Ann Surg Oncol (2010) 17:2459–2464
DOI 10.1245/s10434-010-1111-3

Annals of
SURGICAL ONCOLOGY
OFFICIAL JOURNAL OF THE SOCIETY OF SURGICAL ONCOLOGY

ORIGINAL ARTICLE – HEAD AND NECK ONCOLOGY

Sentinel Node Biopsy in Head and Neck Squamous Cell Cancer: 5-Year Follow-Up of a European Multicenter Trial

Lee W. T. Alkureishi, MRCS[1], Gary L. Ross, FRCS (Plast)[2], Taimur Shoaib, FRCS (Plast)[1], David S. Soutar, ChM[1], A. Gerry Robertson, FRCR[3], Richard Thompson, MD[1], Keith D. Hunter, FRCPath[4], Jens A. Sorensen, PhD[5], Jorn Thomsen, MD[5], Annelise Krogdahl, DMSc[5], Julio Alvarez, MD[6], Luis Barbier, MD[6], Joseba Santamaria, MD[6], Tito Poli, MD[7], Enrico Sesenna, ChM[7], Adorjan F. Kovács, PhD[8], Frank Grünwald, MD[8], Luigi Barzan, MD[9], Sandro Sulfaro, MD[9], and Franco Alberti, MD[9]

[1]Plastic Surgery Unit, Canniesburn Hospital, Glasgow, UK; [2]Plastic Surgery Unit, Christie Hospital, Wilmslow, UK; [3]Beatson West of Scotland Cancer Centre, Glasgow, UK; [4]Department of Oral Pathology, University of Sheffield, Sheffield, UK; [5]Odense University Hospital, Odense, Denmark; [6]Hospital de Cruces, Universidad del Pais Vasco/EHU, Bilbao, Spain; [7]University Hospital of Parma, Parma, Italy; [8]Johann Wolfgang Goethe University Medical School, Frankfurt, Germany; [9]Azienda Ospedaliera "S. Maria degli Angeli, Pordenone, Italy

ABSTRACT

Background. Sentinel node biopsy (SNB) may represent an alternative to elective neck dissection for the staging of patients with early head and neck squamous cell carcinoma (HNSCC). To date, the technique has been successfully described in a number of small single-institution studies. This report describes the long-term follow-up of a large European multicenter trial evaluating the accuracy of the technique.

Methods. A total of 227 SNB procedures were carried out across 6 centers, of which 134 were performed in clinically T1/2 N0 patients. All patients underwent SNB with preoperative lymphoscintigraphy, intraoperative blue dye, and handheld gamma probe. Sentinel nodes were evaluated with hematoxylin and eosin (H&E) staining, step-serial sectioning (SSS), and immunohistochemistry (IHC). There were 79 patients who underwent SNB as the sole staging tool, while 55 patients underwent SNB-assisted elective neck dissection.

Results. Sentinel nodes were successfully identified in 125 of 134 patients (93%), with a lower success rate observed for floor-of-mouth tumors (FoM; 88% vs. 96%, $P = 0.138$). Also, 42 patients were upstaged (34%); of these, 10 patients harbored only micrometastatic disease. At a minimum follow-up of 5 years, the overall sensitivity of SNB was 91%. The sensitivity and negative predictive values (NPV) were lower for patients with FoM tumors compared with other sites (80% vs. 97% and 88% vs. 98%, respectively, $P = 0.034$).

Conclusions. Sentinel node biopsy is a reliable and reproducible means of staging the clinically N0 neck for patients with cT1/T2 HNSCC. It can be used as the sole staging tool for the majority of these patients, but cannot currently be recommended for patients with tumors in the floor of the mouth.

Elective neck dissection (END) remains the current gold standard investigation for staging the cervical lymph nodes in patients with head and neck squamous cell cancer (HNSCC).[1] END is both a staging and a therapeutic procedure, which represents a potential benefit for patients who are subsequently found to harbor occult disease. However, these patients represent only approximately 25% of the population, leading to the possibility of overtreating the remaining 75%.[2] A wait-and-see policy has been advocated; however, the high prevalence of occult disease means that this policy cannot be universally recommended.

Portions of this work have been presented at the American Head and Neck Society Meeting in San Francisco, California (July 2008) and the 3rd Sentinel Node Biopsy Conference in Miami, Florida (March 2007). The content of this written report has not been previously published. The authors have no conflicting interests to declare.

