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Ich bedanke mich bei den unten aufgeführten Kolleginnen und Kollegen für ihre wertvolle Mitarbeit, die sie in den vergangenen drei Jahren geleistet haben.

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Diagnostics of tongue coating using autofluorescence

A clinical study of possibilities and limitations

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tongue coating,
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SUMMARY

In the present study, the VELscope® autofluorescence device was used in addition to the Winkel Tongue Coating Index (WTCI) to evaluate tongue coating; a corresponding index was developed for evaluation with VELscope®. The distinct orange fluorescence of the tongue dorsum caused by autofluorescent bacterially colonized areas motivates halitosis patients to optimize tongue hygiene.

The tongue dorsum of 100 volunteers (35 males, 65 females, average age 51 years) was photographed with and without the autofluorescence device. On the computer, all tongue photographs were divided into sextants. These pictures were evaluated randomly by six investigators (5 inexperienced and the experienced head of the halitosis consultation hour).

Both methods localized the highest coating density in the mid posterior area of the tongue. Significant differences were found between the WTCI and the VELscope® Index ($p<0.001$). While WTCI was more sensitive in discriminating between absence and presence of sparse coating, VELscope® imaging was relatively insensitive to sparse coating, but better detected dense coating than did WTCI. For both methods, inexperienced and experienced examiners achieved comparable results (kappa coefficient without VELscope® 0.654, with VELscope® 0.672).

The VELscope® device can complement tongue coating diagnosis, but it cannot replace the Winkel Tongue Coating Index.

Introduction

During routine examinations of oral mucosa using an autofluorescence device at the School of Dental Medicine/University of Basel, fluorescent orange areas were observed particularly on the tongue dorsum. Based on certain publications (De Veld et al. 2005, Poh et al. 2007), it was postulated that the orange color may be indicative of areas densely colonized by bacteria. A Master's thesis was thus performed in which tongue dorsa were clinically inspected and evaluated with the Winkel Tongue Coating Index (WTCI) as well as irradiated with the autofluorescence device in order to determine whether a correlation exists between tongue coating and the orange color. This may be relevant for the diagnosis and treatment of halitosis.

The autofluorescence method is used in oral mucosa diagnostics to distinguish between normal tissue fluorescence and the loss of fluorescence in altered cells. This method is also applied in gynecology, ophthalmology, dermatology, urology, and gastroenterology. The technique employs blue light with an excitation spectrum of 400–460 nm (Lane et al. 2006); under autofluorescence, normal oral mucosa exhibits a slightly non-homogeneous greenish color. Where dysplastic alterations or carcinomas are present, the light is absorbed and scattered to a greater extent due to the breakdown of the collagen matrix and the associated metabolic changes, which lead to reduced autofluorescence ("fluorescence visualisation loss"), evident as dark areas (Lane et al. 2006, Scheer et al. 2011). When using the autofluorescence device VELscope® (Visual Enhanced Lesion Scope, Fa. LED Dental, Burnaby, Canada), orange areas become evident, instead of the expected greenish color. This was observed as early as 1924 during examination of carcinomas using a Wood's lamp, which emits wavelengths similar to those of the VELscope®. At that time, a correlation between the intensity of the orange color and the severity of cancer was assumed (Ronchese 1954). More recent investigations show an increased porphyrin concentration in carcinomas, which accounts for the red-orange fluorescence in an autofluorescence device. However, there has been much discussion about the provenance of the fluorescent orange porphyrins. Some authors assume that they are produced either by the tumor itself or by bacteria colonizing the tumor's surface (Harris & Werkhaven 1987, Inaguma & Hashimoto 1999, Onizawa et al. 2002, Scheer et al. 2011). Other authors consider the orange color to be correlated with the bacterial infection or the host's response to the porphyrins expressed by the bacteria, or possibly a combination of the two. A fluorescent orange tongue dorsum may thus be an indication of dense bacterial colonization (De Veld et al. 2005, Poh et al. 2007).

