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Sentinel lymph nodes in cancer of the oral cavity: is central step-sectioning enough?

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BACKGROUND: Extended histopathologic work-up has increased the detection of micrometastasis in sentinel lymph nodes in malignant melanoma and breast cancer. The aim of this study was to examine if (A) step-sectioning of the central 1000 μM at 250 μM levels with immunostaining were accurate when compared with (B) step-sectioning and immunostaining of the entire sentinel lymph node at 250 μM levels.

METHODS: Forty patients with T1/T2 cN0 oral cancer were enrolled. Three patients were excluded. In one patient no sentinel lymph node was identified. The remaining two had unidentified sentinel lymph nodes due to lymphoscintigraphic and surgical sampling error. The central 1000 μM of 147 sentinel lymph nodes were step-sectioned in 250-μm intervals and stained with hematoxylin and eosin and CK-KL1. All lymph nodes were recorded as negative or positive for macrometastases or micrometastases. After inclusion of the last patient the residual tissue of the lymph nodes was totally step-sectioned at 250-μm intervals and re-classified. The tumor deposits were divided into macrometastases and micrometastases and ITC.

RESULTS: Method (A) upstaged 17 lymph nodes and 11 patients compared with method (B), which upstaged 22 lymph nodes and 11 patients. Seven of the patients with positive lymph nodes did not change stage. However, four lymph nodes changed from micrometastases to macrometastases. One patient changed from a micrometastasis to four micrometastases. One pN2c patient with bilateral micrometastases did not change stage, but an additional ipsilateral lymph node with a micrometastasis was identified.

CONCLUSION: Larger tumor deposits and more metastases are identified by more extensive sectioning of the sentinel lymph nodes. None of the patients was false-negative due to histopathologic sampling error, but the results indicate that central step-sectioning of the central 1000 μM cannot completely be relied upon for accurate staging of the patients.

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Introduction

The detection of positive sentinel lymph nodes can fail due to non-detected tumor deposits. There seems to be a consensus that step-sectioning and immunohistochemistry should be used (1–3). Extended histopathologic work-up has increased the detection of micrometastasis in both malignant melanoma and breast cancer (1,4). Several studies indicate that this is true for patients with head and neck cancer as well (5–9). However, the ideal amount of sectioning of sentinel lymph nodes in oral cancer has yet to be determined. We wanted to examine the distribution of tumor deposits in sentinel lymph nodes from patients with squamous cell carcinoma of the oral cavity. The classification proposed by Hermanek et al. has been used for description of the tumor deposits: macrometastasis, micrometastasis, and isolated tumor cells (5).

The aim of this study was to examine if (A) step-sectioning of the central 1000 μM at 250 μM levels with immunostaining were accurate when compared with (B) step-sectioning and immunostaining of the entire sentinel lymph node at 250 μM levels.

Methods

Forty consecutive patients, 17 women and 22 men, aged 32–90, with 23 T1 and 16 T2 cN0 squamous cell carcinoma of the oral cavity were enrolled. Three patients were excluded. In patient number 4 no sentinel lymph node was identified. Patients 5 and 6 had unidentified sentinel lymph nodes due to lymphoscintigraphic and surgical sampling error. There were 16

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anterior tongue tumors, 12 floor of the mouth, and nine cheek/gingival. The patients had to be clinical N0 by palpation and were palpated by two observers prior to inclusion. 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. On the day of surgery each patient had 4–6 separate peritumoral submucosal injections of a total volume of 0.2 ml ^{99m}Tc-labeled rheniumsulphide nanocolloid (20 MBq). Injection was done by the surgeon. Planar lymphoscintigraphic images were recorded using a 2-headed gamma camera ('Axis Beacon', Marconi, Philips Medical Systems, Detroit, MI, USA). Lymphatic mapping and sentinel node biopsy were performed in the same day. All the patients were operated by the same surgeon. Five to ten minutes prior to surgery 1 ml of Patent Blue was injected peritumorally at the same sites as the colloid. In most cases the primary tumor was excised before the sentinel lymph node biopsy. The sentinel nodes were removed through one skin incision. Detection and excision of the sentinel nodes were guided by a gamma probe ('Europrobe', Eurorad, France) and Patent Blue. All lymph nodes with activity above background activity and blue nodes were removed.

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 central 1000 μM of the lymph nodes were step-sectioned in 250-μm intervals. Each level was stained with hematoxylin and eosin (HE) and CK-KL1, which is a mixture of cytokeratin 1–8, 10, 13–19. In the middle section was an unstained control. All lymph nodes were recorded as negative or positive for metastases by HE or CK-KL1, or both. Primarily, the tumor deposits were divided into two groups: macrometastases and micrometastases. After inclusion of the last patient the residual tissue of the lymph nodes was totally step-sectioned at 250-μm intervals.

