Available online at www.sciencedirect.com

ScienceDirect

British Journal of Oral and Maxillofacial Surgery 57 (2019) 755–759

BRITISH Journal of Oral and Maxillofacial Surgery

www.bjoms.com

Outcome of sentinel lymph node biopsy in early-stage squamous cell carcinoma of the oral cavity with methylene blue dye alone: a prospective validation study

J.R. Vishnoi [a,b,*], V. Kumar [b], S. Gupta [b], A. Chaturvedi [b], S. Misra [a], N. Akhtar [b], P. Agarwal [c], N. Jamal [d], P. Pareek [e]

[a] *Department of Surgical Oncology, All India Institute of Medical Sciences, Jodhpur, India*
[b] *Department of Surgical Oncology, King George's Medical University, Lucknow, Uttar Pradesh, India*
[c] *Department of Pathology, King George's Medical University, Lucknow, Uttar Pradesh, India*
[d] *Department of Radiodiagnosis, King George's Medical University, Lucknow, Uttar Pradesh, India*
[e] *Department of Radiotherapy, All India Institute of Medical Sciences, Jodhpur, India*

Available online 11 July 2019

Abstract

Our aim was to evaluate the feasibility and role of sentinel lymph node (SLN) biopsy using methylene blue dye alone in identifying occult lymph node metastases in early oral cancer (cT1, T2, and cN0). The study was done from 2013–15 in 94 patients in a large cancer centre. The blue nodes were dissected and sent for frozen section, routine histopathological examination, and immunohistochemical testing for cytokeratin, and was followed by elective neck dissection in all patients. The identification rate was 93.61%. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy for frozen section and haematoxylin and eosin (H&E) staining were 84.6%, 100%, 100%, 93.9% and 95.5%, respectively. Occult lymph node metastasis was seen in 27.6% cases. Biopsy of SLN with blue dye alone might be used successfully with good sensitivity and negative predictive value in countries with limited resources in the developing world. Immunohistochemistry contributes to it by increasing the sensitivity and NPV, and thereby improves the diagnostic value.

Keywords: Early oral cancer; sentinel lymph node biopsy; oral squamous cell carcinoma; blue node; methylene blue dye

Introduction

Squamous cell carcinoma (SCC) of the oral cavity is one of the most common cancers in Southern Asia.[1] When the cervical lymph nodes are involved, the survival reportedly decreases by 50% and the possibility of distant metastases increases.[2] The regional lymphatic spread is therefore an important indicator of poor prognosis in head and neck cancer. The exclusion of the presence of occult lymph node metastases in a clinically and radiographically N0 neck is critical, not only for a proper prognostication but also for planning optimal treatment.[3,4]

The rate of occult metastases in the neck nodes in patients with early (T1 and T2) oral SCC, even when neck nodes are clinically and radiologically clear (N0), is reported to be high (20%-33%).[5] Recently, a randomised controlled trial and systematic review showed that overall survival was improved by elective neck dissection compared with therapeutic neck dissection in these patients.[6,7] However, elective neck dissection would lead to an unnecessary operation for more than two-thirds of cases, with the accompanying risks of functional and aesthetic impairment. It follows that most elective neck dissections are of no therapeutic

* Corresponding author at: Department of Surgical Oncology, All India Institute of Medical Sciences, Basni Industrial Area, Phase II, Jodhpur 342005, India. Tel.: +91 9455061569.
E-mail address: drjvishnoi@gmail.com (J.R. Vishnoi).

https://doi.org/10.1016/j.bjoms.2019.06.025
0266-4356/

benefit and merely provide confirmatory (negative) staging data.[8]

Elective neck dissection results in varying degrees of functional impairment of the shoulder, and correlates with the extent of dissection. Shoulder dysfunction or "shoulder syndrome" is trapezius deficit caused by various degrees of injury to the spinal accessory nerve during neck dissection. It includes shoulder droop, scapular dyskinesia, atrophy of the trapezius, loss of shoulder abduction, and shoulder and neck pain. Apart from these, many other complications are more common in neck dissection, including injury to the vagus, hypoglossal, and marginal mandibular nerves; chylous leak or fistula; and vascular complications.[9] Less extensive surgery therefore means less morbidity and better quality of life.[10]

