated with biopsies and provide a novel approach to increase the pathologic detection rate and early diagnostic rate of the lesions in populations at high risk for NPC.

As another optical imaging technique, narrow-band imaging (NBI) (Olympus Corp., Tokyo, Japan) is an endoscopic technique that involves the use of two filters to select blue and green light with wavelengths of 415 and 540 nm, respectively, to enhance the visualization of epithelial and subepithelial microvascular patterns. Recently, some researchers have reported using NBI to screen patients for head and neck cancers, including for screening and detection of NPC with satisfactory results. Some reports compared the sensitivity and specificity of pCLE with NBI using pathological diagnoses as the gold standard, and found that pCLE is a more sensitive technique with similar rates of specificity. Therefore, we can assume that the combined use of pCLE and NBI can further improve the detection rate and early diagnostic rate of patients at risk for NPC.

Many researchers are confronted with difficulties in looking for biomarkers for NPC, which is useful to study cellular processes and monitor or recognize disruption or alterations in the cellular processes of cancer cells. These studies can reveal why NPC occurs, and they provide specific biological targets for the early diagnosis of NPC. Biomarkers in relation to nanotechnology is a new era of early cancer diagnosis and precise drug delivery. Hence, pCLE optical visualization following the administration of specific biological agents of NPC could potentially facilitate the diagnosis and prognosis of NPC.

### CONCLUSION

This pilot study has demonstrated the feasibility of using pCLE in the diagnostic of NPC. Moreover, pCLE in practice may minimize the sampling error associated with conventional biopsy, improve the detection rate and early diagnostic rate, and assess the effect of treatment with novel chemotherapeutics. The results of this study should be validated by further research on pCLE, especially in the field of otorhinolaryngology, including large-scale, double-blind, randomized controlled trials.

### BIBLIOGRAPHY


