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Autofluorescent Endoscopy as a New Modality in Diagnostics of Laryngeal Premalignant and Malignant Lesions

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ABSTRACT

Objective: In this prospective study, we compared the diagnostic potential of Pentax's System of Autofluorescent Endoscopy (SAFE 1000) with standard microlaryngoscopy in the diagnosis of laryngeal precancerous and malignant lesions.

Methods: We compared the sensitivity and specificity of microlaryngoscopy and SAFE 1000 in a total of 128 patients with various laryngeal pathologies. Fiberoptic endoscopic examinations of the larynx in white light and autofluorescent mode were performed, followed by microlaryngoscopy with obtaining biopsy from suspected areas, performed by another endoscopist. During the microlaryngoscopy, the endoscopist evaluated images provided by SAFE and compared them with the findings of microlaryngoscopy and, if necessary, took biopsies from areas with autofluorescent irregularities. Histopathological examination of biopsy samples was used as a "gold standard" for definitive diagnosis and for comparison of studied methods.

Results: Overall sensitivity of SAFE was higher than the sensitivity of microlaryngoscopy (89.06% vs. 83.59%, $P=.016$). Specificity of detection of defect in autofluorescent signal in cases with laryngeal premalignancies and malignant tumors by SAFE 1000 endoscopy was 81.71%. The results suggest that SAFE has a significantly higher sensitivity than standard MLS in detection and description of precancerous and malignant lesions of the larynx. The specificity of SAFE 1000 was relatively insufficient, suggesting the necessity for histopathological analysis.

Conclusion: SAFE 1000 could be a powerful tool in recognizing and describing premalignant and malignant lesions of the larynx in addition to standard microlaryngoscopy. Authors recommend the simultaneous use of SAFE 1000 and microlaryngoscopy in the diagnostics of laryngeal lesions, in order to obtain the most accurate diagnostic results.

Keywords: Larynx, premalignant lesions, carcinoma, endoscopy, autofluorescence

Introduction

Exact diagnosis, therapeutic choice, and prognosis of pathologic lesions of the larynx are generally dependent on early recognition and precise description of laryngeal lesions, particularly for premalignant lesions and malignant tumors. Microlaryngoscopy (MLS), an endoscopic procedure with usage of operative microscope, performed under general anesthesia, and introduced by Oskar Kleinsasser in 1962,[1] with biopsy and/or complete endoscopic removal of laryngeal lesions, is currently worldwide accepted method for discovering and precise description of laryngeal lesions. Pathohistologic analysis of biopsy specimen usually followed MLS.[1]

In order to improve sensitivity and specificity, as well as to establish a less invasive procedure than MLS, performed without general anesthesia, many efforts to develop other endoscopic techniques for diagnostics of laryngeal lesions were tried.

The term autofluorescence represents the capability of tissue to emit fluorescent light of specified wavelength when it is excited by another light wavelength. The phenomenon of autofluorescence is based on certain biochemical and biophysical variations of metabolism and the presence of some molecules in healthy and malignant tissue. In normal cells, aerobic cycle of glycolysis appears as a major way for producing energy in mitochondria. One of the key coenzyme in aerobic

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pathway of glycolysis is flavin mononucleotide (FMN), which, when oxidized, emits green light during exposure to blue light. Some other substances like elastin, collagen, porphyrins, nicotinamide dinucleotide (NADH), and nicotinamide dinucleotide phosphate (NADP) have the ability to fluoresce in green field of wavelength when exposed to blue light wavelength too.[2] These substances are named fluorophores. In cytoplasm of atypical cells, precancerous and malignant tissues dominate anaerobic cycle of glycolysis with accumulation of lactic acid. Previously mentioned fluorophores do not exist in atypical cells, therefore, these tissues do not emit green fluorescence when exposed to blue light.[3]

The aforementioned biochemical and biophysical features of tissues enabled the progress of new endoscopic techniques, which are based on variations of healthy and neoplastic tissue's metabolism and fluorescence. All of these devices contain different variants of monochromatic blue light source (Xenon lamp with adequate filters or monochromatic laser), which stimulates fluorophores in healthy tissue to emit green fluorescence. Camera captures and intensifies this fluorescent signal, and shows it as a picture on monitor, where healthy tissue is shown as a green area. Green autofluorescence is absent in precancerous and cancerous tissues which appear as a dark field.[2,3]

Devices developed to date which use autofluorescent endoscopy (AFE) are presented in Table 1.

