Head and Neck Sentinel Lymph Node Biopsy: Current State of the Art

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Abstract Sentinel node biopsy is an alternative to elective neck dissection for the management of T1/T2 oral and oro-pharyngeal squamous cell carcinomas and is also finding application to head and neck cancer at other sites. The main clinical aim of sentinel node biopsy is to achieve better staging and there is now evidence that the procedure reduces morbidity. Reported detection rates for sentinel neck nodes are greater than 95% and there is also a negative predictive value of 95% for negative sentinel nodes. Current histopathological protocols have been developed for use in the research setting and are designed to identify all micrometastatic disease. However the use of step serial sectioning at 150 micron intervals with pan-cytokeratin immunohistochemistry is currently advised and appears to upstage nodes by approximately 20% over the initial single routine stained section. Adoption of the UICC/TNM definitions is recommended for future sentinel node studies, but further refinements and descriptions are required. The SENT trial has recruited over 300 cases from 10 European centres and a quality control study of the pathological material is in progress. At the first consensus meeting of the SENT pathology group there were excellent levels of agreement on the diagnosis of positive and negative nodes, and a number of potential pitfalls such as non-malignant inclusions and staining artefacts were identified.

Keywords Oral cancer · Head and neck cancer · Sentinel node · Neck dissection · Histopathology · SENT trial · Lymphoscintigraphy · Guidelines · Pan-cytokeratin · Benign lymph node inclusion

Introduction

The management of the clinically and radiologically negative (N0) neck in patients with small (early) head and neck squamous cell carcinoma remains controversial. Currently most centres undertake routine elective neck dissection for T1/T2 oral and oro-pharyngeal carcinomas to simultaneously stage the neck and remove occult disease. Only 25–30% of patients actually harbour occult disease in the neck [1]. It follows that the majority of neck dissections have no therapeutic benefit and merely provide confirmatory negative staging data. Lymph node metastasis has been shown to be the single most important adverse prognostic factor in oral and oro-pharyngeal squamous cell carcinoma. Accurate staging of the neck is vital to prognostication and is often essential for further treatment planning when using multiple therapeutic modes. Identification of the drainage basins by lymphoscintigraphy combined with detailed histopathological examination of sentinel nodes may provide more accurate staging than current elective neck dissection protocols. Sentinel node biopsy may also be useful for the management of other squamous cell carcinomas of the head and neck, particularly larynx when combined with intra-operative analysis of node status [2].

Several co-operative validation studies (ACOSOG and the University of Miami [3], Sentinel Node European Trial (SENT), the Danish national group [4] and the Brazilian head and neck group [5] are in progress. The published data

On behalf of the SENT trial group.

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so far show a consistent pattern of sentinel node detection rates above 95% and negative predictive values for negative sentinel nodes of 95%. Consequently several individual centres are changing their protocols to employ sentinel node biopsy instead of elective neck dissection and are observing the outcomes. In the future all of these studies will establish the proper role of sentinel node biopsy in the management of head and neck cancer.

Review of the literature reveals scant published information regarding the utility of histopathological techniques for sentinel node examination in the head and neck. Recently guidelines have been proposed but rely on non-randomised studies [6]. In contrast to breast and melanoma, only observational cohort research studies relating to the sentinel node method in head and neck have been conducted to date. The head and neck protocols have typically been designed to detect micrometastases and isolated tumour cells with high sensitivity and have not taken health economic costs into account. There is also an increasing trend towards using intra-operative frozen section of sentinel node in large centres or for specific applications, where lower sensitivity has to be accepted for surgical gain. This review will concentrate on the histopathological aspects of sentinel node biopsy, drawing on the experience of the SENT trial.

Discussion

Clinical Issues

The background to the debate on the role of sentinel node biopsy in head and neck cancer relates to the well established finding that prognosis is closely related to staging. Regional and distant metastases have a powerful influence in reducing overall survival rates [7]. Small oral and oropharyngeal cancers have a locally invasive phenotype and surgery can be curative if clear margins are achieved [8]. Oral and oropharyngeal cancers spread through lymphatic pathways to the cervical lymph nodes. The presence of metastasis in neck nodes is the single most important adverse prognostic factor with overall 5 years survival dropping from 82 to 53% as a result of regional lymphatic involvement [7].