The concept of biopsy of the sentinel lymph node (SLN) was developed so that the regional lymph nodes could be properly evaluated while the consequences of nodal dissections were reduced. A conservative trend in the treatment of patients with early N0 oral SCC has encouraged increasing use of biopsy of the SLN in this subset of patients.[11] Biopsy of the SLN using a combination of radioisotopes and blue dyes is accurate in predicting subclinical neck metastases.[8,12] However, the limited availability of lymphoscintigraphic facilities in developing countries requires the exploration of alternative methods of detection of SLN using blue dye alone, which may be used with similar accuracy. Our aim, therefore, was to evaluate the feasibility and role of biopsy of the SLN using methylene blue dye alone in identifying occult lymph node metastases in early oral cancer (cT1, T2, and cN0).

Patients and methods

Study design

This prospective study was done during the period November 2013 to October 2015 in 94 patients with clinically and radiologically node-negative early oral SCC (cT1/T2 and N0) who were admitted to the Department of Surgical Oncology in a large tertiary care cancer centre in India. Informed written consent was obtained from all patients. Two separate clinicians examined the oral cavity clinically to rule out palpable nodes, and to assess the primary nodes as well as those in the neck. Only when the two agreed about negative neck nodes was it declared as a "clinically node-negative neck". A single radiologist confirmed the clinical findings of negative neck nodes on high-resolution ultrasound. Other preoperative routine investigations were made according to the institutional protocol including orthopantomogram, contrast-enhanced computed tomographic scan, and magnetic resonance imaging as and when required. Those patients who had recurrent disease, or had been given chemotherapy or radiotherapy previously, were not included. The study was approved by the institutional ethics committee of the University.

Surgical technique

The study required that each patient had peritumoural injection of blue dye in four quadrants (methylene blue 1–2 ml) with a 26 G needle in the operating theatre after induction of anaesthesia. After 10–15 minutes, an incision was made into the skin crease of the neck and subplatysmal flaps were raised. Dissection was then initiated along the digastric muscle to identify and resect the blue nodes in the digastric triangle. All the relevant operative details, including (presence or absence of) a blue node, the total number, and the level, were meticulously recorded. After harvesting, the blue node (sentinel node) was sent for frozen section examination immediately, and selective neck node dissection – supraomohyoid neck dissection (SOND), extended SOND, or modified radical neck dissection (MRND) as stated in the institutional protocol were done in all cases. SOND for buccal mucosa and lip, and extended SOND for carcinoma of the tongue, was the minimum extent of elective neck dissection in all patients. Selective neck dissection was extended to functional neck dissection (MRND) in a small number of cases during operation by the operating surgeon's decision made after consulting the reports on the frozen sections of the blue nodes. Neck dissection was followed by resection of the primary tumour and either primary closure of the defect or reconstruction according to our protocol. Final specimens of the primary tumour and neck nodes were sent for detailed histopathological evaluation.

Histopathological protocol

The sentinel node (blue node) and the complete neck dissection specimen were analysed according to the standard protocol in the Department of Pathology. Frozen sections of the sentinel node were made immediately. Irrespective of the result of dissection of a frozen section of the sentinel node, routine analyses were made on the paraffin-embedded, formalin-fixed, material for both haematoxylin and eosin (H&E) staining and pancytokeratin immunohistochemical staining. During routine H&E staining, sentinel nodes were sectioned longitudinally from hilum to periphery at 2 to 3 mm intervals, and placed into cassettes at the individual sites. For immunohistochemical evaluation sections of sentinel nodes were stained for pancytokeratin (Dako) using a standard immunoperoxidase technique (microwave antigen retrieval with the DAB method). All cell clusters that stained for cytokeratin were reviewed for morphology consistent with the presence of SCC. Histopathological examination of the sentinel lymph node was correlated with the final histopathological examination of the neck dissection specimen. An important endpoint in this primary study was concordance

between the sentinel node and the elective neck dissection specimens about metastatic nodal involvement.

Statistical analysis

The chi-squared test was used to compare the significance of categorical variables. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy and positive and negative likelihood ratios were also calculated. The odds ratio (OR) with 95% CI was calculated. Results were considered significant if probabilities were less than 0.05. All the analyses were made with the aid of SPSS for Windows (version 16.0, SPSS Inc).

