The adjunctive role of toluidine blue in detection of oral premalignant and malignant lesions

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Purpose of review

To review the literature on toluidine blue (TBlue) and to discuss the utility of TBlue in assessing and in clinical management of patients with oral mucosal lesions. The literature search was conducted using key word search including oral cancer, oral premalignant lesions, and TBlue and by selecting references from the articles reviewed.

Recent findings

The findings of this review show that TBlue has utility as an adjunct in the detection of premalignant and malignant oral mucosal lesions and in identifying high-risk areas of lesions for biopsy in patients at increased risk of cancer when evaluated by experienced healthcare workers.

Summary

TBlue positive lesions, whether histologically benign or with dysplasia, predict molecular change and behavior of oral premalignant lesions. TBlue may provide information regarding lesion margins, accelerate the decision to biopsy, guide biopsy site selection and treatment of oral premalignant and malignant lesions. These findings support the utility of TBlue as a clinical adjunct in assessment of oral mucosal lesions.

Keywords

early diagnosis, oral cancer, oral premalignant lesions, squamous cell carcinoma, tolonium chloride, toluidine blue

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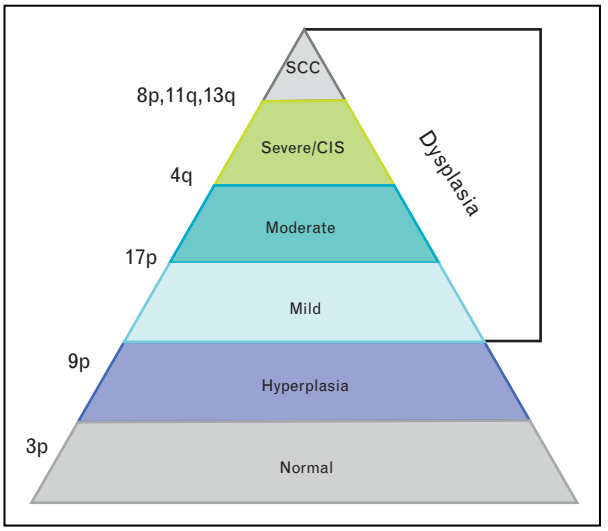
Introduction

Squamous cell carcinoma (SCC) is the most prevalent malignancy in the head and neck, the oral cavity, and pharynx [1–5]. Approximately 300 000 new oral cavity cancer cases and 68 000 deaths worldwide are expected annually [6]. Even though the oral cavity is readily accessible for examination by inspection and palpation, oral SCC (OSCC) is frequently not diagnosed until symptomatic with an advanced stage of disease [7–14]. Patients may not identify oral mucosal changes, and healthcare providers may not perform a thorough head and neck and oral examination that may lead to delay in recognition and diagnosis [2,8]. Approximately two-thirds of OSCCs are diagnosed at stage 3 or 4 disease with spread to adjacent tissues and regional lymph nodes, leading to an overall poor 5-year survival rate [7,8,10,14–17]. Thus, there is a pressing need for early detection of oral premalignant lesions (OPLs) and OSCC. OSCC is most common in patients aged over 45 [6,15]. However, OSCC is becoming more common among younger patients [18–26] who may not have the traditional risk factors of tobacco, alcohol consumption [14,22,27–31], and poor diet [31–36]. Human papilloma virus (HPV) has more recently been identified as a leading etiologic risk factor in oropharyngeal SCC [24,26,37–39].

Oral cancerogenesis

Oral cancer is a genetic process that leads to alterations at the molecular level, followed by phenotypic changes and ultimately presenting as clinically observable changes [2,40–43]. OSCC begins as a focal clonal overgrowth of altered stem cells near the basement membrane, expands upward and laterally, replacing the normal epithelium [44]. With advanced techniques, short DNA sequences repeated throughout the genome (microsatellite markers) can be used to detect imbalance or loss of heterozygosity (LOH) or allelic loss, in the genetic sequence of specific chromosomes [2,42,43,45–47]. LOH is defined as the loss of normal function of one allele of a gene in which the other allele becomes inactivated by mutation and results in a loss of tumor suppressor genes promoting carcinogenesis. Molecular studies of oral carcinogenesis reveal early genetic changes at particular chromosome sites: 3p14 and 9p21 [2,41,45,46,48–54]. Additionally, the risk of progression to cancer increases with genetic losses on additional chromosome arms such as 1p, 4q, 5q, 6q, 8p, 11, 13q, 18q, 21q [2,41,51,55,56], and 17p [2,41,51,56]. DNA overrepresentations at 11q13, on 3q, 8q [55,57], 16p, 11q, 19, 20q, and 22q are also frequently observed in OSCC cases [55]. Oral carcinogenesis occurs with accumulation of key sites of LOH over time

Figure 1 Oral carcinogenesis model, defined by Epstein *et al.*



CIS, carcinoma *in situ*; SCC, squamous cell carcinoma. Adapted from [2].

