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NARROW BAND IMAGING AS SCREENING TEST FOR EARLY DETECTION OF LARYNGEAL CANCER: A PROSPECTIVE STUDY

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CONFLICTS OF INTEREST: NONE

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Abstract: OBJECTIVES: to analyze the specificity and sensibility of Narrow Band Imaging illumination technology in the early detection of laryngeal cancer in the patients' population without previous diagnosis of laryngeal cancer in a screening setting.
DESIGN: unicenter, prospective study
SETTING: One tertiary medical center
PARTECIPANTS: 158 patients completed all protocol steps
MAIN OTUCOME MEASURES: sensitivity, specificity, positive and negative predictive values of NBI in detecting precancerous lesions and early laryngeal cancer
RESULTS: The blind assessment of NBI patterns concurred in 90% of patients. In identifying laryngeal cancer and its precursor lesions, in-office NBI showed a high sensitivity of 97% (CI, 84.2-99.9%), specificity of 92.5% (CI, 79.6-98.4%), PPV of 91.4%(CI, 76.9-98.2%), NPV of 97.4%(CI,86.2-99.9%) and accuracy of 94.5% but intra-operative NBI demonstrated a sensitivity of 97% (CI,84.2-99.9%), a slightly higher specificity of 95%(CI,83.1-99.4%), PPV of 94.1% (CI,80.3-993%), NPV of 97.4% (CI,86.5-99.9%) and accurancy of 95.9%. The comparative ROC curves

confirmed a slightly higher performance for the intra-operative NBI evaluation without any statistical significance (p=0.41).
CONCLUSIONS: Our results confirm the high values of sensitivity and specificity of NBI system in detecting pre-neoplastic laryngeal lesions or early laryngeal cancer in a patients' population selected only by means of risk factors exposure and confirmed the potential role of NBI evaluation as in-office screening tool.

KEYWORDS: Narrow Band Imaging, Early Laryngeal Cancer, Screening Test

Introduction:

In 2012 European Cancer Observatory data estimates incidence from larynx cancer in both sexes of 4.4% and mortality of 2% (cumulative risk, per 100,000) **1**. 85-95% of laryngeal cancer is squamocellular cancer (SCC) and glottic cancer is by far the most common site for laryngeal cancer. In Italy the incidence from laryngeal cancer is estimated of 4.6% and mortality of 1.6% (cumulative risk, per 100,000) (**1-2**). Although the total European population will remain stable in the next years, a 22% increase in the population over 65 years and a 50% over 80 years is expected in the European area. Taking into account the strong association between cancer risk and age, a significant increase in the incidence of cancer is foreseen, independently to the risks factors for specific tumours (**3**). Therefore new diagnostic tools with improved sensitivity and specificity should be introduced to obtain an early detection of premalignant lesions.

Nowadays white light fiberoptic nasolaryngoscopy or direct white light optical laryngoscopy represent the gold standard diagnostic tools applied in laryngeal examination and have overcome the traditional indirect laryngeal mirror examination.

Recently, a new method of endoscopic technology called Narrow Band Imaging (NBI) has been introduced in clinical assessment of the mucosa of aero-digestive tract. It consists of an endoscopic illumination method in which the spectral bandwidth of the filtered light is narrowed. A narrow-band light is converted to a short wave length of 400-430 nm by means of a first optical filter (blue light). In this way the light penetrates only the superficial layer of the mucosa with an enhancement of its vasculature as a consequence. Indeed, a second optical filter generates another narrow-band light of

525-555nm wavelength (green light), which penetrates deeper in the submucosal level, with an enhancement of its vessels. Blue and green light with wavelength of 415 and 540 nm represent the peaks of absorption spectrum of haemoglobin and allow the NBI system to enhance the image of capillary vessels on the surface of mucosa and subepitelial layer. Neoangiogenesis is the most common vascular abnormality detected in the cancerization process of aero-digestive tract mucosa (**4-10**). The most frequent abnormal pattern reported in the intraepithelial layer is the presence of brownish dots with extension, dilatation, weaving, and differing shapes and could be consistent with pre-neoplastic or neoplastic lesions. In 2010 Ni et al. has introduced a more detailed NBI endoscopic classification of laryngeal intraepithelial capillary loop changes, classified into five types (I to V), where types IV and V may identify a pre-neoplastic or neoplastic pattern (**11**) (**Table n°1; Fig. 1 - 3**).