All sections were re-classified and tumor deposits were divided into three groups: macrometastases and micrometastases and ITC according to the classification proposed by Hermanek (5). We evaluated tumor deposits in (A) step-sectioning of the central 1000 μM at 250 μM levels with immunostaining (approximately 14 sections with unstained controls) and (B) step-sectioning and immonustaining of the entire sentinel lymph node at 250 μM levels.

Patients with positive sentinel lymph nodes, macrometastases and micrometastases, were treated with a selective neck dissection, levels I–VI. Patients underwent radiotherapy and follow up as described in the Danish national guidelines (10).

Results

A total of 144 sentinel lymph nodes were removed. Method A resulted in 1930 sections and method B 4898. The mean number of sections cut were 13 (1930/144) by method A and 34 (4898/144) by method B. Method A resulted in 17 metastases in 11 patients compared to method B with 22 metastases in 11 patients. The central HE-stained cross-sections of the lymph nodes were positive in 72% (eight of 11) of positive patients and in 55% (12 of 22) of positive lymph nodes. Additional tumor deposits were identified by method B: (i) isolated tumor cells were identified in three lymph nodes in three pN0 patients, (ii) a tumor embolus in a capillary in the capsule was identified in two lymph nodes in two pN0 patients, and (iii) two lymph nodes harbored apoptotic tumor cells in one pN0 patient. The number of patients with metastasis in the lymph nodes were identical with the two methods, but more and larger tumor deposits were identified by method B. Method A revealed: 2 pN1, 3 pN1mi, 5 pN2b, and 1 pN2C compared with method B: 2 pN1, 2 pN1mi, 6 pN2b, and 1 pN2C and three additional patients had N^{1+} (Table 1). Two patients with pN1 and two pNmi patients did not change. One pNmi patient, changed to pN2b, as four lymph nodes with micrometastases were detected. Five pN2b patients did not change stage; however, four lymph nodes changed from micrometastases to macrometastases. One pN2c patient with bilateral micrometastases did not change stage, but an additional ipsilateral micrometastasis was identified. Three additional tumor-positive lymph nodes all pN1mi were identified in three of 11 neck dissections. None of the patients which was pN0 had a neck recurrence during follow up. Seven patients with lymph node metastases died during follow up, 75% (six of eight) of patients with macrometastases and 33% (one of three) of patients with micrometastases. Mean follow up was 2 years and 4 months, minimum 4 months and maximum 4 years and 6 months.

Discussion

Our current histopathologic protocol, method A, upstaged 12% (17 of 144) of the lymph nodes and 30% (11 of 37) of the patients compared to method B with 15% (22 of 144) of lymph nodes and also 30% of patients. Ross et al. found similar result in head and neck cancer patients, 36% of patients were staged neck positive by HE-staining and increased to 44% after step-sectioning at 150 μM combined with immunohistochemistry (11). Ambrosch et al. (7), Barrera et al. (8), Hamakawa et al. (6), Kwon et al. (12), and Rhee et al. (13) all re-examined lymph nodes from neck dissections, which were pN0 after HE-stained central sections. In all of these studies step-sectioning and immunohistochemistry lead to further upstaging of the patients.

We treat patients with macrometastases and micrometastases in the lymph nodes according to the national Danish guidelines (10). Hence, the evidence regarding clinical implications of micrometastases is scarce. The routine use of the extra workload related to immunostaining and deeper sectioning has been questioned by Van den Brekel et al., because the prognostic significance of micrometastases is unknown (14). This view is supported by Woolgar, who found that the short-term significance of micrometastases seems to be similar

to the N0 neck (15). However, a study by Yamazaki et al. showed that the presence of micrometastases in the cervical lymph nodes is related to a poorer prognosis (16).

In our small study there was a tendency that patients with macrometastases, 75% (six of eight) of patients who died had a worse short-term prognosis compared to patients with micrometastasis 33% (one of three). The only patient with micrometastases who died had multiple positive lymph nodes. Our findings seems to be similar to the ones described by Woolgar (15).

The results also suggest that the consequence of a micrometastasis in a sentinel lymph node, should be a selective neck dissection, as further sectioning often reveals larger and more tumor deposits.

