

ONCOLOGY

# The usefulness of toluidine staining as a diagnostic tool for precancerous and cancerous oropharyngeal and oral cavity lesions

## *Utilità della colorazione con toluidina nella diagnosi delle lesioni precancerose e cancerose dell'orofaringe e del cavo orale*

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### SUMMARY

Toluidine blue stain is used as a marker to differentiate lesions at high risk of progression in order to improve early diagnosis of oropharyngeal carcinomas. This study focused on 45 oral mucosal lesions in 32 patients (13 female, 19 male). In 9 cases, multiple biopsies were collected. Of the 45 lesions examined, 26 (57.0%) were defined clinically benign, while 19 (42.3%) were defined as suspected lesions (pre-malignant or malignant). According to the clinical examination, the sensitivity was 53% (16/30) and for toluidine blue staining 96.2% (26/27) ( $p = 0.0007$ ). The specificity was 80% (12/15) for clinical examination and 77.7% (14/15) for toluidine blue staining ( $p = 0.79$ ). In conclusion toluidine blue stain has been shown to be a reliable aid when clinical examination is unable to differentiate lesions at high risk of progression and then it improves early diagnosis for oral cavity and oropharyngeal cancer.

KEY WORDS: Oral cavity • Oropharynx • Malignant tumours • Precancerous lesions

### RIASSUNTO

*Allo scopo di migliorare la diagnosi precoce dei carcinomi del cavo orale e dell'orofaringe abbiamo ritenuto opportuno verificare l'attendibilità della colorazione con toluidina come marker per differenziare le lesioni ad alto rischio di progressione. Lo studio ha preso in considerazione 45 lesioni del cavo orale e dell'orofaringe che riguardavano 32 pazienti. In 9 casi venivano eseguite biopsie multiple, 19 pazienti erano di sesso maschile e 13 di sesso femminile. Le lesioni definite clinicamente benigne erano 26/45 (57,0%) mentre 19 (42,3%) erano definite sospette (pre-maligne o maligne). Comparando la valutazione dell'esame obiettivo e della colorazione con toluidina delle lesioni con i risultati dell'esame istologico la sensibilità della toluidina è risultata essere più attendibile rispetto all'esame obiettivo, con una differenza statisticamente significativa. La valutazione della specificità non ha mostrato invece differenze statisticamente significative. La colorazione con toluidina sembra dare un contributo significativo nei casi in cui l'esame obiettivo non è sufficiente a differenziare lesioni ad alto rischio di progressione.*

PAROLE CHIAVE: Cavo orale • Orofaringe • Tumori maligni • Lesioni precancerose

Acta Otorhinolaryngol Ital 2009;29:187-190

## Introduction

Prognosis of oropharyngeal squamous cell carcinoma (SCC) (oral cavity and pharynx) depends on early diagnosis, despite advanced surgical techniques and adjuvant treatment, the 5-year survival rate remains ~40-50%<sup>1,2</sup>.

Unfortunately, oral cancer is usually detected when it becomes symptomatic and, at this stage, at least two thirds of the patients present an advanced disease. This requires treatment which gives rise to a high rate of morbidity and mortality and, furthermore, early detection of oropharyngeal pre-malignant lesions is important to improve the survival rate and quality of life (QoL).

In many cases, clinicians have difficulty in recognizing patients at high risk of developing oral cancer.

The major problem is when and where the biopsy should be taken from suspected lesions and this depends on the clinical ability to differentiate pre-malignant lesions from reactive and inflammatory diseases.

Furthermore, in many cases, this may be very difficult because often, when a white patch or plaque, like a clinically defined leukoplakia, is observed, it is difficult to define it as another disorder (inflammatory or reactive)<sup>3</sup>.

In a lesion that appears as a leukoplakia, in 16% of the cases, the lesion is already malignant<sup>4,5</sup> while in the dysplastic leukoplasic lesions, the risk of cancer development

has been reported to be as high as 43%<sup>6</sup> within a mean time of 8.1 years.

Various techniques have been developed to support clinical examinations, with the aim of improving early oropharyngeal cancer diagnosis.