Previously there were two options for the management of the clinically and radiologically negative neck at risk; surveillance or elective neck dissection. However there is a confirmed 25–30% occult metastasis rate and very poor outcomes are associated with detection after surveillance, particularly when extracapsular lymph node spread is present. Thus elective neck dissection has become the gold standard, even though the procedure will provide only negative staging information in 70–75% of cases. Although

the morbidity associated with elective neck dissection is arguably less than dissection at other sites in the body such as axilla or groin, there are still unwanted effects particularly when bilateral dissection is performed. Recent quality of life studies have favoured sentinel node biopsy over elective neck dissection [9]. Detection of occult neck metastasis has improved with advances in imaging and the use in particular of ultrasound with fine needle aspiration can identify many patients requiring neck dissection. However the best methods reach only 80–85% sensitivity and require experienced and skilled operators.

The clinical sentinel node procedure has been described fully elsewhere [7] and briefly consists of injecting a radio-labelled colloid around the primary tumour that drains to the first-echelon lymph nodes. The $^{99}\text{Tc}^{\text{m}}$ -labeled colloid accumulates in the nodes and can be detected by lymphoscintigraphy. A hand held gamma probe is the primary detection method used to identify ‘hot’ nodes and can be combined with static or dynamic imaging. Blue dye can also be injected around the tumour to aid visualisation of the sentinel nodes. Recently the use of SPECT/CT has been advocated and offers better anatomical localisation than planar lymphoscintigraphy but may not improve the outcome of the sentinel node procedure itself [7].

Histopathology Protocols

In the SENT trial and published series, lymphoscintigraphy produces an average of 2.5 lymph nodes per neck side. Thus greater histopathological effort can reasonably be expended on these nodes than on the nodes removed by elective neck dissection which can be numerous and in which the sentinel nodes are not identified.

At present the significance of finding micrometastasis (MMs) and individual tumour cells ITCs) is unknown in head and neck squamous carcinoma. The finding of ITCs and MMs in breast carcinoma sentinel nodes does not always trigger axillary dissection and consequently sentinel nodes blocks for breast cancer are often examined by routine histology at three widely spaced levels only, with the aim of detecting significant metastatic deposits [10]. However every carcinoma has a distinct biological behaviour and the significance of ITCs and MMs in squamous cell carcinoma of the head and neck remains to be ascertained. Recently the effect of adopting the UICC/TNM protocols and definitions (see Boxes 1, 2) on the Canniesburn series has been evaluated [11]. Eighty-six sentinel nodes were reassessed using the UICC/TNM definitions and the results were linked to the pathological findings in the subsequent neck dissections. Additional metastases was found in 46% of patients with MM and only one of out of five ITC patients had subsequent neck metastases. The data are limited but indicate that there is a

Box 1 UICC definitions of metastatic deposits

Metastasis ≥ 2 mm
Micrometastasis >0.2 and <2 mm
Isolated Tumour Cells ≤ 0.2 mm
Single cells, small clusters
No stromal reaction
No contact with vessel wall

Box 2 TNM codes for head and neck sentinel nodes*Generic TNM coding for sentinel nodes*

pNX (sn) Sentinel lymph node could not be assessed

pN0 (sn) No sentinel node metastasis

pN1 (sn) Sentinel node metastasis

Sentinel nodes with micrometastasis only are identified by (mi)

pN1 (sn) (mi) single ipsilateral node with micrometastasis

pN2 (sn) (mi) multiple ipsilateral nodes with micrometastasis

Sentinel nodes with isolated tumour cells are coded separately for morphological and non-morphological techniques such as PCR or flow cytometry

pN0 (i−)(sn) No sentinel lymph node metastasis histologically, negative morphological findings for isolated tumour cells (ITC)

pN0 (i+)(sn) No sentinel lymph node metastasis histologically, positive morphological findings for isolated tumour cells (ITC)

pN0 (mol−)(sn) No sentinel lymph node metastasis histologically, negative non-morphological findings for isolated tumour cells (ITC)

pN0 (mol+)(sn) No sentinel lymph node metastasis histologically, positive non-morphological findings for isolated tumour cells (ITC)

need for elective neck dissection when micrometastasis is found in a sentinel node. Further studies are needed and only when sufficient evidence has accumulated would it be safe to revise the pathology protocols. Meantime the UICC/TNM scheme offers a measure of standardisation for ongoing and future studies.

