ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Detection of early laryngeal cancer and its precursor lesions by a real-time autofluorescence imaging system

MARIOARA DOBRE, MARIOARA POENARU, NICOLAE CONSTANTIN BALICA, CAIUS ION DOROS

Department of ENT, "Victor Babeş" University of Medicine and Pharmacy, Timisoara, Romania

Abstract

The prognosis of laryngeal carcinoma patients can be improved by early diagnosis. The autofluorescence endoscopy has been developed to gain more information about the biologic character of the precancerous or cancerous lesions. The aim of the present study was to evaluate the diagnostic potential and limitations of this imaging technique applied during indirect laryngoscopy and comparing with white light and microlaryngoscopy with biopsy. In a prospective study, 56 patients with suspected precancerous or cancerous lesions were investigated preoperatively by white light and fluorescence endoscopy during indirect laryngoscopy. The procedure was followed by microlaryngoscopy and biopsy. Results were compared to histopathological findings. Normal laryngeal mucosa displayed a typical green fluorescence, moderate to high epithelial dysplasia, *in situ* carcinoma and cancer showed a diminished green fluorescence. In 47 of 56 (84%) cases, we found concordant results. The experience from this study shows that autofluorescence by indirect laryngoscopy may be a useful complementary method for detecting laryngeal malignancies. Its advantages are non-invasiveness, high sensitivity and repeatability.

Keywords: autofluorescence endoscopy, early diagnosis, precursor lesions of larynx, laryngeal cancer.

Introduction

Carcinomas of the upper aerodigestive tract represent a major problem in modern health care. Laryngeal cancer constitutes 1–2% of all malignancies diagnosed worldwide [1].

A recent U.S. study has pointed out that survival rates are significantly higher for early stage carcinomas [1]. Therefore, it is essential to concentrate on the initial steps in tumor development in order to facilitate early detection and timely implementation of suitable therapy [1].

Complete diagnosis of laryngeal pathology includes the detection of their presence, precise determination of their size and boundaries, and providing biopsy from adequate tissue to establish exact histopathological diagnosis [2].

Nowadays, the microlaryngoscopy (MLS) introduced in the sixties by Kleinsasser [3] is a worldwide standard diagnostic procedure for the detection and description of laryngeal pathology, as well as for many endoscopic microsurgical procedures involving the larynx.

Up until today, indirect and direct white light laryngoscopy was considered the standard imaging technique in the assessment of laryngeal cancer and its precursor lesions [4].

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 [6] and many other methods have been developed. However, none of these methods improved diagnostics during MLS [7].

Recently, autofluorescence endoscopy has been developed to enhance endoscopic information of mucosal lesions and has been successfully applied in different medical specialties, *e.g.*, urology, pulmonology and gastroenterology [8–11].

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 [12].

Autofluorescence endoscopy is based on the excitation of tissue-specific fluorescence of the mucosa by short-wave blue light of the visible spectrum (375–440 nm). The causes for changes of the fluorescence characteristics are complex. Changes in metabolism, reactive changes of the adjacent stroma and the frequent thickening of the epithelium are a few possible reasons [13]. Also, atypical and larger nuclei increase the loss of autofluorescence. In addition, autofluorescence is also markedly reduced when elastic fibers in the lamina propria are diminished.

The appearance and degree of autofluorescence depend especially on tissue structure's fluorophores content. Fluorophores are predominantly proteins, *e.g.*, elastin and keratin, as well as nicotinamide adenine dinucleotide (NADH) [14, 15]. In cancer cells, NADH is reduced, and the non-fluorescent oxidative form NAD prevails.

We describe the value and limitation of the method in the diagnosis of precancerous and cancerous lesions of the larynx applied during indirect laryngoscopy and the comparison with white light endoscopy and microlaryngoscopy with biopsy.

