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Abstract

Purpose

To evaluate the individual and combined ability of videostroboscopy (VS), high-speed digital imaging (HSDI), enhanced endoscopy (EE) and saline infusion (SI) to predict neoplasia, defined as glottic precursor lesion (GPL) or T1a glottic cancer, in patients suspected for glottic neoplasia.

Methods

A nationwide prospective cohort study of patients treated by cordectomy for suspected GPL or T1a glottic cancer from August 1st 2016 to October 31st 2018 was conducted in the five Danish University Departments of Head and Neck surgery. Sensitivity, specificity, negative and positive predictive values, and area under Receiver Operating Curves (AUC-ROC) were calculated with 95% confidence intervals with respect to the histological diagnosis. Logistic regression with an imputation model for missing data was applied.

Results

261 patients aged 34-91 years participated; 79 (30.3%) with non-neoplasia (i.e. inflammation, papilloma, hyperkeratosis) and 182(69.7%) neoplasia, hereof 95 (36.4%) with GPL and 87 (33.3%) with T1a glottic cancer. Data from 188 VS, 60 HSDI, 100 preoperative EE, 209 intraoperative EE, and 234 SI were analyzed. In the complete case analysis the AUC-ROC of each diagnostic test was low, but increased when the tests were combined and especially if the combination included EE. However, multinomial logistic regression with imputation showed significant association ($p<0.05$) only between age, male gender, and perpendicular vasculature in intraoperative EE, and the endpoint neoplasia.

Conclusions

Intraoperative EE was the most accurate diagnostic method in detecting neoplasia. The prediction ability of methods applied preoperatively was more limited, but improved when test modalities were combined.

Introduction

Transformation of normal vocal cord mucosa into premalignant lesions (glottic precursor lesions, GPL) and cancer is a multistage process [1,2]. Because of the malignant potential, surgical treatment of GPL is generally recommended. However, GPL or early cancer may present as low-contrast mucosal changes, without obvious characteristics distinguishable from lesions with no malignant potential[3].

Danish national guidelines for handling of neoplasia (defined as GPL or T1a glottic cancer) were agreed in 2012[4]. A one-stage surgical procedure (cordectomy) that provides adequate treatment for neoplasia and allows histological evaluation of the entire lesion was recommended for suspected neoplasia. In such a setup, the risk of damage to the voice must be considered and the extent of surgical intervention balanced according to the potential severity of disease. Pre-surgical evaluation of the malignant potential is crucial[5-7] and a biopsy seems obvious; however this may necessitate subsequently more extensive resections because of the alterations of the normal layered structure of the vocal cord[5]. Furthermore, a punchbiopsy will only allow histological evaluation of a specific point of the lesion, which may or may not represent the most severe part of the lesion. Therefore, several diagnostic methods have been suggested to increase the diagnostic accuracy of pre-surgical assessment. Videostroboscopy (VS) is gold standard in vocal cord examination[8,9,3,10-12] and more studies report a considerable diagnostic value of an impaired mucosal wave in predicting GPL or early glottic cancer[3,13,5,14]. Benign conditions like inflammation, scarring and vocal overuse may however impair the mucosal vibration[1,10] and an edema in the Reinke's space may permit a normal mucosal wave despite microinvasive cancer[1]. Unlike VS, high-speed digital imaging (HSDI) is frequency-independent and one of the key advantages of this technique is its ability to capture recording of the mucosal wave in case of severe dysphonia e.g. very short or aperiodic mucosal vibration that is impossible to capture by VS[15,9,16]. Enhanced endoscopy (EE) such as Narrow Band Imaging (NBI®, Olympus, Tokyo, Japan) or Storz Professional Image Enhancement System (SPIES®, Karl Storz, Tuttlingen, Germany) is designed to visualize the mucosal vasculature and thus the perpendicular neo-angiogenetic vessels in neoplasia[17-20]. Intraoperative saline infusion (SI) in the submucosal space (or "hydrodissection") may be used to evaluate the depth of a glottic lesion and its extension into lamina propria[21,1,5] and has been suggested as

mandatory when performing cordectomy[5]. However, knowledge about accuracy of these diagnostic tools is limited and a combined use of two or more diagnostic methods is suggested to increase the diagnostic accuracy[5,15,22,17,23,7,24-26]. We aimed to evaluate the individual and combined capacity of VS, HSDI, EE, and SI to predict neoplasia in patients suspected for glottic neoplasia.