The purpose of the present study was to determine whether clinical tongue coating examinations during halitosis diagnostics can be complemented or even replaced by the autofluorescence technique in the future.

Materials and Methods

The tongue dorsum of a total of 100 patients between the age of 19 to 91 years (Ø 51 years, SD 17.5; 35 males, 65 females) from the School of Dental Medicine/University of Basel was examined. The subjects were divided into three age categories (<40 years, n=24; 41–55 years, n=25; >55 years, n=51). Patients with oral or systemic diseases were excluded, as were smokers. The recruitment phase lasted a few weeks.

No institutional ethical approval was necessary, since the examination and photodocumentation were explained to the patients (consecutively recruited in the clinic) a priori, and they agreed to participate. They were expressly informed about the study in accordance with the Helsinki Declaration and provided their informed, written consent in keeping with the guidelines.

From all 100 subjects, a photo was taken of their tongue from the tip to the Papillae vallatae (Nikon D90, Tokyo, Japan; flash Macro Speedlight SB-29s, Nikon). Subjects were not allowed to brush either their tongue or their teeth immediately before the picture was taken, because remnants of toothpaste on the tongue could be interpreted as tongue coating. The tongue was photographed again with the same camera but with the VELscope® device attached to it. To do this, the room was darkened and no flash was used. On the computer, all tongue photos were divided into sextants using a grid (PowerPoint Microsoft 2010) analogous to the WTCI (Winkel et al. 2003) (Figs 1 and 2).

These 200 anonymized tongue photos were shown to six examiners in a random sequence to record findings: two dental students, three dentists, and an examiner experienced in tongue coating diagnostics (head of the halitosis consultation hour). This was done in two different sessions one week apart at the same time of day. At each session, 50 photos without VELscope® and 50 with were projected on the screen in a completely dark lecture hall in random order, but never with two photos from the same patient in one session. The examiners evaluated the photos without VELscope® using the WTCI: each sextant of the tongue was first evaluated in terms of coating density: no coating (0), sparse coating (1) and dense coating (2) (Fig. 3). The index was calculated as the sum of individual values, ranging from a minimum of 0 to a maximum of 12 possible points. The color of the tongue coating was not evaluated (Winkel et al. 2003).

The VELscope® images were evaluated using an index developed exclusively for this study; it is similar to the WTCI. Each of the sextants was evaluated separately: no orange color visible (0), little orange color visible (1), and obvious orange color visible (2). In this index as well, the sum of individual values ranged from a minimum of 0 points to a maximum of 12 possible points (Fig. 4).

Before beginning, the six examiners received an introduction in the evaluation of the WTCI and the VELscope® Index.

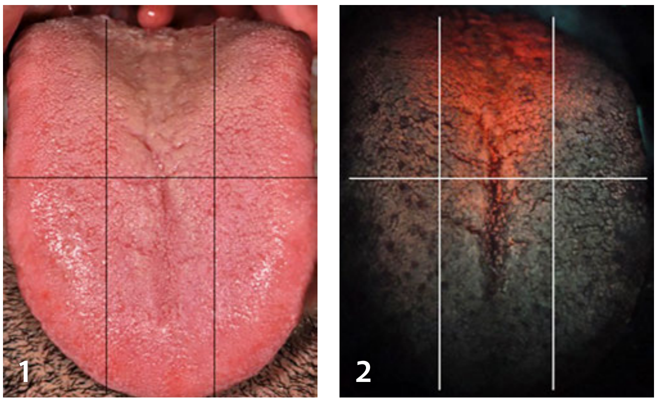
The photos taken with VELscope® (VELscope® Index) and without (WTCI) were evaluated in terms of the distribution of the coating on the tongue dorsum and the congruence of the two indices. In addition, the agreement between the six examiners was determined by calculating the kappa coefficient. An interexaminer agreement/kappa coefficient <0 indicated poor agreement, 0–0.2 slight agreement, 0.21–0.4 sufficient agreement, 0.41–0.6 moderate agreement, 0.61–0.8 considerable agreement, and 0.81–1.00 indicated almost perfect agreement (Landis & Koch 1977). Simply stated, the closer the kappa value approaches 1, the closer the results are to being identical (Everitt 1968).