Patients and Methods

In this prospective pilot study, 56 patients (40 males, 16 female) were examined between December 2013 and July 2014. The mean age was 58 years (35–78 years). All of them suffered from one or more laryngeal symptoms (*e.g.*, hoarseness, dysphagia, foreign body sensation, cough, etc.).

In 40 (71.42%) of them, malignancy was suspected

after mirror laryngoscopy (Table 1). Benign pathology (Reinke's edema, papilloma's, polyps, cysts, nodules) was included for the basic evaluation of the system. Informed consent for the present study was obtained for each patient.

Table 1 – ***Diagnoses and malignancy suspected on mirror laryngoscopy***

Histological diagnosis	Malignancy suspected on mirror laryngoscopy		Total
	No	*Yes*	
Chronic laryngitis (simplex hyperplasia)	7	3	10
Nodules, polyps, cysts, Reinke's edema, papilloma (without dysplasia)	9	0	9
Precancerous lesions, keratosis, atypical hyperplasia, etc.	0	12	12
Laryngeal tumors	0	25	25
Total	16	40	56

All patients were evaluated preoperatively by white light and autofluorescence endoscopy during indirect laryngoscopy. The procedure was followed by micro-laryngoscopy with biopsy.

For white light and autofluorescence endoscopy, we used a 70^0 rigid hypopharyngoscope (Karl Storz, Germany) and the D-LIGHT C/AF System (Karl Storz, Germany). In this system, the excitation blue light source is provided by a Xenon arc lamp with an excitation wavelength ranging from 375 to 440 nm. The remitted excitation light up to 440 nm was blocked out by an integrated optic filter. This system allows real-time white light endoscopy and switches over to autofluorescence endoscopy by pressing a button. We used a high-resolution color charge coupled device (CCD-camera system, Endovision TRICAM SL-PDD and Endovision TELE-CAM SL-PDD, Karl Storz, Germany) camera system. All findings were recorded on a digital video system for documentation and analysis. Intraoperatively obtained tissue samples were taking according to the size of the pathology by excisional biopsy, *e.g.*, CO_2 laser decortication of the vocal fold, CO_2 laser cordectomy, or a biopsy in case of larger tumors. Results were compared then to histopathological findings.

Results

Histopathological findings of 56 cases revealed: 10 (17.85%) cases of hyperplasia, 16 (28.56%) cases of mild dysplasia, eight (14.28%) cases of moderate dysplasia, six (10.71%) cases of severe dysplasia/*in situ* carcinoma and 16 (28.56%) cases of laryngeal cancer.

Normal mucosa of the larynx with no visible alterations in either indirect white light or in autofluorescence laryngoscopy shows the typical homogeneous, pale green fluorescence. Transitional areas with different fluorescent properties between the healthy squamous epithelium of the vocal cords and the respiratory epithelium of the supra- and subglottic region cannot be identified. Vessels were better contrasted to the surrounding tissue due to light absorption by the hemoglobin. Areas of edematous swelling (*e.g.*, Reinke's edema) display normal fluorescence intensities.

In normal light, hyperplastic epithelium with or without mild dysplasia (non-cancerous) presented a milky-white aspect. Keratosis or leukoplakia appeared as a small white warty, sharply circumscribed lesion. According to its high autofluorescence intensity, keratosis appeared as a more-or-less bright green area.

In precancerous or cancerous alterations of the mucosa, the autofluorescence signal changed from green fluorescence to a reddish-violet color (Figures 1–4).

In some cases of severe dysplasia or *in situ* carcinoma, as well as infiltrating cancer, the reddish-violet autofluorescence signal was more intense when compared to moderate dysplastic changes. However, we were not able to differentiate between various higher grades of epithelial dysplasia. Moreover, we could not distinguish *in situ* carcinoma from invasive cancer. In some cases of laryngeal cancer, we were able to detect an orange fluorescence caused by bacterial overgrowth.

The diagnostic accuracy by white light laryngoscopy was 71% (n=40) and that of autofluorescence 84% (n=47).