Narrow band imaging (NBI) and Storz Professional Enhancement System (SPIES) are advanced autofluorescent endoscopic techniques for the evaluation of microvasculature patterns and neoangiogenesis on mucosa surface of the upper aerodigestive tract.[4-7]

In the first autofluorescence study of the larynx by Harries et al.[8] LIFE system was used. LIFE, D-Light AF, and NBI systems were studied in diagnostics of laryngeal lesions by majority of authors. Pentax's SAFE 1000 was used by Mostafa et al.[9] authors of the present article,[3] and Caffier et al.[10] who used a newer variant of the SAFE system (Pentax SAFE 3000).

Objective

This prospective study was conducted to evaluate the diagnostic potential of AFE in comparison to MLS in diagnostics of laryngeal lesions, principally in patients with precancerous and malignant lesions. This was performed by assessment of sensitivity and specificity of Pentax's SAFE 1000 system).

Table 1. Devices for Endoscopic Autofluorescent Diagnostics

Name	Abbreviation	Company	Country
System of Autofluorescent Endoscopy	SAFE 1000	Pentax	Japan
Laser Induced Autofluorescent endoscopy	LIFE, LIAF	Xillix	Canada
D-Light Autofluorescent System	D-Light AF	Storz	Germany
Narrow Band Imaging	NBI	Olympus	Japan
Storz Professional Enhancement System	SPIES	Storz	Germany

Main Points

- Autofluorescent endoscopy (AFE) performed by the Pentax SAFE 1000 system enables statistically higher sensitivity than standard microlaryngoscopy (MLS) in diagnostics of laryngeal premalignant lesions and cancer.
- Specificity of this method is considerably low, due to increased blood content in different lesions of the laryngeal mucosa, which causes false positive results of AFE.
- Procedure of fiberoptic transnasal white light and AFE of the larynx does not require general anesthesia or biopsy and is considerably less traumatic than standard MLS.
- Authors recommend simultaneously performing AFE and standard MLS in order to improve detection and precise evaluation of laryngeal lesions, as well as obtaining more precise biopsy tissue.

Materials and Methods

Pentax SAFE 1000 system contains Xenon lamp of 75 W (Pentax 750AF) as a standard light source for classic white-light endoscopy (WLE). Broadband filter, when turned on, cuts all wavelength of white light from this source, except range 420-450 nm (blue light), which is transmitted by fiberoptic endoscope to endolaryngeal surface. This blue light stimulates fluorophores contained in laryngeal mucosa to produce green light of fluorescence, whose wavelength is around 500 nm. Fluorescence camera integrated in SAFE 1000 captures this green fluorescent signal and contains another specific fluorescence filter which allows only green light of fluorescence to be presented on the monitor. Images could be recorded on digital media too.

Due to the presence of natural fluorophores, normal (healthy) laryngeal mucosa appears as a clear and strong green field (wavelength around 500 nm) on the monitor.

In total, 128 patients (117 male, 11 female) with ages varied from 31 to 81 years (average 57) were studied. All patients had one or more laryngeal complaints (e.g., dysphonia, cough, dysphagia, throat foreign body feeling, etc.), different mirror appearances in the larynx, and had indications for MLS. Following a detailed explanation of the procedures in this study, adequate informed consent was obtained from each patient. The Ethics Committee of our institution approved this research, which was conducted in accordance with the ethical principles of the Helsinki Declaration (Approval No. MFVMA 06/16-18/).

After local use of anesthesia (Lidocaine® spray), transnasal endoscopic examination of the endolarynx with the fiberoptic endoscope Pentax FB-18RX in white light and autofluorescent mode was performed, and findings were recorded on digital media. After fiberoptic examination, another skillful ENT endoscopist performed MLS, noted his observation, and obtained adequate biopsies. At that moment, he had no knowledge about the results of SAFE endoscopy. Then, during the

same MLS, he evaluated images obtained by SAFE, compared them with finding in the larynx during MLS and, if necessary, provided new biopsies from regions with autofluorescence disorders. The same pathologist studied all of these biopsy specimens. If MLS and SAFE endoscopy have not discovered any lesion in larynx, we considered that there was healthy laryngeal mucosa and biopsy was not performed.

