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Sentinel lymph nodes in cancer of the oral cavity – isolated tumour cells

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BACKGROUND: Sentinel lymph node biopsy, step sectioning and immunohistochemistry have changed detection of tumour deposits. Isolated tumour cells (ITC) are detected more frequently than earlier because of a changed level of detection.
METHODS: A total of 108 sentinel lymph nodes from 30 patients with T1/T2 cN0 oral cancer were re-classified histologically to find possible ITC and to describe technical pitfalls.
RESULTS: Primarily we found metastatic spread in 12 of 108 sentinel lymph nodes: five macrometastasis and seven micrometastasis. After re-classification, we found seven lymph nodes with macrometastasis, five with micrometastasis and two with ITC.
CONCLUSION: The ITC are probably precursors of micrometastasis waiting to grow and should be treated as such. Benign inclusions and dendritic cells did not cause problems, but can mimic ITC.
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Keywords: classification; lymph nodes; lymphatic metastasis; neoplasm circulating cells; neoplasm staging; pathology

Introduction

Nodal status is a significant predictor for survival of patients with oral squamous cell carcinoma (1, 2). Sentinel lymph node biopsy, step sectioning of sentinel lymph nodes and immunohistochemistry have changed the detection of tumour deposits in lymph nodes (3).

Tumour deposits in lymph nodes have until recently been divided into macro- and micrometastases. The use of step sectioning and immunohistochemistry has turned the focus towards isolated tumour cells (ITC) (4). Primarily, we did not focus especially on the presence of ITC in the histopathological examination of sentinel lymph nodes, thus none was described. However, in two cases there was doubt about a few immunostained cells. A close follow up was recommended. The aim of this study was to re-classify sentinel lymph nodes histologically to find possible ITC and to describe the technical pitfalls.

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Material and methods

Thirty consecutive patients, 12 women and 18 men, aged 32–90 years, 16 T1 and 14 T2 cN0 squamous cell carcinoma of the oral cavity, were enrolled. T1 tumours are <2 cm and T2 ranges from 2 to 4 cm.

The primary tumour location were: anterior tongue five, posterior tongue 14, floor of the mouth eight and buccal mucosa three. Exclusion criteria were former surgery or radiation therapy to the head and neck. The study was approved by the local ethics committee and was conducted in accordance with the Danish law for scientific ethical committees. All patients gave their informed consent prior to inclusion. The necks of the patients were screened by palpation prior to inclusion. The patients were also examined by magnetic resonance imaging (MRI) and ultrasonography. The results are described in another paper.

Each patient had 20 MBq of tracer in 1 ml divided over four to six peritumoral submucosal injections of ^{99m}Tc-labelled rheniumsulphide nanocolloid (Nanocis/CIS Bio International, Schering, France). Planar scintigraphic images were recorded using a 2-headed γ-camera ('Axis Beacon', Marconi, Philips Medical Systems, Cleveland, OH, USA). The camera heads were positioned in a 90° angle to each other. The region of interest was visualized simultaneously in the anterior and lateral projection. A 5 min acquisition protocol was used. The γ-camera was equipped with low-energy, high-resolution parallel hole collimators operating at a 256×256 matrix. A ^{57}Co-flood field source was placed opposite to each camera head for about 30 s to add a simple 'transmission' of the body contour to the images.

Skin markings were drawn guided by the γ-camera (using dynamic imaging), a ^{57}Co-pointer and a γ-probe ('Europrobe', Eurorad, France).

About 5–10 min prior to surgery 1 ml of Patent blue (Laboratoire Guerbet, Aulnay-Sous-Bois, France) was injected peritumorally at the same sites as the colloid.

Detection of the sentinel nodes was guided by the γ-probe and Patent blue. The surgeon removed the sentinel nodes through one skin incision. All radioactive as well as blue lymph nodes were considered to be sentinel lymph nodes. The location of the sentinel lymph nodes was recorded according to Robbins et al. (5). Lymphatic mapping and sentinel lymph node biopsy were performed in the same day.

In the routine histopathological procedure the removed sentinel lymph nodes were fixed in formalin for 24 h. Lymph nodes with a transverse diameter < 5 mm were embedded in paraffin as a whole and lymph nodes larger than 5 mm were cut through the central cross-section into equal halves.