The pathological protocol used in the Canniesburn trial was devised by the late Professor Gordon MacDonald and has been adapted for the SENT and other trials. The protocol is shown in Boxes 3 and 4 and briefly involves trimming the node into 2 mm slices (or bisecting small nodes through the hilum), step serial sectioning at 150 microns and the use of cytokeratin (AE1/AE3) immunohistochemistry. In the initial series of 125 nodes in 59 clinically negative necks, 13% were positive on initial haematoxylin and eosin stained section and the use of the step serial and cytokeratin staining methods upstaged a further 17% of necks. The need to preserve adjacent sections to allow morphological comparison with immunohistochemical findings was emphasised and a range of non-relevant epithelial and other inclusions were identified. Similar findings and rates have been reported in several

more recent studies [7]. Many of the pathologists in the SENT group have modified the operation of the protocol so that all step serial sections and AE1/AE3 staining is performed in one run to speed turnaround time.

SENT Experience

The SENT trial (CI, Mark McGurk, GKT, London) has recruited over 300 cases from 10 centres in Europe. The SENT pathology group met in Amsterdam in May 2009 to undertake an initial quality control exercise. Histopathological slides contributed by the centres were shown ‘blind’ and there was excellent agreement on the diagnosis of sentinel nodes. These included sentinel nodes that were negative and nodes that contained individual tumour cells, micrometastasis and frank metastasis (Fig. 1). Non squamous carcinoma deposits and inclusions were shown and confirmed by consensus diagnosis. These included lateral thyroid inclusions, metastatic papillary carcinoma of thyroid, parathyroid inclusions, melanocytic naevus, salivary inclusions and other benign epithelial inclusions. The group were surprised by the frequency of finding ‘non-

Box 3 Histopathological protocol for sentinel nodes**Gross sectioning**

Fix basin or node in 10% neutral buffered formalin (or equivalent) for 24 h, as per good laboratory practice

Macroscopic description and dimensions of nodes

Palpate and trim excess fat to reveal node(s) (avoid damage to node periphery)

Sectioning

Nodes too small to divide (less than 2 mm)—process whole

Nodes up to 5 mm—cut through the hilum or longest pole to pole diameter, process both halves *en face*

Nodes greater than 5 mm—cut into 2 mm slices longest pole to pole, process all slices *en face*

Step sectioning

Prepare routine H&E section, report metastatic disease if present and if negative go on to

Mount six exact serial sections, separately number 1–6

Discard 150 μ m (may be saved for research)

Mount further six numbered serial sections, continue through entire block

Stain all number 3 sections by H&E method

Report metastatic disease is present and if negative or equivocal

Immunocytochemistry on all number 2 sections using a pancytokeratin antibody (see below)

Examine for positivity

Compare positivity with “Conclusion” H&E

Use remaining sections if required

Note: Individual laboratories may vary their operating procedures to achieve the protocol such as by performing all levels and immunohistochemistry in one run

Box 4 Reporting procedures for sentinel node*Microscopy*

Sections should be examined using a good quality bright field microscope and equivocal findings discussed with an experienced colleague. Where pancytokeratin positive cells are present it is essential that adjacent sections are stained to allow morphological comparison. Double reporting is considered good practice

Report

The side, number and level of each node basin in the neck must be recorded. A diagram provided by the surgical team should be incorporated into the pathology record where possible

The report must include details of the numbers of nodes found in each individual basin and which nodes were hot, blue, both or unlabelled. The dimensions of each node must be included and the macroscopic appearance of the gross and cut surfaces stated