Further, the influence of age and gender on tongue coating was analyzed.

Statistical analysis was conducted using Statistical package R, version 2.9.2 (The R Foundation for Statistical Computing, Vienna, Austria; www.r-project.org). p-values were calculated with Fisher's Exact Test. The level of significance was set as an error probability of 0.05 (two-sided).

Results

With both techniques, more tongue coating was found in the posterior than anterior tongue area (Fig. 5). The evaluation of coating distribution with and without VELscope® demonstrated that tongue coating and orange color were chiefly located on



Figs 1 and 2 Dividing the tongue dorsum into sextants without (Fig. 1; Winkel et al. 2003) and with the VELscope® (Fig. 2).

the central third of the posterior tongue dorsum. Photos taken without VELscope® displayed the highest density of tongue coating (WTCI score 2; Winkel et al. 2003) in the central posterior third in 33.5% of the cases; photos taken with VELscope® showed 42.3% ($p<0.001$) in the same area (Fig. 5).

Dense tongue coating (strong orange color) was well diagnosable with the VELscope® device. No agreement was found comparing the values of 0, 1 and 2 of the WTCI with the values 0, 1 and 2 of the VELscope® Index ($p<0.001$). However, the evaluation showed that a 2 in the WTCI corresponded to a 1 or 2 of the VELscope® Index. The present results showed that only a dense tongue coating could be imaged with VELscope®. In contrast, if no orange color was visible, this always corresponded to a score of 0 or 1 of the WTCI (Fig. 6).

In evaluating the two methods, clear agreement was found between the five inexperienced examiners and the experienced examiner. For the pictures taken without VELscope®, the kappa coefficient was 0.654 and 0.672 with VELscope® (Tab. I). A kap-