The sensitivity by white light laryngoscopy for detection of precancerous as well as cancerous lesions was 85%, and specificity was 75%. The sensitivity by autofluorescence laryngoscopy was 92% and specificity to 87%.

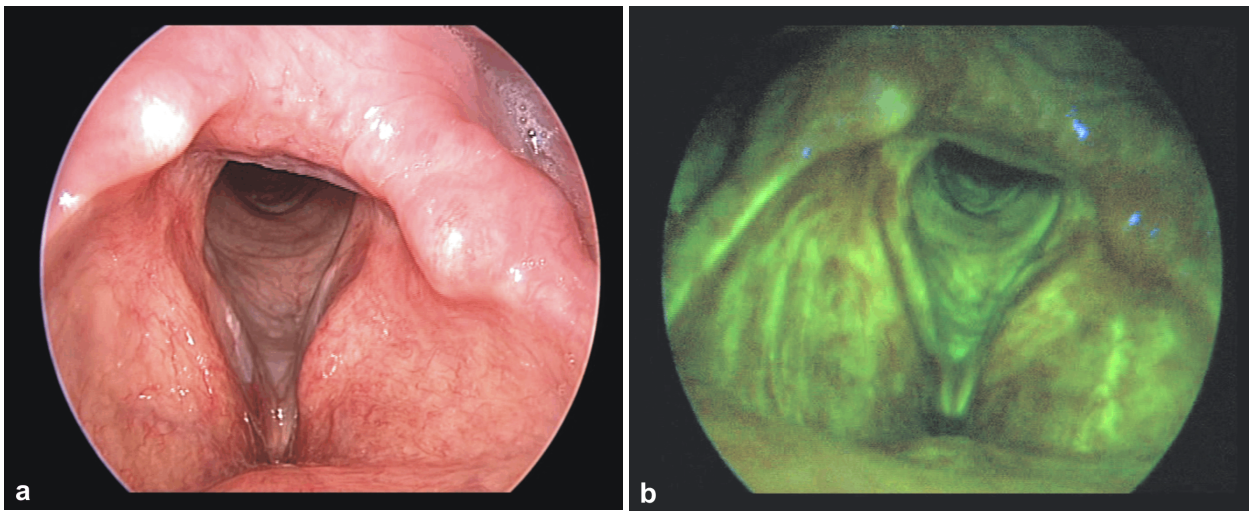

Figure 1 – ***Normal mucosa of the larynx with no visible alterations in either white light (a) or in autofluorescence laryngoscopy (b) displays the bright-green fluorescence.***