Results

Characteristics of patients and tumours

We studied 94 patients (75 men and 19 women, male:female ratio 4:1, median (range) age 45 (20–77) years, and tobacco chewing and smoking were the most important risk factor for the disease. Table 1 shows the clinical and personal variables. We saw no anaphylactic reactions, allergic reactions, or side effects to methylene blue dye.

Table 1
Clinicopathological characteristics. Data are number of patients, except where otherwise stated.

Variable	Total (n = 94)
Median (range) age (years)	45 (20–77)
Sex:	
Male	75
Female	19
Risk factors:	
Smoking	37
Chewing tobacco	80
Alcohol	9
Site of tumour:	
Buccal mucosa	36
Tongue	43
Lip	15
Clinical stage:	
cT1N0	36
cT2N0	58
Operations:	
Wide local excision	79
Wide local excision and marginal mandibulectomy	4
Wide local excision and segmental mandibulectomy	10
Wide local excision and upper alveolectomy	1
Reconstructive procedures	32
Neck dissection:	
Supraomohyoid	40
Extended supraomohyoid	43
Modified radical	11
Side:	
Unilateral	87
Bilateral	7
Pathological characteristics:	
Grade of tumour:	76
Well-differentiated	18
Moderately differentiated	
Perineural invasion	7
Lymphovascular invasion	11

Table 2
Identification rate of sentinel lymph nodes.

Variable	
Sentinel node (n = 88):	
Mean (SD) number identified	1.8 (1.0)
Range	1–7
Buccal mucosa (n = 34):	
Mean (SD) number indentified	1.8 (0.8)
Range	1–4
Tongue (n = 40):	
Mean (SD) number identified	1.8 (0.9)
Range	1–5
Lip (n = 14):	
Mean (SD) number identified	2.0 (1.7)
Range	1–7

Table 3
Distribution of sentinel lymph nodes corresponding to their level in the neck.

Variable	Total (n = 88/94)
Sentinel lymph node distribution:	
IA	3
IB	48
IIA	37
Visible on frozen section or histopathological examination	22
Diagnosed immunohistochemically	25

Sentinel nodes

Table 2 shows the identification rates of sentinel nodes, Table 3 their distribution, and Table 4 their histopathological and immunohistochemical details.

Discussion

There is a large reported variation in the findings of biopsy of the SLN in oral cancer. Combined results of its use as a staging tool for head and neck SCC have reported an identification rate of 95% in a first international conference on SNB with both lymphoscintigraphy and blue dye.[13,14] Ross et al[13] were able to identify the SLN using blue dye alone in 75%, whereas 97% were found with combined techniques in a study by Alkureishi et al.[15] The Sentinel European Node Trial (SENT) reported identification rates and sensitivity of 99.5% and 86%, respectively.[16] We identified 88/94, and the mean (SD) was 1.83 (1.03), range 1–7. We want to emphasise that precise dissection at onset is helpful in identifying the blue-draining lymphatics, which lead to the SLN, and this ultimately improves the identification rate.

A large study of the combined technique reported an overall sensitivity of 93% (floor of mouth 80%, and the rest of the tumours 100%); almost similar sensitivity was also reported in a diagnostic meta-analysis.[15,17] Our sensitivity of frozen section and routine H&E was 84.6%, which

Table 4
Diagnostic evaluation of sentinel lymph nodes using various methods.

Factors	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Accuracy	Likelihood ratio positive	Likelihood ratio negative
General:							
Histopathological	84.6	100.0	100.0	93.9	95.5	–	0.15
Immunohistochemical	96.2	100.0	100.0	98.4	98.9	–	0.03
Buccal mucosa:							
Histopathological	80.0	100.0	100.0	92.3	94.1	–	0.20
Immunohistochemical	100.0	100.0	100.0	100.0	100.0	–	0.0
Lip:							
Histopathological	66.7	100.0	100.0	91.7	92.9	–	0.33
Immunohistochemical	100.0	100.0	100.0	100.0	100.0	–	0.0
Tongue:							
Histopathological	92.3	100.0	100.0	96.4	97.5	–	0.08
Immunohistochemical	96.3	100.0	100.0	96.77	97.4	–	0.08

was increased to 96.2% when combined with immunohistochemistry (which helps to increase sensitivity by identifying additional micrometastasis and isolated tumour cells). Although we know of no substantial evidence, cervical micrometastases of oral cancer should be considered to be as important as macrometastases and should, therefore, warrant completion of neck dissection by removing levels I to IV.[18,19]