The lymph nodes were step-sectioned at 250-micron intervals. Each layer was stained with haematoxylin and eosin (H & E) and cytokeratin (CK1). In the middle section was an unstained control (Fig. 1). All lymph nodes were recorded as negative or positive for metastases. Tumour deposits were divided into two groups: macrometastases and micrometastases (Fig. 2). Patients with positive sentinel lymph nodes underwent a modified radical neck dissection. The neck dissection specimens were examined by standard H & E staining. All patients were examined clinically every 3 month for the first 2 years and every 6 month the following 3 years.

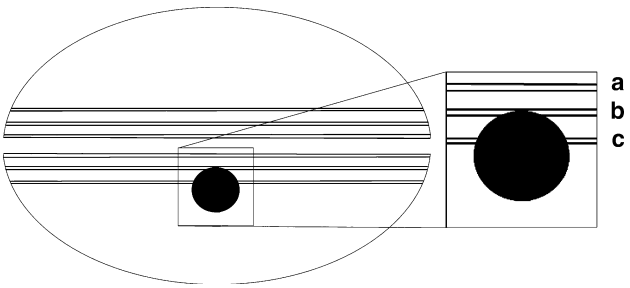

Figure 1 Lymph node cross-sectioned and step-sectioned three times in 250-micron intervals. One haematoxylin and eosin (H & E) section and one immunostained section per step. One section was an unstained control. The sections show: (a) no tumour deposits, (b) an isolated tumour cells (ITC), (c) a micrometastasis. A macrometastasis could in theory be missed.

The sentinel lymph nodes were re-examined according to the classification proposed by Hermanek et al. (4) and the tumour deposits were divided into three groups: macro- and micrometastases and ITC. Accordingly, micrometastases were tumour deposits smaller than 2 mm, where implantation, extravasation and proliferation of tumour cells had occurred in the lymph nodes. In most cases, there was also a stromal reaction. Tumour deposits was only classified as ITC, if there were no contact with vessels or lymph sinus walls, no extravasation, no stromal reaction and no extravascular tumour cell proliferation. The re-classification was performed after inclusion and treatment of the last patient.

Results

By routine histopathological examination we found 12 metastases in 108 sentinel lymph nodes: five were macrometastases and seven micrometastases. No ITC were described.

The 108 sentinel lymph nodes were located in level I (18), level II (37), level III (36), level IV (12) and level V (five). Nine of 30 patients had bilateral sentinel lymph nodes. Eight patients had metastases in the sentinel lymph nodes. One patient had bilateral metastases. Three patients with tumour-negative sentinel lymph nodes had a recurrence in level I during follow up. Eleven neck dissections were performed. One patient did not want surgery and was treated with radiation therapy. Four additional tumour-positive none-sentinel lymph nodes was found in the neck dissections.

After reclassification tumour deposits were found in 14 sentinel lymph nodes, of which seven were macrometastases, five micrometastases (two of these with ITC in different sections) and two ITC. The total number of necks containing tumour deposits increased from 8 to 10. Two sentinel lymph nodes with micrometastases were converted to macrometastases (Table 1). We found ITC in four of the sentinel lymph nodes. One with ITC in two different sections. We observed benign epithelial inclusions and severe CK staining of dendrite cells in several lymph nodes. We did not register the number of

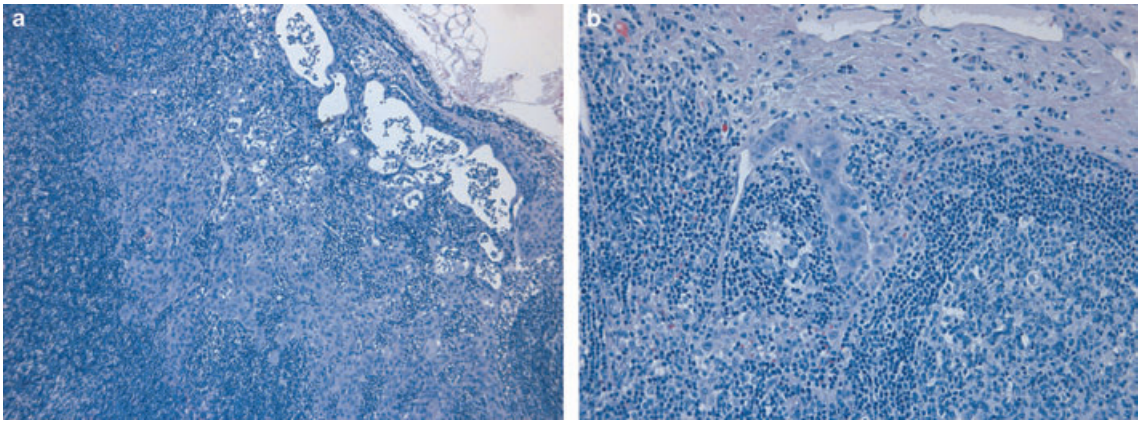

Figure 2 Haematoxylin and eosin (H & E)-stained sentinel lymph nodes: (a) macrometastasis, (b) micrometastasis.