Two patients with tumor emboli in sentinel nodes had a T-recurrence and died after 9 and 20 months (Fig. 1). These two cases could be due to venolymphatic spread, described as a plausible alternative pathway to the lymphatic channels by Ivanov et al. (17). These tumor emboli can be described in two ways: (i) venolymphatic spread, a lymph node metastasis N1 or (ii) as viable tumor cells passing through the lymph node becoming a distant metastasis M1. However, the presence of a tumor emboli seems to be related to a bad prognosis. Two lymph nodes from one patient revealed large areas with apoptotic tumor cells and only one viable tumor cell, which could indicate, that a severe immunological reaction can cause apoptosis (Figs 2 and 3). The patient was lost to follow up after 18 months. Two other patients with isolated tumor cells only are still alive. The prognostic significance of isolated tumor cells is unclear as concluded by Stöeckli et al. (9). However, they may in some cases be precursors of micrometastases (18) and potentially worsen the patients prognosis. The prognostic relevance of isolated tumor cells is unknown in other types of cancer. For breast cancer, Weaver et al. concludes that identification of isolated tumor cell

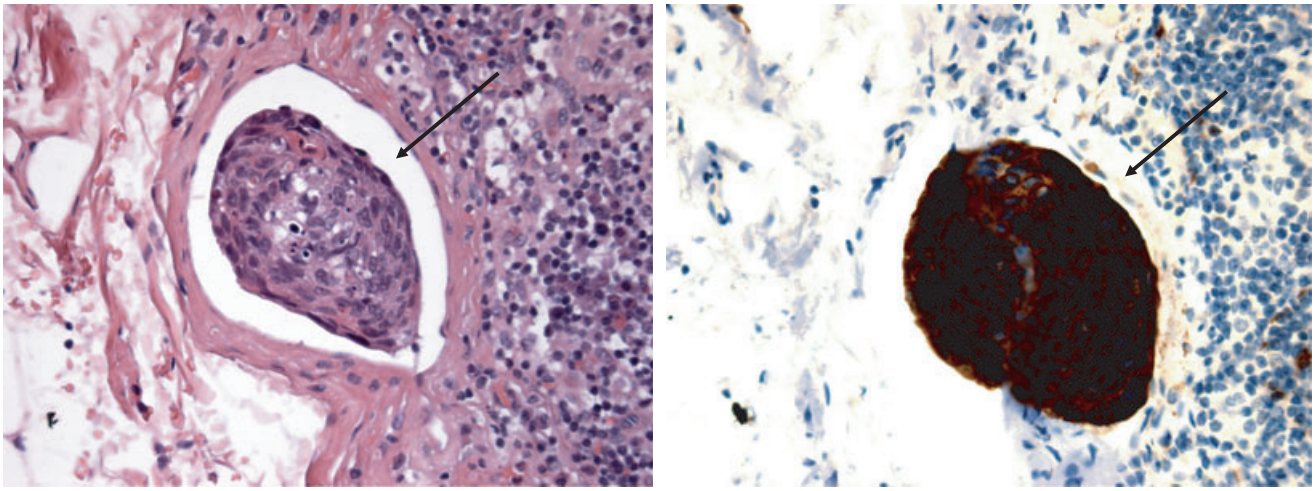

Figure 1 Patient 17 was pN0. After re-sectioning a tumor embolus was detected in a capillary in the capsule of the lymph node. These were the only tumor cells in the sentinel lymph nodes from this patient.