Materials and methods

Study design

Data were prospectively included from the five Danish University Departments of Head and Neck surgery and registered in a national REDCap[27] (Research Electronic Data Capture) database. The inclusion criteria for this diagnostic accuracy study were cordectomy performed for suspected neoplasia in patients ≥ 18 years of age between August 1st 2016 and October 31st 2018. Patients were excluded if they had had surgery on the affected vocal cord more than once or during the last year, had intraoperatively unexpectedly exhibited signs of extensive disease (>T1a glottic carcinoma) or if anatomical or technical limitations made cordectomy impossible.

The study is reported according to the STARD-2015 guidelines[26].

Preoperative VS, EE with NBI or SPIES, and HSDI (when available) was performed in the outpatient clinic. The patient was offered cordectomy in general anesthesia if neoplasia was suspected (e.g. suspicious morphology, persistent erytro- or leukoplakia, reduced mucosal waves or perpendicular vessels) and a limited cordectomy was expected to be sufficient treatment. In the operating room, EE was repeated, and SI performed. Based on this test-battery the surgeon chose the type of cordectomy necessary to secure radicality (European Laryngological Society type I-III)[28]. Pre- and intraoperative assessment as well as surgery was performed by a limited number of trained ENT-doctors at each department.

Index tests

Measured variables are provided as Appendix. For the four different index-tests, pre-specified cut-offs were applied to achieve binary outcomes as follows:

VS: normal versus reduced/absent mucosal wave, lesion elevated/exophytic or not

HSDI: normal versus reduced/absent mucosal wave

EE: visible perpendicular vessels or not

SI: complete lifting ("ballooning") of mucosa versus partly/complete fixation of mucosa

Reference standard

Histological assessment served as reference standard. Analyses were performed with a pre-specified cut-off of the reference standard, into neoplasia (GPL or T1a glottic cancer) vs. non-neoplasia (NNP).

Clinical information was available to the performers of the index test, because of the clinical setting, but the reference standard was not known at the time of inclusion and assessment of index test.

Clinical information and index test results were hypothetically available to the assessors of the reference standard, but not used as a part of the daily clinical routine.

Statistical analysis

Summary statistics for demographic and clinical characteristics were determined. Sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), and area under the Receiver Operating Curves (AUC-ROC) were estimated with 95% confidence intervals (CI) for VS, HSDI, EE and SI with respect to the final histological diagnosis as reference standard, with the above-mentioned cut-offs. The performance of the applied cut-offs was assessed using AUC-ROC for each test as well as for clinically relevant combinations of tests. The combined diagnostic set-up was further analyzed by multinomial logistic regression to investigate the relationship between outcome and multiple simultaneous clinical and demographical covariates. Indeterminate index test results (explanatory variables) were handled as missing data while there were no indeterminate or missing reference standard results. Missing data on the index tests were handled using a Multivariate Imputation by Chained Equations (MICE) model[29].

Statistical analysis was done using the Stata software (Version 15, College Station, TX, USA: StataCorp, 2017). P-values< 0.05 were considered statistically significant. Power calculation was not performed since no valid data concerning accuracy for the established methods was available before this study.

Results

Data from the 261 patients including 188 VS, 60 HSDI, 234 SI, 100 preoperative EE, and 209 intraoperative EE were registered and analyzed (215 had at least one EE performed). The demographic and clinical characteristics of the patients are presented in Table 1. The distribution of final histological diagnoses was 79 (30.3%) NNP (i.e. inflammation, papilloma, hyperkeratosis) and 182 (69.7%) neoplasia, hereof 95 (36.4%) GPL and 87 (33.3%) T1a glottic cancer.

The sensitivity, specificity, NPV, PPV, and AUC- ROC for each test modality in detecting neoplasia are shown in Table 2. In the complete case analysis, the AUC-ROC of each diagnostic test was low, but the AUC-ROC increased when the tests were combined and especially if the combination included EE (Table 3). The point estimates of logistic regression with and without MICE imputation were not very different. However, the use of imputation narrowed the confidence intervals and thus made associations apparent that were not seen in the complete case analysis (Table 4). HSDI was omitted from the regression analyses because of a limited number of observations almost all from one center (57 of 60). Multinomial logistic regression with imputation showed significant association ($p<0.05$) between age, male gender, and perpendicular vasculature in intra-operative EE, and neoplasia. The logistic regression model was tested for goodness of fit and was acceptable ($p=0.69$).

The time interval between the index tests and reference standard was not calculated, but all of the included patients were handled in a clinical setting in which preoperative assessment was followed by surgery within approximately a month and subsequent histological assessment within an additional two weeks.