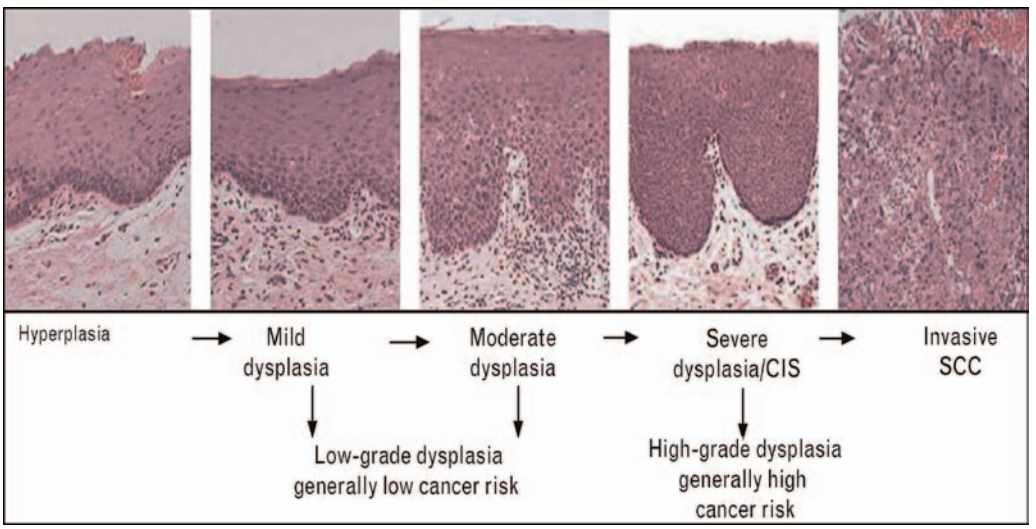
(Figs 1 and 2) [2,58]. Even though the exact factors that influence the patient's response to adjuvant therapy are yet to be explained [59], initial evidence of genetic mutations (such as *p53* gene) within the postoperative residual squamous tumor tissues may cause resistance to radiotherapy of the primary cancer [42,59,60].

The molecular model of multistep carcinogenesis indicates that an accumulation of genetic alterations forms the basis for the development of OSCC with genetic heterogeneity [42,43,46,54,61–63]. In addition, the genotype results in phenotypic change, later seen with histologic abnormality, and finally clinically detected change [61]. Regional molecular change has been shown in cases in which tissue distant from primary main tumor, detected with TBlue, harbors molecular change, findings that are critical in treatment planning [64]. 'Field cancerization' is a concept that proposes the presence of genetic aberrations required for carcinogenesis throughout the upper aerodigestive tract including the oral mucosa of high-risk populations [8,42,46,65]. Field cancerization may develop either when the oral mucosa is exposed to etiological agent(s) that causes independent transformation of epithelial cells at separate sites or may result in the transformation of a single oral epithelial cell that produces expanding clones that spread through the oral mucosa. Additionally, primary OSCC may have a paracrine effect on the adjacent oral mucosa and increase risk of cancer development [65].

Clinical manifestation of oral squamous cell carcinoma lesions

Clinically, OSCCs may appear as red, white, or mixed patches; a mass with or without ulceration, which may develop in an area of clinically normal mucosa or arise from an OPL [1,2,6,13,14,29,66] (Figs 3–5). The most common sites of OSCC are the lower lip, the lateral border of the tongue, and the floor of the mouth [1,6,8,67], which contain relatively thin epithelium, minimal keratinization and, thus, may be more susceptible to environmental carcinogens [8]. They may have benign clinical appearances and may be asymptomatic or present with few symptoms making it difficult for clinicians to differentiate OPLs and early stage OSCCs from common benign lesions [8,68,69]. Even though the

Figure 2 The progression of oral squamous cell carcinoma from epithelial hyperplasia to oral squamous cell carcinoma through varying steps of dysplasia



CIS, carcinoma *in situ*; SCC, squamous cell carcinoma. Courtesy of BC OCPP (www.orcanet.ca) [58].