We compared diagnostic parameters (sensitivity and specificity) of SAFE and MLS, where pathohistologic result of biopsy samples was considered as a "gold standard" for definitive diagnosis. True and false results have been noted for each diagnosis and for both methods. Sensitivity and specificity of both of these methods for all pathologic lesions in the study were compared separately. For statistical evaluation of results, Fisher's exact test in data analysis statistical software PASW 18 was used.

Results

Normal (healthy) mucosa of the larynx, chronic laryngitis, mild (SIN I), moderate (SIN II), and severe (SIN III) epithelial dysplasia, keratosis, papilloma, vocal cords hematoma, and invasive carcinoma were discovered in this study. Results of sensitivity (Sn) of both methods in all studied pathology of the larynx (MLS and SAFE) are presented in Table 2, where true positive result is labeled as "+," and false negative as "–."

Overall sensitivity of autofluorescent mode in SAFE endoscopy for all laryngeal pathology noted in this study was 89.06%, while sensitivity of MLS was 83.59%. For the overall sensitivity of SAFE and MLS in recognizing of total 128 cases of laryngeal lesions, Fisher's exact test gave $P=.016$, so there was a statistically significant difference.

High statistical significance was noted by using studied methods in diagnostics of mild and moderate dysplasia, but in those cases, MLS showed much better sensitivity (7/7) than SAFE endoscopy (0/7), $P=.000$. None of these lesions showed any autofluorescence disturbance. In those cases, highly statistically significant difference ($P=.000$) was in favor of MLS.

SAFE endoscopy achieved statistically significant better sensitivity in detecting the premalignant lesion—severe dysplasia SIN III ($P=.026$) and invasive carcinoma ($P=.028$) than standard MLS.

Statistically significant difference in sensitivity of studied methods was not found in diagnostics of laryngeal papilloma ($P=.228$), keratosis ($P=.221$), submucosal hematoma ($P=1.000$), and chronic laryngitis ($P=1.000$).

A defect of autofluorescence was discovered in 82 patients. All cases with autofluorescence deficiency are listed in Table 3. True positive results were found in total of 67 cases (39 with invasive squamocellular carcinoma and 28 with severe epithelial dysplasia—SIN III). False positive results as a defect of the autofluorescent signal were provided by SAFE endoscopy in 2 cases of laryngeal subepithelial hematoma, 7 cases of papilloma, and 6 cases with chronic laryngitis—in a total of 15 patients (Table 3). Specificity of detection of defect of autofluorescent signal in cases with laryngeal premalignancies and malignant tumors by SAFE endoscopy was 81.71%. False positive results in the sense of defect of autofluorescence appeared in 18.29% of all studied cases.

Discussion

Healthy laryngeal mucosa, due to presence of previously mentioned fluorophores, was presented as a clear green autofluorescent signal emitted from mucosa of all structures of the larynx (Figure 1a and b).

Laryngeal mucosa covered with epithelium with mild and moderate degree of epithelial dysplasia (SIN I and II) did not show any autofluorescence disturbances too (sensitivity 0%), but all of these 7 lesions were recognized only by MLS (sensitivity 100%) as a variety of slight irregularities on mucosa surface.

Table 2. Sensitivity of MLS and SAFE Endoscopy in Diagnostics of Laryngeal Lesions

		Detected Cases (True Positive) and Sensitivity (Sn %)				SAFE +	SAFE +	SAFE –	
		SAFE		MLS					
Pathohistological Analysis	No	No	Sn %	No	Sn %	MLS +	MLS –	MLS +	*P*
Healthy laryngeal mucosa	9	9	100	9	100	9	0	0	1.000
Chronic laryngitis	11	11	100	11	100	11	0	0	1.000
Hematoma	2	2	100	2	100	2	0	0	1.000
Keratosis	23	23	100	18	78.26	18	5	0	.221
Papilloma	9	7	77.78	9	100	7	0	2	.228
SIN I and II dysplasia	7	0	0	7	100	0	0	7	.000
SIN III dysplasia	28	25	89.28	19	67.86	17	9	2	.026
Invasive carcinoma	39	37	94.87	32	82.05	32	5	2	.028
Carcinoma and SIN III total	67	62	92.54	51	76.12	49	14	4	.028
Total	128	114	89.06	107	83.59	96	20	12	.016

Sn, Sensitivity; +, true positive; – false negative.