First Received: 26 March 2009;
Published Online: 15 June 2010

L. W. T. Alkureishi, MRCS
e-mail: lee_alkureishi@hotmail.com

The considerable morbidity associated with neck dissection reinforces the importance of finding alternative means of accurately staging the clinically negative (cN0) neck.[3,4]

Sentinel node biopsy (SNB) potentially allows for staging of the cervical lymph nodes without the morbidity of a neck dissection.[5] The combination of preoperative peritumoral injection of radiotracer and intraoperative injection of blue dye provides a means of selecting the small number of nodes most likely to receive the initial drainage from the region of the primary tumor, and the accuracy of the procedure is dependent on the concept that these "sentinel" nodes will accurately reflect the remainder of the nodal basin.[6] The ability to limit the pathological evaluation to a small number of nodes potentially offers an additional benefit: It is possible to use more detailed pathological techniques to identify micrometastatic deposits, a process that would be impractical for a complete neck dissection specimen.[2]

The acceptance of SNB for HNSCC has been slower than that for melanoma; however, initial results have shown considerable promise. Early phase I validation studies have consistently reported sensitivities and negative predictive values of greater than 90%, paving the way for larger multicenter trials such as the current study.[7–9]

Preliminary results of this study, based on an interim analysis at 2 years of follow-up, were published in 2004 and demonstrated the feasibility of the technique as a staging tool.[10] Sentinel nodes were successfully identified and harvested in 93% of patients, and the overall sensitivity of the technique was found to be 93%. Patient accrual for this study was completed in 2002, and all patients have now reached at least 5 years of follow-up.

PATIENTS AND METHODS

Patients were recruited from June 1998 until November 2002; 6 Plastic and Maxillofacial surgery units contributed patients from the United Kingdom, Germany, Denmark, Italy, and Spain. Before commencement of the study, local and multicenter research ethics committee (REC) approvals were obtained.

Inclusion Criteria

The full details of this study's inclusion/exclusion criteria and study protocol have been published previously.[11,12] In brief, patients with clinically staged T1/T2 N0 oral/oropharyngeal SCC underwent either SNB alone or SNB-assisted elective neck dissection (SNB-assisted END) in order to stage the clinically negative neck. All SNB procedures used the triple diagnostic technique of preoperative lymphoscintigraphy (LSG), intraoperative injection of patent blue V dye (Laboratoire Guerbet, Aulnay-Sous-Bois, France), and handheld gamma probe radiolocalization, and follow-up was at least 5 years (mean 5.4 years).

Pathologic Evaluation

Harvested sentinel nodes were trimmed into blocks a maximum of 2.5 mm thick. One initial section from each block was examined with H&E staining. Negative nodes were subsequently examined with H&E at 150-μm step-serial sections (SSS). Nodes that remained negative were then evaluated with cytokeratin AE1/3 immunohistochemistry (IHC), and IHC-positive slices were compared with adjacent sections to confirm viable tumor cells. Patients in whom a sentinel node was positive by step-serial sectioning/immunohistochemistry with no further evidence of disease in the neck dissection specimen were deemed to have micrometastatic disease and were staged pN1mi.[13–15] Pathologic evaluation of neck dissection specimens was with H&E-only.

Statistics

Descriptive statistics were given as mean, percentage, and range. The unpaired *t* test was used to compare means between groups, and the chi-square test was used for categorical data. Kaplan-Meier survival curves were generated, with log-rank and Breslow tests for comparison of survival between groups. A P value <0.05 was considered significant. All analysis was carried out in SPSS 12 for Windows (SPSS Inc., Chicago, IL).

RESULTS

A total of 134 patients were included, with 79 patients undergoing SNB alone and 55 undergoing SNB-assisted END. Age, sex, and T-stage distribution did not differ significantly between groups. Full details of this patient population have been previously published.[10]

Sentinel nodes were successfully harvested in 125 of 134 patients (93%), with lower identification rates observed for floor-of-mouth (FoM) tumors (88% vs. 96%, $P = 0.14$) and for SNB-alone (72 of 79, 91% versus 53 of 55, 96%). These findings did not reach statistical significance.

Of the 125 cN0 patients, 42 were upstaged by SNB (34%), with 10 patients demonstrating micrometastatic disease detectable only by SSS ($n = 2$) or IHC ($n = 8$). There were no differences in upstaging between SNB-alone or SNB-assisted END groups, or between tumor sites. cT2 tumors were upstaged twice as often as cT1 tumors (51% vs. 20%; $P = 0.001$). Also, 19 patients underwent bilateral SNB, giving 144 neck sides.