Figure 3 Oral squamous cell carcinoma lesion developed on the fornix of posterior mandible

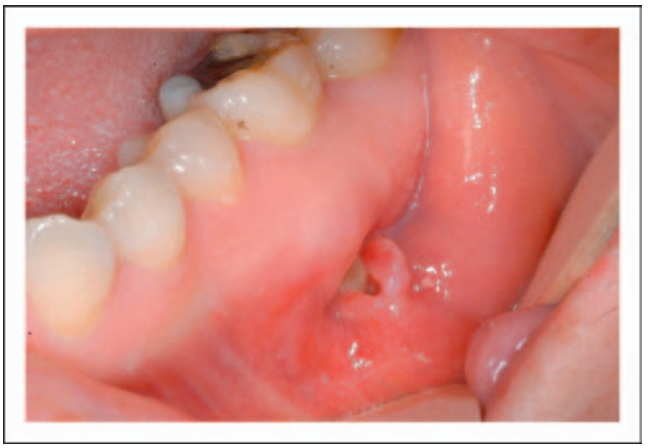
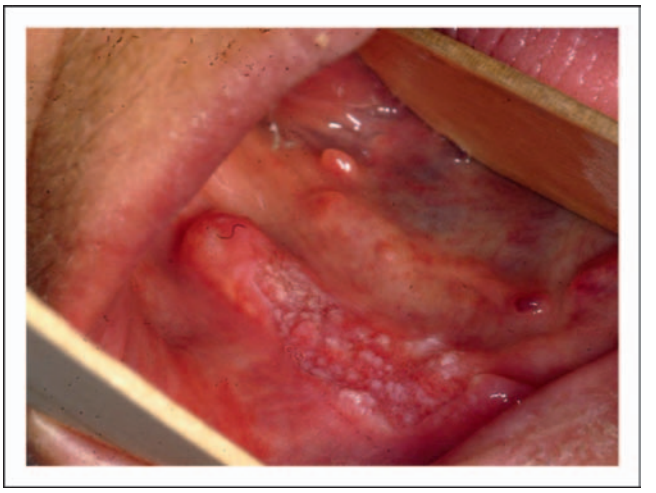


Figure 5 Oral squamous cell carcinoma on the mandibular right alveolar ridge, with white components



histopathological characteristics of OSCC lesions are well known, pathologic diagnosis is subject to intra-examiner and interexaminer variability [8,70,71] (Fig. 6). Studies may combine all grades of dysplasia as 'positive' lesions, whereas others include high-grade dysplasia or carcinoma *in situ* (CIS)/SCC as 'positive', thus complicating the interpretation of the diagnostic efficacy of examination adjuncts in lesions with a high risk for malignant transformation versus lesions with lower risk [72••].

Toluidine blue staining

In order to facilitate detection and identification of at risk lesions, clinical adjunctive methods have been introduced [68,69,72••]. Vital tissue staining with TBlue is the most studied method that may promote early detection of OPLs/OSCC. TBlue is a cationic metachromatic dye that may selectively bind to free anionic groups such as sulphate, phosphate, and carboxylate radicals of large molecules [1,73]. In pathology laboratories, TBlue has been compounded and used as an in-vitro nuclear stain because of binding ability to phosphate groups of nucleic acids. *In vivo*, TBlue stains deoxyribonucleic and nucleic acids and may be retained in intracellular spaces of dysplastic epithelium [13,64,72••,74,75]. Dysplastic and malignant tissues may retain TBlue due to the loss of tumor suppressor genes that predict progression of OPLs to OSCC or may represent OSCC at diagnosis [12,52,76,77]. Epstein *et al.* [9] demonstrated increased

Figure 4 Expanding oral squamous cell carcinoma developed on the ground of oral lichen planus