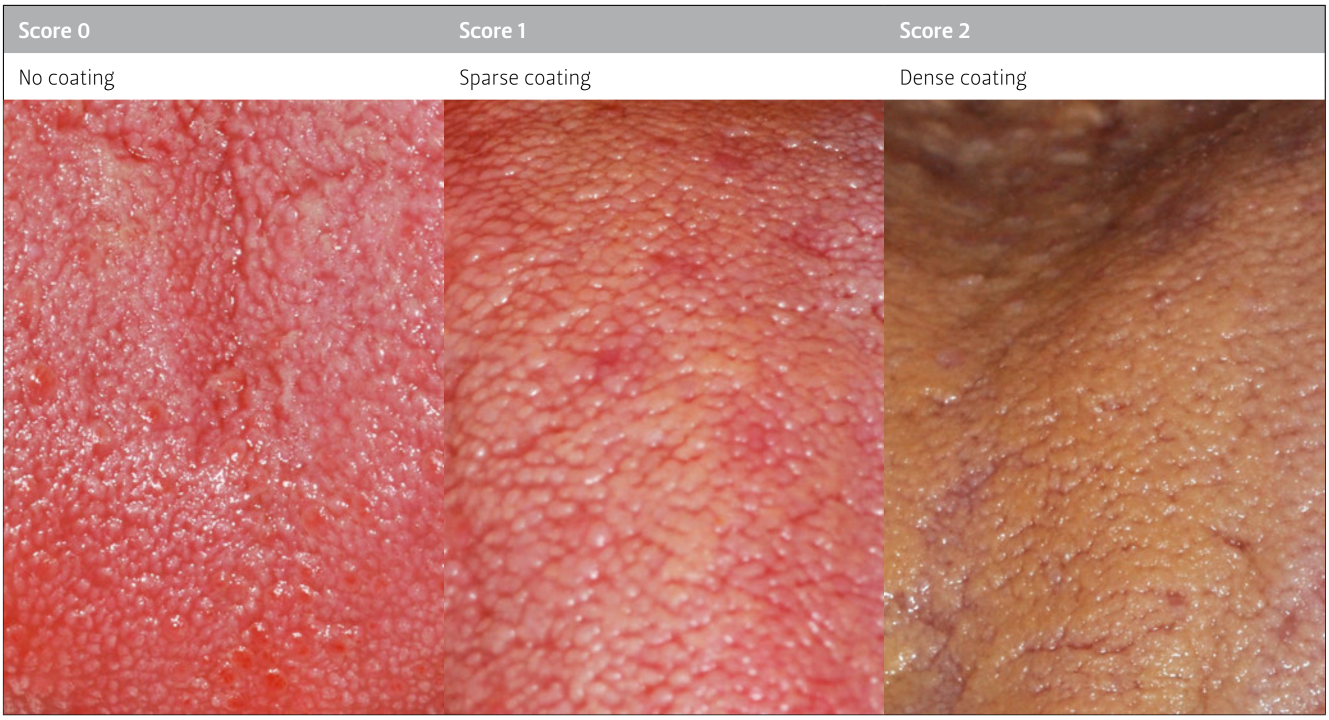


Fig. 3 Winkel Tongue Coating Index (Winkel et al. 2003). Each of the sextants (see Figs 1 and 2) is evaluated according to the following scores: no coating (0, left-hand image), sparse coating (1, center), dense coating (2, right-hand image). The summed scores yield the index (minimum 0, maximum 12 points).

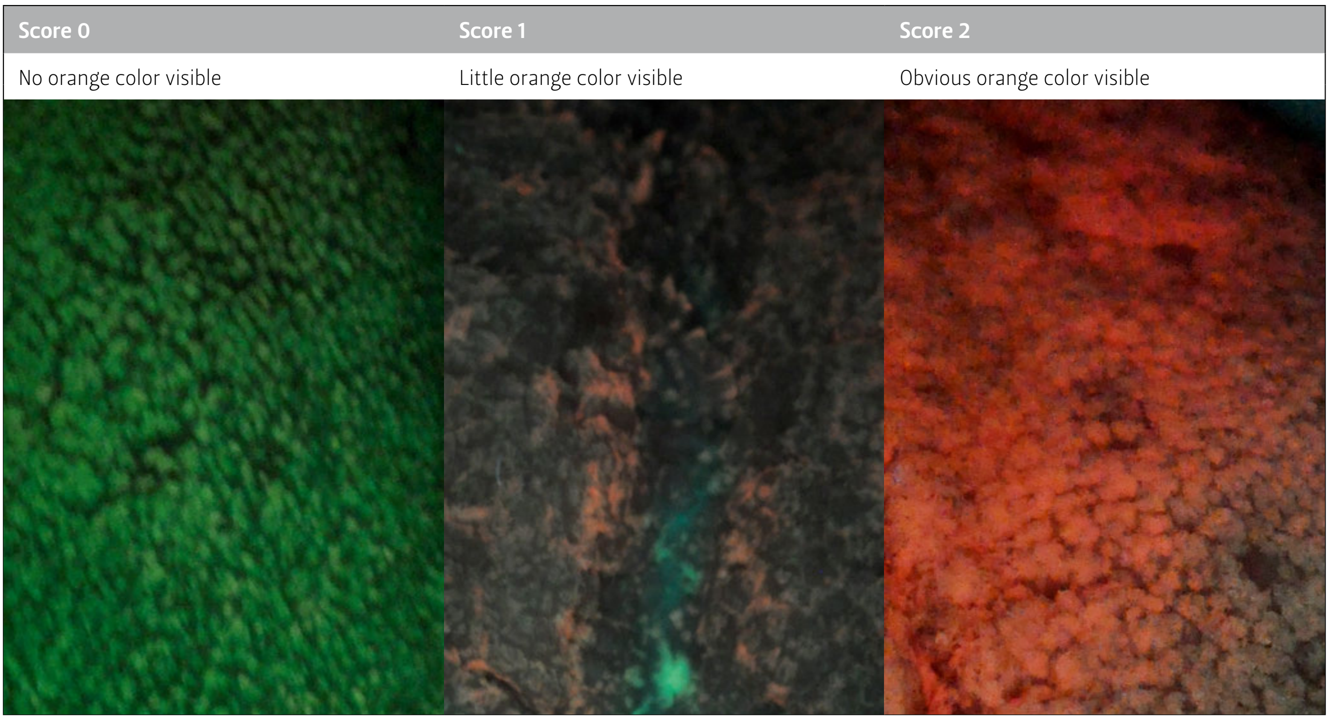


Fig. 4 The VELscope® Index, developed for the study. The summed scores yield the index (minimum 0, maximum 12 points).