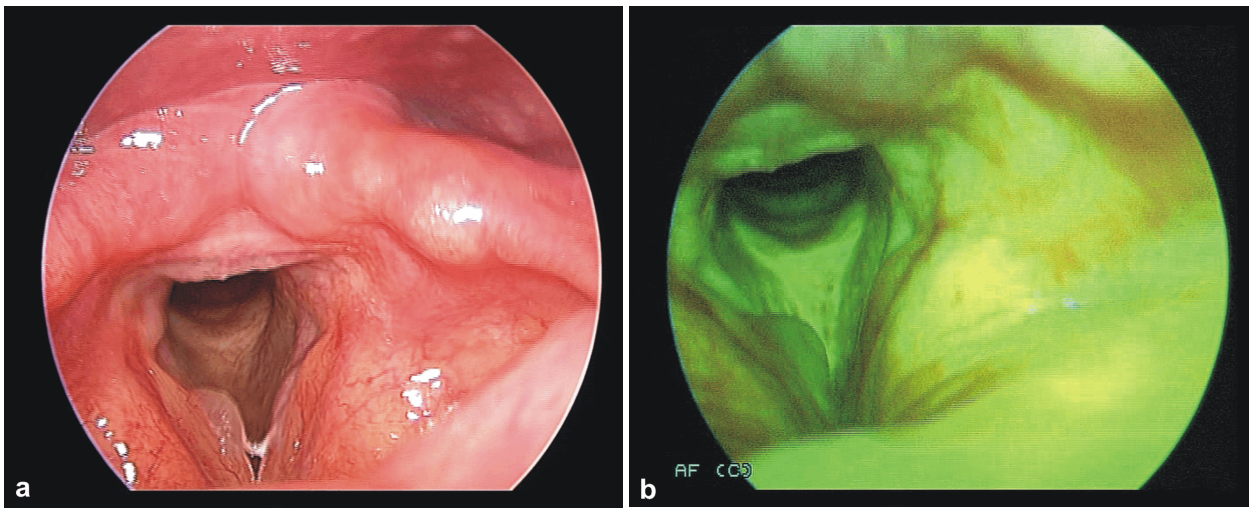

Figure 2 – ***Laryngeal cyst: White light endoscopy (a); Autofluorescence endoscopy (b) shows a homogeneous intensity and distribution.***

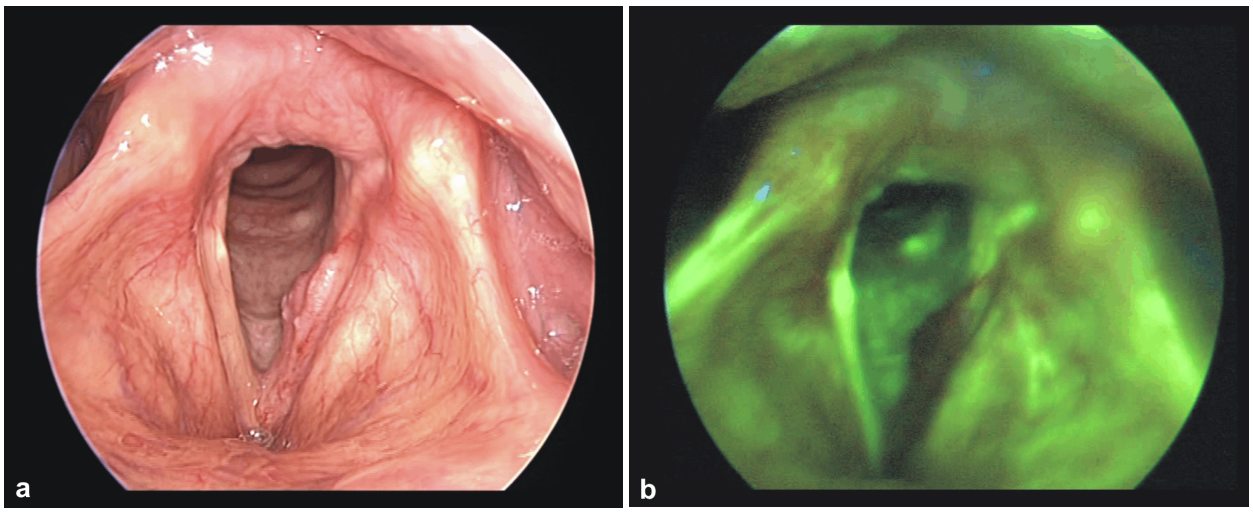

Figure 3 – ***Invasive carcinoma (pT1a): White light endoscopy (a) demonstrates a bulging tumor of the left vocal fold; Autofluorescence endoscopy (b) shows a marked loss of autofluorescence, whereas induced fluorescence endoscopy.***