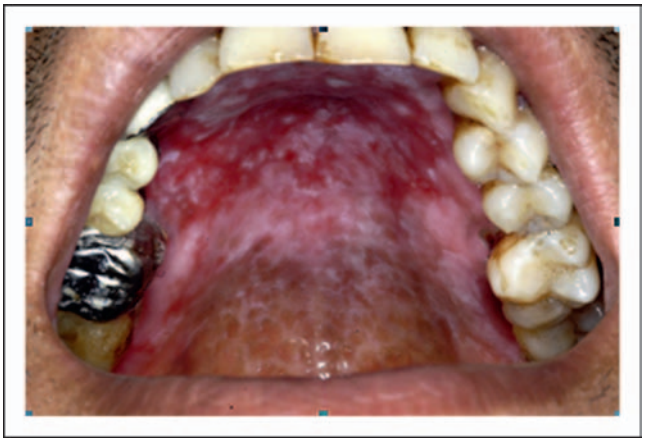
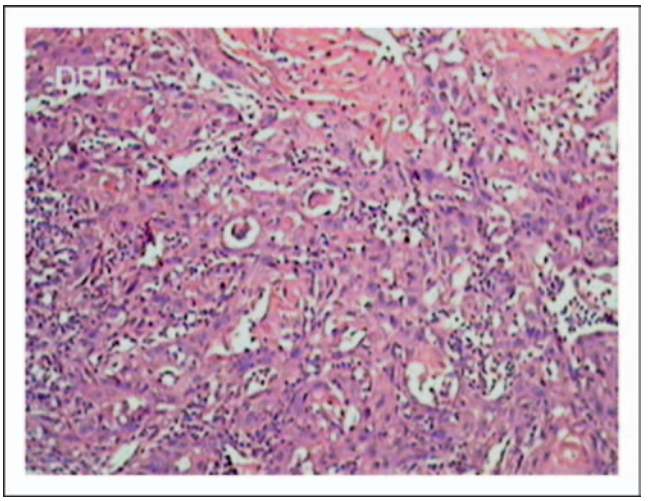


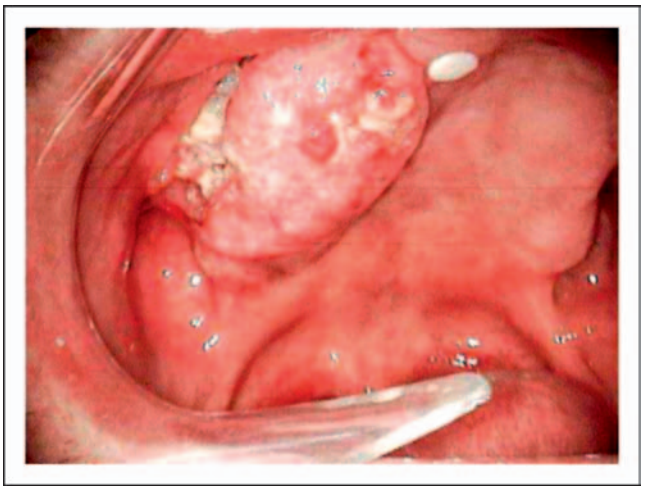
Figure 6 Histopathological example of an oral squamous cell carcinoma lesion, revealing numerous large and atypical nuclei with irregular pattern which were densely stained by hematoxylin–eosin



sensitivity of clinical examination after TBlue application for detection of malignant oral mucosal lesions in high-risk patients following cancer treatment. In another study, Epstein *et al.* [12] investigated the molecular profiles of TBlue positive and negative OPLs and evaluated the staining intensity variations with molecular aberrations. LOH at 3p14 and 9p21, 17p13 and presence of more than one site of loss were reported more frequently in TBlue positive samples and correlated with risk of progression to OSCC or recurrence. All stained lesions showed LOH, with higher frequency in dysplastic lesions. TBlue uptake correlated with allelic loss and with histologic progression of the lesions [12]. Guo *et al.* [52] examined 80 biopsies from 46 patients, after the lesions were evaluated with a pharmaceutical grade TBlue, the stained areas and adjacent negative staining areas within a 5 mm margin of the clinical lesion were biopsied. When microsatellite markers for LOH at 3p21, 9p21, and 17p13 (TP53) were examined, in addition to all SCC cases, 82% of carcinoma-*in-situ* or dysplasia and 59% of cases without dysplasia showed LOH in at least one marker. Three-quarters of the lesions identified by TBlue were clonal and therefore had the potential to progress to malignancy [52]. As weakly stained areas had significantly increased LOH at sites associated with progression to cancer compared with TBlue negative samples, close clinical observation for these lesions was suggested [52]. Zhang *et al.* [77] monitored 100 patients with OPLs in a longitudinal prospective study for a mean of 44 months at 6-month intervals. TBlue positive OPLs harbored a higher frequency of LOH and multiple LOH, indicating the efficacy of TBlue in identifying areas that harbor abnormal molecular change. Further, the progression of OPLs to SCC was significantly higher in stain positive areas, with a four-fold higher risk of progression to SCC even in lesions with benign histopathology or mild dysplasia. After 44 months, 33% of the TBlue positive OPLs with or without dysplasia progressed to SCC, but only 5% of the TBlue negative mucosal lesions progressed to cancer ($P = 0.0002$) [77]. Zhang *et al.* [77] also assessed the responses of the lesions to therapy in this prospective study and found that 81% of TBlue negative lesions at the baseline remained negative; however, three negative cases progressed to cancer and became TBlue positive prior to histologically proven progression [77].

