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Autofluorescent diagnostics in laryngeal pathology

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Abstract Autofluorescent diagnostics are based on the ability of oxidized flavin mononucleotide (FMN) in normal cells to emit green fluorescence when exposed to blue light. Neoplastic cells have significantly lower concentrations of FMN and do not emit green fluorescence. Autofluorescent endoscopy is designed for early, accurate and minimally invasive diagnostics for laryngeal pathology. This procedure has the ability to give information about the nature of laryngeal lesions without the devastation of tissue and has important advantages over standard biopsy. In our investigation we used the System of AutoFluorescent Endoscopy (SAFE 1000) designed by Pentax. We examined 38 patients using the SAFE 1000 system, and then all of the patients underwent laryngomicroscopy (LMS). In LMS, a biopsy was taken, and the diagnostic sensitivity of these two methods was compared according to the pathohistologic diagnosis. For statistical evaluation we used Fisher's exact test. We found that autofluorescent endoscopy has greater sensitivity in the detection of precancerous and malignant conditions in the larynx than standard laryngomicroscopy. We believe that autofluorescent endoscopy in addition to laryngomicroscopy gives a more accurate diagnosis of laryngeal pathology than laryngomicroscopy alone.

Keywords Autofluorescence · Diagnosis · Larynx

Introduction

Early detection and accurate determination of the localization and extent of benign growths, particularly precancerous lesions and malignant tumors of the larynx, are extremely important for their therapy and prognosis [6, 17]. Nowadays, laryngomicroscopy (LMS) with biopsy is a worldwide standard diagnostic procedure for the detection and exact description of laryngeal pathology. However, the detection and accurate description of laryngeal lesions can be a difficult task, requiring an experienced laryngologist. During LMS, endoscopists may experience problems trying to detect the existing changes and determining exactly their extent within the larynx because of its small diameter, the localization in the larynx, the submucosal position and visual characteristics similar to the surrounding tissue. Often it is difficult to take the most representative specimen for biopsy to give an accurate pathohistologic diagnosis. The diagnosis of different lesions in the larynx treated by surgery, radiotherapy and after some diseases and injuries is difficult [10].

LMS with biopsy is an invasive procedure performed under general anesthesia and is associated with a certain devastation of the delicate and specialized structures of the larynx, particularly the vocal cords. This problem is more difficult if it is necessary to repeat the LMS and biopsy for disease control, especially in patients who have undergone surgery and/or radiotherapy.

Because of this, the attempts to optimize diagnostic procedures to achieve more sensitive detection and accurate description of laryngeal pathology remain a challenge for the otolaryngologist [14]. Each diagnostic procedure that is able to give exact information about the nature of laryngeal lesions without devastation of the tissue has important advantages over standard biopsy. To improve the recognition of precancerous lesions and cancer, supravital staining of mucosa with toluidine blue [5], selective demonstration of tumor tissue using Lugol's solution [4] and many other methods have been tried. However, none of these methods improved diagnostics during LMS [7].

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Autofluorescence is a natural capacity of tissue to fluoresce when exposed to a certain light wavelength. This feature is a consequence of the presence of some substances in the tissue that fluoresce when exposed to a narrow wavelength range [3].

In 1924, Policard [16] observed the ability of tissue to fluoresce under certain conditions. In 1933, Sutro and Burmann described the phenomenon of the different fluorescences of normal and tumorous tissue. Alfano in 1984 [2] reported the possibility of differentiating between healthy and malignant tissue by means of their fluorescent characteristics.

The German biochemist Otto Warburg observed some biochemical differences between normal and abnormal cells. Neoplastic cells have predominant anaerobic cycles of glycolysis with subsequent accumulation of lactic acid [1]. Flavin mononucleotide (FMN) is present in normal cells as a coenzyme in the aerobic glycolytic cycle, but not in the anaerobic glycolytic pathway. When it is excited with blue light, the oxidized FMN emits green fluorescence.