Literature data reported high sensitivity and specificity of the NBI in detection of early mucosal cancer of the oropharynx and hypopharynx as well as in the surveillance of cancer of the head and neck (**4-10**).

In this paper the authors investigated the sensitivity, specificity, positive and negative predictive values of NBI in detecting precancerous lesions and early laryngeal cancer in the population afferent to our institution, in order to evaluate the NBI system as screening diagnostic tool.

Material And Methods

The study is designed to investigate the sensitivity, specificity, positive and negative predictive values of NBI in detecting precancerous lesions and early laryngeal cancer in a screening setting(primary endpoint). The potential upstaging power of NBI system during the general anaesthesia panendoscopy was analyzed (second endpoint).

This study includes 381 consecutive patients referred to our Institution during screening campaigns for laryngeal cancer from October 2010 to October 2014. In this perspective, we organized specific seminars for General Practitioners and Otolaryngologists in order to optimize the patients' selection

process. Inclusion criteria were patients from 18 to 80 years old, with a declared tobacco and/or alcohol use, professional exposure to chemical substance related to Head and Neck cancer (HNC), any grade of dyphonia and symptoms related to laryngopharyngeal reflux.

Exclusion criteria were previous history of any surgery or chemo-radiotherapy for a HNC, or a biopsy-proven laryngeal cancer of the current lesion. A written informed consent was obtained from all patients.

All in-office NBI endoscopy has been carried out by the same otorhinolaryngologist (ADV). All patients underwent a video-recorded endoscopy with an ENF-V2 transnasal flexible fiberscope connected to an Evis Exera II CV 180 B light source (Olympus Medical Systems, Tokyo, Japan). Indirect laryngoscopy was carried out with the patient in a seated position and locally anaesthetized with lidocaine. The endoscopic evaluation was initially performed with white light and then with the NBI filter. Then, each author (ADV and CV) separately conducted a blinded revision of each patient's video-recording. The video evaluations were made according to the classification of the microvascular endoscopic patterns of Ni et al. (**11**) (**Table n°1**). When a mismatch in interpreting NBI patterns was found among authors, the video was collegially watched and discussed. Endoscopic NBI findings were classified into 5 types (I–V) according to the intraepithelial papillary capillary loop features. Type I lesions correspond to thin, oblique, and arborescent vessels without any intraepithelial papillary capillary loop. Type II lesions show enlarged, oblique, and arborescent vessels without any intraepithelial papillary capillary loop. In type III lesions, vessels are obscured or seen indistinctly by white mucosa. In type IV lesions, intraepithelial papillary capillary loop can be recognized as regular, small, and dark brown spots. In type V lesions, intraepithelial papillary capillary loop appears as solid or hollow, with a brownish, speckled pattern, and various shapes (type Va) or as irregular, tortuous, line-like shapes (type Vb), or as brownish speckles or tortuous, line-like shapes with irregular distribution, scattered on the tumor surface (type Vc). According to other authors (**12-13**), type I to III lesions were considered benign and type IV-V precancerous or malignant lesions.

In case of a type IV-V or other type with high risk factors (such as smoking, heavy drinker, severe dysphonia, fixed vocal cord etc.), the patients were suggested to undergo general anaesthesia panendoscopy with excisional biopsy or simple biopsy of the lesion (NBI positive group). In case of types I-III and no high risk factors, we recommended the patients to perform a further in-office NBI assessment after 6 months (NBI negative group). This method was universally accepted to overcome the limitation of an endoscopic negative pattern without a biopsy-proven histology (7). Whether the 6-month NBI evaluation confirmed any pattern, the patients were addressed to a panendoscopy; otherwise whether any suspicious lesions and patterns disappeared, we recommended to perform a yearly scheduled NBI endoscopy.

All patients who underwent panendoscopy signed a written informed consent. The procedures were conducted in general anaesthesia, and a new video-endoscopic evaluation with NBI filters was done using 0, 30, and 70 degrees rigid endoscopes (Karl Storz, Tuttlingen, Germany) connected to an Evis Exera high-definition video camera (Olympus Medical Systems). Then the lesions were excised with CO_2 laser or simple biopsied if a deep infiltration, especially in the muscular layer, was suspected. The video-recordings were separately evaluated by each single author in the same manner as described above but blindly to histopathologic report.