Second primary oral cancers or recurrence of OSCC in previously treated patients impact long-term survival of patients [8,46,73]; thus, early detection is critical in these high-risk patients. These lesions may arise as persistent or recurrent disease or from cells adjacent to the primary index tumor or both or in a field of molecular change [46,61]. However, detection is more difficult in previously treated cancer patients due to posttreatment mucosal changes resulting from radiation and surgery.

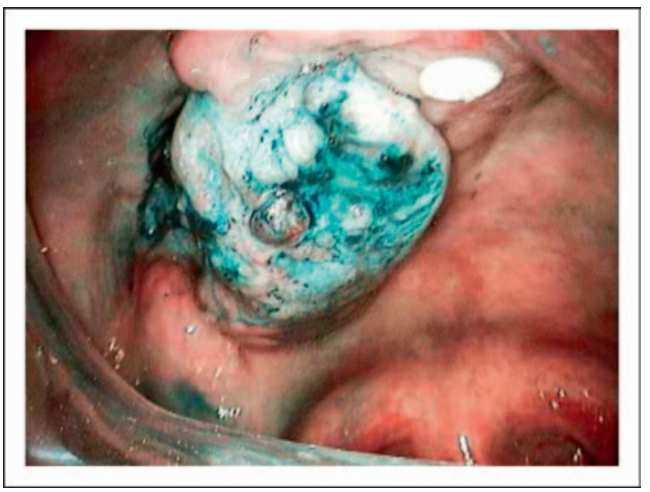
Figure 7 An exophytic lesion located on the maxillary right alveolar mucosa (the white disc has been used as a calibration material to standardize the brightness of the image)



As molecular abnormalities precede phenotypic change and may be present at margins of lesions that are histologically normal, residual disease may remain in excised lesion sites or extend beyond radiation fields, condemning the patient to recurrence [2,42,43,59,72••]. Zhang *et al.* [77] suggested that staining intensity might provide important data due to TBlue binding to molecular changes that predict malignant risk.

TBlue has been recommended as an adjunctive method to assist in early detection of OPLs and OSCC, to assist biopsy site selection, for assessment of margins of OPLs/OSCC and in determining OPLs at risk of progression to OSCC [64,68,69,74,76,78,79] (Figs 7 and 8).

Figure 8 Appearance of the same lesion after toluidine blue application



Toluidine Blue (TBlue) is recommended for the following reasons:

(1) determine OPLs at risk of progression to OSCC (data from [64,68,69,74,76–79]);
(2) identify mucosal lesion with the presence of high-risk molecular patterns that have the potential for progression to cancer (for both low-grade and high-grade dysplasia) (data from [12,52,53,77]);
(3) assess the extent of a lesion and assess margins of OPLs/OSCC (data from [80]);
(4) assist in biopsy site selection and to accelerate the decision to biopsy (data from [8,9,80,81]);
(5) assess the outcome of treatment of oral dysplasic lesions and follow-up postcancer treatment (data from [77]).

False negative staining is very rarely observed in OSCC, particularly in modern series [9,12,76,77,79,82–84], in which improved study designs and pharmaceutical grade TBlue are used. Nevertheless, binding of TBlue to the nucleic acids may occur in mucosal ulcerations, granulation tissue [68,69,74,79,83,84], and in inflammatory lesions that can contribute to false positive outcomes [9,76,84,85]. However, unlike malignant lesions, the blue appearance of these traumatic/benign lesions may not persist as long in the tissue [74] and may localize at the periphery of the ulceration, presenting subjective differences in assessment of stain retention that may guide clinical impression [86]. In order to reduce positive outcomes in inflammatory lesions, a 2-week review of lesions not felt to be at high risk of cancer at first evaluation is recommended [2,74]. On the contrary, TBlue does not stain all early stage dysplastic lesions [12], which suggests that some dysplastic lesions may not have the LOH associated with TBlue retention [80]. Recent studies showed that histologically benign lesions that were TBlue positive have revealed molecular abnormalities and were therefore at increased risk of progression to cancer [52,77], suggesting that 'false positive' TBlue results may represent molecularly true positive lesions with high risk of progression to OSCC [52].

TBlue may be applied with a cotton applicator or swab [9,82,83,85,87,88] or can be used as a rinse to cover the oral mucosa within the mouth [8,12,72••, 73,81,85,86,88–90]. Mashberg [87] identified second primaries in oral mucosa, which were not detected upon clinical examination and were identified after TBlue rinse extending beyond the area of the lesion. Therefore, using TBlue as a mouth rinse was suggested [65,87].