These biochemical and biophysical characteristics of tissue led to the development of diagnostic devices for autofluorescent endoscopy, which is based on the difference in the autofluorescence of normal and neoplastic tissue. These devices have a blue light source, which excites FMN in normal tissue to emit green fluorescence. A highly sensitive camera receives and amplifies this fluorescence and gives a video pseudocolor image on a high-resolution monitor in real time. In this image, normal tissue is presented as a green field, while precancerous and cancerous tissue does not emit green fluorescence, so it is presented as a dark field.

Pentax developed a device called System of AutoFluorescent Endoscopy (SAFE 1000) that uses a 75-W xenon lamp as a cold light source. Based on the observations of Palcic et al. [15] that it is possible to detect bronchial precancerous and cancerous lesions under blue light illumination, Xillix Corporation of Canada with the British Columbia Cancer Agency developed the Laser-Induced Autofluorescent Endoscopy (LIFE) system. This system uses a helium-cadmium laser as a source of monochromatic blue light (442 nm). Storz designed the D-Light AF System, which uses a 300-W xenon lamp as the excitation light.

Several studies on the usage of autofluorescence in the differentiation of normal and cancerous tissue have been published. The majority of authors have used the LIFE system for their studies. Autofluorescent endoscopy began with the evaluation of the tracheobronchial system [9, 13]. Evaluation of the larynx with the LIFE system was begun in the year 1995 by Harries and al. [8]. There was no available literature about the usage of the SAFE 1000 device in the examination of laryngeal pathology. Zargi et al. in 2000 [18] published that the sensitivity of autofluorescence in laryngeal pathology diagnostics was 87%, while specificity was 71%. The LIFE mode achieved better results in sensitivity, but there was no significant difference between the white-light and LIFE modes. The same authors stated that the image gained during autofluorescent endoscopy

was relatively unclear in comparison with standard LMS. They also reported that the presence of blood, bacterial plaques and necrotic tissue could produce a reduction of autofluorescent intensity and lead to false positive findings. Kulapaditharom et al. [12] found that the sensitivity of autofluorescence in the detection of cancer of the upper aerodigestive tract was 100%, while the sensitivity of white-light procedures for examination of the cavities of the head and neck was 87.5%. Kulapaditharom and Boonkitticharoen [11] stated that this procedure had great potential in the detection of head and neck unknown primary lesions with metastatic cervical lymph nodes.

Materials and methods

This pilot study was designed to examine the possibilities of the Pentax SAFE 1000 system in the diagnostics of laryngeal pathology. The SAFE 1000 device contains the light source Pentax LX-750AF with a Xenon lamp of 75 W, which is used for standard white-light endoscopy (WLE). In the autofluorescent (AF) mode the examiner switches on the special broadband excitation filter (EX filter), which allows only a blue light wavelength of 420 to 450 nm to pass through a fiberoptic instrument (bronchoscope) to the laryngeal mucosa. This light wavelength excites FMN in exposed tissue to emit green fluorescence. A video camera system incorporated in the SAFE 1000 contains an endoscopic CCD TV camera and fluorescence TV camera with a fluorescent filter, as well as an image intensifier that amplifies faint fluorescent signals. A switcher that inserts the EX filter simultaneously changes cameras for the WLE and AF modes [1]. The image intensifier controller (Pentax SAFE-1000c) processes the fluorescence images and transmits them to the high-resolution color monitor and video cassette recorder. Healthy laryngeal mucosa is presented by a strong signal in the green wavelength range (around 500 nm), but the signal is significantly decreased or absent in precancerous and cancerous tissues.

In this prospective pilot study, 38 patients (37 males, 1 female) were examined between November 2002 and March 2003. The age of the patients ranged from 35 to 73 years (average 53). All of them suffered from one or more laryngeal symptoms (e.g., hoarseness, dysphagia, foreign body sensation, cough, etc.).

