The use of toluidine blue in the detection of pre-malignant and malignant oral lesions

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INTRODUCTION: The purpose of this study was to evaluate the efficacy of the toluidine blue (TB) test as a diagnostic tool in the detection of malignant and dysplastic lesions of the oral cavity. This study was carried out because of a lack of consensus among different authors on the utility of TB, as well as to determine useful adjuncts to detect oral pre-cancer and cancer.

MATERIALS AND METHODS: The study included 160 patients with oral mucosal disorders that included suspicious or malignant lesions detected at clinical visual examination, confirmed by histopathological evaluation. All lesions were submitted to TB staining.

RESULTS: The sensitivity and specificity for the detection of malignant or dysplastic lesions by this test were 65.5% and 73.3%, respectively. Overall, the detection rate with TB (sensitivity) was slightly lower compared with those reported by other authors but the specificity was comparable to several reports. Positive predictive value (35.2%) was also lower than previous studies, whereas negative predictive value (90.6%) was similar.

CONCLUSIONS: The simplicity of the test procedure and the validity of derived values suggest TB staining can be a valuable adjunct to the diagnostic process, as long as it is carefully correlated with the clinical characteristics of the mucosal disorder and histopathological diagnosis.

Keywords: dysplastic lesions; early detection; malignant disorders; oral cancer; toluidine blue

Introduction

The relative frequency of oral cancer in Europe is lower compared to other cancers, but its importance lies in its reported high mortality. In fact, approximately 50% of patients with oral cancer will die within 5 years mainly because of delay in diagnosis (1). Nevertheless, if oral cancer is diagnosed during stage I, the survival rate improves to 86% (2). In the oral cavity, there are numerous mucosal disorders designated as potentially malignant, whose diagnosis and treatment may favour early detection or prevention of cancer thereby diminishing its incidence and mortality (3).

There is no agreement regarding the efficacy of conventional oral examination in the detection of potentially malignant disorders and early oral cancers. Some authors have reported a high sensitivity, specificity and a favourable predictive value, while others have not (4, 5). There are several tests and adjunctive diagnostic techniques that are now commercially available and are promoted for use in dental and hospital settings.

Wilson and Junger (6) provided guidelines for an ideal screening test. In general terms, it would be desirable to have a test of high sensitivity and specificity; in other words, the test outcome should lead to few false positives (FP) and few false negatives (FN).

The most frequently used adjunctive diagnostic technique to assess oral mucosal disorders is the toluidine blue (TB) dye test. TB is an acidophilic metachromatic dye of the thiazine group. In solution, it takes on a blue-violet colour (7). Its main characteristic is that it selectively stains acidic tissue components. The test is based on the fact that dysplastic cells may contain quantitatively more nucleic acids and a dysplastic epithelium also has some loss of cohesion. These features facilitate the penetration through the epithelium and retention of the dye in cancer cells, which are replicating in vivo, whereas normal mucosa fail to retain the dye.

Starting in 1964 and because of the impetus given by Niebel and Chomet (8), TB has been increasingly employed by numerous authors for diagnostic purposes. However, studies published over the last three decades using TB to identify dysplastic and malignant lesions of the oral mucosa have reported very disparate data resulting in controversies for its use. This justifies further experimental studies on this diagnostic tool (9).
The main objective of this study was to determine whether TB application would aid in the diagnosis of oral malignancies and dysplastic lesions. To this end, we computed the sensitivity, specificity, and predictive values of the test for all oral lesions that on clinical grounds required biopsy.

Patients and methods

One hundred and sixty patients referred to the Department of Oral Medicine, Faculty of Dentistry, Complutense University of Madrid, Spain were selected for this study. There were 77 men and 83 women with a median age of 55.3 ± 16.1 years (range 13–100 years). All patients were Caucasian. Among the 160 patients, 34% were smokers and 27% consumed alcohol regularly. These patients presented with 160 mucosal lesions, which required biopsy evaluation to establish a definitive diagnosis.

Application and validation of the diagnostic test

All patients were subjected to a clinical history taking and extra-oral/intra-oral routine examination. Any lesion detected by clinical oral examination was described, charted and photographed, and a working clinical diagnosis was established using WHO Criteria (1980) (10). These data were recorded on a proforma (available from the authors on request). After clinical examination, informed consent was obtained from all patients, and those volunteering were included in the study. Then, TB was applied as a mouth rinse using the protocol described by Mashberg, with 1% aqueous acetic acid applied initially as a mucolytic agent and after TB rinsing to remove excess stain. (11, 12). All lesions were immediately rephotographed following staining. The stain was considered positive when the surface mucosa took on a blue colour, either if the entire lesion was stained or just a portion of it. Those that do not took colouration or with equivocal findings were considered negatives. The test outcome was subjected to clinical evaluation, by four experienced oral pathologists previously calibrated in pairs.

