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The diagnostic performance parameters of Narrow Band Imaging: A preclinical and clinical study

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ABSTRACT

Objectives: The oral carcinoma is a widespread pathology and still presents poor prognosis. Among the available procedures for its early detection, Narrow Band Imaging technique allows to assess potential vascular network abnormalities. The reliability of this technique in the detection of dysplastic and neoplastic oral lesions was evaluated in a preclinical and clinical study.

Materials and methods: In the preclinical study, a chemical oral carcinogen was administered to 50 mice to induct both dysplastic and neoplastic oral lesions. In the clinical study 91 patients, bearing suspicious premalignant and malignant oral lesions, have been included. Images of animals' and patients' lesions were acquired under white and Narrow Band Imaging light prior to biopsy. Two expert raters examined the images and classified lesions, which were eventually compared to the histological diagnosis. The diagnostic performance included sensitivity, specificity, positive likelihood ratio, positive and negative predictive values, accuracy, percentages and degree of agreement between raters' evaluation and the histological report.

Results: In the preclinical study sensitivity ranged from 0.57 to 1, specificity from 0.85 to 0.99, positive likelihood ratio from 6.54 to 65.04, positive predictive values from 0.32 to 0.96, negative predictive values from 0.91 to 1 and accuracy from 0.86 to 0.98. In the clinical study sensitivity ranged from 0.63 to 0.99, specificity from 0.89 to 1, positive likelihood ratio from 8.45 to 61.47, positive predictive values from 0.59 to 0.96, negative predictive values from 0.78 to 1 and accuracy from 0.82 to 0.99.

Conclusion: Narrow Band Imaging is an accurate technique, which holds a great potential for tumour angiogenesis evaluation and for the subsequent early detection of suspicious premalignant and malignant oral lesions.

Introduction

The oral squamous cell carcinoma (OSCC) is the most widespread malignant tumour of the oral cavity, representing a major burden in terms of clinical impact, incidence, prevalence, and mortality rate [1]. Despite advances in the its treatment over the past 30 years, the five-year survival rate has remained unchanged, around 50%, becoming 15% when patients present with advanced cancers associated to cervical lymph nodes invasion [2]. Early detection of OSCC at dysplastic or neoplastic in situ stages improves morbidity and mortality, as the risk of metastasis is very low [3,4]. Moreover, it also allows to avoid painful, invasive and disfiguring treatments, which negatively affect patients' quality of life requiring complex and expensive therapeutic processes [5,6]. The overall, essential key in OSCC treatment is represented by its early diagnosis. To achieve early detection, several diagnostic methodologies have been suggested: the easiest and mostly applied diagnostic procedure is the evaluation by a naked eye. This approach has a sensitivity of 85% and a specificity of 97% [7].

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Sensitivity can be further improved by toluidine blue in vivo coloration and by brush biopsy. Studies assessing toluidine blue have shown higher results in term of sensitivity and specificity ranging from 93.5% to 97.8% and 73.3% to 92.9%, respectively [8,9]. The brush biopsy technique reaches a sensitivity of 86% and a specificity of 81%, according to recent meta-analysis [10–12].

During the later years, new diagnostic imaging techniques have been proposed: ViziLite®, VELscope® and Narrow Band Imaging (NBI) examination. ViziLite® has high sensitivity and negative predictive value (NPV) up to 100% with a very low specificity (30%) and positive predictive value (PPV, 26%) [13]. VELscope® has sensitivity and specificity values of 30% and 63%, respectively [14]; whereas, NBI reaches the highest values, going from percentages of 93.75% to 98%, and from 91% to 99%, respectively [15–17]. NBI exploits specific optic filters selecting two wavelengths specific for the haemoglobin absorbance: 415 nm and 540 nm [18], thus enhancing the visibility of lesion's vascular network. Considering the well described property of neoplastic tissues to activate the sprouting of new vessels from the pre-existent capillary network increase the nourishment to cancer cells in response to hypoxic stimuli, NBI is expected to unveil the presence of altered mucosal areas, usually invisible by a naked eye or under white light [19–24]. NBI aims at the early detection of the angiogenic switch, which depends on a finely tuned equilibrium between pro-angiogenic and anti-angiogenetic factors [25]. Often, tumor-associated vessels are characterized by a peculiar morphology, such as tortuous course, dilated and variable diameter, numerous branches and interconnections, thus creating an unsettled and chaotic hematic flow [26]. Being able of absorbing the high content of haemoglobin within vessels, NBI enhances their contours and patterns, as vascular structures result darker and present a higher contrast to the surrounding mucosa [27–29]. NBI highlights vessel abnormalities as brown spots, which are classified as Takano and collaborators explain [28]. Thanks to the specific vessel morphology, it is possible to achieve an earlier diagnosis of dysplastic and neoplastic oral lesions, even for lesions smaller than 5 mm^2 in size [30–32]. While NBI is commonly used in other medical fields [4,5,33,34], mounting evidences support its ability in the diagnosis of recurrent malignancies in patients subdued to resections for oesophageal, pharynx and OSCC [35], and in radio-treated patients [36]. This technology is often restricted to specialist and tertiary referral centres since it requires a dedicated training. Moreover, high cost, large size, and limited portable capacity of NBI system makes it undesirable for general practitioners [5,37].

