Clinical evaluation of an autofluorescence diagnostic device for oral cancer detection: a prospective randomized diagnostic study

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The prognosis for patients with oral squamous cell carcinoma remains poor despite advances in multimodal treatment concepts. Early diagnosis and treatment is the key to improved patient survival. A device (VELscope) that uses autofluorescence technology, allowing direct fluorescence visualization of the oral cavity, might be a useful tool for oral cancer detection or as an adjunct to standard clinical examination. A total of 289 patients with oral premalignant lesions were randomly divided into two groups for clinical examination of precancerous oral lesions. In group 1, 166 patients were examined conventionally with white light, and in group 2, 123 patients were examined with the autofluorescence visualization device (VELscope) in addition to the white light examination. Biopsies were obtained from all suspicious areas identified in both examination groups ($n=52$). In the first step, baseline characteristics of the two groups (only white light vs. white light and VELscope) were compared to exclude selection bias. In the second step, for the group examined with white light and VELscope (123 patients), the diagnostic strategies were compared with regard to sensitivity and specificity using biopsy as the gold standard. The results showed that using the VELscope leads to higher sensitivity (100% instead of 17%), but to lower specificity (74% instead of 97%). Thus, we can conclude that the VELscope is a useful new diagnostic device for detection of oral cancer diseases. *European Journal of Cancer Prevention* 00:000–000 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Oral and oropharyngeal cancer is a significant health problem throughout the world. It is the eighth most common cancer worldwide with 300 000 new cases reported every year (Parkin *et al.*, 2005). Many countries feature incidence rates of oral cancer that vary in men from one to 10 cases per 100 000 population (Stewart and Kleihues, 2005). Developing countries suffer from higher incidence rates of oral cancer compared with developed countries (Petersen, 2003). It is a cause for worry that the incidence of the disease is reportedly increasing in most countries, such as central and eastern Europe and the USA (Petersen, 2003; Stewart and Kleihues, 2005). The 5-year overall survival rate for patients with oral cancer has been stagnating for the last 20 years (Bray *et al.*, 2002). The survival rate is only 54% in industrial countries, one of the lowest rates of all major cancers. Five-year survival rates in developing countries barely reach 30% (American Cancer Society, 2005). Smoking (Newcomb and Carbone, 1992) and immoderate consumption of alcohol (Merletti *et al.*, 1989), and human papillomavirus are the main risk factors for oral cancer. The association of the two main risk factors has a synergic effect, boosting the risk of developing oral cancer by 30 times (Blot *et al.*, 1988). Most of the oral carcinomas develop from oral premalignant lesions, particularly leukoplakia, erythroplakia, and lichen planus (Scheifele and Reichart, 2003). According to literature data, premalignant lesions might turn into carcinoma in a percentage varying between 5 and 18% of cases; hence early identification of potentially malignant disorders is important to prevent the onset of tumors (Lumerman *et al.*, 1995). Early diagnosis of tumor significantly increases survival rates and reduces impairment of health and quality of life through surgical therapy (Burzynski *et al.*, 1997; Howaldt *et al.*, 1999; Palmer and Grannum, 2011). Nevertheless, most oral carcinomas are currently detected at a late stage. The main reason for this delay is not only the lack of awareness of the symptoms and risk factors among the public (Mashberg, 2000) but also the lack of prevention and early detection by healthcare providers (Mignogna *et al.*, 2001). Early detection is also impeded by the lack of typical clinical characteristics, such as ulceration, induration, or pain at early carcinoma stages (Mashberg and Samit, 1995). Early malignant lesions are often indistinguishable from normal-looking mucosa, making them harder to detect even for experienced examiners (Shugars and Patton, 1997). Currently,