Follow-up

In the SNB-assisted END group, 22 of 53 patients were upstaged. One patient with a FoM tumor, staged negative by SNB, was subsequently found to have tumor within the END specimen, giving a sensitivity of 96% (22 of 23) and negative predictive value of 97% (30 of 31) for the SNB-assisted END group. No patients in this group have developed nodal recurrence.

In the SNB-alone group, 20 patients were staged SNB-positive and 52 SNB-negative. Of the SNB-negative patients, 3 developed cervical node involvement during the 5-year follow-up period, giving a sensitivity of 87% (20 of 23) and negative predictive value of 94% for SNB alone. Overall sensitivity for sentinel node biopsy in patients with cT1/T2 cN0 HNSCC was 42 of 46 patients (91%), with a negative predictive value of 95%.

One patient with a right-sided FoM tumor close to, but not involving, the midline demonstrated only ipsilateral drainage on preoperative LSG. He underwent ipsilateral SNB alone, which proved positive, and was treated with a right-sided MRND. At 4 years follow-up, he developed contralateral (left-sided) nodal recurrence and was subsequently treated with left-sided neck dissection. When considering neck sides, the overall sensitivity of SNB was 44 of 49 (90%) and NPV was 95 of 100 (95%).

Of the 5 false-negative patients, 4 had floor-of-mouth tumors. The sensitivity was 80% (12 of 15 patients) for FoM tumors compared with 97% (30 of 31 patients) for other tumor sites ($P = 0.034$). Similarly, the NPV for FoM tumors was 88% compared with 98% for tumors in other sites. The lower sensitivity for FoM tumors was more pronounced in the SNB-alone group (67% vs. 94%, $P = 0.016$) compared with the SNB-assisted END group (89% vs. 100%, $P = 0.391$) (Tables 1, 2). Negative predictive values were lower for FoM patients in both groups (91% vs. 97% and 75% vs. 100%, respectively).

A number of patients were excluded from further follow-up. Of the 52 SNB-alone patients staged SNB negative, 11 developed second primary aerodigestive SCC tumors, 5 died due to unrelated causes, 4 developed local recurrence, 1 presented with distant metastasis, and 3 patients were lost to further follow-up. Of the 52 patients, 28 remained disease-free throughout the follow-up period, while 7 patients are currently disease-free following additional treatment.

Kaplan-Meier survival analysis was used to compare locoregional disease-free survival between SNB-positive and SNB-negative patient groups. The curves appear to demonstrate poorer survival in the SNB-positive group, but this finding did not reach statistical significance (log-rank statistic 0.37, $P = 0.55$). Comparison between SNB alone and SNB-assisted END demonstrated no significant survival difference for patients with tumors outside of the floor of the mouth (Fig. 1).

TABLE 1 Upstaging of patients by primary tumor site

Tumor site	Patients		Neck sides	
	SN+	Total	SN+	Total
Anterior tongue	16 (33%)	48	17 (31%)	54
Floor of mouth	12 (32%)	37	13 (28%)	47
Posterior tongue	5 (56%)	9	5 (56%)	9
Retromolar trigone	6 (55%)	11	6 (55%)	11
Buccal	1 (20%)	5	1 (20%)	5
Lower alveolus	1 (17%)	6	1 (14%)	7
Hard palate	1 (33%)	3	1 (25%)	4
Soft palate	0	2	0	3
Lip	0	1	0	1
Tonsil	0	2	0	2
Upper alveolus	0	1	0	1
Total	42 (34%)	125	44 (31%)	144

No significant differences in likelihood of upstaging were found between primary tumor sites

DISCUSSION

Management of the clinically negative neck in patients with early HNSCC remains a controversial subject. The choice between elective neck dissection or wait-and-see policy is seldom an easy one, based primarily on the characteristics of the primary tumor.[16] This approach is imperfect, with approximately 25% of clinically negative patients reported to harbor occult metastases.[17,18] Conversely, the considerable morbidity associated with END precludes it from being universally employed in this patient group.[4]

The emergence of sentinel node biopsy provides the possibility of accurate pathological staging of the cervical node basin, while minimizing the invasiveness of the procedure and its associated morbidity.[6,19] The use of preoperative lymphoscintigraphy has the additional advantage of identifying aberrant drainage pathways, potentially guiding the surgeon in planning further treatment.[20]

The results of phase I trials have proven encouraging, with several small single-center studies reporting technical success rates, sensitivities, and negative predictive values greater than 90%.[7–9] While these results suggest the feasibility of SNB, larger phase II and III trials are required before the technique can be recommended as a true alternative to END in this population. To date, this large multicenter study provides the most compelling evidence to support the use of SNB as a staging tool.