Macrometastasis

- Largest dimension of the metastatic deposit in each node
- Whether extracapsular spread is present or not

Micrometastasis

- Should be recorded, even in the presence of macrometastasis
- The largest dimension should be recorded
- Anatomical location within the node: capsular, subcapsule, parenchymal
- Unifocal or multifocal: it is often not possible to be confident of the exact numbers
- Extracapsular spread. This is permitted if the deposit is peripherally located and is associated with a reactive stromal response

Individual Tumour Cells

- If evident, should be recorded, even in the presence of macro or micrometastasis
- If cohesive: the size of the largest deposit should be stated
- If dispersed: anatomical distribution
- Benign inclusions including naevus cells, salivary inclusions and false positive cytokeratin artefacts (e.g. dendritic cells or scattered non-viable anucleate cells) should be recorded

relevant' epithelium in sentinel nodes. The presence of such epithelium could potentially limit the usefulness of the PCR technique, although the use of specific cytokeratin sequences may overcome these limitations. However the group favoured morphological interpretation as the gold standard. Problem areas such whether to classify non-nucleated cytokeratin positive cells (thanatosomes [12]) as ITCs, how to classify multiple micrometastatic deposits in the same node, whether extracapsular spread can be diagnosed in a micrometastasis and the significance of finding tumour cells in lymphatic vessels outside an otherwise uninvolved node were discussed. It was agreed that the latter do not represent micrometastasis but that their presence should be recorded (Fig. 2). Where multiple micrometastatic deposits were present in a single node, many members favoured upstaging to macrometastasis, particularly where continuity between the deposits was likely,

Fig. 1 AE1/AE3 stained section showing individual nucleated tumour cells related to the lymph node sinus in a sentinel node

Fig. 2 Group of squamous carcinoma cells in a lymphatic vessel outside a node. This was only seen in one level and illustrates a limitation of the pathology protocol. If section quality is poor at any level, there is no opportunity to examine further material from the block

although such nodes were recorded for analysis in the SENT trial as 'multiple micrometastasis'. The possible significance of the relationship of tumour cells to the lymph node sinus and the presence of stromal reaction were considered [7, 13]. Some members of the group felt that extracapsular spread could be reported in a micrometastasis if there was stromal response, whereas others had never encountered this situation. The SENT trial data recorded so far includes only seven sentinel nodes where extra-capsular spread was found altogether. These nodes have yet to be reviewed by the consensus group. Cases had also been encountered where groups of cells that were suspicious for metastasis in the haematoxylin and eosin stained sections proved to be AE1/AE3 cytokeratin negative. The cells were

Fig. 3 Group of suspicious cells close to the periphery of a sentinel node. These cells proved to be cytokeratin negative and were confirmed as macrophages by further immunohistochemistry

Fig. 4 Cytokeratin positive artefacts. Dendritic cells stained by AE1/AE3 and extrinsic positively stained desquamated squames are commonly encountered in sentinel node preparations

subsequently confirmed as macrophages by CD68 immunohistochemistry (Fig. 3) and were regarded as an incidental granulomatous response. The experience of the group emphasised the finding of the late Professor MacDonald that a series of adjacent sections are necessary at each level examined to allow correlation of the haematoxylin and eosin sections with AE1/AE3 and sometimes other immunohistochemical markers. Unusual cases that had been encountered during the SENT trial were also discussed. Examples included a case where abnormal tissue that proved to be metastatic squamous carcinoma was identified by a surgeon adjacent to a ‘hot blue sentinel’ node. The node was negative on histology and the interpretation agreed was that obstruction of a true sentinel node by metastatic carcinoma could render it negative to

lymphoscintigraphy and divert the colloid to an adjacent node. A similar case of metastasis to a floor of mouth node that was negative on lymphoscintigraphy had been encountered in the London cohort. Slides showing cytokeratin positive artefacts were discussed and included background staining of dendritic cells by AE1/AE3, extrinsic desquamated cells and keratinous debris (Fig. 4). The SENT group plans to review all positive cases and a proportion of negative cases prior to publication of the trial which should finish accrual soon. There is a need for better morphological descriptions of the patterns of metastasis and the various artefacts, inclusions and deposits that may be encountered in head and neck sentinel nodes, and also for more detailed reporting guidelines.