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biopsy is considered as the gold standard for the diagnosis of oral carcinoma, because the grade of epithelial dysplasia can only be diagnosed in a histopathological specimen (Natarajan and Eisenberg, 2011). The standard method for oral cancer screening is a conventional oral examination (COE) using normal (incandescent) light (Patton *et al.*, 2008). Numerous publications indicate that COE may have limited value as a method for detecting precancerous lesions (Silverman, 1988; Downer *et al.*, 2004). Additional screening aids for improving the detection rates of oral cancer are needed and are being marketed by the industry. There are several studies assessing the diagnostic value of the different new diagnostic methods (Lingen *et al.*, 2008; Patton *et al.*, 2008; Fedele, 2009; Trullenque-Eriksson *et al.*, 2009; Seoane Leston and Diz Dios, 2010). Toluidine blue is a vital dye to improve the visibility of lesions during visual examination, but it has a relatively low specificity. The technique was reviewed in the literature with data for sensitivity ranging from 38 to 98% and specificity ranging from only 9 to 93% (Patton *et al.*, 2008; Epstein and Guneri, 2009). Another visual adjunct for oral examination is the ViziLite from Zila Pharmaceuticals Inc. (Phoenix, Arizona, USA). Blue light emitted by a disposable chemiluminescent light source illuminates the oral tissue, apparently improving the brightness and sharpness of oral premalignant lesions (Epstein *et al.*, 2006). However, some studies concluded that examination with the ViziLite did not change the diagnosis (Ram and Siar, 2005; Farah and McCullough, 2007). The use of autofluorescence imaging is a similar noninvasive approach for improving the detection of potentially malignant oral cavity lesions (Lane *et al.*, 2006). As these systems do not represent a complete diagnostic device, they have to be supplemented by additional hardware devices. In consequence, the handling of these systems is of an experimental nature, and the detection of oral malignant lesions is not feasible in daily routine. The VELscope System by LED Medical Diagnostics (White Rock, British Columbia, Canada; VELscope: the Oral Cancer Screening System, LED Dental Inc., Burnaby, British Columbia, Canada), a novel fluorescence technology allowing direct fluorescence visualization of the oral cavity, might be a useful tool. The purpose of this clinical study was to establish and clinically evaluate a novel, user-friendly diagnostic device for oral cancer prevention as an adjunct to standard clinical examination in a clinical setting with regard to the sensitivity and specificity of the autofluorescence examination in comparison with COE alone.

Materials and methods

The study was approved by the local ethics committee at the Hannover Medical School, Germany (EK 5586/2009). Study participants were enrolled in a clinical protocol reviewed and approved by the institutional cancer board. Before beginning the study, written informed consent was obtained from each patient.

Patients

Patients were enrolled from the Hannover Medical School, Department of Craniomaxillofacial Surgery. A total of 289 patients with oral premalignant lesions were randomly divided into two groups for clinical examination of oral cancer lesions (COE). In group 1, 166 patients were examined with conventional white light, and in group 2, 123 patients were examined with an autofluorescence visualization device, VELscope (autofluorescence visualized examination), in addition to the white light examination. Biopsies were obtained from all suspicious areas identified in both examination groups ($n = 52$).

Study inclusion criteria and protocol

Only patients with an oral premalignant lesion (leukoplakia, erythroplakia, lichen planus, or pemphigus vulgaris) were included in this study. Potential participants were excluded from the study if they had a history of oral cancer or cancer recurrence, possibility of missing follow-up examination, were pregnant, nursing, had undergone recent operations, or had diseases of the heart and circulation, infections, systemic and malignant diseases, or immune system-affecting diseases, or blood coagulation disorders and allergic reactions to pharmaceuticals and antibiotics. The clinical inclusion and exclusion criteria are shown in Table 1.

All patients provided informed consent and completed a detailed questionnaire, which included information on demographics, smoking and alcohol use, current medications, and general health and dental care history. Oral health-related quality of life was evaluated for all patients of completing the abbreviated German version of the Oral Health Impact Profile (OHIP-G-14). A lesion protocol based on the topographical classification system of Roed-Petersen and Renstrup (1969) was applied. All patients were examined using standardized methods and techniques. The visual and VELscope examination was carried out by experienced examiners. To avoid bias, patients

Table 1 Study inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Oral premalignant lesion: leukoplakia, erythroplakia, lichen planus, or pemphigus vulgaris	Tumor or tumor recurrences missing operability foreseeable missing opportunity of follow-up examination
Age 18–75	Pregnancy, heart disease, pulmonary disease, liver disease, kidney disease, and chronic pain syndrome, nursing, drug addiction, recent operations, and diseases of the heart, metabolism, central nervous system, circulation, infections, systemic, malignant and immune system-affecting diseases, as well as blood coagulation disorders and allergic reactions to pharmaceuticals and antibiotics
Written informed consent	Dermatological diseases of the face

were examined in two different examination rooms, and the examiner using the VELscope was unaware of the results of the conventional group.