The results of this study have demonstrated that sentinel node biopsy is a viable alternative to elective neck

TABLE 2 Comparison of floor-of-mouth tumors (FoM) with tumors in other sites

	SNB alone						SNB-assisted END						All SNB				
	Success	Total	(%)	SN +	All +	Sensitivity/NPV	Success	Total	(%)	SN +	All +	Sensitivity/NPV	Success	Total	(%)	SN +	All +	Sensitivity/NPV
FoM tumors	25	30	83%	4	6	67%/91%	12	12	100%	8	9	89%/75%	37	42	88%	12	15	80%/88%
Other tumors	47	49	96%	16	17	94%/97%	41	43	95%	14	14	100%/100%	88	92	96%	30	31	97%/98%
All tumors	72	79	91%	20	23	87%/94%	53	55	96%	22	23	96%/97%	125	134	93%	42	46	91%/95%
Statistical significance	$P = 0.098$			$P = 0.016$			$P = 0.3$			$P = 0.391$			$P = 0.138$			$P = 0.034$		

SNB sentinel node biopsy, *END* elective neck dissection, *FoM* floor-of-mouth

A lower identification rate and sensitivity are seen in patients undergoing SNB alone for FoM tumorsdupe

dissection for staging the clinically negative neck in patients with early head and neck squamous cell cancer. While there is undoubtedly a learning curve associated with the procedure, the 93% successful SN identification rate, 91% sensitivity, and 95% negative predictive values observed in this study are in keeping with previous reports and highlight the feasibility of the technique across multiple institutions.[2,7–9,20–23] Perhaps most importantly, patients undergoing sentinel node biopsy alone were not demonstrated to have a significantly different long-term survival compared with patients undergoing elective neck dissection in this study.

Sentinel node biopsy has the benefit of drastically reducing the number of lymph nodes for pathological evaluation, by superselecting only those nodes most likely to reflect the disease status of the rest of the neck.[2,6] This allows more in-depth evaluation of the small number of sentinel nodes, using SSS and immunohistochemistry for the detection of micrometastatic deposits.[23] The term "micrometastasis" was applied to any disease that was not detected by routine H&E staining of the initial blocks and was found in 10 patients. These represent 11% of the patients who were initially staged SNB negative, and this relatively high proportion illustrates the importance of these additional pathological techniques for the detection of occult disease. The size criteria outlined by Hermanek et al. were not employed in this study.[15] These criteria are not based on robust evidence, and there remains considerable debate regarding the size of SLN metastasis that is clinically significant.[24] As such, we have included all viable occult metastases in our analysis. While the significance of micrometastatic disease has yet to be fully determined, it is currently recommended that these patients should be treated in the same way as pN1 patients until clear evidence dictates otherwise.[9,13,15]

However, it is clear from our results that sentinel node biopsy may not be appropriate for every patient in this population. Specifically, SNB demonstrates lower technical success rates and poorer accuracy for patients with tumors in the floor of the mouth—findings that were evident in our interim results in 2004 and have also been reported by other groups.[10,25] The close proximity of the primary tumor to the draining lymph node basin causes difficulties for both preoperative lymphoscintigraphy and intraoperative radiolocalization, because of the well-described phenomena of "shine-through" radioactivity and scatter from the primary site.[26] These problems were more pronounced in the group of patients undergoing SNB-alone, where limited access through a small incision can add significantly to the technical difficulty of the procedure. The improved access afforded by raising flaps for neck dissection may go some way toward mitigating the difficulties related to the proximity of the primary tumor