Since dysplasia and *in situ* carcinoma contain much more DNA and RNA than the normal surrounding epithelium, the use of *in vivo* staining, by means of toluidine blue dye, is based on the fact that it is an acidophilic dye that selectively stains acidic tissue components such as DNA and RNA.

Toluidine blue staining is considered to be sensitive in identifying early oro-pharyngeal premalignant and malignant lesions<sup>7-11</sup>.

In the present study, the use of toluidine blue staining was taken into consideration to identify clinically doubtful oro-pharyngeal lesions and to compare the clinical evaluation with toluidine blue stain and with the histological evaluation.

### Material and Methods

The study focuses on 45 oral mucosa lesions from 32 patients (13 female, 19 male, mean age 59 years, range 42-82), coming under observation at the Department of Otolaryngology- Head and Neck Surgery, at the University of Catanzaro. In 9 cases, multiple biopsies were made. All lesions were examined by an otolaryngologist (A.G.) with a long experience in head and neck cancer lesions and, according to the visual inspection 26 lesions were considered benign while 19 were suspected as premalignant or malignant.

The lesions varied in size from 0.5 cm to 1.5 cm in minimum diameter (mean 0.8 cm) to 2.5 cm in maximum diameter (mean 1.8 cm). The site of the lesion was tongue in 11 cases, buccal mucosa in 9, floor of the mouth in 8 and hard or soft palate in 4.

Informed consent from the patients, to perform a biopsy, was obtained prior to application of toluidine blue stain.

Patients rinsed the oral cavity with water for 20 sec. to remove debris prior to rinsing with 1% acetic acid for 20 sec. Toluidine blue (1% W/W) was applied as an oral rinse for 20 sec. and then 1% acetic acid was used for 20 sec to eliminate mechanically retained stain<sup>12-15</sup>.

Lesions that showed dark blue staining were considered to be positive for premalignant or malignant tissue, while those with light staining, or totally not coloured, were considered negative.

The biopsies were performed under local anaesthesia by punch biopsy, all specimens were labelled with a progressive number and in a separate book, for each specimen the clinical examination and the result of the toluidine blue staining were reported.

The pathologist examining all the biopsies was not informed regarding the clinical or staining evaluation of each sample.

Histopathologic diagnoses were referred as: non-neoplastic (hyper-keratoses, hyper-para-keratoses, etc), mild dysplasia, moderate dysplasia, severe dysplasia, *in situ* carcinoma, invasive carcinoma<sup>16</sup>.

Sensitivity and specificity were determined from true-positive and true negative results. Positive predictive value was calculated as true positive/true positive + false positive and negative predictive value as true negative/false negative+true negative.

For the statistical analysis, we used histopathologic assessment as the gold standard with which to compare clinical examination and toluidine blue stain retention.

To assess statistical significance, for sensitivity and specificity of toluidine blue versus clinical examination, Mc Nemar’s approximate chi-squared test was used<sup>16-19</sup>.

The same test was used for positive and negative predictive value.

This study was approved by the Institutional Review Board of “Magna Grecia” Catanzaro University.

### Results

Of the 45 lesions examined 26 (57.0%) were defined as clinically benign. While 19 (42.3%) were defined as suspected lesions (pre-malignant or malignant).

Histological examinations revealed that 15/45 (33.4%) were benign lesions (hyperkeratosis, hyperparakeratosis, papillomatosis) and 30/45 (66.6%) were precancerous or cancerous lesions, 8 (26.6%) of the latter were mild dysplasia, 5 (16.6%) moderate dysplasia, 6 (29.0%) severe dysplasia, 4 (13.3%) *in situ* carcinomas and 7 (23.3%) invasive carcinomas.

**Table I.** Correlation between clinical examination and histology in 45 oral lesions.

Histology	Clinically benign (%)	Clinically suspected (%)
Benign	12 (46.1)	3 (15.7)
Mild dysplasia	6 (23.0)	2 (10.5)
Moderate dysplasia	3 (11.5)	2 (10.5)
Severe dysplasia	3 (11.5)	3 (15.7)
Carcinoma in situ	1 (3.8)	3 (15.7)
Invasive carcinoma	1 (3.8)	6 (31.5)
Total	26	19