We present the first combined preclinical, and clinical study on the assessment of NBI technique reliability. Concerning the preclinical analysis, we established a dedicated mouse model of oral carcinogenesis, and in parallel patients affected by suspicious premalignant and malignant oral lesions have been screened in an oral medicine and pathology unit. As outcome of the study we disclose an accurate analysis of NBI diagnostic accuracy parameters.

Materials and methods

The study involving patients has been conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki (version, 2002 www.wma.net/e/policy/b3.htm) and the additional requirements, after acquisition of a written consent by all recruited patients. Animal care and treatment were conducted in conformity with institutional guideline in compliance with national and international laws and policies (EEC Council Directive 86/609, OJL 358, 12 December 1987), upon approval by the ICGEB Animal Welfare Board and Ethical Committee as well as by the Italian Minister of Health. The number of ethical approval is 135/2016-PR.

Mouse model

50 female C57BL/6 mice with a mean weight of 20 g, 6-week-old, were used for this study. Animals were housed under controlled environmental conditions for 5 days with a 12-h light/dark cycle. The 4-NQO carcinogen (Sigma–Aldrich, St. Louis, MO, USA) was dissolved in propylene glycol (4 mg/ml) and then diluted in the drinking water to a final concentration of 50 μg/ml. It was replaced once a week and administered to all mice for 16 weeks. Starting from the 17th week it was replaced by regular water. Mice were allowed to access the drinking water and chow diet ad libitum during the treatment. All mice were weighed every 4 weeks. During the 20th week mice underwent video endoscopic screening using white and NBI light endoscopic imaging by using a Visera Elite system (OTV-S190 video processor and CLV-190 light source, OTV-S7Pro-10E HDTV camera; Olympus Medical Systems Corp, Tokyo, Japan) with a rigid endoscope with a viewing angle of 0°. Before animal sacrifice, pictures of the affected areas were also recorded using an ImageXpress Micro high-content screening microscope (Molecular Devices, Sunnyvale, CA). Inclusion criteria were: presence of potentially malignant and malignant oral lesions, the acquisition of focused images and presence of a clear and visible IPCL network. Both white and NBI acquired images were collected in a Power Point Template file and shown to the raters expert in the use of NBI for clinical screening. By examining images acquired under NBI light, the raters were asked to provide a diagnosis among the following options: mild dysplastic (D1), moderate dysplastic (D2), severe dysplastic (D3) and neoplastic (K). According to the World Health Organization (WHO) 2005 classification [38], three independent pathologists classified the biopsied lesions, sectioned and stained with haematoxylin and eosin, as: hyperplastic or inflammatory (0), mild dysplastic (D1), moderate dysplastic (D2), severe dysplastic (D3) and neoplastic (K). Finally, raters' evaluations were compared to the histological diagnosis in order to evaluate the diagnostic performance of the NBI technique.

Patient population

Between July 2012 and July 2014 at the Division of Oral Medicine and Pathology (Dental Science Department, Ospedale Maggiore, Trieste, Italy) a total of 114 patients, affected by suspicious premalignant and malignant oral lesions, have been investigated. The first visit consisted in a preliminary conventional visual examination under white light and the record of the affected areas by using a digital reflex camera (Nikon® D100). These patients also underwent video screening using white and NBI light endoscopic imaging by using a Visera Elite system with a rigid endoscope with a viewing angle of 0°. Patients were examined in a seated position and all procedures were recorded on video and on followed by the acquisition of images.

Inclusion criteria were: presence of suspicious premalignant and malignant oral lesion, acquisition of focused images both with digital camera and NBI technology, which allowed the presence of a clear and visible IPCL network. Exclusion criteria were: previous diagnosis of premalignant or malignant oral lesion in the same or in different oral sites, prior event of surgical intervention for OSCC, history of chemotherapy and/or of head and neck radiotherapy. Fig. 1 reports examples of patients affected by NBI positive lesions, according to Takano's IPCL classification. The white light endoscopic examination enhanced visibility more than conventional oral examination, highlighting lesion colour (white, red or red-white), clinical appearance (homogeneous, non-homogeneous, lichenoid, ulcerative) and border distinctness (diffuse or sharp). Positive areas were considered: red, red-white non-homogeneous, ulcerative and elevated lesions. Areas considered as NBI positive consisted in regions of hypercaptation,