

Methylene Blue Staining Test in Assessing Disease Free Margins in Head and Neck Cancers

ALEXANDRA LOSTUN¹, GABRIEL LOSTUN^{2,3*}

¹ Transilvania University Brasov, Faculty of Medicine, Department of Anatomic Pathology, 56 Nicolae Balcescu Str., 500019, Brasov, Romania

² Transilvania University Brasov, Faculty of Medicine, Department of Anatomy, 56 Nicolae Balcescu Str., 500019, Brasov, Romania

³ Regina Maria Military Hospital Brasov, 9 Pietii Str., 500007, Brasov, Romania

Despite remarkable advancements in medicine, cancers remain one of the most challenging pathologies for doctors. Head and neck neoplasms especially raise serious management problems due to the often advanced stage at the moment of diagnosis, associated with difficult curative interventions that impose significant changes in the quality of life of patients. Whenever possible, a complete removal of the tumor with disease free margins significantly increases the rate of survival. During surgery, assessing the limit of resection up to healthy tissue is crucial in order to attempt a curative intervention. The purpose of this paper is to evaluate the use of the methylene blue staining test in assessing the margins of resection during interventions for head and neck cancers.

Key words: methylene blue, disease free margins

Head and neck cancers represent an important health problem due to the difficult interventions and associated therapy that are necessary for achieving a disease free status [1-4]. This is especially true when the diagnosis is made in an advanced local case, as it often happens due to the non-specific symptoms [5]. Although the technology has advanced greatly, providing more and more accurate diagnosis and treatment tools, curative resections in the head and neck area can still be debilitating and difficult to accept for the patient, especially when applying the principles of oncologic surgery. The failure to set an early diagnosis entails the need to use systemic or local therapies, most of the times accompanied by complex adverse drug reactions [6, 7]. The management of these adverse drug reactions sometimes requires team work between a medical oncologist and a dermatologist [7]. Various tumors in the head and neck area might also be treated locally depending on their extension and histological types [6, 8, 9]. Extension assessment of various neoplasms is attempted with the help of serum markers [10, 11]. The seric value of matrix metalloproteinases (MMPs) was demonstrated to be predictive for tumor grading and tumor progression in the head and neck area [11].

The most commonly incriminated risk factors for head and neck malignancies are alcohol and tobacco abuse [12, 13]. The association between these two, corroborated with long-term exposure, has proved to be one of the most often encountered independent risk factor for these patients [14, 15]. Knowing the risk factors and eliminating them when possible is important in managing patients with cancers of the head and neck area. Viral infections that are associated with malignant degeneration, such as Epstein-Barr infection for cancers of the rhinopharynx [16] or cancer-related strains of human papilloma virus for laryngeal cancers [17-19], must be diagnosed and, if possible, treated in order to improve the overall outcome. Other risk factors, such as occupational exposure to wood dust or heavy metals and asbestos, can no longer be influenced, but should be known, as each is associated with a specific location [20, 21]. Dental problems of these patients may be seen as risk factors of cancer onset in the

head and neck area and sometimes cause serious concerns to the surgery team. Dental treatments in these cases call for multidisciplinary approach and require the use of materials with high quality morphology and functional properties [22].

Head and neck cancers include multiple locations, from the nose to the larynx, each with its symptoms according to location and extension. Most tumors are squamous cell carcinomas [23], but other types, such as sarcomas, lymphomas or melanomas can also be encountered. Taking into account that the symptoms in early stages vary from nasal obstruction and recurrent sinusitis for nose and sinus involvement, persistent lesions of the oral cavity with pain, a foreign body sensation, sore throat and difficulties in swallowing if the tumor affects the pharynx or persistent dysphonia whenever the larynx is affected, it is easy to understand why patients may ignore these complaints at first [24]. The more locally advanced the disease, the more persistent and disturbing the symptoms will become. Another disturbing symptom, which may prompt the patient to seek medical assistance, is represented by lymph nodes involvement, which can occur for all head and neck cancers, but it appears more often with pharyngeal tumors.