Table 3. Specificity (Sp)—True and False Positive Findings of Autofluorescence Defect

Pathohistological Diagnosis with Defect of Autofluorescent Signal		No of Patients					
		n			%		
True positive	Invasive carcinoma	39	Σ	67	47.56	Σ	81.71 (Sp)
	Severe epithelial dysplasia (SIN III)	28			34.15		
False positive	Subepithelial haematoma	2	Σ	15	2.44	Σ	18.29
	Chronic laryngitis	6			7.32		
	Papilloma	7			8.54		
Σ			82			100	

Severe epithelial dysplasia (SIN III) is considered as a premalignant lesion, which demonstrated significantly decreased intensity or total defect of autofluorescence due to the absence of fluorophores (Figure 2a and b), and was detected in 25 of 28 cases (sensitivity 89.28%), while19 of these lesions were recognized by classic MLS (sensitivity 67.86%). Autofluorescent endoscopy achieved significantly better sensitivity than classic MLS in these cases.

Keratosis was recognized during the MLS as leukoplakia in 18 of 23 cases (sensitivity 76.26%), while these lesions in SAFE mode were presented as a field with intensive autofluorescence (Figure 3a and b) in all cases (sensitivity 100%), due to presence of collagen in keratotic plaques. Collagen has the capability to release strong autofluorescence of around 450 nm wavelength. Thus, any lesion covered with keratotic plaques could be camouflaged and give a false negative result during AFE.

Two patients with vocal cord hematoma were diagnosed in the present study. The MLS revealed both cases of these lesions as blue fields at the vocal cord surface. In autofluorescent mode, they both were presented as an obvious total defect of autofluorescent signal (dark field). Hemoglobin is a powerful absorber for many autofluorescence wavelengths released from mucosa (Figure 4a and b). Any blood collection within laryngeal mucosa could be the cause of false positive result in diagnostics of laryngeal premalignant lesions and cancer.

Some of laryngeal papilloma presents high level of stromal vascularization. All cases of papilloma in our study were without epithelial dysplasia. Microlaryngoscopy recognized these tumors in all cases (9/9—sensitivity 100%), while SAFE

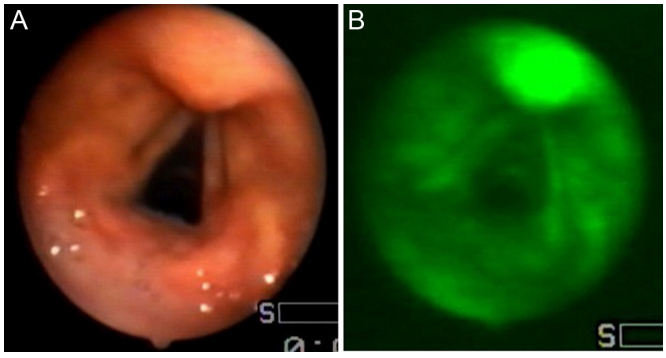

Figure 1. (a) White-light appearance of healthy larynx. (b) Autofluorescent appearance of healthy larynx.

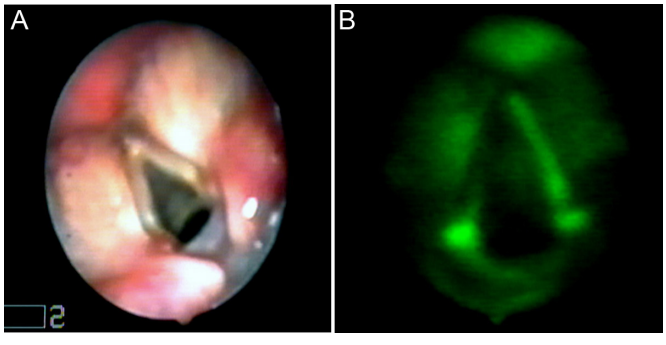

Figure 2. (a) White-light appearance of SIN III dysplasia of left vocal cord. (b) Autofluorescent appearance of SIN III dysplasia of left vocal cord.

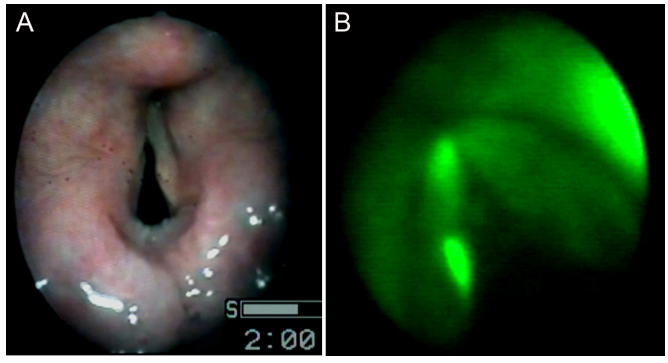

Figure 3. (a) White-light appearance of left vocal cord keratosis. (b) Autofluorescent appearance of left vocal cord keratosis.