Conventional method for oral cancer screening

For conventional oral cancer screening of the oral cavity, a dental chair examination light was used (15V/200W, OSRAM, OSRAM AG, Munich, Bavaria, Germany). The standard clinical examination includes visual inspection of the oral mucosa, followed by palpation of suspicious lesions. Initial clinically abnormal lesions were noted and photographed with a digital reflex camera (Pentax *ist DS, Pentax Imaging Systems GmbH, Hamburg, Germany) equipped with a Pentax 100-mm Macro Lens and Macro Ring Lite. After photo documentation the suspicious lesion was biopsied under local anesthesia. All specimens were placed in 4% buffered formalin for fixation and sent for histopathological examination. The presence or absence of dysplasia in the biopsy specimen was recorded by an experienced oral pathologist.

Additional autofluorescence examination using the VELscope

The additional autofluorescence visualised examination was carried out in a dark environment using the VELscope V2 device supported by Mectron Inc. (Cologne, Germany). Patients wore protective glasses during the entire examination. Suspected lesions were photographed using the above-mentioned camera equipment without the ring flash being mounted on the back of the hand piece using an adapter (Photo Med VELscope Photography System, Photomed International, Los Angeles, California, USA). After photo documentation and noting of lesions, biopsy was taken in the above-mentioned manner. To reduce the rate of false-positive results, a follow-up visit 2 weeks after the first examination was implemented if there was any suspicion that the lesion was of acute inflammatory origin. Possible causes of inflammation (sharp teeth, edges of insufficient fillings, poorly fitting set of dentures, etc.) were eliminated by then. Persisting lesions required a biopsy (Thumfart *et al.*, 1978). Following the manufacturer's advice (LED Dental Inc., 2009), a diascopy (Rudd *et al.*, 2001) was performed on any suspicious lesion to reduce the rate of false-positive results. Applying soft pressure with a clear tongue depressor may restore normal autofluorescence in inflammatory lesions by reducing the pathologically increased blood flow (LED Dental Inc., 2009). Fluorescence loss in malignant or premalignant lesions is not modified by this test.

VELscope device

The VELscope is a device for the direct visualization of changes in tissue fluorescence in the oral cavity. It consists of a bench-top casing containing a 120 W metal-halide arc lamp plus a system of filters and reflectors optimized for producing near-ultraviolet/blue light between 400 and 460 nm and a coupled handheld unit for direct observation (Lane *et al.*, 2006). If needed, a camera can be connected to the hand piece for the purpose of documentation. Digital image processing of wide-field autofluorescence images can be used to outline suspicious regions in real time. The autofluorescence observed in wide-field images of the normal oral mucosa originates primarily from stromal collagen. Oral neoplasia is associated with a loss of stromal autofluorescence. Benign lesions, such as inflammation, are also associated with loss of stromal autofluorescence, which may limit diagnostic specificity, especially in low-risk populations.

Technique of autofluorescence visualization

The autofluorescence of tissue and its potential use in cancer detection were described first in 1924 (Policard, 1924). Naturally occurring fluorochromes (e.g. collagen, elastin, keratin, FAD, NADH) (Richards-Kortum and Sevick-Muraca, 1996) that are located in the epithelial cell lining and submucosa of the oral cavity show fluorescence in the green spectral range when excited with light between 375 and 440 nm (Betz *et al.*, 1999). Malignant or dysplastic alteration causes complete loss of the normal tissue fluorescence (fluorescence visualization loss) because of the disturbance in the distribution of these fluorochromes (Svistun *et al.*, 2004; Lane *et al.*, 2006). According to the literature, autofluorescence spectroscopy has a sensitivity and specificity higher than 95% for differentiating malignant tumors from healthy oral tissue. Adding autofluorescence imaging to conventional clinical examination could possibly improve sensitivity and specificity (Kulapaditharom and Boonkitticharoen, 2001; Betz *et al.*, 2002). Recent studies have criticized the failure of the VELscope to discriminate high-risk lesions from low-risk lesions (Awan *et al.*, 2011) and its high rate of false-positive results (Balevi, 2007).

Statistical analysis

The sample size for the study was planned using the data of a pilot study ($n = 30$). In this pilot study, the white light examination showed a sensitivity of 50% and a specificity of 100% and for white light plus VELscope the result showed a sensitivity of 100% and a specificity of 96%. The aim of the study was to prove that, with the additional use of the VELscope, the sensitivity is significantly higher and the specificity is not relevantly lower (a loss of specificity of more than 20% is considered relevant). Because both hypotheses had to be rejected for the success of the study, type one error did not have to be adjusted (two-sided 5%); however, the power had to be set to 90% for each hypothesis. With an assumed incidence of 10%, this led to a sample size of 150 patients.