Discussion

This is the first nationwide study to evaluate the diagnostic accuracy of diagnostic methods applied before surgical intervention, as part of the pre- and intraoperative evaluation of suspected glottic neoplasia. The estimated sensitivity, specificity, PPV, NPV and AUC-ROC of the individual and combined diagnostic tests were highly variable. Our results suggest that none of the evaluated test modalities (VS, HSDI, EE or SI) are accurate enough to stand alone and that combinations of test modalities increase diagnostic accuracy.

Intraoperative EE was found to be superior to the remaining tested methods, especially if used as a part of a combined diagnostic approach.

Every test modalities evaluated in this study scored relatively poor compared to other reported series. However, most of these series are smaller dimensioned, single-institutional, involving only one or a few expert assessors, and/or performed retrospectively[20,1,30,31,18,22,9] which may have increased their estimated diagnostic accuracy. In our setup the methods were applied prospectively in a national multicenter organization with diverse clinical traditions and preferences reflecting "a real-world situation". The setup was chosen to improve generalizability (external validity) of our results.

Histology served as reference standard for estimates of diagnostic performance. For the study period in question, the World Health Organization classification WHOC 2005[32] was used in Denmark dividing laryngeal precursor lesions into mild-, moderate-, or severe dysplasia, or carcinoma in situ. The revised WHOC 2017[33], dividing laryngeal precursor lesions into low vs. high grade, was not fully implemented in Denmark and therefore not used in this study.

To make our results relevant for the clinical setting we chose to analyze the diagnostic accuracy in neoplasia (GPL according to WHOC 2005 or T1a glottic cancer) vs. non-neoplasia (NNP). This prespecified cut-off supported the Danish national guidelines[4] after which GPL and T1a glottic cancer are managed in a similar way with cordectomy whereas NNP usually is handled by other surgical or non-surgical treatment methods.

Certain morphological features like heterogenous color, irregular texture, thickness or asymmetry may cause a "typical neoplastic appearance" and some authors suggest that diagnostic criteria should include morphological abnormalities[11,25,34]. We only had very limited data on morphological features. The finding of an elevated or exophytic lesion, as opposed to a flat lesion, seemed to be a reasonably sensitive, but not very specific finding for neoplasia (n=185, sensitivity 0.85, specificity 0.26, data not shown elsewhere). However, such finding is not strictly related to any specific imaging modality. Finally, also common non-neoplastic lesions (e.g. granulomas or polyps) very often appear elevated or exophytic. Therefore, we chose to omit data on morphological features from further calculations.

VS remain the initial, basic procedure in laryngological diagnostics for early detection of neoplastic tissue[3]. By merging of still-pictures extracted from several successive vibration cycles, VS allows for

visualization of the mucosal wave, which, in real time, occurs too fast for the human eye to perceive[14]. A reduced or absent mucosal wave raises the suspicion of infiltration into the deeper layers of the mucosa and thus an invasive lesion[3,25,13,11,14]. There are diagnostic challenges in the assessment of GPL[14], but most authors agree that normal, symmetrical mucosal waves excludes extensive vocal ligament invasion[1,13,11,14]. A fundamental limitation of VS is the dependency on a fairly stable phonation frequency and a periodic vocal fold vibration frequency to activate the strobelight. Unlike VS, the HSDI technique is frequency-independent and allows recording of aperiodic or aphonic sequences of vibration[9,16] and also in patients with very short phonation time or a strong gag reflex. Studies comparing VS and HSDI in patients with normal voices have shown good agreement between the two methods[16], whereas studies of abnormal voices have showed some important differences[9,16]. The drawbacks of HSDI compared to VS are a limited availability, a higher price, shorter duration of the recording, poorer image resolution and the compatibility only with rigid endoscopes. Based on the possible advantages of HSDI in the assessment of suspected neoplasia[15,24], HSDI was agreed on as a supplementary diagnostic method, in the initial phase of our study. However, due to limited access to the equipment, HSDI was only used in two of the centers. We defined a reduced/absent mucosal wave in VS or HSDI as a pathological finding. Our results regarding VS are in line with previous reports of a sensitivity as high as 0.86-1.00, but a much more variable specificity of 0.07-0.93[14,22]. Comparable results for HSDI are not available, but Volgger et al[24] reported a sensitivity of 100% and a specificity of 85.7% in the distinction between malignant and non-malignant in 34 lesions. We found a more moderate accuracy of HSDI in predicting neoplasia.