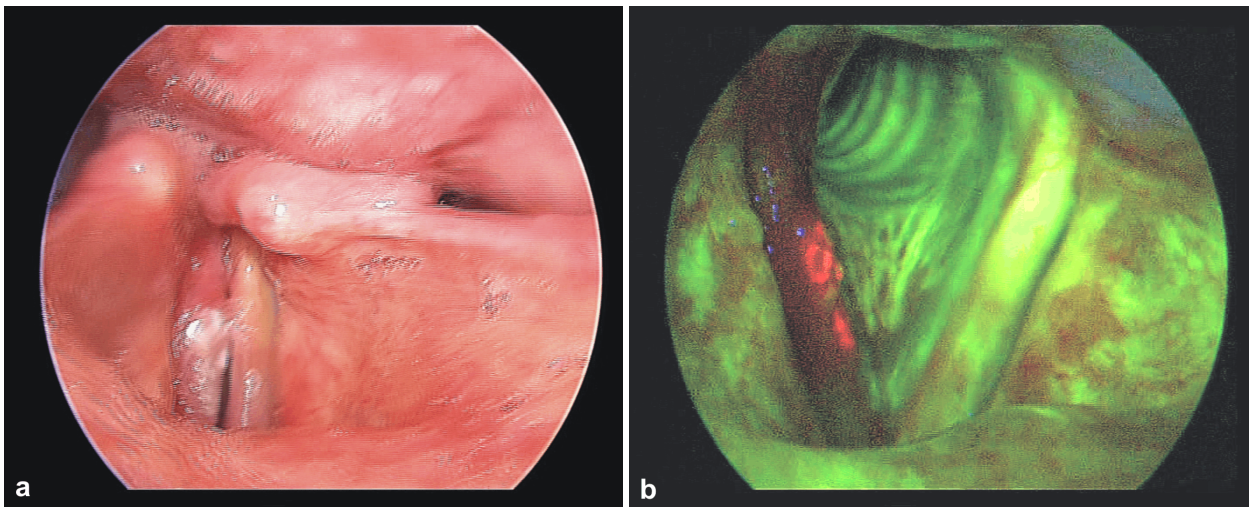

Figure 4 – ***Moderate differentiated squamous cell carcinoma of the right vocal fold with lesions of carcinoma in situ (pT1a): White light endoscopy (a); Autofluorescence endoscopy (b) shows a marked loss of autofluorescence, whereas induced fluorescence endoscopy.***

Discussion

Optimization of early diagnosis of laryngeal carcinomas represents an objective of ENT specialists. It is not only the key to reducing the mortality rate of oncological patients, but also the condition for improving the modalities of minimally invasive treatment. Early detection and preoperative assessment are important to a curative and function-preserving therapy, because the

treatment of laryngeal cancer and its precursor lesions has a great impact on important basic functions of daily life such as breathing, verbal communication and swallowing.

The AFE method exploits the fact that cancer cells have a different metabolism from that of normal cells. A specific molecular mechanism in the tumor leads to the accumulation of fluorescent substances in the tumor; the defective cells will fluoresce and show up in the image. Demarcation of precursor lesions and dysplastic margins of carcinomas are intensified by the color contrast. In such a manner, AFE can contribute to guided biopsy and surgical resection as well as oncological follow-up of precancerous and cancerous lesions.

The AF system can only detect changes on laryngeal epithelium, but not in deeper structures. For these 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. If a reduction of autofluorescent intensity appears, it is necessary to consider this area as a suspicious, and to take a biopsy.

In 1924, Policard [16] observed the ability of tissue to fluoresce under certain conditions. Alfano *et al.*, in 1984 [17], reported the possibility of differentiating between healthy and malignant tissue by means of their fluorescent characteristics. In 1933, Sutro and Burmann described the phenomenon of the different fluorescences of normal and tumor tissue. In 1995, Harries *et al.* [18] in a pilot study of eight cases with carcinoma of the vocal folds concluded that the technique can increase the accuracy of staging of cancer of the larynx and allows earlier diagnosis of tumors and their recurrence.

In 2002, Malzahn *et al.* [19] in a report of a case series of 127 patients, of which 111 were suspected of having precancerous and cancerous lesions, concluded that: normal laryngeal mucosa displays a typical green fluorescence signal, moderate and severe epithelial dysplasia, CIS and invasive carcinoma shows a diminished green fluorescence. They reported two false-negative cases and eight false-positive cases, and a sensitivity of 97%, while specificity was 83%.