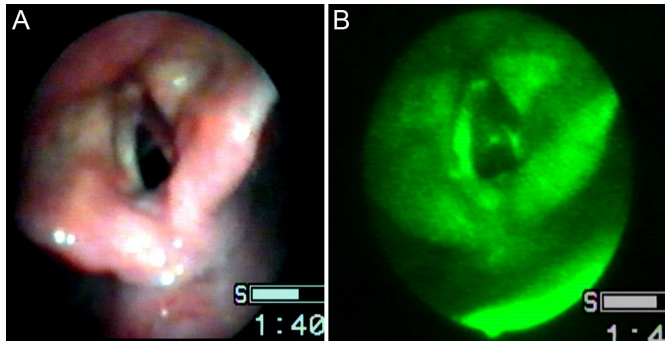

Figure 4. (a) White-light appearance of right vocal cord hematoma. (b) Autofluorescent appearance of right vocal cord hematoma.

endoscopy achieved positive findings in 7 of 9 cases, as a reduction of autofluorescent signal due to the presence of increased blood and hemoglobin amount in these tumors, which absorbed green autofluorescence. Because of that, we assumed that SAFE endoscopy provided false positive resul ts—autofluorescence defect in these 7 cases.

In 6 of the total 11 cases of chronic laryngitis, we established obviously reduced autofluorescent signal, also due to the presence of increased blood content in chronically inflamed mucosa and submucosa (hyperemia due to chronic inflammation). This appearance was interpreted as false positive results of SAFE endoscopy because there was no severe dysplasia or malignant tumor.

Investigators who used devices for AFE in the diagnostics of premalignancies and malignant tumors of the larynx generally stated that AFE has significantly higher rate of false positive results (low specificity), which is the main drawback of the AFE of larynx.[3,11,12] Any amount of blood on the surface, within, or under laryngeal mucosa could cause reduction of autofluorescence and lead to a false positive finding for the presence of a premalignant or malignant lesion. In the literature, it was published that false positive results of AFE were founded in cases with mucosal hyperemia, inflammation, scar formations, and granulomas after different endoscopies, open surgical procedures, and irradiation.[3,11-13]

Very good sensitivity (94.87%) of SAFE method was found in the detection of laryngeal carcinoma (37 of 39 cases) in our study (Figure 5a and b). In 5 of 39 cases with MLS visible laryngeal carcinoma, histologic examination of provided biopsy material did not verify the expected malignant disease. Each of these 5 malignant tumors was exactly identified in SAFE mode. In those cases, histopathological examination in the next biopsy taken from the area with a defect of autofluorescence has reported the presence of squamocellular carcinoma. Autofluorescent endoscopy can serve as a guide for taking biopsy.

Two undetected cases of laryngeal carcinoma during AFE were covered with keratotic layer, and in this way, gave intensified autofluorescent signal, camouflaging underlying carcinoma. In our and other studies, keratosis of laryngeal mucosa was noted as a single factor that led to false negative results in the detection of carcinoma and premalignant lesions during AFE endoscopy.[3,11]

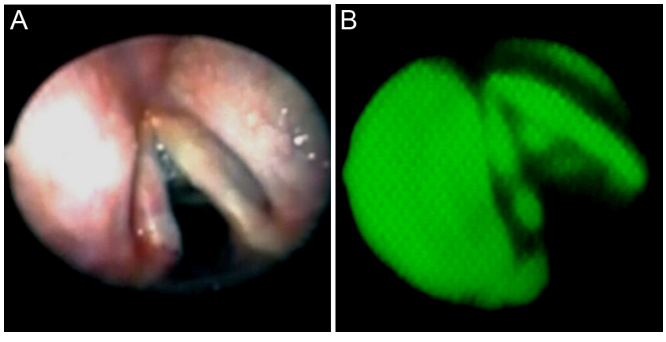

Figure 5. (a) White-light appearance of left vocal cord carcinoma. (b) Autofluorescent appearance of left vocal cord carcinoma.