The use of SI with cordectomy was described by Zeitels[21] and adapted by other authors[5,1,6]. SI eases the phonosurgical dissection, prevents thermal trauma to the underlying vocal ligament in laser-resection and helps predict invasion of cancer into the vocal ligament, by leaving a depression in the mucosal surface caused by the incomplete lifting of the mucosa. Dependence of proper training in the procedure as well as the possible normal “ballooning” of the mucosa in cancers with very limited invasion of the basal membrane or an incomplete lifting of mucosa with benign scar tissue or in lesions very anteriorly or posteriorly has been described as diagnostic limitations of SI[5,21]. We defined an incomplete “ballooning” (partial or complete fixation of the mucosa) as a pathological finding of SI. There are only few reports on the value of SI for

comparison. Peretti et al.[5] reported a comparably high specificity (90 %), but a considerably higher sensitivity (83%) of SI in predicting malignancy in 52 patients, than we did in predicting neoplasia (32%). This difference may be explained by the use of different endpoints in the two studies, since our endpoint (neoplasia) included non-invasive GPL which are not expected to hamper the mucosal "ballooning" like an invasive, malignant lesion would.

A structured assessment of the vascular patterns of the vocal folds is now recommended as an integrated part of the evaluation of laryngeal lesions[17,35]. Vascular patterns hardly seen with white light, becomes better contrasted by EE and thus more visible. The NBI system centers the emitted wavelengths at 415 nm (blue light) and 540 nm (green light), which limits the penetration depth and facilitates the visualization of superficial vessel[23,34] whereas the SPIES system uses a standard light unit combined with a camera system capable of enhancing selected wavelengths of light in five different spectral ranges[35].

Two recent meta-analyses have shown a combined sensitivity of 81% and a specificity of 92% of NBI in the distinction of laryngeal cancer[36] and a combined sensitivity of 82-89% and a specificity of 82-93% in the distinction of neoplasia[19]. Studies of preoperative EE with flexible laryngoscopes have reported high sensitivities of NBI in diagnosing severe dysplasia, CIS or invasive cancer of 92.9-93.3% and specificities of 88.9-97.3%[30,37]. In diagnosing CIS or invasive cancer, sensitivities of 88-97 % and specificities of 89.5-96% have been reported[38,18] whereas Shoffel-Havakuk et al[7] reported a more moderate performance of preoperative NBI in predicting invasive cancer with a sensitivity of 58.6% and a specificity of 61.2%. The authors of the latter study explained their estimates with evaluation of images taken relatively far from the lesion, poorer image quality of flexible endoscopy compared to rigid endoscopy and independent assessment by the observers. Those factors may also have influenced our comparable estimates of the preoperative EE.

Intraoperative rigid endoscopy with NBI was evaluated by Sifrer et al[39] who reported a very high sensitivity of 100% and a specificity of 95% in the distinction of severe dysplasia, CIS or invasive cancer and by Ni and colleagues[40] who found a high sensitivity of 88.9% and a specificity of 93.2% in the distinction of CIS or invasive cancer. Pre- and intraoperative EE have been compared with variable results. Piazza et al[41] found a significantly higher sensitivity of intraoperative vs. preoperative EE with NBI (sensitivity 98% vs. 61%), but comparable PPV (86% vs. 83%) whereas De Vito and colleagues[20] found

no statistically significant differences in the diagnostic performance (preoperative sensitivity/specificity 97%/92.5% vs. intraoperative 97%/95 % (p=0.41)). Stanikova et al[42] compared preoperative EE with NBI to intraoperative EE with SPIES in laryngeal or hypopharyngeal lesions and found no significant differences in diagnostic performance. The SPIES and the NBI technique was also compared in a study of combined EE and contactendoscopy and the authors found that both techniques allowed for the recognition of neoangiogenesis found in neoplasia and both required a degree of experience to avoid misinterpretation[35]. In our study, the intraoperative EE was superior to the preoperative EE in predicting neoplasia.

Several classification systems of vascular changes have been suggested. The so-called Ni-classification from 2011[40] is well-documented[38,43,20,44,18] and subdivides vascular patterns into five types I-V, of which type I-III represents longitudinal vessels and type IV and V represents visible, perpendicular intraepithelial papillary capillary loops (IPCLs) with increasing irregularity caused by a carcinogenic stimulus[19,17]. In 2016 the European Laryngological Society (ELS) proposed a descriptive guideline of vascular changes suggesting a dichotomous distinction between benign (longitudinal vessels) and premalignant or malignant (perpendicular) vessels[17]. The ELS-guideline was subsequently evaluated to be reliable in predicting neoplasia[39,19]. This simplistic and practical approach and its distinction between NNP and premalignant/malignant (neoplasia) was compatible with the Danish national treatment strategy and therefore, applied in our study. The intraoperative finding of visible perpendicular vessels was the most accurate indicator of neoplasia in our study.