Table 1 Histopathological findings and changes before and after re-classification

	Histopathology		
	Before	*After*	*Change*
Macrometastases	5	7	2
Micrometastases	7	5	2
Isolated tumour cells (ITC)	0	2	2

stained dendrite cells, since these are seen in at least 50% of the lymph nodes. Table 1 shows the changes before and after re-classification.

Discussion

We found ITC in four of 108 sentinel lymph nodes (Fig. 3a). If an ITC was to be considered as a micrometastasis, the consequence would be stage migration in two patients. One was pN0 at the primary histopathological examination, the second patient had a macrometastasis on the left side of the neck and an ITC on the right. The consequence could thus have been two more neck dissections. However, the patients were not treated, since the tumour deposits were not described as ITC primarily, but had been described as atypical cells. The first patient is recurrence-free after 21 months. The other patient had a recurrence at the primary tumour site.

Two other patients had sentinel lymph nodes with ITC, which also contained a micrometastasis, thus no change occurred because of ITC.

However, this could indicate that ITC is merely a very small micrometastasis waiting to grow and that there is a risk that lymph nodes containing ITC may harbour more tumour cells which have not been revealed.

Three different studies in head and neck cancer patients, colorectal cancer patients and non-small cell lung cancer patients support this (6–8). These studies show that patients with immunohistochemical detected ITCs in lymph nodes have a poorer prognosis than patients without ITCs. The results from studies of colorectal cancer and non-small cell lung cancer (NSCLC) cannot be transferred directly to head and neck cancer, but it emphasizes that ITC in lymph nodes may be of prognostic relevance for cancer patients. The significance and prognostic relevance of ITC has been questioned (3). However, ITC might have prognostic significance and we will treat patients harbouring ITC as other patients with metastatic spread. The discussion of ITC is probably just a new step on the ladder concerning the significance of micrometastases and the relevance of treating these patients (2, 9–11). However, we will registrate ITC separately.

For those who adapt a wait and see policy for ITC, it is important to ensure that the ITC are not the periphery of a micrometastasis as described above. This can be carried out by cutting two unstained sections next to every stained section. If an ITC is detected, the unstained sections can then be stained and examined.

We found a few benign inclusions and they did not cause problems. A benign inclusion is depicted in Fig. 3b. In this case, the section leaves no doubt that it is a benign inclusion. However, if cut more peripheral, only a few cells would have been seen and could have imitated ITC (12).

Dendritic cells expressing CK are frequently seen in the immunostained sentinel lymph nodes (Fig. 3c). When severely stained they can be difficult to differentiate from ITC. This differentiation is mainly up to the experience of the pathologist (13, 14).

Are we examining enough sections of the lymph nodes or do we miss metastases by our current technique? One study by Diaz et al. shows that metastases are typical localized near the inflow junction of the afferent lymphatic vessels (15). These metastases can in most cases be identified by a central cross-section through the lymph node. In another study by Cserni, central cross-sectioning failed to detect metastases in eight of 26 lymph nodes (16).

In our study, we examined to a depth of 500-micron from the central cross-section in each lymph. The study by Cserni (16) indicates that there might be more metastases in deeper levels of the lymph nodes. Other studies have shown that more metastases can be found by more intense sectioning (9–11), but none of these studies have specified the spatial distribution of these metastases in the lymph nodes.

We cannot examine all the lymph nodes in detail. A simple calculation shows that a lymph node with a diameter of 6 mm cut into 5-micron sections would amount to 1200 sections. The relationship between the size of a metastasis and number of section that needs to be cut to detect the metastases in a lymph node with a transverse diameter of 6 mm is illustrated in Fig. 4.