The diagnosis is suggested by a visual evaluation of the tumor. Advanced optic technologies allow today early stage diagnosis, the gold standard being represented by a transnasal flexible endoscopy. The association of this examination with light filters, such as Narrow Band Imaging (NBI) [25] or SPIES enhances the diagnostic accuracy. Other methods of examination, such as videocontact endoscopy or acid-induced fluorescence, have proved their value in assessing these patients. However, none of the so-called *optical biopsies* can replace at this point the histopathological examination of a biopsy sample.

Imagistic evaluations of patients with head and neck cancers are mandatory [26]. According to the location and suspected extension of the tumor, the physician can rely on computer tomography and MRI for a complete local assessment of the lesion. Also, neck lymph nodes involvement will be evaluated by means of imagistic

* email: gabriellostun@gmail.com; Phone: +40 740163109

All authors have contributed equally to this paper.

investigations. These evaluations are important in the staging process.

One of the most important aspects of a surgical intervention is obtaining a complete resection with disease free margins. This translates into an overall increased survival rate, with a better prognosis for patients with head and neck cancers. The narrow spaces involved in this pathology create a challenge for the ENT surgeon, that must associate the need for oncological resection with the desire to preserve as much healthy tissue as possible in order to have a superior functional result. The oncologic principles are more important for a curative resection, but the current trend focuses on healthy tissue preservation whenever possible. At this point, a correct evaluation of the resection margins can be crucial for the outcome of the intervention. The gold standard remains an extemporaneous examination, but whenever this is not available, staining tests have gained importance in orienting the surgeon.

Experimental part

The purpose of this paper is to present the use of the methylene blue staining test as a method of evaluating the resection margins in patients with head and neck cancers, taking into account the advantages, disadvantages and reliability of the method.

Patients diagnosed with tumors of the head and neck area, with a confirmed diagnosis of malignancy, were included in this observational study. We did not evaluate patients in which a complete resection was not possible.

With patients under general anesthesia, we first washed the tumor area with saline solution, then with 1% acetic acid. After the area was completely dried, we colored the entire tumor and the surrounding tissue with 1% methylene blue solution using a moist sponge. Once the average waiting period of about 3 min completed, the excess methylene blue was cleaned with 1% acetic acid. As a first step, we observed the shape and contour of the tumor, the shades of blue present after the coloring process and the staining pattern compared to adjacent tissues. For all tumor areas, the color was more intense, a shade of dark blue, whereas the surrounding presumably normal tissue had various shades of light blue.

Once this step completed, the respective tumor was completely resected. Afterwards, the same staining process was repeated, this time at the level of the rejection margins. For all cases in which any abnormal staining pattern occurred, in color intensity or tissue appearance, the resection limit was enlarged and biopsy samples from the new safety margins were obtained. All resected tissue was sent for histopathological examination.

Results and discussions

The methylene blue staining method has already proved to be a reliable method in assessing resection margins for laryngeal cancers, especially associated with microscopic control and advanced optical technologies, such as NBI or videocontact endoscopy [27-29]. Following our observational study, we could determine that this method is valuable in terms of assessing the disease free margins in tumor resection for other areas as well. It can be successfully used for tumors of the oral cavity or pharynx, being a reliable and safe method. We can also perform this examination for tumors of the nasal fossae, as long as these tumors have their origin in the mucosa and do not extend to bony structures. In such cases, we can perform the test under endoscopic control. However, this method has proved to be difficult to use in such cases. Due to

anatomic particularities, we could not use it for tumors located in the sinuses.

Another advantage when using the methylene blue staining test is represented by the properties of the substance. It facilitates the scaring process and has associated bacteriostatic and antioxidant action. The adverse reactions are minimal, even if accidentally swallowed, compared to other staining agents, such as toluidine blue, which also have their proven indications [30-32].

These type of evaluations do not replace the histopathological examination, or the advantages of molecular and cellular analysis of tumor specimens, which allow a more thorough evaluation [33].