pa coefficient of 0.61–0.8 indicates considerable agreement (LANDIS & KOCH 1977).

The evaluation of photos using the WTCI in respect of the three age groups showed that patients over 55 years old had significantly less tongue coating (p=0.012) than did patients under 40 and between the ages of 41 and 55. Evaluating the photos using the VELscope® Index showed higher values with increasing age, but this increase in older vs younger patients was not significant (p=0.20).

No significant difference between men and women was found either with the WTCI (p=0.12) or the VELscope® Index (p=0.22).

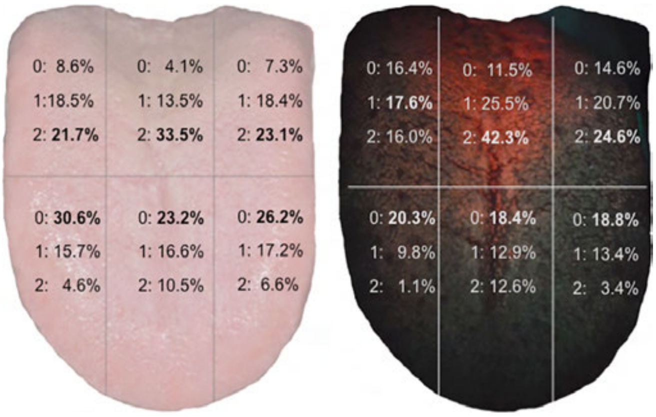


Fig. 5 Percent distribution of all degrees of tongue coating in all sextants according to the Winkel Tongue Coating Index (left; WINKEL ET AL. 2003) and VELscope® Index (right).

Discussion

In some cases, the evaluation of tongue coating using the WTCI was not unambiguous: with a WTC score of 1, the question arose as to whether the finding was actually tongue coating; if instead it was hyperkeratosis of the filiform tongue papillae, this resulted in a false positive result. LUNDGREN ET AL. (2007) studied the reproducibility of the WTCI and found it conducive to eliminate score 1. Where only scores of 0 (no coating) and 2 (dense coating) were employed, the reproducibility was better. It proved simple to detect dense tongue coating with the VELscope® device. In this study, a VELscope® score of 1 or 2 corresponded to a Winkel Tongue Coating score of 2. A VELscope® score of 0 correlated with Winkel Tongue Coating scores of 0 or 1. Evaluation was easier even for inexperienced examiners if the two methods were combined.

Dividing the tongue's dorsum into sextants enabled even inexperienced examiners to describe the distribution of the coating. Statistical analysis demonstrated good agreement between the experienced examiner and those lacking experience.