**Table II.** Correlation between clinical examination and toluidine blue staining in 45 oral lesions.

Histology	Toluidine blue negative (%)	Toluidine blue positive (%)
Benign	14 (77.7)	1 (37.0)
Mild dysplasia	3 (16.6)	5 (18.5)
Moderate dysplasia	1 (5.5)	4 (14.8)
Severe dysplasia	0 (0)	6 (22.2)
Carcinoma <i>in situ</i>	0 (0)	4 (14.8)
Invasive carcinoma	0 (0)	7 (25.9)
Total	18	27

Of the 26 lesions clinically evaluated as benign, 12/26 (46.1%) were histologically benign while 17 of the 19 (89.4%) lesions, defined as clinically suspected, were confirmed, at the histological examination, as precancerous or cancerous.

The correlations between clinical examinations and histological results are shown in Table I.

Lesions that showed dark blue staining, after toluidine blue application, were 27 out of the 45 (60%) while those considered negative were 18 (40%).

Furthermore, 14 (77.7%) out of the total 18 negative lesions to toluidine blue staining were histologically benign lesions while 26 (96.3%) out of the 27 staining toluidine blue positive were histologically defined as pre-cancerous or cancerous lesions.

The results of the toluidine blue staining and histological findings are outlined in Table II.

The results of the clinical evaluation, the toluidine blue test and histology, were compared in order to calculate the sensitivity (true-positivity) and specificity (true-negatives) (Table III).

According to the clinical examination, sensitivity was 53% (16/30) while for toluidine blue staining, it reached 96.2% (26/27) ( $p = 0.0007$ ).

Specificity was 80% (12/15) for the clinical examination and 77.7% (14/15) for toluidine blue staining ( $p = 0.79$ ).

The positive predictive value for clinical examination was 84.2% (16/19) and 86.6% (26/30) for toluidine blue staining ( $p = 0.85$ ).

The negative predictive value for clinical examination was 46.1% (12/16) and 93.3% (14/15) for toluidine blue staining ( $p = 0.0073$ ).

## Discussion

We revealed that 46.1% of the lesions defined as clinically benign were histologically benign while 89.4% of the lesions defined as clinically suspicious were confirmed as pre-cancerous or cancerous lesions.

Regarding toluidine blue staining, 77.7% of the negative lesions were confirmed as histologically benign while 96.2% of the lesions, toluidine blue positive, were histologically pre-cancerous or cancerous lesions.

The sensitivity and specificity of toluidine blue staining, observed in our study, is similar to that reported recently<sup>21,22</sup>.

The difference in sensitivity between toluidine blue versus clinical examination was statistically significant ( $p < 0.001$ ), while specificity was not statistically different.

These data show that when we have a clear objective neoplastic lesion there is no difference between the clinical examination and the toluidine blue staining whereas when we have lesions in which the clinical examination appears negative, then toluidine blue staining is more sensitive in identifying suspected lesions.

The analysis between positive and negative predictive values confirms this hypothesis. No statistically significant difference was found between the clinical examination and toluidine blue staining for positive predictive value ( $p > 0.05$ ) while a significant statistical difference was present for the negative predictive value ( $p < 0.005$ ).

This means that there is a 53.9% probability that clinically negative specimens could be histologically positive and a 6.7% probability that a toluidine blue negative sample could be histologically positive.

Furthermore, it has recently been demonstrated that le-

**Table III.** Histopathologic evaluation compared with clinical examination and toluidine blue.

Clinical examination	Histologically positive	Histologically negative	Total
Positive	16	3	19
Negative	14	12	26

  

Toluidine blue	Histologically positive	Histologically negative	Total
Positive	26	4	30
Negative	1	14	15

sions positive for toluidine staining showed genetic alterations associated with multiple sites of loss of heterozygosity, frequently implicated in the multistep of head and neck carcinogenesis<sup>21</sup> and, furthermore, Zhang et al.<sup>23</sup> demonstrated the potential value of toluidine blue to detect precancerous and cancerous oral lesions with

molecular features at high-risk of clinical progression. In conclusion, in our opinion, toluidine blue stain could be a useful aid when the clinical examination shows dubious lesions in order to establish whether the lesions are at high risk of progression and to contribute to an early diagnosis of oropharyngeal cancer.

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Received: July 12, 2008 - Accepted: June 29, 2009

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