Biopsies were performed in all cases who underwent the test. For lesions with a positive toluidine test, the biopsy was taken from the stained area. To avoid any interexaminer variability, the biopsies from this study were evaluated by the same pathologist to determine the presence and degree of dysplasia, or malignancy.

Following histopathological diagnosis, all lesions were classified in two groups: non-dysplastic/non-malignant lesions, when there were no signs of dysplasia or histological malignancy and dysplastic/malignant lesions, when dysplasia or invasion was present.

Global validation of the test result was established by calculating the sensitivity, specificity and both the positive and negative predictive values (13).

Results

The case series consisted of 160 subjects with benign lesions or clinically suspicious pre-malignant or malig-

### Table 1 Clinical description of included cases

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>White lesions</td>
<td>45</td>
<td>38</td>
<td>83</td>
</tr>
<tr>
<td>Red lesions</td>
<td>11</td>
<td>23</td>
<td>34</td>
</tr>
<tr>
<td>Exophytic lesions</td>
<td>14</td>
<td>15</td>
<td>29</td>
</tr>
<tr>
<td>Ulcers</td>
<td>7</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>77</td>
<td>83</td>
<td>160</td>
</tr>
</tbody>
</table>

### Table 2 Results of TB staining compared with histological diagnosis

<table>
<thead>
<tr>
<th>Histological diagnosis</th>
<th>TB (+)</th>
<th>TB (−)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign disorders with no dysplasia or malignancy</td>
<td>35 (FP)</td>
<td>96 (TN)</td>
<td>131</td>
</tr>
<tr>
<td>Dysplastic lesions</td>
<td>7 (TP)</td>
<td>9 (FN)</td>
<td>16</td>
</tr>
<tr>
<td>Malignant lesions</td>
<td>12 (TP)</td>
<td>1 (FN)</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>54</td>
<td>106</td>
<td>160</td>
</tr>
</tbody>
</table>

FN, false negatives; FP, false positives; TB, toluidine blue; TP, true positive.
planus (LP), 18 of them stained with TB. As the objective of the study was to detect epithelial dysplasia or cancer, LPs that stained positive were considered FP in this study.

Discussion

The sensitivity and specificity rates regarding the efficacy of TB reported in published studies over the last decade range from 64% to 100% and 9% to 100%, respectively (8, 11, 12, 14–30) (Fig. 1). A meta-analysis by Rosenberg and Cretin in 1989 (31) studied the ability of TB to detect cancer or pre-cancer. The sample size of these studies varied between 20 and 1190 subjects, with a mean of 250 patients per study. The meta-analysis revealed a sensitivity rate of 97.7 ± 4.65%. The specificity rate was lower, with a mean of 90.8 ± 9.34% (31). A later systematic review by Gray et al. (32), of 75 published studies, produced a range of sensitivity for detection from 0.78 to 1.00 and specificity from 0.31 to 1.00. In our study, the sensitivity (65.51%) and specificity (73.28%) values were lower than the mean estimates reported by Rosenberg and Cretin (31).

This may be because of several factors. The first is the fact that inflammatory and ulcerative lesions (irrespective of malignancy status) tend to retain the dye because of greater cell activity and mechanical retention and consequently yield a higher number of FP. On the contrary, hyperkeratotic lesions do not facilitate dye penetration to the deeper epithelial layers, and thus cellular changes at the depth of the epithelium may be missed by the dye, producing FN results.

According to Gandolfo et al. (30), another factor would be the misinterpretation of the staining results based on the intensity of stain, type of lesions on which TB is used and the staining procedure (mouth rinse or application).

Low specificity generally leads to a larger number of false positive results because of retention of the stain in inflammatory or traumatic areas of mucosa. In the hands of a generalist, this could lead to unnecessary biopsy and increased anxiety to the patient. A low sensitivity could be because of lower penetration of the dye through the white patches. This suggests that TB may not be suitable for primary care use where a high proportion of white patches encountered are benign disorders.