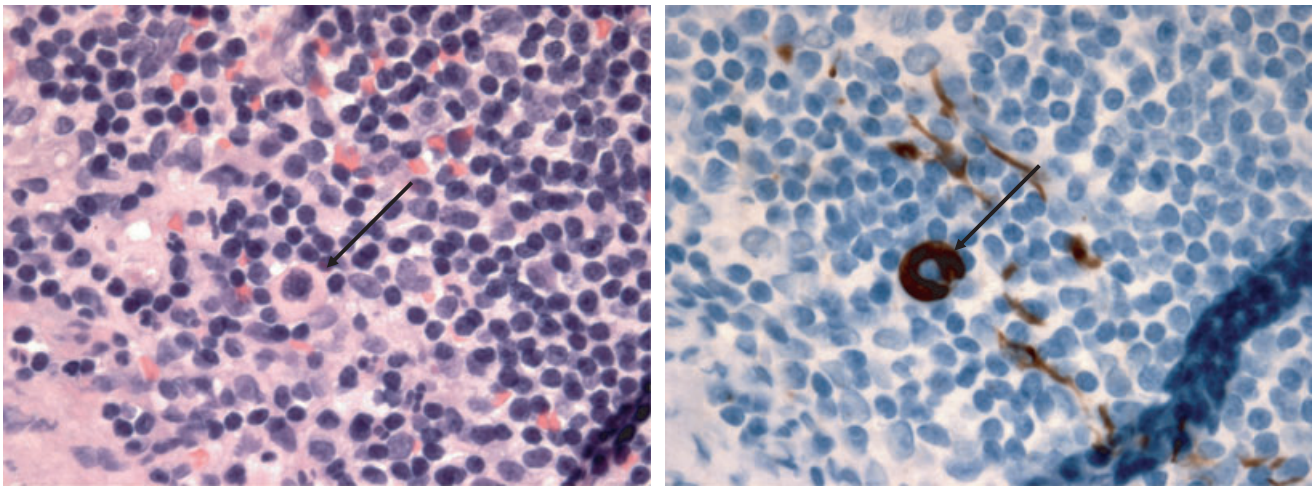

Figure 2 In patient 7 only one isolated tumor cell was found. The tumor cell was detected in both the HE and immunostained sections.

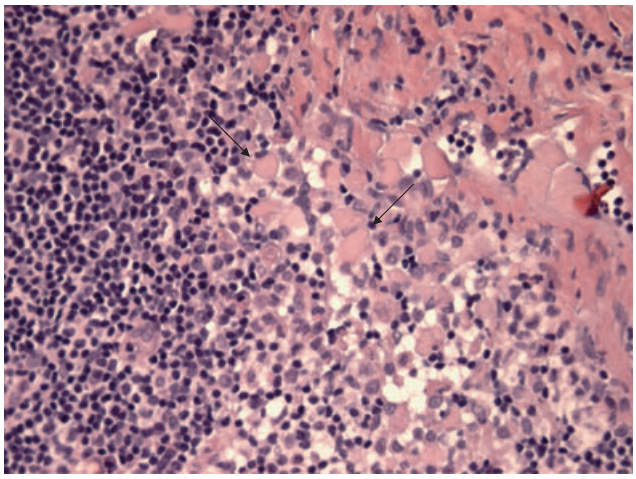

Figure 3 Apoptotic tumor cells in a lymph node from patient 7.

Table 1 Patient stages after examination of HE-stained central cross-sections, central 1000 μM, entire lymph node, and follow-up

Stage	*N0*	*pN1*	*pN1mi*	*pN2b*	*pN2c*	*Other*	*Total*
Inclusion	37	–	–	–	–	–	37
Central 1000 μM	28	2	3	5	1	0	37
Entire lymph node	28	2	2	6	1	3^{I+}	37

clusters may not be identifying a group of patients with outcome statistically worse than node-negative patients (19). Klevesat et al. also questions the prognostic relevance and states that the value of immunohistochemistry is limited until the prognostic significance of isolated tumor cells has been determined (20). Lee et al. examined patients with colorectal cancer and found that ITC did not have an influence on the prognosis (21). The same tendency is seen in gastric cancer, where patients with ITC in the lymph nodes did not show different survival when compared to cases without metastases (22).

Other studies indicate that isolated tumor cells may have prognostic significance and are merely a very small micrometastasis waiting to grow, which is supported by the findings of three studies by Kubuschok et al. (23), Nieuwenhuis et al. (24), and Rosenberg et al. (25).

When we started this study in 2001, we only had experience with sentinel lymph node biopsy in malignant melanoma and breast cancer in our institution. The step-sectioning width was 250 μM for both. The fact that we already had experience with 250 μM and a literature review, which can be summed up by Meyers article from 1998 about strategies for histopathologic examination of sentinel lymph node specimens (26). Meyer concluded that three microsections prepared repeatedly at intervals of 250 μM appears to be practical. Two sections from each level can be examined by routine staining and the third by immunohistochemical stain. However, we changed this to two microsections per 250 μM. In the studies published about sentinel lymph node biopsy in head and neck cancer there has not been any consensus as to how step-sectioning should be performed. In 21% (four of 19) of studies there were no specific information about the histopathologic work-up (27–30), 26% (five of 19) used step-sectioning >100–250 μM (11, 31–34), and 53% (10 of 19) >500 μM (9,35–44). However, a consensus was reached at the second international conference on sentinel node biopsy in mucosal head and neck cancer that 150 μM should be used (45). Since then we have changed our protocol to step-sectioning at 150 μM in three levels from 2 mm blocks of each sentinel lymph node.

In conclusion, larger tumor deposits and more metastases are identified by more extensive sectioning of the sentinel lymph nodes. None of the patients was false-negative due to histopathologic sampling error, but the results indicate that central step-sectioning of the central 1000 μM cannot completely be relied upon for accurate staging of the patients.

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