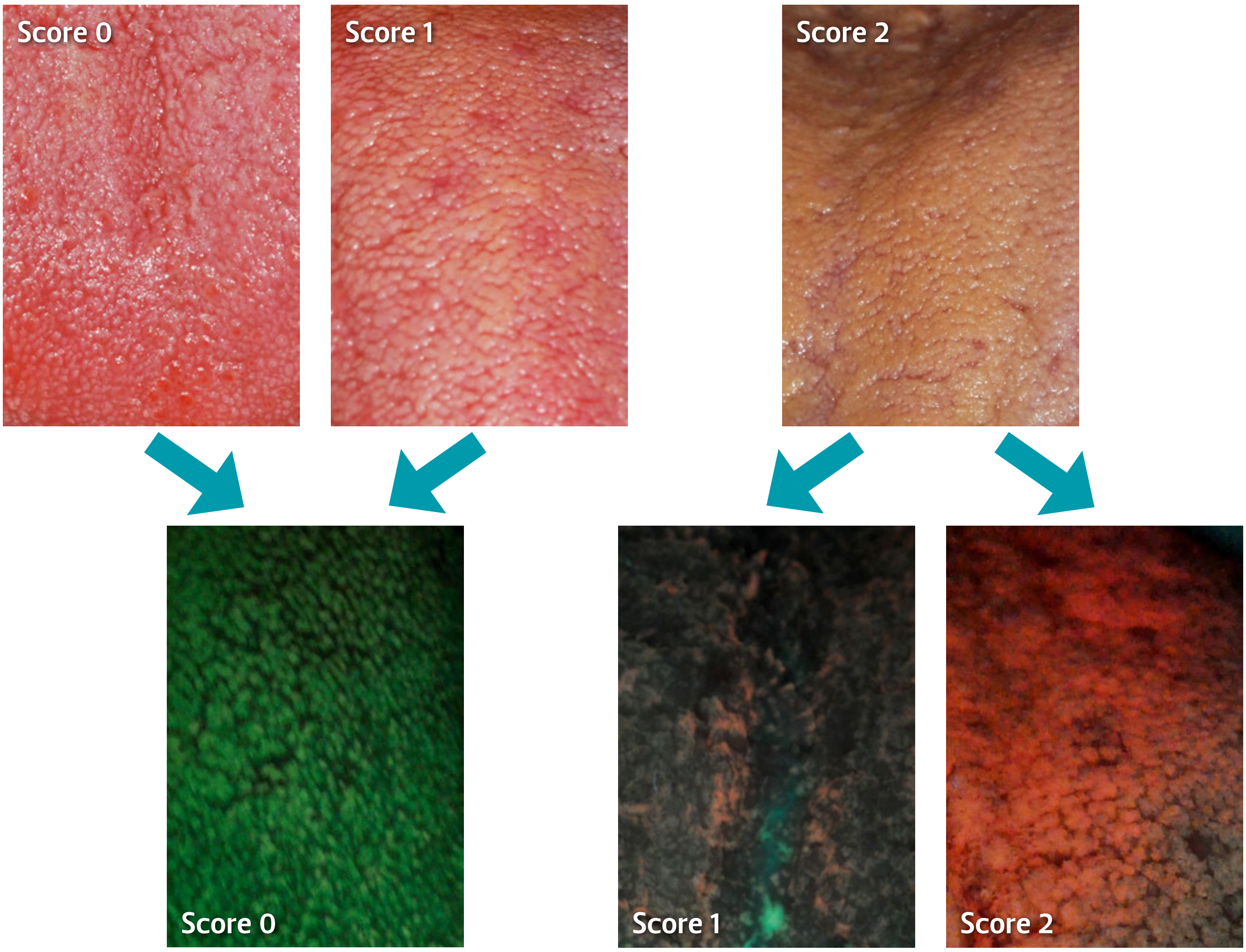


Fig. 6 Comparison of the two tongue coating indices per sextant. Scores 0 or 1 of the Winkel Tongue Coating Index (top) correspond to score 0 of the VELscope® Index (bottom), while score 2 of the Winkel Tongue Coating Index corresponds to scores 1 or 2 of the VELscope® Index.

Tab. I Kappa coefficients of the two examination methods compared between the experienced examiner and the inexperienced examiners (dentists 1–3, students 1 and 2).

Clinical manifestation			
Comparison	**Kappa**	**lower 95%CI**	**upper 95%CI**
Dentist 1 vs experienced examiner	0.68	0.59	0.77
Dentist 2 vs experienced examiner	0.64	0.55	0.73
Student 1 vs experienced examiner	0.74	0.65	0.84
Dentist 3 vs experienced examiner	0.64	0.55	0.74
Student 2 vs experienced examiner	0.57	0.48	0.66
VELscope®			
Comparison	**Kappa**	**lower 95%CI**	**upper 95%CI**
Dentist 1 vs experienced examiner	0.64	0.58	0.70
Dentist 2 vs experienced examiner	0.77	0.70	0.84
Student 1 vs experienced examiner	0.55	0.48	0.61
Dentist 3 vs experienced examiner	0.65	0.58	0.72
Student 2 vs experienced examiner	0.75	0.69	0.82

vs: versus

Both indices diagnosed more coating on the posterior part of the tongue. This distribution resulted from less abrasion by food and lack of contact between teeth and the tongue's posterior dorsum, compared to the anterior regions.