In a multi-institutional trial, Civantos et al reported the NPV of 94% and 96% with additional sectioning and IHC.[20] The present study showed a NPV of 93.9%, and immunohistochemistry increased this value to 98.4%. Similarly, a smaller study also reported findings with NPV of 95.8% with blue dye alone, which was increased to 100% with immunohistochemistry.[21] We found occult lymph node metastasis in 27.6% of cases, which was comparable to the results of a systemic review and meta-analysis (26.85%).[22] The rate of occult lymph node metastases was reported to be 33% in early oral cancer also by Shah in his large series from the Memorial Sloan Kettering Cancer Center.[23]

We found invaded lymph nodes in level IA (5.7%), IB (48.6%), IIA (37.1%), and III (8.6%). We found no lymph node metastases to level IIB, IV, and V. According to published studies, the lip drains primarily to level I and the buccal mucosa and tongue to levels I and II. Shah reported no lymph node metastases to levels IV and V for early cancers of the buccal mucosa.[23] However, Woolgar[5] and Byers[24] reported 15.8% skip metastasis to the level IV in patients with cancer of the tongue.[5,24]

To the best of our knowledge, this is the largest study of SLN biopsy in early oral cancer using methylene blue dye alone. The studies done previously were either in the form of pilot projects or were smaller samples. Our study was a well-designed, and systematically conducted, prospective observational study in a cancer centre with a large turnover of patients. Methylene blue dye is inexpensive, readily available and safe.[25] Limitations to its use are spillage of dye in tissue planes, and the need for an incision in the skin crease of the neck. One ml of blue dye (instead of the usual two ml) is adequate, and minimises spillage.

Conclusion

Biopsy of the SLN with methylene blue dye alone in early oral SCC is feasible. It can be used successfully with good sensitivity and negative predictive value in countries with limited resources such as India. Immunohistochemistry contributes to the sensitivity and negative predictive value of biopsy of SLN, and improves diagnostic value. A larger prospective study is needed to compare elective neck dissection with biopsy of the SLN alone in patients with clear nodes to assess its reliability and true applicability.

Conflict of interest

We have no conflicts of interest.

Ethics statement/confirmation of patients' permission

The study was approved by the institutional ethics committee of the University. Informed written consent was obtained from all patients. No information has been given that could be used to identify patients.

Support

No financial support was granted for this article.

Prior publication

The abstract was presented in the award session at 2nd Indian Cancer Congress, Bengaluru in November 2017 and selected as one of the best papers and sent to the Society of Surgical Oncology 2018, Chicago. There it was presented as Global Partner Poster on 21st to 24th March 2018.

Acknowledgement

Our sincere thanks to Prof. Ravikant, Vice Chancellor, King George's Medical University, Lucknow for guiding and submitting to present this article.

