Disease Free Margins Assessment Using Enhanced Methylene Blue Video Contact Endoscopy with SPIES Filters in Vocal Fold Malignancies

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Vocal fold carcinomas represent a pathology that has an important incidence. Recent studies showed that 70% of the dysplastic lesions can skip the phases of mild and severe dysplasia directly to carcinoma. For that reason early detection of a malignancy of the vocal fold is mandatory. If the lesion is discovered in an early stage it must be aggressively treated using CO2 LASER surgery and disease free margins must be obtained. Because frozen sections are not always available in all medical centers we are proposing a new protocol to assess disease free margins during CO2 LASER assisted laryngoscopy. Methylene blue contact endoscopy is not a new method, as it was introduced in laryngology by professors Oscar Diaz and Mario Andreea in 1994. We are proposing a protocol designed to assess disease free margins using an enhanced video contact endoscopy with SPIES software filters. The SPIES filters technology is a relatively new technology proposed by Karl Storz in 2013. A critical analysis of the method will be done comparing the results with paraffin histopathology exam.

Keywords: methylene blue, vocal fold carcinoma, contact endoscopy, SPIES filters, disease free

Video contact endoscopy is not a new method in laryngology, where it was introduced in 1994 by two professors Mario Andreea and Oscar Diaz [1-3]. In The Institute of Phonoaudiology and Functional ENT Surgery we started using this method in 1997. First the method was designed to detect early malignancies on the vocal fold. Great focus was on the cellular field modifications, such as: uniformity of the cellular field, the ratio between the nucleus and cytoplasm, size and shape of the cell and nuclear abnormalities.

The cellular abnormalities can only be identified using 150X video contact endoscope and it is necessary at least a 3-5 years learning curve for a surgeon to learn to read and to interpret the cellular field's modifications.

Surgery with oncological safety limits offers a better long-term survival. Thus the relapse rate decreases, as well as the risk of distant metastasis. Unfavorable evolution is associated with the need to use multiple lines of oncological treatment with a wide range of immediate and remote side effects [4,5].

In 2008 we proposed during the European Congress of Laryngology in Barcelona [6] a new approach using methylene blue video contact endoscopy. We wanted to move the focus from the abnormalities of the cellular field to the pattern of the superficial vascular network. We have done that having in mind that a 1 mm malignant tumor will start to produce endothelial growth factor in order to get more vessels and nutrients needed for the accelerated development of the tumor [7-10]. That will lead to vascular network pattern modifications that can be easily identified by the surgeon. Also, new types of delivery systems for cytotoxic drugs have been deviced for unresectable lesions in the oral cavity and for other local tumor therapies [11, 12]. The results can also be correlated with complex oncological investigations. New serum markers are being tested in order to establish the existence of mucosal damages and tumor angiogenesis [13].

So far, the methylene blue videocontact endoscopy enhanced with other light filters, like NBI (Narrow Band Imaging) has shown promising results [14].

Experimental part

The study was performed in The Prof. Dr. Dorin Hociota Institute of Phonoaudiology and Functional ENT Surgery.

We enrolled in our study 32 patients with confirmed malignant lesion of the vocal fold in stages T1 and T2b. The age of the patients was between 39 and 68 year old. The patients group consisted of 24 males and 8 females. The patients were enrolled in the study after performing trans nasal flexible endoscopy using white and Narrow Band Imaging light.

The surgery was performed under general anesthesia with oral intubation. The glottis plan was exposed using a medium Kleissaser laryngoscope.

Rigid endoscopy was performed using white and SPIES filtered light. We used all SPIES filters, but according to our earlier studies concerning the SPIES filters we have taken into account only the filters CHROMA and SPECTRA B.

First, a methylene blue video contact endoscopy was performed using normal light, after which the enhancing SPIES filters and a combination between these filters was used, respecting the following protocol (only CHROMA and SPECTRA B).