TBlue for topical application has historically been made by compounding laboratory grade powder [84,86]. In 2005, the US Food and Drug Administration (FDA) cleared the only pharmaceutical grade TBlue available for oral use in the United States. TBlue, as part of a light source examination kit (ViziLite Plus) indicated for use as a diagnostic auxiliary to conventional oral cancer screening, is produced by Zila Pharmaceuticals, Inc. (Phoenix, Arizona, USA). The TBlue is part of a swab system to be used at the discretion of the healthcare provider to physically mark oral mucosa lesions differentially identified during ViziLite examination. Since 2008, TBlue has been available in Canada, the United Kingdom, Ireland, France, Germany, Spain, Portugal, and Andorra with distribution agreements in place for Greece, Cyprus, Russia, and Belarus. This system is registered with the Medicines and Healthcare Products Regulatory Agency in the UK as a 'Conformité Européenne' marked that certifies that a product has met EU health, safety, and environmental requirements of a medical device enabling the company to marketing to all European Union (EU) member states. Introduction is planned in Italy in 2009. Regulatory approval is also being sought in China, Korea, India, and Australia.

Zila Pharmaceuticals, Inc., also holds licenses for marketing authorization in the United Kingdom, Belgium, Portugal, Luxembourg, Finland, the Netherlands, and Greece for a pharmaceutical grade TBlue product in a rinse form called OraTest. Distribution is planned in 2009. OraTest is a diagnostic kit, indicated as an adjunct method to clinical examination in the initial diagnosis and treatment of malignant lesions and conditions of the oral mucosa as well as previously treated OSCC patients.

The laboratory grade TBlue, unlike the pharmaceutical grade, may vary between batches, manufacturers, composition, stability, and purity with an unknown shelf life. These differences may be a reason for variability in findings in older studies. Taste is a greater issue with the rinse application than cotton swab application to localized areas.

As is the case in all diagnostic tools and adjuncts, sensitivity and specificity of oropharyngeal application of TBlue is impacted by the prevalence of disease in a population [9]; therefore, outcomes in high-risk patients may not be replicated in lower risk populations [72••]. Additionally, inclusion of equivocal stain results either as positive or negative also impacts the above-mentioned characteristics in some studies. In order to assess sensitivity and specificity of a diagnostic adjunct, all lesions at entry must be assessed using the gold standard test of biopsy. Some trials did not complete biopsies of TBlue negative tissue and, therefore, assessing the sensitivity and specificity of TBlue in these trials cannot be

Table 1 Sensitivity and specificity of toluidine blue staining reported in the literature, which vary with the evaluation criteria of the pale blue lesions

															When TBlue ± was considered as TBlue +						When TBlue ± was considered as TBlue −					
		Lesions with HE[a]			SCC (*n*)		SCC and TBlue+		SCC and TBlue−		SCC and TBlue±		NM[a] (*n*)	NM and TBlue+		NM and TBlue−		NM and TBlue±		True positives		True negatives		False positives		False negatives	
Reference	Lesions (*n*)	*N*	Percentage		*N*	Percentage	*N*	Percentage	*N*	Percentage	*N*	Percentage		*N*	Percentage	*N*	Percentage	*N*	Percentage	*N*	Percentage	*N*	Percentage	*N*	Percentage	*N*	Percentage
[90]	145	86	59.31	18	20.93	18	100.00	0	0.00	0	0.00	68	40	58.82	21	30.88	7	10.29	18	100.00	21	30.88	47	69.12	0	0.00	18	26.47	28	41.18	40.00	58.82	0	0.00
[82]	59	59	100.00	40	67.80	37	92.50	3	7.50	0	0.00	19	7	36.84	12	63.16	0	0	37	92.50	12	63.16	7	36.84	3	7.50	37	194.74	12	63.16	7.00	36.84	3	7.50
[9]	81	81	100.00	27	33.33	22	81.48	0	0.00	5	18.52	54	11	20.37	28	51.85	15	27.78	27	100.00	28	51.85	26	48.15	0	0.00	27	50.00	43	79.63	11.00	20.37	5	18.52
[83]	50	50	100.00	7	14.00	7	100.00	0	0.00	0	0.00	43	16	37.21	27	62.79	0	0	7	100.00	27	62.79	16	37.21	0	0.00	7	16.28	27	62.79	16.00	37.21	0	0.00
[12]	39	39	100.00	25	64.10	9	36.00	9	36.00	7	28.00	14	2	14.29	5	35.71	7	50.00	16	64.00	5	35.71	9	64.29	9	36.00	16	114.29	12	85.71	2.00	14.29	16	64.00
[84]	46	31	67.39	14	45.16	14	100.00	0	0.00	0	0.00	17	8	47.06	9	52.94	0	0	14	100.00	9	52.94	5	29.41	0	0.00	14	82.35	9	52.94	8.00	47.06	0	0.00
[79]	18	18	100.00	9	50.00	9	100.00	0	0.00	0	0.00	9	4	44.44	0	0.00	5	55.56	9	100.00	0	0.00	9	100.00	0	0.00	9	100.00	5	55.56	4.00	44.44	0	0.00
[77][a]	100	100	100.00	17	17.00	16	94.12	1	5.88	NA	NA	83	20	24.10	63	75.90	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
[85][b]	97	97	100.00	20	20.62	15	75.00	0	0.00	5	25.00	77	24	31.17	40	51.95	13	0.17	20	100.00	76	98.70	37	48.05	0	0.00	15	19.48	53	24.00	24.00	31.17	5	25.00
Total	635	561	88.35	177	31.55	147	83.05	13	7.34	17	9.60	384	132	34.38	205	53.39	47	12.24	148	83.62	178	46.35	159	41.41	12	6.78	136	35.42	189	49.22	112	29.17	29	16.38