References

1. Misra S, Chaturvedi A, Misra NC. Management of gingivobuccal complex cancer. *Ann R Coll Surg Engl* 2008;**90**:546–53.
2. Layland MK, Sessions DG, Lenox J. The influence of lymph node metastasis in the treatment of squamous cell carcinoma of the oral cavity, oropharynx, larynx, and hypopharynx: N0 versus N+. *Laryngoscope* 2005;**115**:629–39.
3. Byers RM, El-Naggar AK, Lee YY, et al. Can we detect or predict the presence of occult nodal metastases in patients with squamous carcinoma of the oral tongue? *Head Neck* 1998;**20**:138–44.
4. Misra S, Chaturvedi A, Misra NC. Oral carcinoma. In: Johnson C, Taylor I, editors. *Recent advances in surgery 25*. London: RSM Books; 2002. p. 71–86.
5. Woolgar JA. Pathology of the N0 neck. *Br J Oral Maxillofac Surg* 1999;**37**:205–9.
6. D'Cruz AK, Vaish R, Kapre N, et al. Elective versus therapeutic neck dissection in node-negative oral cancer. *N Engl J Med* 2015;**373**:521–9.
7. Ding Z, Xiao T, Huang J, et al. Elective neck dissection versus observation in squamous cell carcinoma of oral cavity with clinically N0 Neck: A systematic review and meta-analysis of prospective studies. *J Oral Maxillofac Surg* 2019;**77**:184–94.
8. Kurokawa H, Yamashita Y, Takeda S, et al. Risk factors for late cervical lymph node metastases in patients with stage I or II carcinoma of the tongue. *Head Neck* 2002;**24**:731–6.
9. Kuntz AL, Weymuller EA. Impact of neck dissection on quality of life. *Laryngoscope* 1999;**109**:1334–8.
10. Payakachat N, Ounpraseuth S, Suen JY. Late complications and long-term quality of life for survivors (&5 years) with history of head and neck cancer. *Head Neck* 2013;**35**:819–25.
11. Thompson CF, St John MA, Lawson G, et al. Diagnostic value of sentinel lymph node biopsy in head and neck cancer: A meta-analysis. *Eur Arch Otorhinolaryngol* 2013;**270**:2115–22.
12. Schiefke F, Akdemir M, Weber A, et al. Function, postoperative morbidity, and quality of life after cervical sentinel node biopsy and after selective neck dissection. *Head Neck* 2009;**31**:503–12.
13. Ross GL, Shoaib T, Soutar DS, et al. The first International Conference on sentinel node biopsy in mucosal head and neck cancer and adoption of a multicenter trial protocol. *Ann Surg Oncol* 2002;**9**:406–10.
14. Singh S, Gaud U, Dwivedi AN, et al. Sentinel lymph node biopsy using blue dye technique in carcinoma of the buccal mucosa. *World J Surg Med Radiat Oncol* 2012;**1**:1–8.
15. Alkureishi LW, Ross GL, Shoaib T, et al. Sentinel node biopsy in head and neck squamous cell cancer: 5-year follow-up of a European multicenter trial. *Ann Surg Oncol* 2010;**17**:2459–64.
16. Schilling C, Stoeckli SJ, Haerle SK, et al. Sentinel European Node Trial (SENT): 3-year results of sentinel node biopsy in oral cancer. *Eur J Cancer* 2015;**51**:2777–84.
17. Govers TM, Hannink G, Merkx MA, et al. Sentinel node biopsy for squamous cell carcinoma of the oral cavity and oropharynx: a diagnostic meta-analysis. *Oral Oncol* 2013;**49**:726–32.
18. Melkane AE, Mamelle G, Wycisk G, et al. Sentinel node biopsy in early oral squamous cell carcinomas: A 10-year experience. *Laryngoscope* 2012;**122**:1782–8.
19. Kumar V, Vishnoi J, Kori C, et al. Primary malignant melanoma of oral cavity: a tertiary care center experience. *Natl J Maxillofac Surg* 2015;**6**:167–71.
20. Civantos FJ, Zitsch RP, Schuller DE, et al. Sentinel lymph node biopsy accurately stages the regional lymph nodes for T1–T2 oral squamous cell carcinomas: results of a prospective multi-institutional trial. *J Clin Oncol* 2010;**28**:1395–400.
21. Ramamurthy R, Kottayasamy Seenivasagam R, Shanmugam S, et al. A prospective study on sentinel lymph node biopsy in early oral cancers using methylene blue dye alone. *Indian J Surg Oncol* 2014;**5**:178–83.
22. Hassan O, Taha MS, El Mehairy H. Sentinel lymph node biopsy versus elective neck dissection in evaluation of cN0 neck in patients with oral and oropharyngeal squamous cell carcinoma. Systematic review and meta-analysis study. *Egypt J Ear Nose Throat Allied Sci* 2015;**16**:25–34.
23. Shah JP. Patterns of cervical lymph node metastasis from squamous carcinomas of the upper aerodigestive tract. *Am J Surg* 1990;**160**:405–9.
24. Byers RM. Modified neck dissection. A study of 967 cases from 1970 to 1980. *Am J Surg* 1985;**150**:414–21.
25. Varghese P, Abdel-Rahman AT, Akberali S, et al. Methylene blue dye—a safe and effective alternative for sentinel lymph node localization. *Breast J* 2008;**14**:61–7.