Conclusion

The clinical use of sentinel node biopsy for head and neck cancer is becoming more widespread. Future clinical trials are planned for situations where bilateral neck dissection is currently indicated. Increasingly intra-operative analysis by frozen section will be indicated, for example in laryngeal cancer and in high volume centres where a single operative procedure is preferred for logistical reasons. It is likely that case selection criteria for sentinel node biopsy will be refined and that health economic considerations will play a greater role in selecting methods used. The use of combined imaging modalities and fine needle aspiration will improve case selection, by better identification of patients who would benefit from elective neck dissection at the outset.

Until more data become available, the use of the SENT pathology protocol is advised (see Boxes 3, 4) with UICC/TNM definitions (see Boxes 1, 2). On average 2.5 sentinel node basins are submitted per neck side. The protocol may produce up to 12 levels per node. If three nodes are present in each basin there could be 180 slides to examine. On the other hand comprehensive examination of an elective neck dissection where all tissue is processed to reveal small nodes and soft tissue deposits can produce over 120 slides (Ken MacLennan, personal communication, [14]). However the SENT protocol has the advantage that it is able to detect all micrometastatic deposits with certainty. Also the majority of sentinel nodes are small and the nodes from a single basin can often be grouped into one cassette (using ink to identify ‘hot’ and ‘blue’ nodes) saving laboratory time and effort. According to Cochran’s principle, metastatic deposits tend to cluster in the plane of the hilum, and some authors argue for examination of bisected sentinel nodes at three or six levels only [15]. Although this practice can theoretically miss a micrometastasis (see Thomsen et al. [16]) future trial data may reveal whether there is a

real need to sample through the blocks beyond the main central levels. Several studies have confirmed the original findings of the Canniesburn study that sentinel nodes are upstaged by step serial sectioning with cytokeratin immunohistochemistry, for example recently Bilde et al. [4] demonstrated upstaging from 4 to 22% using the SENT protocol.

Little has been published on the cost effectiveness of sentinel node biopsy in head and neck cancer. One analysis of the sentinel node procedure versus elective neck dissection that included an extra 60 min of time for pathological examination demonstrated that that cost savings could be made by using the sentinel node procedure [17]. However immunohistochemistry was not used in that study and the laboratory costs for step serial sections with immunohistochemistry combined with additional examination time could potentially tip the economic balance. A prospective cost effectiveness trial is underway in France (G Mamelle, personal communication) and will provide better quantitative health economic data than are presently available. Intra-operative frozen section (as used in the French trial) is increasingly popular and is potentially less costly than the SENT protocol. An important health economic consideration which may ultimately determine the choice of laboratory protocol relates to the clinical operating schedule as allude to above. In centres where operating list times are pre-scheduled, detailed examination of the fixed sentinel node followed by elective neck dissection at a later time (within 2 weeks) may be most effective. However in large centres with simultaneous flexible operating lists, then a single stage procedure with frozen section of the sentinel node may be a more economic use of operating room time.

Questions such as the biological significance of individual tumour cells in head and neck cancer remain unanswered at present. In other situations such as breast cancer and melanoma there is currently considerable controversy about whether comprehensive regional lymph node clearance should be undertaken or not when ITCs are found in the sentinel node. Centres vary in their practice [18]. However if it is proven that ITCs are of no biological significance in long term follow studies, then they can be considered as ‘false positives’ if they trigger regional lymph node dissection and this will profoundly affect cost benefit health economic analysis [18].

One of the major aims of sentinel node biopsy in head and neck cancer is to achieve better staging and thorough examination of the nodes is a mandatory requirement for this. There is a need not only for larger studies but also for the adoption of carefully standardised protocols for the histopathological assessment of sentinel lymph nodes to enable future meta-analysis. Only with robust data can the

role of the sentinel node procedure in head and neck cancer be determined.

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