A combined use of two or more diagnostic methods has been suggested of many authors to increase the diagnostic accuracy[5,15,9,22,26,6,17,23,7,24,25] and assessment of macroscopic appearance, mucosal vibration, and vascular changes[17] with all of the findings put into relation has been suggested in order to get the most reliable pre- and intraoperative assessment[17]. Our results support this suggestion.

Strengths and limitations:

Our study is prospective with involvement of five tertiary institutions and more than 20 doctors with varying levels of experience though reflecting a common clinical setting, and assesses several diagnostic tools used in daily clinical laryngological practice. For these reasons, the external validity of the study is high.

The study is however limited by some factors. A tertiary hospital setting may hamper the generalizability of the results to other types of institutions and the varied level of expertise among the involved doctors may have affected our results, bearing in mind an expected learning curve[45]. Furthermore, our dataset is not complete, and the missing data thus caused less precise estimates of diagnostic accuracy with wide confidence intervals. The use of imputed data compensated partly for this. Finally, the pre- and intraoperative assessment was not blinded, since the doctors who performed the evaluations were involved in the clinical care of the patients.

Conclusion

Our study reveals considerable diagnostic challenges in the pre-surgical prediction of neoplasia. None of the evaluated diagnostic test modalities seems adequate to stand alone. Based on our results we suggest that a pre- and intraoperative diagnostic set-up includes more than one diagnostic test modality and preferably visualization of the vasculature by EE. Future research in automated, less subjective diagnostic methods should be encouraged to further improve the diagnostic accuracy and thereby benefit patients with suspected neoplasia.

Compliance with ethical standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of the 1964 Helsinki declaration and its later amendments. According to Danish legislation no Ethical approval was needed. Informed consent was obtained from all individual participants included in the study.

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Table 1 Demographic and clinical characteristics of the entire study population (n =261)

Age at diagnosis, years	Mean, sd	64.9 (11.6)
	Median, range	66.2 (34.2-91.6)
Distribution among centers, n	Center 1	18
	Center 2	14
	Center 3	82
	Center 4	80
	Center 5	67
Gender (%)	Male	204(78.2)
	Female	57(21.8)
Tobacco use (%)	Never	20(7.7)
	Ex-smoker [a]	78(29.9)
	Current smoker	149(57.1)
	Unknown	14(5.4)
Histological diagnosis (%)	Non-neoplasia	79(30.3)
	Mild dysplasia	31(11.9)
	Moderate dysplasia	24(9.2)
	Severe dysplasia	30(11.5)
	Carcinoma in situ	7(2.7)
	Dysplasia, NOC	3(1.2)
	Invasive cancer	87(33.3)

sd: standard deviation, NOC: not otherwise classified

[a] *smoking cessation >3 months ago*

Table 2. Testmodalities and their ability to identify neoplasia. Estimates of sensitivity, specificity, negative and positive predictive values, and accuracy of each diagnostic test and relevant predictive findings along with 95% confidence intervals

Test modality	**Predictor**	**N**	**Sens (%)** (95% CI)	**Spec (%)** (95% CI)	**PPV (%)** (95% CI)	**NPV (%)** (95% CI)	**AUC-ROC** (95% CI)
VS	**Impaired mucosal wave**	**169**	**85** (78-91)	**34** (22-48)	**74** (66- 81)	**51** (34-69)	**0.60** (0.52-0.67)
HSDI	**Impaired mucosal wave**	**60**	**89** (75-96)	**19** (4-46)	**75** (61- 86)	**38** (9-76)	**0.54** (0.43-0.65)
EE, Preoperative	**Visible perpendicular vessels**	**100**	**43** (31-56)	**62** (45-78)	**66** (49-80)	**39** (27-53)	**0.53** (0.43-0.63)
EE, Intra-operative	**Visible perpendicular vessels**	**209**	**58** (49- 66)	**76** (64-85)	**83** (73- 89)	**47** (38-57)	**0.67** (0.60-0.73)
SI	**Fixation of mucosa**	**237**	**32** (25-40)	**87** (77- 94)	**86** (74-93)	**35** (28-43)	**0.60** (0.54-0.65)

VS: videostroboscopy, HSDI: high speed digital imaging, EE: enhanced endoscopy, SI: saline infusion, sens: sensitivity, spec: specificity, NPV: negative predictive value, PPV: positive predictive value, AUC: area under curve, ROC: Receiver Operating Characteristic, CI: confidence interval.