Statistical analysis

Associations between variables and endpoints were tested with the Fisher exact or *t* tests, as appropriate. A 2-tailed P value less than 0.05 was regarded as statistically significant. The diagnostic performance of NBI was assessed by using receiver operating characteristic (ROC) curve and the area under the receiver operating characteristic (AUC) curve analysis; sensitivity, specificity, positive predictive value (PPV), and negative predictive value(NPV) were reported with 95% with the 95% confidence interval (CI). Statistical analysis was performed with STATA 12.0 software (Stata Corp., College Station, TX, USA).

Ethical considerations

The study was conducted in compliance with the our Institutional Review Board requirements (ORL-10-01) and it was designed and conducted in compliance with the principles of Good Clinical Practice regulations and the Helsinki Declaration. Informed consent was obtained from each patient before inclusion in this study.

RESULTS

Fifty-one (13.4%) of 381 patients met the exclusion criteria whilst 163(42.8%) patients did not show any suspicious laryngeal lesion and then all of them ruled out from the study. Six (1.6%) patients refused to undergo panendoscopy after the 6 months evaluation and were excluded from the analysis. However, we invited these patients to perform scheduled endoscopic follow-up every 6-8 months. Three (7.9%) patients with type I-II lesions, who were suggested a further evaluation after 6 months, did not perform the scheduled endoscopy and then were excluded from the study. Thus, 158(41.5%) patients completed all protocol steps. The study population characteristics are described in **Table 2**. A mismatch in interpreting NBI patterns was not found among authors with a concurrence in 90% of patients during the blind assessment of NBI patterns.

According to our protocol, all patients who showed type IV-V lesions during the screening sessions (n=36) were addressed to a general anaesthesia panendoscopy as well as 27(22.1%) out of 122 type I-III patients with high risk factors. However, 4 patients refused to undergo panendoscopy preferring to have a further in-office endoscopy after 6 months. All of these patients showed the same staged lesion during the second evaluation and they accepted to perform a panendoscopy. The remaining 95 type I-III patients were carefully evaluated after 6 months, but a laryngeal lesion persisted in only 10 (10.5%) subjects. These patients were suggested to undergo panendoscopy and all of them accepted the procedure. Finally, 73(46.2%) out of 158 patients had a panendoscopy with excision or biopsy of the lesion and final histopathologic report (**Table 3**).

During the panendoscopy, a further NBI evaluation was done for each patient. An upstaging of in office NBI evaluation was find in 5(8.2%) type I to type II, 1(5.2%) type III to type IV, 4(44.4%) type IV to type V. Moreover, a downstaging was showed in 2(4.8%) type II to type I, 1(5.2%) type III to type I, 1(5.2%)type III to type I, 1(11.1%) type IV to type III, 1(11.1%) type IV to type 2, 3(11.1%) type V to type IV. In identifying laryngeal cancer and its precursor lesions, in-office NBI showed a high sensitivity of 97%(CI, 84.2-99.9%), specificity of 92.5%(CI, 79.6-98.4%), PPV of 91.4%(CI, 76.9-98.2%), NPV of 97.4%(CI, 86.2-99.9%) and accuracy of 94.5% but the intra-operative NBI demonstrated a sensitivity of 97%(CI, 84.2-99.9%), a slightly higher specificity of 95%(CI, 83.1-99.4%), PPV of 94.1%(CI, 80.3-99.3%), NPV of 97.4%(CI, 86.5-99.9%) and accuracy of 95.9%. The comparative ROC curves confirmed a slightly higher performance for the intra-operative NBI evaluation without any statistical significance(p=0.41)**(Fig.4).**

DISCUSSION

Laryngeal cancer represents one of the most common head and neck cancer, even though its decrease of incidence has been recently worldwide reported (14). Computed Tomography (CT) and/or Magnetic Resonance Imaging (MRI) have an essential role in assessment of laryngeal cancer and its loco-regional spread, but do not allow us to identify superficial mucosal pathological patterns. White light fiberoptic nasolaryngoscopy or direct white light optical laryngoscopy still represent the standard pre-operative methods for diagnosis of precancerous lesions and early cancer of the larynx and direct microlaryngoscopy is the most important tool for general anaesthesia biopsy or treatment of such lesions. However, these techniques may not always be sufficient to assess premalignant lesions and new diagnostic techniques have been applied to overcome the limits of the traditional diagnostic methods such as autofluorescence (**15-18**) and contact endoscopy (**19-22**).