Statistical analyses were carried out using SPSS for Windows version 18.0 (SPSS Inc., Chicago, Illinois, USA). In the descriptive analysis for quantitative variables, boxplots were drawn to decide whether normal distribution could be assumed. If the distribution was symmetric,

mean and SD were calculated, and the two-sample *t*-test was used for comparison between the groups. If the distribution was nonsymmetric, the median (minimum and maximum) was calculated and the Mann–Whitney *U*-test was used for the two-group comparison. For categorical variables, absolute and relative frequencies were calculated and the χ^2-test and Fisher's exact test were used for comparison, respectively.

In the first step, the two groups (with or without the additional use of the VELscope) were compared with the above-described descriptive analyses. Afterwards, for the group with additional use of the VELscope, the baseline characteristics and the OHIP score were analyzed descriptively for the patients with and without cancer lesions.

For the primary analysis, the differences (white light plus VELscope vs. white light only) in the sensitivities and specificities of the two diagnostic approaches, with the corresponding two-sided 95% Agresti confidence intervals, were calculated. Superiority with regard to sensitivity was concluded if the lower limit of the corresponding confidence interval was above 0, and noninferiority with regard to specificity was concluded if the lower limit of the corresponding confidence interval was above -0.2. As a secondary analysis, the sensitivities and specificities with the corresponding two-sided Agresti confidence intervals were calculated for the two tests separately.

Results

Because of time restrictions in the daily diagnostic process, only 123 of 269 patients fulfilling the inclusion and exclusion criteria could be examined with the VELscope additionally. The selection of the patients for this group was determined randomly on the basis of the availability of the VELscope, and there were differences between the two groups regarding alcohol intake and frequency of biopsy (Table 2).

In contrast to the assumption for the sample size calculation, the incidence in the population was 5% instead of 10%. This led to six patients with cancer lesions and 117 patients without cancer lesions. The two groups (with vs. without cancer lesion) were compared descriptively. The alcohol intake of patients with and without cancer lesions was different; however, the other parameters were distributed similarly in the two subgroups (Table 3).

The results of the evaluation of the diagnostic accuracy are shown in Table 4. As expected, the additional use of the VELscope led to a higher sensitivity (100% instead of 17%), but to lower specificity (74% instead of 97%) (Figs 1–3).

The loss of fluorescence in all examined lesions is shown in Table 5.

Discussion

Early detection of oral cancer is one of the most efficient ways to reduce the high mortality due to this disease. It can minimize the morbidity of the disease and its treatment, which is associated with a severe loss of function, disfigurement, depression, and poor quality of life. There is increasing demand for additional useful tools for cancer detection to supplement conventional white light oral examination (Balevi, 2007). Our study evaluated the diagnostic accuracy of the VELscope device. In conclusion, the additional use of the VELscope

Table 2 Baseline characteristics of the two groups (with or without VELscope)

	White light plus VELscope (*n*=123)	White light (*n*=166)	*P*-value
Age, mean ± SD	62.5 ± 10.81	63.83 ± 12.75	0.35
Sex, *n* (%)			0.841
Male	46 (37.4)	64 (38.6)	
Female	77 (62.6)	102 (61.4)	
Smoking, *n* (%)			0.897
Never	22 (18.5)	26 (16.9)	
Previous	33 (27.7)	41 (26.6)	
Actual	64 (53.8)	87 (56.5)	
Alcohol, *n* (%)			0.026
Never	46 (37.4)	36 (22.2)	
≤ 20 g/day	41 (33.3)	80 (49.4)	
21–40 g/day	24 (19.5)	31 (19.1)	
41–60 g/day	10 (8.1)	9 (5.6)	
61–80 g/day	0 (0)	1 (0.6)	
Unknown	2 (1.6)	5 (3.1)	
Biopsy taken, *n* (%)			0.044
Yes	92 (74.8)	140 (84.3)	
No	31 (25.2)	26 (15.7)	
Cancer lesion, *n* (%)			0.33
Yes	6 (4.9)	4 (2.4)	
No	117 (95.1)	162 (97.6)	