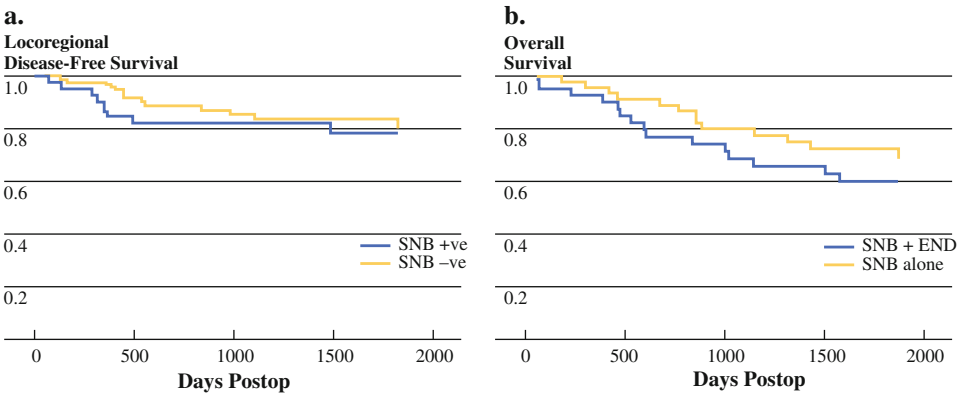

FIG. 1 Survival. (a) SNB-positive patients demonstrate lower locoregional disease-free survival compared with SN negative, although not statistically significant (log rank = 0.37, $P = 0.545$). (b) SNB-alone patients do not demonstrate significantly different overall survival compared with SNB-assisted-END (log rank = 1.10, $P = 0.293$)

site. As a result, it is not possible to advocate SNB as the sole staging tool for patients with FoM tumors. It is possible that combining the SNB with a level I node clearance may improve detection rates and sensitivity, though this will require further study before it can be universally recommended.

Future of SNB in HNSCC

The outcomes of this study provide further weight to the argument for the use of sentinel node biopsy in selected patients with early HNSCC. However, its exact role in the management of these patients remains largely undefined. At present, there are 2 ongoing multicenter trials whose outcomes may prove of considerable importance: the SENT and ACOSOG Z0360 trials.[25,27]

The European Sentinel Node Trial (SENT) is a large prospective study, which builds upon data collected from the present study, the Swiss experience, and a number of other experienced European centers.[27] The mean follow-up for this dataset is currently at 27 months. The American College of Surgeons Oncology Group (ACOSOG) Z0360 trial is a prospective multicenter validation study that completed accrual of 137 oral SCC patients from 25 institutions in 2006. The trial is currently in the follow-up phase and reported an interim analysis of their data in 2007.[25] Based on preliminary pathology with H&E staining alone, the authors reported a negative predictive value of 94%. Notably, the ACOSOG trial also described poorer results for patients with FoM tumors, in keeping with the results of the present study.

In conclusion, the role of sentinel node biopsy in the management of patients with early HNSCC has yet to be fully elucidated. However, evidence favoring its use as a staging tool continues to grow, and the results of this study provide the strongest supporting argument to date. While SNB may not be universally applicable in this patient population, its potential benefits are clear and the upcoming results of ongoing multicenter studies will hopefully go some way toward clarifying its exact role.

REFERENCES

1. Pitman KT, Johnson JT, Myers EN. Effectiveness of selective neck dissection for management of the clinically negative neck. *Arch Otolaryngol Head Neck Surg.* 1997;123:917–22.
2. Werner JA, Dunne AA, Ramaswamy A, Dalchow C, Behr T, Moll R, et al. The sentinel node concept in head and neck cancer: solution for the controversies in the N0 neck? *Head Neck.* 2004;26:603–11.
3. Alvi A, Johnson JT. Extracapsular spread in the clinically negative neck (N0): implications and outcome. *Otolaryngol Head Neck Surg.* 1996;114:65–70.
4. Sobol S, Jensen C, Sawyer W 2nd, Costiloe P, Thong N. Objective comparison of physical dysfunction after neck dissection. *Am J Surg.* 1985;150:503–9.
5. Schiefke F, Akdemir M, Weber A, Akdemir D, Singer S, Frerich B. Function, postoperative morbidity, and quality of life after cervical sentinel node biopsy and after selective neck dissection. *Head Neck.* 2009;31:503–12.
6. Morton DL, Wen DR, Wong JH, Economou JS, Cagle LA, Storm FK, et al. Technical details of intraoperative lymphatic mapping for early stage melanoma. *Arch Surg.* 1992;127:392–9.
7. Shoaib T, Soutar DS, MacDonald DG, Camilleri IG, Dunaway DJ, Gray HW, et al. The accuracy of head and neck carcinoma sentinel lymph node biopsy in the clinically N0 neck. *Cancer.* 2001;91:2077–83.
8. Taylor RJ, Wahl RL, Sharma PK, Bradford CR, Terrell JE, Teknos TN, et al. Sentinel node localization in oral cavity and oropharynx squamous cell cancer. *Arch Otolaryngol Head Neck Surg.* 2001;127:970–4.
9. Stoeckli SJ, Steinert H, Pfaltz M, Schmid S. Sentinel lymph node evaluation in squamous cell carcinoma of the head and neck. *Otolaryngol Head Neck Surg.* 2001;125:221–6.
10. Ross GL, Soutar DS, MacDonald DG, Shoaib T, Camilleri I, Roberton AG, et al. Sentinel node biopsy in head and neck cancer: preliminary results of a multicenter trial. *Ann Surg Oncol.* 2004;11:690–6.
11. Ross GL, Shoaib T, Soutar DS, MacDonald DG, Camilleri IG, Bessent RG, 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.
12. Shoaib T, Soutar DS, Prosser JE, Dunaway DJ, Gray HW, McCurrach GM, et al. A suggested method for sentinel node