Autofluorescence allows to analyse the reduction of the green fluorescence emission by the neoplastic cells, because of the lower concentration of the oxidized flavin mononucleotide (FMN), which is detected in the normal cells, when exposed to the blue light.

A recent meta-analysis confirms the high effectiveness of fluorescence endoscopy in identifying pre-neoplastic, early laryngeal cancer and its recurrence (**15**), even though scarring, marked hyperkeratosis and inflammation may limit the predictive value of the method (**16-18**).

Contact endoscopy consists of endoscopic examination of laryngeal mucosa after its staining by means of methylene blue 1%. This technique allows to observe the cells, nuclei and cytoplasm and the grade of abnormality using specific contact endoscopes (**19-22**). Arens et al. reported promising results in a series of 323 cases with different stages of laryngeal dysplasia, carcinoma in situ and laryngeal carcinoma (**21**). Warnecke et al. obtained a sensitivity of 90% and a specificity of 93.75% of the method, by applying the tool for the evaluation of 42 specimens of precancerous and cancerous pharyngo-laryngeal lesions and remarking that contact endoscopy offers valuable support for rapid intra-operative evaluation of oropharyngeal, hypopharyngeal, and laryngeal mucosa, but it does not replace biopsy sampling (**22**).

Literature data reported high values of the specificity, sensibility, PPV, NPV of the NBI illumination technology (**4**, **9**, **24-26**). Muto et al. first reported the importance of NBI endoscopic assessment in early detection of superficial oropharyngeal and hypopharyngeal preneoplastic lesion or carcinoma at early stage (**4**) and Watanabe et al remarked the role of Narrow Band Imaging (NBI) in the identification process of synchronous or metachronous lesions of head and neck (**6**). More recently, Peretti et al performed the NBI endoscopic evaluation in 279 consecutive patients affected by laryngeal squamous cell carcinoma or previously treated for the same disease, confirming the high sensitivity and specificity of NBI system; particularly if coupled with a HDTV system, which is available for rigid telescopes only (**24**). Iraja et al reported the same results, applying NBI endoscopy in their patients' population, 77% of whom in a follow-up program after previous diagnosis of head and neck carcinoma (**25**).

Our results showed the high value of NBI sensitivity and specificity in detecting premalignant laryngeal lesion or early cancers in a screening setting. Further, according to Ni classification (**11**), IV-V type lesions are likely to be cancer or a precursors (32/35 cases, 91.4%). Comparing the PPV of

the in-office and intra-operative, no statistical differences were found thus the in-office NBI might be considered a valuable tool in a laryngeal carcinoma screening campaign. Similar to our results, other authors (**12**) found that NBI has a significantly higher sensitivity (97% vs 79%) rather than white light alone with a comparable specificity (96% vs 95%). In an other study, investigators demonstrated that the application of Ni classification (**11**) in their NBI practice increased the specificity by 38.6% and the PPV by 12.2% with a statistical significance compared to the in-office NBI endoscopies without the use of this classification (**13**).

However, we experienced that 3 IV-type lesions were histologically proven not to be neither a dysplasia or a cancer (florid ulceration, tuberculoma, amyloidosis). These pitfalls might be related to a high vascular pattern such as in florid ulceration or a cancer mimiking-lesions such as tuberculoma or amyloydosis which could led the authors to define those lesions on the morphological basis rather than on the NBI pattern.

In fact, occasionally, false-negative and false-positive results occur (**6**). Severe hyperkeratosis may cover a subjacent cancerous lesion, and thus lead to a false-negative result. Therefore, the immediate surroundings should always be examined for vascular atypias (**25**). Additionally, small vocal fold cancers can be confused with isolated respiratory papillomas, which was also true in our study (**6**,**13**) At the beginning of the learning curve, there is a risk of an increased number of false-positive results with subsequent unnecessary biopsies. The latter are usually caused by inflammatory or post-radiation changes. With appropriate experience, these can be distinguished from cancerous lesions by blurred edges as well as more regular and smaller spots (**25**).