After the patients received local epimucous anesthesia, the fiberoptic bronchoscope Pentax FB-18RX was introduced into the larynx. Endoscopic findings in the larynx under white light and under the autofluorescent mode were recorded onto VHS videotape. The representative images from the recorded material were captured via Win Coder software in a personal computer. These images were carefully evaluated and described by an ENT specialist. After SAFE endoscopy, another experienced ENT specialist performed LMS, noted the findings and then took biopsies from the pathologically changed areas. At that time the endoscopist had no knowledge about the findings of the previously performed SAFE endoscopy. After that, the endoscopist analyzed the images gained by SAFE and took biopsies from the non-biopsied areas that had presented autofluorescent disturbances. These biopsy specimens were evaluated by the same pathologist who had established the definitive pathohistologic diagnosis. If both of the methods did not demonstrate any pathologic change in the larynx, we assumed that there was healthy laryngeal epithelium.

The sensitivity of SAFE and LMS were compared according to the pathohistologic diagnoses from biopsy specimens. Which method gave true or false detection of the lesion was noted in each case.

At first, we compared the overall sensitivity of both methods in all pathologic diagnoses. Then we compared the sensitivity of methods for single pathologic conditions where the difference between the number of exact detections using SAFE endoscopy and LMS appeared. We used Fisher's exact test for statistical evaluation of results.

Table 1 Results of laryngeal epithelium examination by SAFE endoscopy and LMS.
+ True positive finding;
– false negative finding

Pathohistology analysis	No	Detected (true positive)		SAFE + LMS +	SAFE + LMS –	SAFE – LMS +	<i>P</i>
		SAFE	LMS				
Healthy epithelium	5	5	5	5	0	0	1.00
Abnormal hyperplasia	12	10	8	6	4	2	0.32
Atypical hyperplasia	9	8	6	5	3	1	0.29
Parakeratosis	4	4	4	4	0	0	1.00
Invasive carcinoma	8	8	5	5	3	0	0.10
Total	38	35	28	25	10	3	–

Results

Table 1 presents the results of the examination. Healthy epithelium of the larynx, abnormal hyperplasia, atypical hyperplasia, parakeratosis and invasive carcinoma were found in this study. The overall sensitivity of the SAFE mode for all examined pathology of the larynx was 92.10%, while sensitivity of LMS was 73.68%. Three cases were diagnosed as false negatives by SAFE and 10 by LMS. There were no false positive findings.

Healthy laryngeal mucosa did not demonstrate any disturbance in autofluorescent intensity during SAFE endoscopy. Laryngeal lesions with abnormal and/or atypical hyperplasia showed significantly decreased intensity, but not a total defect of autofluorescence. Different changes on the laryngeal mucosa (hyperemia, edema, rough surface of vocal cords, etc.) were seen during LMS in cases with abnormal and atypical hyperplasia. Mucosa covered with keratin layers (parakeratosis) was seen during LMS as leukoplakia, and in the SAFE mode as a field with more intensive autofluorescence. With the SAFE mode, carcinoma was seen as a demarcated area with a defect of the autofluorescent signal (black field).

The difference in sensitivity of these methods appeared in the detection of abnormal hyperplasia, atypical hyperplasia and invasive carcinoma. Fisher's exact test for the overall difference of sensitivity of SAFE and LMS in diagnosing all 38 evaluated cases of laryngeal pathology was $P=0.03$, so a statistically significant difference between these two methods really existed. However, using the same test we have not found statistically significant differences between SAFE and LMS in the diagnosing of abnormal hyperplasia ($P=0.32$), atypical hyperplasia ($P=0.29$) and laryngeal cancer ($P=0.10$). For other lesions of the larynx there was no difference in the sensitivity of these two methods.