Table 3. Ability of clinically relevant combinations of test modalities in predicting neoplasia

Test modality	Predictor	N	AUC-ROC (95% CI)
VS & EE preoperative	VS: impaired mucosal wave EE: visible perpendicular vessels	86	0.66 (0.53-0.78)
HSDI & EE preoperative	HSDI: impaired mucosal wave EE: visible perpendicular vessels	24	0.56 (0.28-0.85)
VS, HSDI & EE preoperative	VS: impaired mucosal wave HSDI: impaired mucosal wave EE: visible perpendicular vessels	24	0.71 (0.47-0.96)
VS preoperative **EE intra-operative**	VS: impaired mucosal wave EE: visible perpendicular vessels	147	0.70 (0.61-0.78)
VS preoperative **SI intra-operative**	VS: impaired mucosal wave SI: Fixation of mucosa	154	0.67 (0.58-0.75)
VS preoperative **EE&SI intra-operative**	VS: impaired mucosal wave EE: visible perpendicular vessels SI: Fixation of mucosa	136	0.71 (0.62-0.80)

VS: videostroboscopy, HSDI: high speed digital imaging, EE: enhanced endoscopy, SI: saline infusion, AUC: area under curve, ROC: Receiver Operating Characteristic, CI: confidence interval

Table 4 Logistic regression modelling of relevant explanatory variables in predicting neoplasia. Estimates of odds ratios for neoplasia are presented with and without imputation for handling missing data by use of a Multivariate Imputation by Chained Equations model.

HSDI was omitted because of few observations almost all from one center

		Complete case analysis (n=68)		**Analysis with imputation (n=261)**		
		Odds ratio (95% CI)	**P-value**	**Number of imputed observations**	**Odds ratio (95% CI)**	**P-value**
Age		1.04 (0.98-1.11)	0.191	0	1.04 (1.01-1.07)	0.007
Male gender		0.16 (0.04-0.66)	0.012	0	0.22 (0.11-0.44)	0.000
Smoking	Never	(empty)		-	-	-
	Previously[a]	1.16 (0.26-5.24)	0.844	14	0.73 (0.19-2.83)	0.653
	Currently	- [b]		14	0.70 (0.19-2.54)	0.586
Impaired/absent mucosal wave in VS		3.12 (0.74-13.12)	0.120	92	1.84 (0.83-4.07)	0.133

Perpendicular vessels in preoperative EE	0.64 (0.13-3.24)	0.592	161	0.73 (0.30-1.79)	0.498
Perpendicular vessels in intra-operative EE	4.98 (0.73-33.90)	0.101	52	2.56 (1.04-6.31)	0.041
Partly or complete mucosal fixation in SI	0.61 (0.07-5.38)	0.655	24	1.51 (0.58-3.88)	0.397
Baseline odds	*0.05 (0.00-2.84)*	*0.145*		*0.72 (0.06-8.49)*	*0.797*

VS: videostroboscopy, EE: enhanced endoscopy, SI: saline infusion, CI: confidence interval.

[a] *smoking cessation >3 months ago*

[b] *omitted because of collinearity*

Value of pre-and intraoperative diagnostic methods in suspected glottic neoplasia

European archives of otorhinolaryngology

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Appendix . Measured variables

Patient characteristics

Age, gender, tobacco use, inclusion date, surgical department

Preoperative diagnostic set-up (explanatory variables marked with *)

VS:

Lesion type (elevated yes/no, exophytic yes/no)*

Mucosal wave (normal/reduced/absent)*

HSDI:

Mucosal wave (normal/reduced/absent)*

EE:

vascular changes (perpendicular vessels yes/no/unknown)*

Surgical procedure: (explanatory variables marked with *)

Intraoperative EE used (yes/no)

If yes: vascular changes (perpendicular vessels yes/no/unknown)*

SI used (yes/no)

If yes: lesion adherent to ligament (yes/partly/no)*

Histology (response variable):

Non-neoplasia, mild dysplasia, moderate dysplasia, severe dysplasia, carcinoma in situ, invasive carcinoma, other invasive cancer