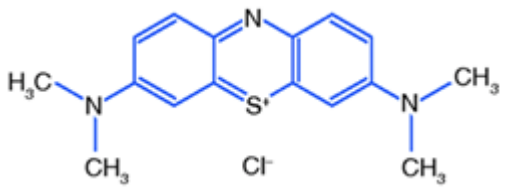

Fig. 1. Chemical formula of methilene blue

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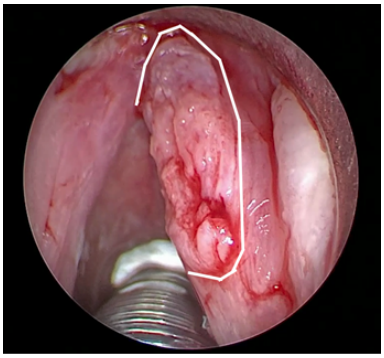

Fig. 2. Virtual map with the resection plan obtained from the methylene blue video contact endoscopy with white light

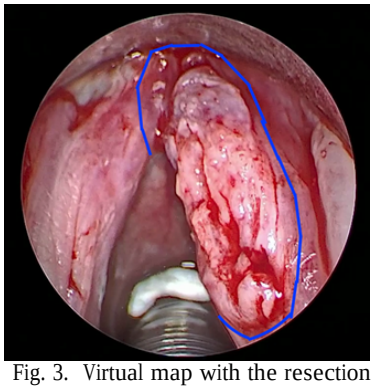

Fig. 3. Virtual map with the resection plan obtained from the methylene blue video contact endoscopy enhanced with CHROMA SPIES filter

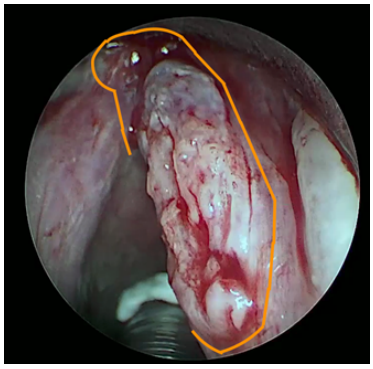

Fig. 4 Virtual map with the resection plan obtained from the methylene blue video contact endoscopy enhanced with SPECTRA B SPIES filter

Type II cordectomy	Type III cordectomy	Type IV cordectomy	Type V cordectomy
3	8	11	10

Table 1
ELS II TO ELS V CORDECTOMIES IN OUR PATIENT GROUP

Table 2
RESULTS OF THE COMPARISON BETWEEN THE VIRTUAL MAP FOR DISEASE FREE MARGINS UNDER WHITE LIGHT AND SPIES ENHANCED METHYLENE BLUE ENDOSCOPY

	Free disease margins	False positives	False negatives
Microscopic resection virtual map	62.31%	14,6%	63.2%
Video contact endoscopy virtual map	68.12%	26.3%	42.3%
SPIES enhanced VCE virtual map with CHROMA filter	75.32%	62.34%	14.54%
SPIES enhanced VCE virtual map with SPECTRA B filter	84,43%	65.45%	10.26%

We used the 0 degree 60X magnification contact endoscopy laryngoscope [15]. First we obtained information concerning the normal vocal fold and then the vocal fold with the lesion was evaluated. A virtual map with the resection was designed using the information from the methylene blue video contact endoscopy in white light (fig. 2).

A second resection virtual map was designed using the information obtained using the enhanced methylene blue video contact endoscopy with SPIES filters (fig. 3 and fig. 4).

The resection of the lesion was performed as in the virtual map obtained with white light. Multiple biopsies were performed. In the second step the resection plan continued according to the virtual plans obtained using the SPIES enhanced methylene blue video contact endoscopy. Again, multiple biopsies were sent for pathologic examination.

We performed ELS II to ELS V type cordectomy as indicated in table 1.

The percentage of disease free margins was analyzed comparing the numbers obtained from the virtual resection map designed using methylene blue contact endoscopy and from the virtual map using the enhanced methylene blue video contact endoscopy using CHROMA and SPECTRA B SPIES filters.

Results and discussions

Comparing the virtual map for disease free margins under white light and SPIES enhanced methylene blue endoscopy we obtained the results shown in Table 2.

Both types of methylene blue SPIES enhanced video contact endoscopy provide a better percentage of disease free margins compared to the normal microscopic virtual resection map.

SPIES software filters enhanced methylene blue video contact endoscopy provides the surgeon the ability to obtain more accurate disease free margins when performing the resection of the tumor. The CHROMA filter provides a good percentage of disease free margins, the false positive results are relatively high, but the false negative numbers are at an acceptably low value compared to the classic microscopic resection plan. The SPECTRA B filter provides the best numbers as far as disease free margins are concerned, with a high value of false positives and low false negatives.

Conclusions

SPIES software filter enhancing of the methylene blue contact endoscopy using CHROMA and SPECTRA B filters will provide an increased number of disease free margins compared to the classic microscopic resection plan or even to the methylene blue contact endoscopy plan using white light.