Discussion

Healthy laryngeal epithelium (Fig. 1a, b) in the SAFE mode presented neither a reduction nor a defect of autofluorescent light intensity. Epithelium with some degree of dysplasia like abnormal or atypical hyperplasia has revealed some deficit, but not a total defect of autofluorescence. This feature was seen as numerous nuances between green and

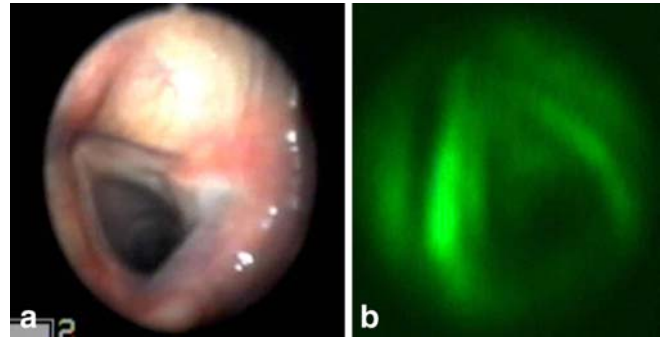


Fig. 1 **a** White-light fiberoptic (bronchoscopic) image of a normal larynx. **b** Autofluorescent (SAFE) image of the same normal larynx. There are no disturbances in autofluorescence intensity

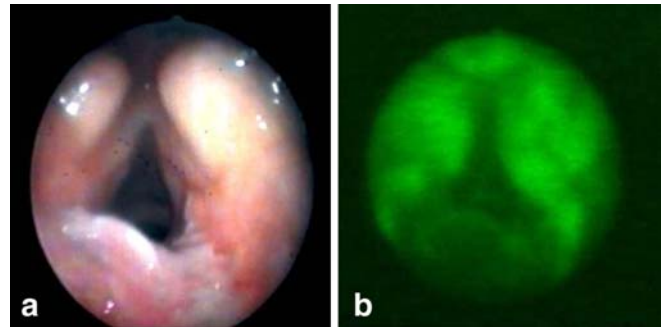


Fig. 2 **a** White-light image of atypical hyperplasia in the interarytenoid and left arytenoid regions. **b** Autofluorescent (SAFE) image of the same view. Atypical hyperplasia is presented as significant autofluorescent intensity reduction

gray color. Any lesion in the larynx covered with abnormal and/or atypical epithelium has demonstrated some degree of autofluorescence-decreased intensity (Fig. 2a, b).

The SAFE system can only detect changes on laryngeal epithelium, but not in deeper structures. For this reason, laryngeal nodule, polyp, Reinke's edema, papilloma and other lesions covered by normal epithelium will not result in any disturbance in the autofluorescent signal. However, if a reduction of autofluorescent intensity appears, it is necessary to consider this area as suspicious, and to take a biopsy.

Parakeratosis of the laryngeal mucosa was presented as a strongly demarcated field with more intensive autofluorescence (Fig. 3a, b). A possible problem could appear

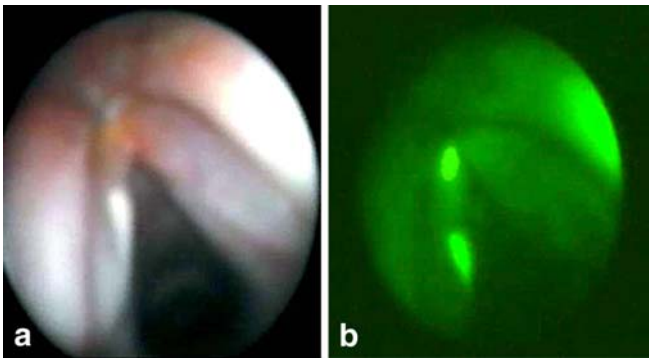


Fig. 3 **a** White-light image presenting left vocal cord leukoplakia (parakeratosis). **b** Autofluorescent (SAFE) image of the same view showing significantly increased autofluorescence intensity on the left vocal cord. This feature is associated with parakeratosis