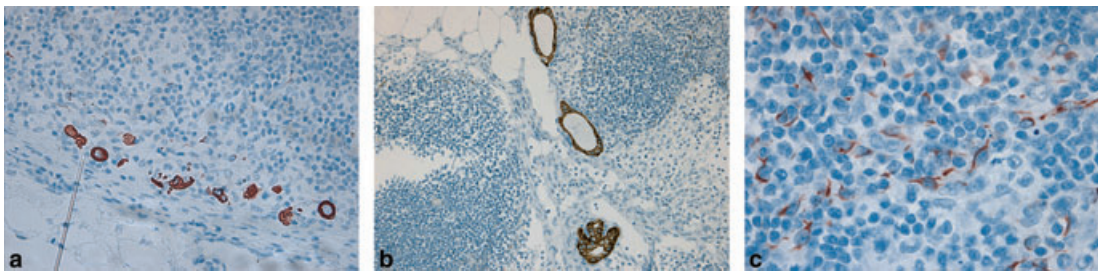

Figure 3 Immunostained sentinel lymph nodes: (a) isolated tumour cells (ITC), (b) benign inclusions, (c) dendritic cells.

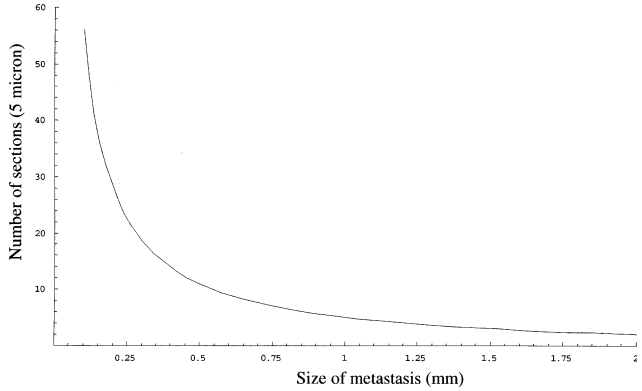

Figure 4 Relationship between the size of a metastasis and the number of sections that needs to be cut to detect the metastasis in a lymph node with a transverse diameter of 6 mm.

What level of detection should we choose? If we make one section stained with H & E throughout a lymph node with a transverse diameter of 6 mm at 250-micron it would amount to 24 sections. By adding immunohistochemistry and an unstained control it would increase to 50 sections. We averaged 3.6 sentinel lymph nodes per patient. This would equal 180 sections per patient. The pathologist uses about 1 min per section resulting in about 3 h examination per patient. This is simply not acceptable considering cost, work and more importantly we would only identify about half of all metastases 0.1 mm in diameter if random distribution of spherical metastasis within lymph nodes is assumed (17). The consequence is that we will not aim to find all tumour cells, but try to balance detection rate and workload. An 1-h examination time per patient is reasonable in a daily routine. With 3.6 sentinel lymph nodes per patient, this equals 16–17 min per lymph node (60 min/3.6).

With our current histopathological examination we cut 14 sections in each lymph node. Only one of six of a lymph node with a transverse diameter of 6 mm is examined (Fig. 1). In theory, a micrometastasis can be missed with this protocol. However, this can be avoided by cutting four more sections per lymph node as illustrated in Fig. 5. We have planned to re-examine the sentinel lymph nodes once again to see if we have missed any tumour deposits in the residual lymph node tissue.

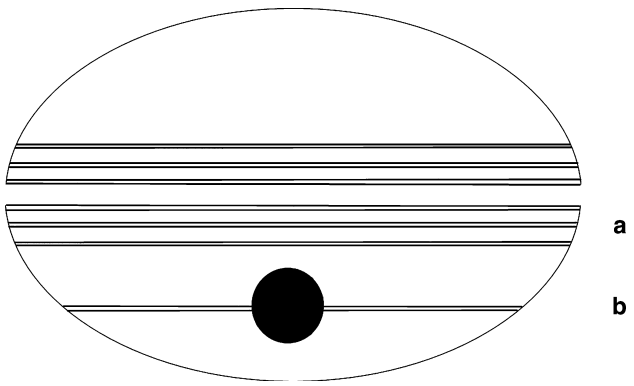

Figure 5 Lymph node cross-sectioned and step-sectioned in three 250-micron intervals. Two extra-sections are cut at a deeper level. The current technique reveals no tumour deposits (a). The two extra-sections [one haematoxylin and eosin (H & E) and one immunostained] ensures that no larger tumour deposits are missed (b).

Conclusion

The ITC are probably precursors of micrometastasis waiting to grow and should be treated as such. When ITC are described more sections have to be examined to ensure that the cells do not represent benign inclusions, dendritic cells or the periphery of a micrometastasis. With the current histopathological techniques it is not possible to find all tumour deposits.

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