Following the meta-analysis, Rosenberg and Cretin (31) concluded that if TB is used as a screening method for high-risk populations, then FN results will be significantly low, whereas FP will increase. Tests with high false positive rates although are more appropriate for selected high-risk groups are not suitable for primary care or a community setting screening (33). Epstein et al. (27) promoted using TB for the management of patients with a prior history of upper aerodigestive tract cancer based on their results of a multicentre trial. They concluded that TB was more sensitive than their clinical examination in detecting carcinomas in this high-risk group of patients. A statement published by the World Dental Federation (Fédération Dentaire Internationale, FDI) recommended that TB should only be used when screening high-risk groups.

When using vital dyes, in this case TB, it is important to consider that the results may be influenced by the amount of dye retention (i.e. intensity of the stain) that helps to classify the lesion as positive or negative; a differentiation that is not clearly stated in most of the published data. Only study so far to address this issue

![Figure 1](https://example.com/figure1.png)  
**Figure 1** Sensitivity, specificity of toluidine blue test and the number of cases investigated in different studies.
was by Gandolfo et al. (30) who reported that dark royal blue staining is significantly related to the nuclear uptake by the dye and indicated more serious disease. Their study was, however, limited to 18 patients and needs revalidation.

In our study, 92% of SCC were confirmed positive by the dye. The single SCC that was missed by the dye had the clinical appearance of an ulcer but was localized in the glosopalatine arch (anterior pillar of fauces). Probably our staining technique, which used a 10 ml TB mouth rinse, failed access to the site. Mashberg (19) in his early studies on TB highlighted the significance of the volume of the mouth rinse to enable the dye to flow to posterior part of the oral cavity.

In the limited number of dysplastic lesions included in the study, no real difference was evident whether TB may stain more severe dysplasias compared to those with mild changes. Other authors such as Epstein et al. (34) and Zang et al. (35) have reported similar outcomes comparing mild vs. moderate dysplasia but Epstein et al. (9) in an earlier study indicated that all severe dysplasias retained the dye. It has also been remarked that those retaining the dye may have a higher risk of developing cancer (9, 35). Such claims need to be further verified in further prospective trials.

It is important to assess the value of a diagnostic test, so that one may not subject patients to unnecessary diagnostic procedures that misinterpret the true nature of the disorder. For this, one needs to establish the positive predictive value and the negative predictive value by applying the test to a spectrum of disease grades (36). This was established in our study by examining benign, dysplastic and malignant cases.

The positive predictive value of our study (35.2%) is lower than studies than studies by Onofre et al. (25), 43.5% and Epstein et al. (34), 37%. This may be because of the fact that prevalence (dysplastic/malignant lesions) of our study, 18.1%, is lower than those previously mentioned by Onofre et al. (25), 26%, and Epstein et al. (34), 55.7%.

The study by Zhang et al. (35) showed a higher values of prevalence, 81%, and predictive positive value, 86%.

The negative predictive value of our study, 90.2%, is similar to Onofre et al. (25), 88.9%, and lower than the published by Epstein et al. (34), 100%. On the contrary, the value obtained by Zhang et al. (35) was much lower, 22%.

This suggest that if prevalence is high, a positive result tends to confirm the presence of the disease, whereas if prevalence is low, a positive result does not permit its confirmation. Then, if the test is used in high-risk population, there will be few FP, but FN may occur.

On the other hand, when prevalence is low, a negative result permits rule out the disease more safely, and a positive result does not permit to confirm the diagnosis. Hence, if TB is used in general population, the test will produce more FP.

Problems with studies of TB were recently highlighted by Lingen et al. (37). We have taken account of some of these deficiencies noted in earlier studies by including both benign, dysplastic and malignant cases in our case series and subjecting all cases to histopathology.

Our study, as many reported previously, was conducted by hospital specialists and therefore does not answer the feasibility of using TB in community/primary care settings.

Conclusions

In our study, the rates of sensitivity (65.1%) and specificity (73.3%) were lower than some previously published in a review. Nevertheless, since 1992, the reported values regarding sensitivity and specificity have fallen and are more in agreement with our findings.

In our experience, any lack of continuity of the mucosal surface may vary the result of the test generating therefore false positive results. Besides, the hyperkeratotic areas do not permit the penetration of the dye, generating FN.

According to our results, TB staining per se is of questionable value in the detection of dysplastic lesions although most malignant lesions of the oral cavity will retain the dye. The simplicity of the test procedure is attractive for routine use. The findings suggest the test can be a valuable adjunct to the diagnostic process, at least as a guideline, as long as it is carefully correlated with the clinical characteristics of the lesion, and histopathological diagnosis.

References