In 2006, Arens *et al.* [20] in a prospective study of 116 cases with suspected precancerous or cancerous lesions of the larynx with "Indirect Autofluorescence Laryngoscopy" and comparing the images with direct autofluorescence reported concordant results in 103 cases. It was therefore concluded that the technique is a promising tool in the diagnosis of early laryngeal cancer. They have reported a sensitivity of 97%, while specificity was 82%.

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 AF mode and WL mode was 92% and 85%, respectively. Indirect autofluorescence endoscopy improved the sensitivity by 7% and the specificity by 12% compared to normal white light endoscopy.

Some conditions in the larynx can produce false positive and false negative findings. A dark field in an image may be the results of a shadow from an anatomic structure over this field. Hyperemia, scars, hemangioma 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.* [21] confirm these statements.

False-positive findings (decreased autofluorescence with a nonmalignant histopathology) were relatively common and resulted mainly from scar tissue or pronounced local inflammatory reactions of the mucosa. False-negative findings [negative autofluorescence despite (pre)malignant histopathology] were generally rare and resulted mostly from pronounced hyperkeratosis of the examined areas that caused optical "masking" of clinically significant lesions. In attention to avoid false positive and false negative findings, we recommended simultaneous careful comparison of white light and autofluorescent images of same view.

The AFE procedure provides a better evaluation of the horizontal extension in cancerous lesions then white light endoscopy alone. It 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.

In this study, we found that the AF imaging system was a useful complementary procedure for detecting laryngeal malignancies. However, when used alone it did not significantly improve the diagnostic evaluation in comparison with microlaryngoscopy. From our study, we can say that AFE and WLE do improve diagnostic findings when used in combination with MLS.

Conclusions

Indirect autofluorescence laryngoscopy is a quick, reproducible, non-invasive imaging technique, without drug application, which facilitates the detection and delineation of precancerous lesions, *in situ* carcinoma, and cancer of the larynx more accurately than white light examination alone. AFE could be an important additional diagnostic method, which coupled with the standard MLS could significantly improve the diagnostic efficacy of laryngeal pathology.

Acknowledgments

The study was financed by the "Victor Babeş" University of Medicine and Pharmacy, Timişoara, Romania; Doctoral Grant No. 978/31.01.2013.

References

[1] Ferlito A, Doglioni C, Rinaldo A, Devaney KO, *What is the earliest non-invasive malignant lesion of the larynx?* ORL J Otorhinolaryngol Relat Spec, 2000, 62(2):57–59.

[2] Baletic N, Malicevic H, Petrovic Z, Marinkovic-Eric J, Peric A, *Advantages and limitations of the autofluorescent diagnostics of the laryngeal cancer and precancerosis*, Eur Arch Otorhinolaryngol, 2010, 267(6):925–931.

[3] Kleinsasser O, *Die Laryngomikroskopie (Lupenlaryngoskopie) und ihre Bedeutung für die Erkennung der Vorerkrankungen und Frühformen des Stimmlippenkarzinoms*, Arch Ohrenheilk, 1962, 180:724–727.

[4] Von Stuckrad H, Lakatos I, *Über ein neues Lupenlaryngoscop (Epipharyngoskop)*, Laryngol Rhinol Otol, 1975, 54:336–340.

[5] Epstein JB, Oakley C, Millner A, Emerton S, van der Meij E, Le N, *The utility of toluidine blue application as a diagnostic*