Both CHROMA and SPECTRA B filters provide an important percentage of false positives that will lead to a more extensive resection plan than it is necessary, but we think that the most important thing is the low false positive results concerning the disease free margins obtained in our study.

We have been using the SPIES filters technology for 1,5 year, so we are at the beginning of our learning curve. As in all other areas, we consider that the more experienced we will get, the better results we will achieve.

Frozen sections remain the gold standard in assessing disease free margins, but these are not available in all centers at all time. In that cases we consider that SPIES enhanced methylene blue video contact endoscopy is a viable alternative to frozen sections.

Still the video contact endoscopy system and the SPIES capable high definition camera are expensive state of the art technologies that need a learning curve in order to achieve good results.

The main disadvantage of the so-called *optical biopsies* is that we do not have any information about the depth of the lesion, but they remain a viable alternative, still in need of further research.

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References

1. M. ANDREA, O. DIAS, A. SANTOS, Acta Oto-Laryngologica, **115**, nr. 2, 1995, p. 314–316.
2. M. ANDREA, O. DIAS, A. SANTOS, Annals of Otology, Rhinology and Laryngology, **104**, nr. 5, 1995, p. 333–339.
3. O. R. HUGHES, N. STONE, M. KRAFT, C. ARENSS, M. A. BIRCHALL, Head & Neck, **32**, nr. 11, 2010, p. 1544–1553.
4. C. NITIPIR, M.A. BARBU, L.G. POPA, M.M. MIHAI, I. RADU, D. MIREA, C. GIURCANEANU, R.V. SCAUNASU, Revista Farmacia, **63**, nr. 6, 2015, p. 805-810.
5. M. YIPEL, M.G. ALBU, A. SPOIALA, M. RADULESCU, D. FICAI, A. FICAI, C. BLEOTU, C. NITIPIR, Current Organic Chemistry, **20**, 2016, p. 2934-2948.
6. V. ZAINEA, R. HAINAROSIE, N. POPESCU, V. POSTELNICU, A. ILIESCU, A. IOAN, I. POSTOLACHE, C. IONITA, M. TUSALIU, C. STOICA, M. CRIVEANU, R. UDRESCU, M. LUPASCU. European Archives of Oto-Rhino-Laryngology**, 266,** nr.4, 2009.
7. BONHIN RG, ROCHA VB, CARVALHO GM, GUIMARES AC, CRESPO AN, CHONE CT, AMSTALDEN EM. Braz J Otorhinolaryngol., **81**, nr.1, 2015, p.58-62.
8. ZHANG LP, CHEN HL. J Laryngol Otol., **131**, nr.1, 2017 Jan; p.44-50.
9. QIU Y., ZHOU H. Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi., **28**, nr.6, 2014, p.389-393.
10. HAINAROSIE, R., ZAINEA, V., HAINAROSIE, M., PIETROSANU, C., IONITA, I., Rev. Chim. (Bucharest), **68**, no. 7, 2017, p. 1532
11. C. NITIPIR, M.G. ALBU, G. VOICU, A. FICAI, M.A. BARBU, L.G. POPA, D. MIREA, C. LEVAI, S. LAZAR, M.V. GHICA, Journal of Chemistry, **66**, nr. 8, 2015.
12. M.V. GHICA, M.G. ALBU, D.A. KAYA, L. POPA, S. OZTURK, L.C. RUSU, C. DINU-PIRVU, C. CHELARU, L. ALBU, A. MEGHEA, C. NITIPIR, Korean Journal of Chemical Engineering, **33**, nr. 4, 2016, p. 1325-1330.
13. VOIOSU T., BALANESCU P., BENGUS A., VOIOSU A., BAICUS C.R., BARBU M., LADARU A., NITIPIR C., MATEESCU B., DICULESCU M., VOIOSU R., Clin Lab, **60**, nr. 3, 2014, p. 505-10.
14. D.C. STEFANESCU, O. CEACHIR, V. ZAINEA, M. HAINAROSIE, C. PIETROSANU, I.G. IONITA, R. HAINAROSIE, Rev. Chim. (Bucharest), **67**, no. 8, 2016, p. 1558-1559.
15. C. PIAZZA, D. COCCO, F. DEL BON et al., Oral Oncology, **46**, nr. 4, 2010, p. 307–310.

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