HE, histological examination; NA, not applicable; NM, nonmalignant; SCC, squamous cell carcinoma; TBlue, toluidine blue staining.

[a]Oral premalignant lesions with high-grade dysplasia were considered as 'positive/malignant' group whereas lesions without dysplasia and with low-grade dysplasia were considered as 'nonmalignant' group.

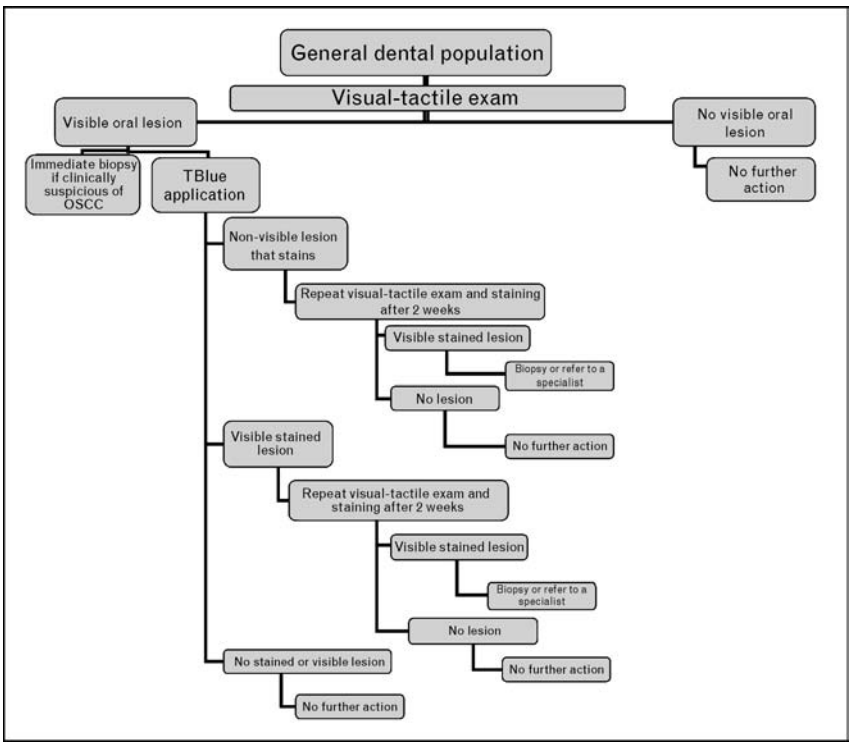
[b]Lesions with severe pathology (severe dysplasia, carcinoma *in situ*, or SCC) were examined as the 'positive/malignant' group whereas lesions with no dysplasia, mild or moderate dysplasia were considered as the 'negative/nonmalignant' group.

completed [72••,79,84,89]. Further, as histopathologic interpretation is subjective, a panel of blinded pathologists should provide outcome data. Studies that have used these methods provide more creditable data to guide clinical utilization [2,9,77,85,86,90] (Table 1).

A recent systematic review of adjunctive examination aids included 2400 lesions in studies varying from 18 to 1030 lesions, stained with TBlue with histologic outcomes [72••]. The sensitivity and specificity of TBlue varied from 38 to 98% (median 85%) and 9 to 93% (median 67%), respectively, whereas the positive predictive value (PPV) ranged from 33 to 93% (median 85%) and the negative predictive value (NPV) from 22 to 92% (median 83%) [72••].