Conclusions

Up to this moment, as multiple studies have shown, the most reliable method for assessing the safety margins of a resection remains the extemporaneous examination. However, for all cases in which this exam is not available, we consider the use of adjacent methods to be a viable alternative, that helps guide the surgeon in the resection lines. The methylene blue staining test is a simple method that offers clear advantages: it is a cheap, easy to repeat test with practically no adverse effects. We can perform it on all patients, in a minimum time.

Our observational study showed that this method is a reliable one in assessing the disease free margins for endocavitary tumors of the head and neck areas. The biopsy samples of the resection margins after the staining test proved negative, which means the resections were complete. However, we consider further studies are necessary, on a larger number of patients, in order to statistically analyze our results.

In all patients with head and neck cancers, where a complete resection is possible and advisable, it is preferable to remove the tumor entirely during the first intervention. Positive resection margins will require a reintervention, which means increased risks and morbidity for the patient. That is why all methods that help guide the surgeon are valuable and should be considered as a safety net for such interventions.

One of the main advantages of this method is the fact that both the evaluation and obtaining the results are processes that happen in real time. The result is available immediately, during the same surgical intervention, with the investigation performed *in vivo*.

We must also underline one of the problems associated with the methylene blue staining test used for assessing the disease free margins, which is the subjective factor. The surgeon requires a learning curve in order to be able to interpret the lesion. Even after the learning process is completed, the results can still be influenced by experience.

However, taking all the data into account, we consider that the use of the methylene blue staining test for assessing the disease free margins in endocavitary head and neck tumor resection can be a valuable tool, especially when the extemporaneous examination is not available. It guides the surgeon as to the limits of the tumor and increases the chances of a successful *per primam* resection.

References

- 1.S.C. LEE, I.P. TANG, S.P. AVATAR, N. AHMAD, K.S. SELVA, K.K. TAY et al., *Med J Malaysia*, **66**, 2011, p. 101-104.
- 2.L.P. KOWALSKI, A.L. CARVALHO, *Oral Oncol*, **37**, 2001, p. 94-98
- 3.M. MCGURK, C. CHAN, J. JONES, E. O'REGAN, M. SHERRIFF, *Br J Oral Maxillofac Surg*, **43**, 2005, p. 281-284