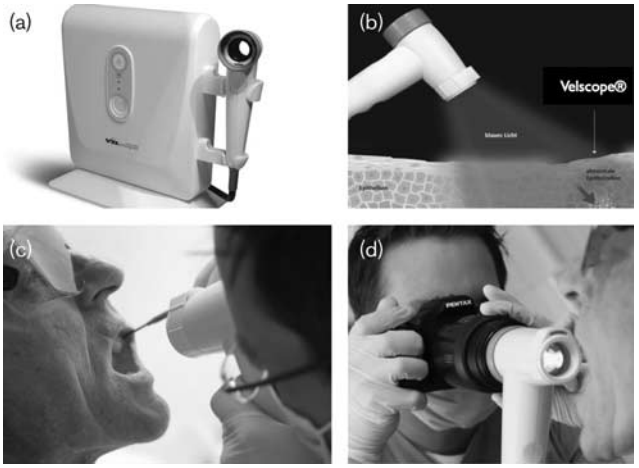
Table 3 Baseline characteristics of the two subgroups (with VELscope, with or without cancer lesion)

	With cancer lesions (*n*=6)	Without cancer lesion (*n*=117)	*P*-value
Age, mean ± SD	58 ± 9	63 ± 11	0.298
OHIP score, median (min–max)	3 (0–15)	4 (0–32)	0.511
Sex, *n* (%)			0.195
Male	4 (67)	42 (36)	
Female	2 (33)	75 (64)	
Smoking, *n* (%)			1.000
Never	1 (17)	21 (19)	
Previous	2 (33)	31 (27)	
Actual	3 (50)	61 (54)	
Alcohol, *n* (%)			0.065
Never	3 (50)	43 (37)	
≤ 20 g/day	1 (17)	40 (34)	
21–40 g/day	–	24 (21)	
41–60 g/day	1 (17)	9 (8)	
61–80 g/day	–	–	
Unknown	1 (17)	1 (0.9)	
Frequency of examination, *n* (%)			0.779
Twice a year	1 (17)	34 (29)	
Once a year	5 (83)	75 (64)	
More than twice a year	–	8 (7%)	

Table 4 Sensitivity and specificity with corresponding 95% confidence intervals for the two diagnostic procedures

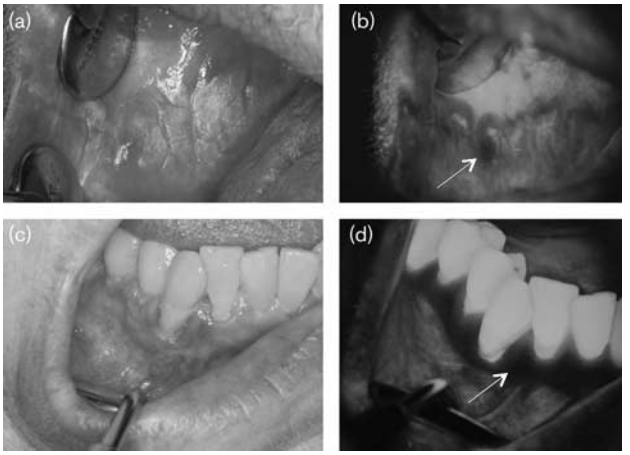
	Sensitivity ($n=6$)	95% CI	Specificity ($n=117$)	95% CI
Difference (white light and VELscope – white light)	83%	28%; 100%	–22%	–46%; 1%
White light	17%	0%; 49%	97%	93%; 100%
White light and VELscope	100%	61%; 100%	74%	67%; 82%

Fig. 1



(a) VELscope device, (b) autofluorescence light, (c) examination with the VELscope, (d) procedure of photo documentation.

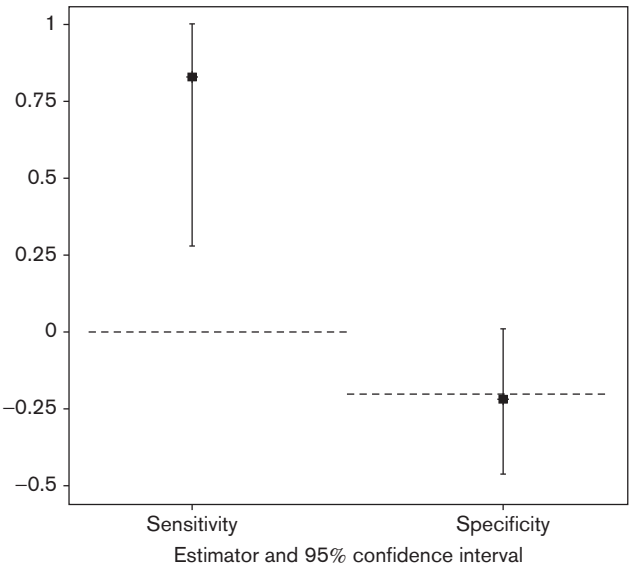
Fig. 2



(a) Oral cavity with precancerous lesion of planum buccale, (b) red arrow shows loss of fluorescence in oral cavity with VELscope examination, (c) precancerous lesion of the gingiva, (d) red arrow shows loss of fluorescence with VELscope examination of the gingiva.