The correlation between the intensity of TBlue staining and the severity of dysplasia has been debated. The variability in the reporting of TBlue staining patterns was seen in different studies, in which some reported only 'a royal-blue' intense stain as positive, whereas others reported any staining as positive [72••]. Gandolfo *et al.* [79] reported that all OSCC stained TBlue positive and that none of the OSCC lesions stained pale blue. However, in a detailed review, Gray *et al.* [1] showed that when equivocal staining was included in positive lesions, sensitivity of TBlue staining was as low as 40% and as high as 100%. This observation was also reported by Missmann *et al.* [64]. If equivocal stained lesions were considered negative, the sensitivity rate was from 100 to 81% [1]. When equivocal staining was accepted as positive, the specificity of using TBlue in potentially malignant lesions was reported as low as 31% – less than 50% [1,9,82,90] and as high as 93% [1,9,64]. Conversely, accepting light blue staining as negative increased the specificity rates significantly in some studies [1,9]. In a multicenter study of 84 patients, with 97 clinically suspicious lesions, all subjected to biopsy, TBlue was shown to provide 100% true positive results and to reduce clinically determined false positive rate by 55.26% with 100% NPV. This finding showed that TBlue might provide utility in reducing the number of biopsies by approximately half while identifying all lesions representing severe dysplasia and OSCC [91••]. Additionally, Zhang *et al.* [77] conducted a longitudinal study, assessing TBlue staining characteristics among high-risk primary OPLs. TBlue negative staining was recorded in 14 out of 19 OPLs without dysplasia, 49 out of 64 OPLs with low-grade dysplasia and one out of 17 OPLs with high-grade dysplasia [77]. TBlue positive staining was observed in five out of 19 OPLs without dysplasia, 15 out of 64 OPLs with low-grade dysplasia and 16 out of 17 OPLs with high-grade dysplasia [77]. The data revealed that 36% of the OPLs were TBlue positive, and 64% of the OPLs were TBlue negative. At last follow-up, 15 out of 100 of all OPLs progressed to cancer and of these 15 lesions,

Figure 9 The decision tree for toluidine blue staining of a suspicious oral lesion



CIS, carcinoma *in situ*; SCC, squamous cell carcinoma; TBlue, toluidine blue. Adapted from [1].

three were TBlue negative, 12 were positive. Among 83 OPLs with mild or no dysplasia, 20 were stained with TBlue, and five of these progressed in cancer. Of the remaining 63 TBlue negative OPLs with minimal or no dysplasia, three lesions developed cancer during follow-up [77]. It is recommended that any staining with TBlue should elevate the index of suspicion [72••], and any lesion with TBlue staining should be considered a candidate for biopsy [77].

Conclusion

The TBlue literature shows that it is a practical, rapid, inexpensive, and effective adjunct diagnostic tool in mucosal disease clinics and cancer centers with experienced providers in high-risk patients. TBlue may assist in detection of oral mucosa with molecular changes with or without phenotypic changes on biopsy that are associated with OPLs or OSCC [7,12,52,72••,75–77,83,90]. TBlue used in addition to clinical examination increased efficacy in detecting OSCC or premalignant lesions or both in high-risk clinics [64,76,85,91••]. In other prospective studies, the use of TBlue is reported to be more sensitive than clinical examination alone in high-risk clinics in detecting premalignant or malignant lesions in patients who had been previously treated for carcinoma of the upper aerodigestive tract and in identifying oral mucosal lesions with high-risk molecular features [76,77].

TBlue is an adjunct to a detailed visual and digital head and neck examination and is useful in raising or confirming clinical suspicion, and when stain is retained, all suspicious lesions should undergo biopsy [9,77,92,93]. A decision tree for TBlue staining of a suspicious oral lesion is presented in Fig. 9 [1].

It is not known whether the more widespread use of TBlue in general practice as an examination adjunct will result in increased diagnosis of dysplasia and malignancy or will lead to increasing numbers of biopsies of benign mucosal changes. However, Epstein *et al.* [91••] showed use in high-risk patients examined by experienced providers reduced the number of biopsies of benign lesions by approximately 50% and identified all severe dysplasia and OSCC lesions would occur with TBlue as part of the clinical protocol. TBlue is recommended as an adjunct to the clinical examination of oral mucosal lesions, specifically in high-risk patients by expert providers. When TBlue is used in general practice and suspicious mucosal lesions are identified, referral to centers experienced in the diagnosis and treatment of OPLs and OSCC is recommended.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 132–133).

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