- 4.X.D. BROUHA, D.M. TROMP, R. KOOLE, G.J. HORDIJK, J.A. WINNUST, J.R. DE LEEUW, *Oral Oncol*, **43**, 2007, p. 551-556
- 5.A.W. DEBES FELIPPU, E.C. FREIRE, R. DE ARRUDA SILVA, A.V. GUIMARAES, R.A. DEDIVITIS, *Brazilian Journal of Otorhinolaryngology*, **82**, nr. 2, 2016, p. 140-143.
- 6.YIPEL M, GHICA M, KAYA M et al. *Current Organic Chemistry*, **20**, nr. 28, 2016, p. 2934-2938.
- 7.NITIPIR, C, BARBU, MA, POPA, L.G. *Farmacia*, **63**, nr. 6, 2015, p. 805-810.
- 8.NITIPIR, C, ALBU, MG, VOICU, G. *Rev. Chim.(Bucharest)*, **66**, no. 8, 2015, p. 1169
- 9.GHICA MV, ALBU MG, KAYA DA. *Korean Journal of Chemical Engineering*, **33**, nr.4, 2016, p. 1325-1330.
- 10.VOIOSU T, BALANESCU P, BENGUS A. et. al., *Clinical Laboratory*, **60**, nr. 3, 2014, p. 505-510.
- 11.STANCIU AE, ANTON AZC, STANCIU MM, POPESCU CR, GHEORGHE DC, NITIPIR C. *Romanian Biotechnological Letters*, **22**, nr. 2, 2017, p. 12419-12426.
- 12.S. GANDINI, E. BOTTERI, S. IODICE, *International Journal of Cancer*, **122**, nr. 1, 2008, p. 155-164.
- 13.M. HASHIBE, P. BOFFETTA, D. ZARIDZE, *Cancer Epidemiology, Biomarkers and Prevention*, **15**, nr. 4, 2006, p. 696-703.
- 14.A.J TUYNS, J. ESTEVE, L. RAYMOND, *International Journal of Cancer*, **41**, nr. 4, 1988, p. 483-491.
- 15.M. HASHIBE, P. BRENNAN, S.C. CHUANG, *Cancer Epidemiology, Biomarkers and Prevention*, **18**, nr. 2, 2009, p. 541-550.
- 16.Y.C. CHIEN, J.Y. CHEN, M.Y. LIU, *New England Journal of Medicine*, **345**, nr. 26, 2001, p. 1877-1882.
- 17.A.K. CHATURVEDI, E.A. ENGELS, R.M. PFEIFFER, *Journal of Clinical Oncology*, **29**, nr. 32, 2011, p. 4294-4301.
- 18.D.J. ADELSTEIN, J.A. RIDGE, M.L. GILLISON, *Head and Neck*, **31**, nr. 11, 2009, p. 1393-1422.
- 19.M.L. GILLISON, G. D'SOUZA, W. WESTRA, *Journal of the National Cancer Institute*, **100**, nr. 6, 2008, p. 407-420.
- 20.M.C. YU, J.M.YUAN, *Seminars in Cancer Biology*, **12**, nr. 6, 2002, p. 421-429.
- 21.P. BOFFETTA, L. RICHIARDI, F. BERRINO, *Cancer Causes and Control*, **14**, nr. 3, 2003, p. 203-212.
- 22.PODARIU, A.C, JUMANCA, D, GALUSCAN, A, POPOCI, RA, PODARIU, A.S, NITIPIR, C., CHISCOP I., BARLEAN, L.M., *Mat. Plast.*, **52**, no. 4, 2015, p. 604
- 23.R.J. SANDERSON, J.A.D. IRONSIDE, *BMJ*, **325**, nr. 7368, 2002, p. 822-827.
- 24.H. JOSHI, D. MCPARTLIN, *The British Journal of General Practice*, **62**, nr. 597, 2012, p. 182.
- 25.X.G.NI, S. HE, Z.G.XU, L. GAO ET.AL., *The Journal of Laryngology and Otology*, **125**, nr.3, 2011, p. 288-296.
- 26.M. BECKER, P. ZBAREN, H. LANG, C. STOUPIIS, B. PORCELLINI, P. VOCK, *Radiology*, **194**, 1995, p. 661-669.
27. HAINAROSIE, R., ZAINEA, V., CEACHIR, O., HAINAROSIE, M., PIETROSANU, C., STEFANESCU, C.D., *Rev. Chim.(Bucharest)*, **68**, no. 1, 2017, p. 16
28. STEFANESCU, D.C., CEACHIR, O., ZAINEA, V., HAINAROSIE, M., PIETROSANU, C., IONITA, I.G., HAINAROSIE, R., *Rev. Chim.(Bucharest)*, **67**, no. 8, 2016, p. 1558
29. STEFANESCU, D.C., CEACHIR, O., ZAINEA, V., HAINAROSIE, M., PIETROSANU, C., IONITA, I.G., HAINAROSIE, R., *Rev. Chim.(Bucharest)*, **67**, no. 7, 2016, p. 1327
30. STEFANESCU, D.C., CEACHIR, O., ZAINEA, V., HAINAROSIE, M., PIETROSANU, C., IONITA, I.G., HAINAROSIE, R., *Rev. Chim.(Bucharest)*, **67**, no. 7, 2016, p. 1255-1256.
31. HAINAROSIE, R., CEACHIR, O., ZAINEA, V., HAINAROSIE, M., PIETROSANU, C., ZAMFIR, C., STEFANESCU, D.C., *Rev. Chim.(Bucharest)*, **68**, no. 2, 2017, p. 226
32. MCMAHON J, DEVINE JC, MCCAUL JA, MCLELLAN DR, FARROW A. *Br J Oral Maxillofac Surg*. 48, 2010, p.84-87
- 33.G.G. PETRICA-MATEI, F. IORDACHE, R. HAINAROSIE, M. BOSTAN, *Rom J Morphol Embryol*, **57**, nr.2 Suppl, 2016, p. 791-799

Manuscript received: 21.04.2017