Tongue coating increases with age (Yaegaki & Sanada 1992, Kikutani et al. 2009). However, this could not be confirmed in the present study, perhaps because the individual age groups were not sufficiently representative, since over half of the participants were in the > 55 years group.

As expected, no gender-based difference in the distribution and density of tongue coating was observed in this study, but the sample size may have been too small to yield representative results.

Unfortunately, the literature contains little data on the use of the autofluorescence method in halitosis diagnostics and on fluorescent bacteria on the tongue dorsum. After a literature search, it was not possible to conclusively answer the question of which bacteria fluoresce orange using the autofluorescence method and why. In a microbiological investigation, the bacteria on the tongue dorsum which fluoresced orange under the VELscope® were sampled and identified as *Actinomyces oris* and *Actinomyces naeslundii* (Saladino 2013).

The literature describes *A. naeslundii* as producing compounds containing porphyrin (Koenig et al. 2000) and exhibiting orange-fluorescent behavior (Lennon et al. 2006). However, the most recent studies show that although the autorfluoescence method can detect *A. naeslundii* as a fluorescent orange micro-organism, the fluorescence only becomes evident when blood is added. Apparently, not only certain species of bacteria but also metabolic products of biofilm are responsible for fluorescence (Volgenant et al. 2013).

Thus, further studies are necessary to identify micro-organisms which show fluorescence and are associated with halitosis.

Conclusion

The VELscope® device is a suitable instrument for detecting tongue coating and can complement tongue coating examinations as part of halitosis diagnostics. Thanks to the orange color of tongue coating illuminated by the VELscope®, dentists can graphically impress upon patients the importance of optimizing their tongue hygiene. Due to the presently high acquisition costs (about € 4000), chiefly patients at university clinics will benefit from tongue coating examinations using an autofluorescence device.

Résumé

Dans la présente enquête, le dépôt sur la langue a été examiné d'une part à l'aide de l'indice dit «Winkel Tongue Coating Index» et d'autre part il a été complété avec le dispositif d'autofluorescence VELscope®, pour lequel un indice a été créé. L'autofluorescence permet de visualiser clairement la coloration orange de la langue et ainsi, à l'avenir, montrer distinctement aux patients souffrant d'halitose les zones de la langue contaminées, avec pour but de les encourager à optimiser leur hygiène buccale.

La surface de la langue de 100 patients (35 h, 65 f, moyenne d'âge 51 ans) a été photographiée à l'aide de ce dispositif d'autofluorescence et sans celui-ci. Toutes ces images ont été découpées en six zones à l'ordinateur sous forme de quadrillage. six personnes (5 novices et le directeur du service) ont analysé les images de manière aléatoire.

Ces deux techniques d'imagerie ont permis de diagnostiquer un dépôt bactérien plus important situé principalement au niveau de la partie centrale postérieure de la langue. Une comparaison effectuée entre l'indice «Winkel Tongue Coating Index» et l'indice VELscope® a révélé des différences notables ($p<0{,}001$). Il a été démontré qu'un dépôt lingual important pouvait être mieux diagnostiqué et représenté à l'aide du dispositif VELscope®. Toutes les personnes ayant effectué cette étude, qu'elles soient expérimentées ou non, sont parvenues à des résultats comparables pour les deux méthodes (valeur kappa sans VELscope® 0,654, avec VELscope® 0,672).

Il s'est avéré que le dispositif VELscope® complète un diagnostic du dépôt lingual sans toutefois remplacer l'indice «Winkel Tongue Coating Index».

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