Nevertheless, after an adequate 6-month training also performed in high-volume centre, the value of NBI is undisputed in malignant and premalignant lesions. Thus, NBI is helpful in the early detection of laryngeal cancer and its precursor lesions, determination of their horizontal extension, differentiation between high-grade and low-grade dysplasia, identification of unknown primaries, and definition of an adequate strategy. The potential benefits of the use of NBI in a screening campaign is evaluated by our study with compelling results. Early detection of laryngo-pharyngeal cancer is

always desirable because it permits a higher rate of therapeutic success and, consequently, a higher survival rate. On the basis of our results, we have routinely adopted the use the NBI also in screening sessions albeit further investigations are required. Futhermore we would highlight that the NBI system is connected with a High Definition (HD) endoscopic camera, which represents one of the main technological innovation during the last years in Upper Airways evaluation in comparison with indirect laryngoscopy and traditional fiberoptic-endoscopic laryngoscopy. We suggest an appropriate training and experience with HD systems, if available, before approaching the laryngeal assessment by means of NBI system. This is more true for younger ENT, if an overestimation of laryngeal lesions has to be avoided.

CONCLUSION

Considering the strong association between age rising and cancer development as well as the risks factors constantly present in the European society, endoscopic screening or follow-up programs should be developed to improve the identification of earlier neoplastic lesions, if better prognosis and reduction of health service cost have to obtained. The ideal diagnostic tool for targeting biopsies should be easy to perform, accurate and inexpensive but, up to now, this is not available yet. Our results suggest that the NBI system endoscopic assessment of the larynx could represent a valid screening diagnostic tool, mainly for its high sensitivity and specificity reported.

Table 1. Ni N.B.I. endoscopic classification	
Type I	lesions correspond to thin, oblique, and arborescent vessels without any intraepithelial papillary capillary loop.
Type II	lesions show enlarged, oblique, and arborescent vessels without any intraepithelial papillary capillary loop.
Type III	vessels are obscured or seen indistinctly by white mucosa
Type IV	lesions, intraepithelial papillary capillary loop can be recognized as regular, small, and dark brown spots.
Type Va	lesions, intraepithelial papillary capillary loop appears as solid or hollow, with a brownish, speckled pattern, and various shapes.
Type Vb	or as irregular, tortuous, line-like shapes.
Type Vc	or as brownish speckles or tortuous, line-like shapes with irregular distribution, scattered on the tumor surface**.**

Table 2. Characteristics of the study population.			
	No.(%)		No.(%)
Sex • Male • Female	102(64.6) 56(35.4)	Dysphonia	49(31)
		Laryngopharyngeal reflux symptoms	31(19.6)
		NBI findings according to Ni et al.[11] classification at first screening endoscopy	
Age (*mean±SD)*	*61.4±11.4*	• Type I	61(38.6)

Smoking Status		• Type II	42(26.6)
• No	80(50.6)	• Type III	19(12)
• Light smoker	13(8.2)	• Type IV	9(5.7)
• Moderate smoker	14(8.9)	• Type V(a, b, c)	27(17.1)
• Heavy smoker	32(20.3)	SD= Standard Deviation; NBI= Narrow Band Imaging	
• Former smoker	19(12)		
Alcohol consumption			
• No	117(74.1)		
• Light drinker	27(17.1)		
• Moderate drinker	7(4.4)		
• Heavy drinker	7(4.4)		

Table 3. Correlation between in-office endoscopic NBI classification and definitive histopathology (n=73).

Histopathology	Ni type I	Ni Type II	Ni Type III	Ni Type IV	Ni Type V	Total No.(%)
Malignant Lesions						21(28.8)
• Invasive SCC	-	-	-	-	12	12
• Microinvasive SCC	-	-	-	-	7	7
• In situ SCC	-	-	-	1	1	2
Premalignant lesions						12(16.4)
• Severe dysplasia	-	-	-	2	1	3
• Moderate dysplasia	-	-	1	-	3	4
• Mild dysplasia	-	-	-	2	3	5
Benign Lesions						40(54.8)
• Laryngeal edema	11	1	-	-	-	12
• Chronic laryngitis	3	2	-	-	-	5
• Hyperkeratosis	3	6	3	-	-	12
• Respiratory papilloma	-	1	2	-	-	3
• Florid ulceration	-	-	1	1	-	2
• Vocal fold nodule	1	1	-	-	-	2
• Amyloidosis	-	-	1	1	-	2

• Granuloma	1	-	-	-	-	1
• Tuberculoma	-	-	-	1	-	1

NBI= Narrow Band Imaging; Ni type I= thin oblique arborescent vessels; Ni type II= enlarged oblique arborescent vessels; Ni type III= vessels obscured or seen indistinctly by white mucosa; Ni type IV= regular small dark brown spots; Ni type V= irregular dark brown spots or tortuous line-like shapes; SCC= Squamous Cell Carcinoma.