increased sensitivity from 17 to 100% compared with COE alone in detecting malignant lesions of the oral mucosa but reduced specificity significantly from 97 to 74%. A loss of fluorescence was detected in 100% of all dysplastic lesions, which shows the ability to detect high-risk lesions (Table 5). However, 37.84% of all cases of leukoplakia/erythroplakia and the majority (81.08%) of all clinically diagnosed cases of lichen planus showed loss of tissue fluorescence (Table 5). A recent study by Awan *et al.* (2011) showed similar results and criticized the lack of specificity of the technique. Our results indicate that autofluorescence examination can help to identify dysplastic lesions but cannot differentiate benign oral lesions such as inflammation or oral lichen from malignant lesions reliably. It is disappointing that 64.23% of all examined lesions showed a loss of fluorescence, whereas only 4.88% of the lesions could be identified as dysplasia. This could lead to overdiagnosis if the VELscope is used by a nonspecialist. In our experience, the findings of the VELscope are very subjective, and both clinical experience and training are needed to accomplish good test results. In our study, the relatively low specificity of the device led to a rather large number of false-positive test results (26 patients with 32 biopsies), although a strict examination protocol was applied for the autofluorescence examination (use of diascopy, follow-up visit). This is not acceptable for clinical purposes. False-positive examination results not only frighten patients but also

Fig. 3



Demonstration of the difference between white light and VELscope with white light according to sensitivity and specificity.

increase morbidity risks because of unnecessary biopsy. A similar conclusion was made in an up-to-date study by Balevi (2011). The high rate of false-positive test

Table 5 Loss of fluorescence in the VELscope group

Loss of fluorescence in the VELscope group					
Lesion	Leukoplakia	Lichen	Ulcer	Candida	Others
Total patients (*n*)	37	74	2	2	8
Normal tissue (FVL−)	23	14	0	0	7
Loss of fluorescence (FVL+)	14	60	2	2	1
Positive diascopy (FVL+)	0	46	0	0	0
Negative diascopy (FVL+)	14	14	2	0	1
Biopsies taken, *n* (%)	14	14	2	0	1

results was also highlighted by Scheer *et al.* (2011) in a recent study. There are several studies that support the ability of the VELscope to identify areas of dysplasia (Lingen *et al.*, 2008; Patton *et al.*, 2008). Another study resembling our results for the high sensitivity of the device was published in 2006 by Lane *et al.* (2006). Using histology as the gold standard, the device achieved a sensitivity of 98% in discriminating normal mucosa from severe dysplasia or carcinoma *in situ*. Therefore, the author recommended this device as a suitable adjunct for oral cancer screening. This study also showed excellent test results of 100% for the specificity of the device. The clear difference from the mere 74% specificity of our study could be because only high-risk patients with a former oral cancer diagnoses were examined in that study, whereas our study population consisted of patients with different histologic diagnoses. Different studies commended the VELscope for biopsy guidance in superficial lesions of the oral mucosa (De Veld *et al.*, 2005; Kois and Truelove, 2006). Our clinical experience during the examinations was similar. To conclude, VELscope is a simple, noninvasive examination test of the oral mucosa with the ability to help locate malignant oral lesions and find the right location for a biopsy. However, its results have to be interpreted carefully, and a good examination protocol and documentation is very important to decrease false-positive results. It cannot replace histological evaluation of the oral tissue as a gold standard.

Conclusion

Early diagnosis of oral cancer is a major requirement for multidisciplinary oncologic physicians. Detection should lead to less damage from cancer therapy and to better prognosis. The VELscope device, which uses visible light of 430 nm wavelength to cause fluorescent excitation of certain compounds in the tissues, will play a major part in prevention of oral cancer diseases.

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Conflicts of interest

There are no conflicts of interest.

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