aid in patients previously treated for upper oropharyngeal carcinoma, Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 1997, 83(5):537–547.
[6] Chisholm EM, Williams SR, Leung JW, Chung SC, Van Hasselt CA, Li AK, *Lugol's iodine dye-enhanced endoscopy in patients with cancer of the oesophagus and head and neck*, Eur J Surg Oncol, 1992, 18(6):550–552.
[7] Gale N, Kambic V, Michaels L, Cardesa A, Hellquist H, Zidar N, Poljak M, *The Ljubljana classification: a practical strategy for the diagnosis of laryngeal precancerous lesions*, Adv Anat Pathol, 2000, 7(4):240–251.
[8] Hung J, Lam S, LeRiche JC, Palcic B, *Autofluorescence of normal and malignant bronchial tissue*, Lasers Surg Med, 1991, 11(2):99–105.
[9] Lam S, Kennedy T, Unger M, Miller YE, Gelmont D, Rusch V, Gipe B, Howard D, LeRiche JC, Coldman A, Gazdar AF, *Localization of bronchial intraepithelial neoplastic lesions by fluorescence bronchoscopy*, Chest, 1998, 113(3):696–702.
[10] Kriegmair M, Baumgartner R, Knüchel R, Stepp H, Hofstädter F, Hofstetter A, *Detection of early bladder cancer by 5-aminolevulinic acid induced porphyrin fluorescence*, J Urol, 1996, 155(1):105–109; discussion 109–110.
[11] Panjehpour M, Overholt BF, Vo-Dinh T, Haggitt RC, Edwards DH, Buckley FP 3rd, *Endoscopic fluorescence detection of high-grade dysplasia in Barrett's esophagus*, Gastroenterology, 1996, 111(1):93–101.
[12] Betz V, Schneckenburger H, Alleroder HP, Sybrecht GW, Meyer JU, *Evaluation of changes in the NADH level between carcinogenic and normal tissue samples by use of fluorescence spectroscopy*, Proc SPIE, 1994, 2324:284–291.
[13] Dhingra JK, Zhang X, McMillan K, Kabani S, Manoharan R, Itzkan I, Feld MS, Shapshay SM, *Diagnosis of head and neck precancerous lesions in an animal model using fluorescence spectroscopy*, Laryngoscope, 1998, 108(4 Pt 1):471–475.
[14] Fryen A, Glanz H, Lohmann W, Dreyer T, Bohle RM, *Significance of autofluorescence for the optical demarcation of field cancerisation in the upper aerodigestive tract*, Acta Otolaryngol, 1997, 117(2):316–319.
[15] Richards-Kortum R, Sevick-Muraca E, *Quantitative optical spectroscopy for tissue diagnosis*, Annu Rev Phys Chem, 1996, 47:555–606.
[16] Policard A, *Etude sur les aspects offerts par des tumeurs expérimentales examinées à la lumière de Wood*, C R Soc Biol (Paris), 1924, 91:1423–1424.
[17] Alfano R, Tata D, Cordero J, Tomashefsky P, Longo F, Alfano M, *Laser induced fluorescence spectroscopy from native cancerous and normal tissue*, IEEE J Quant Electron, 1984, 20(12):1507–1511.
[18] Harries ML, Lam S, MacAulay C, Qu J, Palcic B, *Diagnostic imaging of the larynx: autofluorescence of laryngeal tumours using the helium-cadmium laser*, J Laryngol Otol, 1995, 109(2):108–110.
[19] Malzahn K, Dreyer T, Glanz H, Arens C, *Autofluorescence endoscopy in the diagnosis of early laryngeal cancer and its precursor lesions*, Laryngoscope, 2002, 112(3):488–493.
[20] Arens C, Reussner D, Neubacher H, Woenckhaus J, Glanz H, *Spectrometric measurement in laryngeal cancer*, Eur Arch Otorhinolaryngol, 2006, 263(11):1001–1007.
[21] Zargi M, Fajdiga I, Smid L, *Autofluorescence imaging in the diagnosis of laryngeal cancer*, Eur Arch Otorhinolaryngol, 2000, 257(1):17–23.

Corresponding author
Marioara Dobre, MD, PhD Student in Otorhinolaryngology, Department of ENT, "Victor Babeş" University of Medicine and Pharmacy, 2 Eftimie Murgu Square, 300041 Timişoara, Romania; Phone +40786–330 040, e-mail: mary2dobre@yahoo.com

Received: February 12, 2014

Accepted: December 26, 2014