LETTER TO THE EDITOR

Current evidence on confocal laser endomicroscopy for noninvasive head and neck cancer imaging

Evidenze scientifiche attuali sulla endomicroscopia confocale laser nell'imaging del tumore non invasivo del distretto testa e collo

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Dear Editor,
We read with interest the article entitled "Probe-based confocal laser endomicroscopy in detecting malignant lesions of vocal folds" recently published in *Acta Otorhinolaryngologica Italica* by Goncalves et al. [1]. The authors determined the diagnostic value and inter-rater reliability of confocal laser endomicroscopy (CLE) by comparing 58 video sequences of 3 patients with squamous cell carcinomas (SCC) and 4 patients with benign alterations of the vocal folds [1]. CLE imaging features of SCC compared with the benign alterations were well characterised [1], but the current evidence on CLE imaging in noninvasive detection of HNSCC is making progress and needs to be significantly expanded upon (Tab. I). This Letter aims to provide the reader with an up-to-date review of the literature on CLE in the setting of HNSCC. We classify the research topics of current investigations into CLE head and neck imaging, and briefly discuss current practices and challenges that implicate future directions.
In studies before 2014, the main objective of preliminary studies was to investigate the CLE imaging characteristics of head and neck cancer tissue compared to normal mucosa and surgical margins (reviewed in Abbaci et al. [2]). Earlier studies have shown that by using CLE, micro-anatomical structures of normal mucosa/margins and cancerous lesions can be well identified, allowing for differentiation of malignant and benign mucosal. However, these results should be interpreted prudently for several reasons: very small sample size, different measurement devices used, lack of diagnostic criteria based on micro-imaging of CLE. In addition, field of view and depth penetration of this technology have not yet been well addressed [2].
Starting in 2014, the main objective of prior studies was to evaluate whether CLE is useful in diagnosing HNSCC [3-8]. According to preliminary data [1,3,6-8], the sensitivity and specificity of diagnosing SCC was reported to

How to cite this article: Yang X, Liu W. Current evidence on confocal laser endomicroscopy for noninvasive head and neck cancer imaging. Acta Otorhinolaryngol Ital 2020;40:396-398. https://doi.org/10.14639/0392-100X-N0801

be 85.0-95.3% and 72.0-100%, respectively. Additionally, the interobserver accuracy and reliability of CLE for discrimination of head and neck lesions from normal mucosa has also been investigated [4-6]. Intriguingly, the feasibility of automated analysis and classification of cancerous tissue in CLE head and neck imaging using deep learning has been demonstrated [7,8]. Although the results are promising, they are limited to mainly small descriptive studies. The standardised diagnostic protocols as well as clinically relevant classification systems for head and neck diseases have not yet been described. Moreover, the integration between pathologist and clinician/surgeon in the review process of CLE imaging has not been elucidated.

One of the greatest advantages of CLE with fluorescence is its potential for multiplex analyses in which morphological information can be combined with molecular and/or functional markers. Alterations in molecular and/or functional properties of a cancerous tissue can be translated into significant and optically measurable changes in fluorescent signals. In vivo molecular imaging of gastrointestinal cancer using CLE by targeting EGFR and VEGF has been demonstrated; this concept was recently applied to CLE head and neck cancer imaging with EpCAM and EGFR antibodies conjugated to fluorescent labels [9,10]. When combined with the molecular imaging capabilities of CLE at the cellular level, these new fluorescent targets can contribute to the currently pursued topic of personalised medicine in the field of head and neck cancer, by making it possible to predict the cells' response to the molecular imaging guided diagnosis and therapy.

In summary, the noninvasive point-of-care CLE for head and neck imaging at the cellular level, as a new emerging science, is of great promise for research, especially in molecular targeted chairside diagnosis and intraoperative normal margins. Multi-institutional studies on the three aforementioned research classifications are warranted to overcome the drawbacks and consolidate the value of CLE. The dynamic observation of early malignant changes at the cellular level is a crucial element in the understanding of patient-specific information and today, noninvasive CLE is probably the most versatile technology to face this challenge. Nevertheless, substantial researches are still needed in order to promote fluorescence molecular imaging techniques to the status of routine use in clinical practice for HNSCC.

Table I. Summary of the English-language literature of confocal laser endomicroscopy for diagnostic assessment of head and neck squamous cell carcinoma (SCC).

Year of publication	First author	Country	Manufacturer	No. of subjects and location	Subject setting	Aim of study	Sensitivity %	Specificity %
2019	Goncalves et al. [1]	Germany	CellVizio, France	Vocal folds	4 benign vs. 3 SCC	Diagnostic assessment and interobserver agreement	91.4-96.6	100
2014	Nathan et al. [3]	USA	CellVizio, France	Oral	12 leukoplakia vs. 9 SCC	Diagnostic assessment [a]	85.7	100
2016	Moore et al. [4]	USA	CellVizio, France	Oral	6 non-dysplasia vs. 7 dysplasia vs. 11 SCC	Interobserver agreement	NA	NA
2016	Linxweiler et al. [5]	Germany	CellVizio, France	HN	50 normal vs. 135 SCC	Interobserver agreement [b]	NA	NA
2016	Oetter et al. [6]	Germany	CellVizio, France	Oral	45 normal 50 SCC	Diagnostic assessment and interobserver agreement	95.3	88.9
2016	Dittberner et al. [7]	Germany	CellVizio, France	Oral, oropharynx, others	Self control of 12 normal margins and SCC	Automated Diagnostic assessment	85.0	72.0
2017	Aubreville et al. [8]	Germany	CellVizio, France	oral	Self control of 12 normal margins and SCC	Automated Diagnostic assessment	86.6	90.0
2017	Englhard et al. [9]	Germany	CellVizio, France	HN	5 normal vs. 11 SCC	EGFR/EpCAM-targeted micro-imaging	NA	NA
2019	Watermann et al. [10]	Germany	Optiscan, Australia	NA	Gingiva normal vs. Oropharynx SCC	EGFR nanoparticles-targeted micro-imaging	NA	NA

HN: head and neck sites being not-specified; NA: not available. [a] SCC vs. non-dysplasia. [b] using formalin-fixed tissue specimens.

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