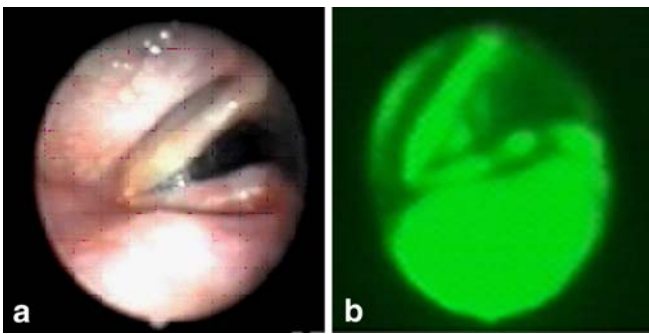


Fig. 4 **a** White-light image of the presenting tumor on the posterior third of the left vocal cord. Pathohistologic analysis revealed the presence of squamous cell carcinoma. **b** Autofluorescent (SAFE) image of the same view. On the posterior third a defect of autofluorescence is presented with the central field with increased autofluorescent intensity. There was a parakeratotic field on the tumor's surface

when a pathologic condition with a defect or reduction of autofluorescence is masked by the keratotic layer. Carcinoma in situ was not found in this study.

Undoubtedly, the most important fact is that all of the eight cases of laryngeal carcinoma were visualized in the SAFE mode as an obvious defect (black field) of autofluorescence (Fig. 4a, b). Three of the patients had undergone LMS before SAFE endoscopy, when the tumor was seen. However, pathohistologic analysis has shown an absence of the expected malignant disease. All of these cases were exactly detected by SAFE endoscopies as a defect of autofluorescence. Pathohistologic analysis of biopsy specimens from the field with defects of autofluorescence, provided by repeated LMS, reported the presence of squamocellular carcinoma.

Most of the authors have found that autofluorescent endoscopy is more sensitive in detecting laryngeal changes than the classic white-light endoscopy (78–100%). We found that the overall sensitivity of the SAFE mode and LMS was 92.10% and 73.68%, respectively.

Autofluorescent endoscopy of the larynx is a minimally invasive procedure that can be performed on an outpatient

basis using local epimucous anesthesia. The procedure is short, easy to perform and without complication. Biopsy is not required, so there is no trauma to the delicate laryngeal structures, which is important to patients who have had partial laryngectomies or received radiotherapy.

However, autofluorescent endoscopy of the larynx using the flexible endoscope (bronchoscope) has some disadvantages. The autofluorescent mode provides pseudocolor images of laryngeal structures, not the real image that can be gained under white light. Thus, the image is relatively unclear, and the method is insufficient for fine visualization of relatively complex laryngeal anatomic structures, particularly in patients who have had morphological changes of the larynx because of massive local disease, partial laryngectomy or applied radiotherapy. Zargi et al. [18] stated that the flexible endoscope is not appropriate for examination of laryngeal autofluorescence. They have used the rigid laryngeal telescope for this purpose.

Some conditions in the larynx can produce false positive and false negative findings. A dark field in an image may be the result of a shadow from an anatomic structure over this field. Blood does not contain FMN, and does not fluoresce. Hyperemia, haemangioma or hemorrhage on laryngeal mucosa may significantly reduce the intensity of autofluorescence. Bacterial plaques and necrotic tissue can lead to a defect of autofluorescence that leads to false positive findings. Zargi et al. [18] confirm these statements.

This is why the examiner must always pay attention to avoid false positive and false negative findings. The authors recommend simultaneous careful comparison of white-light and autofluorescent images of same view.

General anesthesia was not applied during SAFE endoscopy in this study, and the patients were not relaxed. During the procedure, there were some reactions to the presence of fiberoptic instruments, such as cough, deglutition and hypersalivation, which caused significant difficulties for some patients.

Conclusion

We found that SAFE endoscopy generally gives better results in the detection of laryngeal pathology than LMS, particularly in cases with laryngeal precancerosis and cancer. However, at this time, we do not have enough cases to evaluate the potential of SAFE endoscopy in the diagnostics of laryngeal pathology. It is necessary to achieve more experience in performing this procedure. Laryngomicroscopy with biopsy and pathohistologic examination is still a gold standard for the exact diagnosis of laryngeal lesions. Nevertheless, results of this study support a potential application of the SAFE system as an adjunct to conventional diagnostic methods for the larynx. The combination of autofluorescent endoscopy and LMS can greatly improve the diagnostics of laryngeal lesions. Autofluorescent endoscopy of the larynx provides minimally invasive detection of early cancer and precancerous lesions of the larynx and precise guided biopsy of these lesions.

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