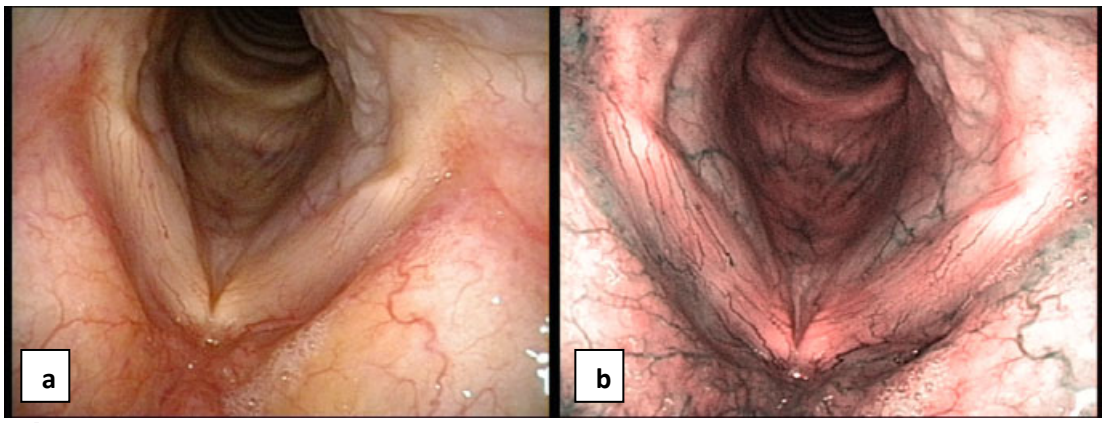

Figure n°1(a Whit Light-b NBI) TYPE I Ni classification endoscopic views of vocal folds: thin, oblique and arborescent vessels are interconnected. Intraepithelial papillary capillary loops are invisible.

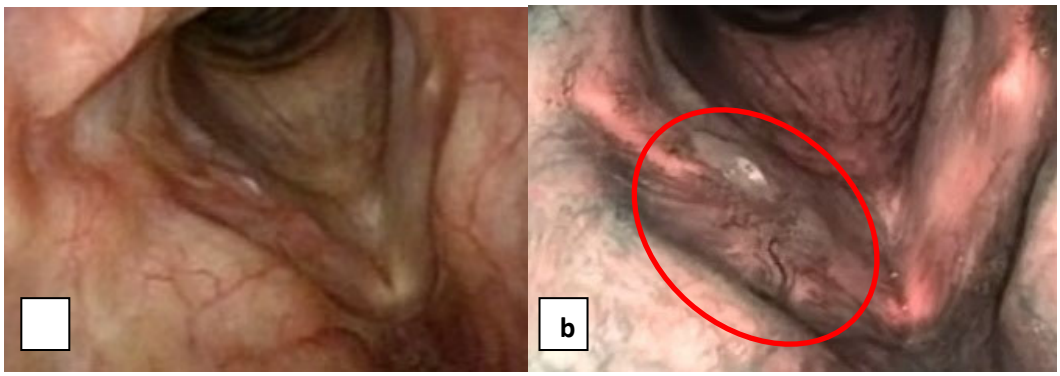

Figure n°2(a Whit Light-b NBI) TYPE IV Ni classification endoscopic views of vocal folds: intraepithelial papillary capillary loops can be recognized as small dots, with afferents vessels