Laryngeal carcinomas in our study were detected by MLS in 82.05% of cases. Other authors in that sense reported sensitivity of MLS between 69% and 96%.[3,11-13]

The majority of authors reported that autofluorescent endoscopic diagnostics is more sensitive (sensitivity 78%-100%) for discovering premalignant or malignant laryngeal lesions than standard WLE.[3,11-13] We have established that the sensitivity of autofluorescent mode and MLS in diagnostics of these lesions was 92.54% and 76.12% respectively.

Most authors also stated that a few false negative findings during AFE were mostly due to the presence of keratosis on mucosa which is considered previously.[3,11,13]

Mostafa et al[9] published similar sensitivity of SAFE 1000 in the detection of laryngeal cancer.

Caffier et al[10] used a newer variant of SAFE system (Pentax SAFE 3000) in the study of vocal cords pathology and achieved a sensitivity of 94% and a specificity of 69% in reference to histopathological diagnosis. Authors noted substantial defect of autofluorescence not only in malignant lesions but also in chronic inflammation, severe dysplasia, granulomas, vascular polyps, and papillomatosis.[10]

Zargi et al[11] in diagnostics of premalignant lesions and malignant tumors of the larynx, using LIFE system, reported a sensitivity 87% and a specificity of 71%. Microlaryngoscopy in the same patients' group provided these parameters at 77% and 81%.[11]

Succo et al[12] reported the sensitivity of autofluorescent mode was 96.5% with a specificity of 98.5%. Authors suggested that AFE could improve the precise assessment of tumor boundaries and enable tumor-free surgical margins after laser resection of the tumor.[12]

In the literature review, Kraft et al[13] found that all variants of autofluorescence endoscopy devices show a significantly higher sensitivity, specificity, and accuracy than WLE alone in detecting malignant and premalignant lesions of the larynx.

Wacławek et al[14] used autofluorescent NBI endoscopy in diagnostics of malignant neoplasms of hypopharynx and larynx and achieved sensitivity of 90.48% and high specificity of 91.14%.

Saraniti et al[4] in their literature review reported that NBI is an effective diagnostic tool for recognizing premalignant and malignant laryngeal lesions, particularly for evaluation of resection margins after different surgical procedures. They also stated that this procedure is suitable in cases where leukoplakia covers a possible malignant tumor.[4]

Similar statement was published by Piazza et al.[5] They found that NBI could serve as a good additional diagnostic tool for the endoscopic evaluation of patients with laryngeal and hypopharyngeal malignancies.

Wu et al in literature review found that the various optical imaging procedures in the head and neck oncologic patients (fluorescence imaging, high-resolution contact endoscopy, NBI, and the Raman spectroscopy) have similar advantages and limitations we have found in our study.[7]

Duration of fiberoptic AFE of the larynx is short, easy to perform, atraumatic (no necessity for general anesthesia and biopsy), and complications were not reported.

However, autofluorescent mode during SAFE endoscopy delivers "pseudo-color" picture of laryngeal mucosa in real time, which is relatively unclear. This endoscopic technique could be inadequate for satisfactory and precise imagining of laryngeal structures, predominantly in patients who have extensive morphological lesions within the larynx (massive tumors, inflammation, etc.), or underwent any reconstructive surgery or radiotherapy. The procedure is performed in local anesthesia, without relaxation, which could cause swallowing, coughing, and hypersalivation during endoscopy.

To achieve the best diagnostic results, we recommend synchronized evaluation of the larynx by MLS and autofluorescent mode.

Conclusion

In our study on large number of patients, we established that autofluorescent diagnostics in recognizing laryngeal premalignancies and carcinomas provided significantly superior results than standard MLS. Autofluorescent mode, simultaneously with MLS, could serve as a useful diagnostic tool, particularly for early visualization, exact determination of boundaries of lesions, and for more exact obtaining biopsy material for histologic evaluation.

Due to the aforementioned reasons, autofluorescent mode has some disadvantages, mainly because of lower specificity. Authors recommend that AFE should be performed carefully by a skilled laryngologist, simultaneously with classic MLS to achieve more precise diagnostics. However, histologic diagnosis remains the "golden standard" in laryngeal pathology diagnostics.

The authors also recommend the use of AFE as an addition to all other endoscopic methods for laryngeal pathology. A combination of WLE and AFE could substantially improve these diagnostics.

Ethics Committee Approval: Ethics Committee Approval was received for this study from the Ethics Committee of the Military Medical Academy, Belgrade, Serbia (Approval MFVMA 06/16-18/).

Informed Consent: Written informed consent was obtained from all subjects who participated in this study.

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