biopsy in squamous cell carcinoma of the head and neck. *Head Neck.* 1999;21:728–33.
13. Sobin LH, Wittelind Ch, eds. International union against cancer: TNM classification of malignant tumours. 6th ed. New York: Wiley, 2002.
14. Ross GL, Shoaib T, Soutar DS, Camilleri IG, Gray HW, Bessent RG, et al. The use of sentinel node biopsy to upstage the clinically N0 neck in head and neck cancer. *Arch Otolaryngol Head Neck Surg.* 2002;128:1287–91.
15. Hermanek P, Hutter RVP, Sobin LH, Wittekind C. Classification of isolated tumour cells and micrometastasis. *Cancer.* 1999;86: 2668–73.
16. Yuen AP, Lam KY, Chan AC, Wei WI, Lam LK, Ho WK, et al. Clinicopathological analysis of elective neck dissection for N0 neck of early oral tongue carcinoma. *Am J Surg.* 1999;177:90–2.
17. van den Brekel MW, Stel HV, Castelijns JA, Nauta JJ, van der Waal I, Valk J, et al. Cervical lymph node metastasis: assessment of radiologic criteria. *Radiology.* 1990;177:379–84.
18. Alex JC, Krag DN. The gamma-probe-guided resection of radiolabeled primary lymph nodes. *Surg Oncol Clin N Am.* 1996;5: 33–41.
19. Dünne AA, Külkens C, Ramaswamy A, Folz BJ, Brandt D, Lippert BM, et al. Value of sentinel lymphonodectomy in head and neck cancer patients without evidence of lymphogenic metastatic disease. *Auris Nasus Larynx*. 2001;28:339–44.
20. van der Veen H, Hoekstra OS, Paul MA, Cuesta MA, Meijer S. Gamma probe-guided sentinel node biopsy to select patients with melanoma for lymphadenectomy. *Br J Surg.* 1994;81:1769–70.
21. Mozzillo N, Chiesa F, Botti G, Caracò C, Lastoria S, Giugliano G, et al. Sentinel node biopsy in head and neck cancer. *Ann Surg Oncol.* 2001;8:103S–5S.
22. Thomsen JB, Sørensen JA, Grupe P, Karstoft J, Krogdahl A, et al. Staging N0 oral cancer: lymphoscintigraphy and conventional imaging. *Acta Radiol.* 2005;46:492–6.
23. Ambrosch P, Brinck U. detection of nodal micrometastasis in head and neck cancer by serial sectioning and immunostaining. *Oncology (Huntingt).* 1996;10:1221–6.
24. Atula T, Hunter KD, Cooper LA, Shoaib T, Ross GL, Soutar DS. Micrometastases and isolated tumour cells in sentinel lymph nodes in oral and oropharyngeal squamous cell carcinoma. *Eur J Surg Oncol.* 2009;35:532–8.
25. Civantos F, Zitsch R, Bared A. Sentinel node biopsy in oral squamous cell carcinoma. *J Surg Oncol.* 2007;96:330–6.
26. Krag D, Weaver D, Ashikaga T, Moffat F, Klimberg VS, Shriver C, et al. The sentinel node in breast cancer—a multicenter validation study. *N Engl J Med.* 1998;339:941–6.
27. Ross GL, On Behalf of the Sentinel European Node Trial (SENT) Organising Committee. Sentinel node biopsy for squamous cell carcinoma of the oral cavity: preliminary results of the SENT trial. Presented at the annual meeting of the American Head and Neck Society (AHNS), San Francisco, CA, July 2008.