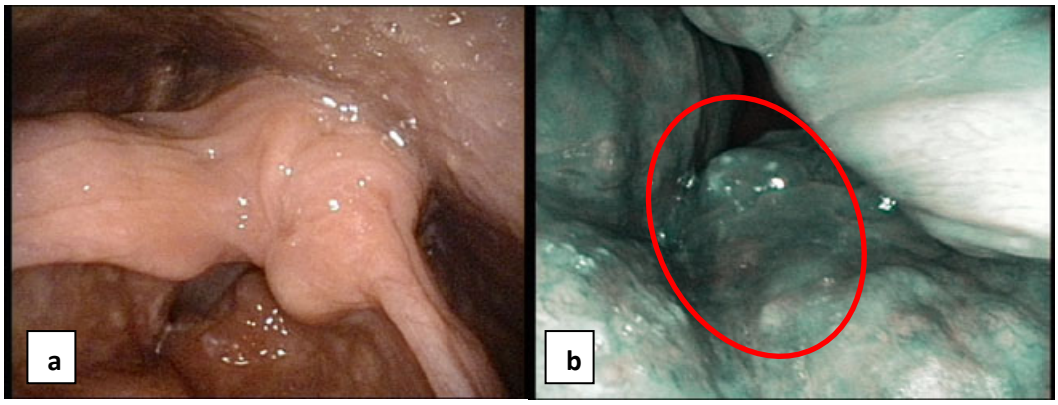

Figure n°3(a Whit Light-b NBI) TYPE Vc Ni classification endoscopic views of vocal folds: intraepithelial papillary capillary loops appear as brownish speckles or tortuous, line-like shapes with irregular distribution, scattered on the tumour surface

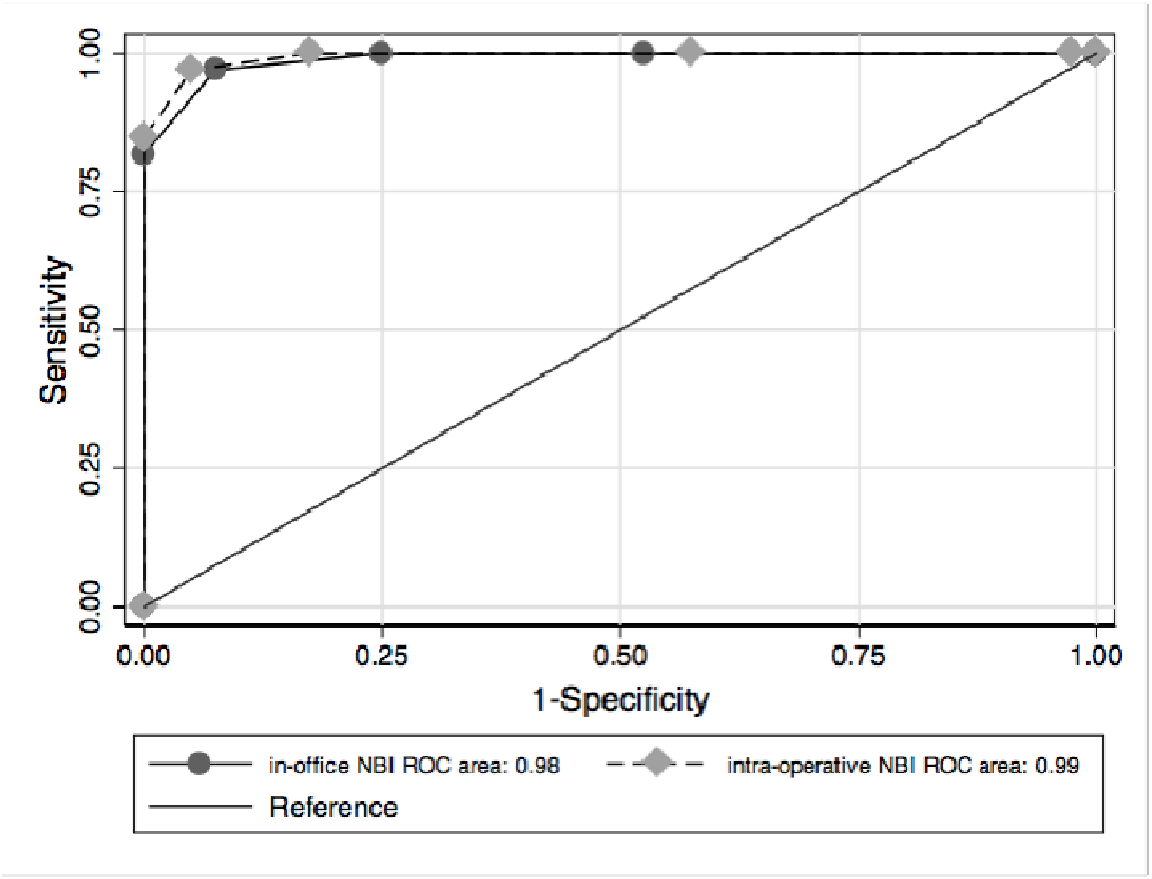

Figure n°4: Comparative ROC curves which confirmed a slightly higher performance for the intra-operative NBI evaluation without any statistical significance(p=0.41)