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CLINICAL TRIAL

Absence of ectopic epithelial inclusions in 3,904 axillary lymph nodes examined in sentinel technique

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Abstract Intraoperative examination of sentinel axillary lymph nodes can be done by imprint cytology, frozen section, or, most recently, by PCR-based amplification of a cytokeratin signal. Using this technique, benign epithelial inclusions, representing mammary tissue displaced along the milk line, will likely generate a positive PCR signal and lead to a false-positive diagnosis of metastatic disease. To better appreciate the incidence of ectopic epithelial inclusions in axillary lymph nodes, we have performed an autopsy study, examining on 100 μm step sections 3,904 lymph nodes obtained from 160 axillary dissections in 80 patients. The median number of lymph nodes per axilla was 23 (15, 6, and 1 in levels 1, 2, and 3, respectively). A total of 30,450 hematoxylin-eosin stained slides were examined, as well as 8,825 slides immunostained with pan-cytokeratin antibodies. Despite this meticulous work-up, not a single epithelial inclusion was found in this study, suggesting that the incidence of such inclusions is much lower than the assumed 5% reported in the literature.

Keywords Benign epithelial inclusions · Sentinel lymph node · Polymerase chain reaction · Autopsy · Axillary lymph nodes

Introduction

Removing metastatic axillary lymph nodes is currently considered standard of care in patients with invasive breast carcinoma. Intraoperative examination of the sentinel

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lymph node by frozen section or imprint cytology allows to decide on the spot who should benefit from immediate axillary dissection (in case of a metastasis in the sentinel lymph node) or who can be spared this procedure and hence the risk of lymph edema. However, due to technical limitations and time constraints, intra-operative sentinel node examination suffers from a rather high false-negativity rate, resulting in re-operations in a substantial number of patients [1, 2].

An alternative to the microscopic search for nests of cytologically atypical tumor cells is the search for cytokeratin signatures by polymerase chain reaction (PCR) in homogenized lymph nodes [3]. Using quantitative real time (RT)-PCR, cut-offs have been defined which should allow to differentiate between "irrelevant" isolated tumor cells/sub-micrometastases (<200 μm) and "relevant" metastases (micro-metastases and metastases; Ref. [3]). At least two different companies are now marketing the PCR-based technology for intraoperative examination of sentinel lymph nodes (GeneSearch from Veridex, Raritan, NJ and OSNA from Sysmex, Norderstedt, Germany).

Ectopic epithelial tissue/benign glandular inclusions in lymph nodes can occasionally lead to diagnostic surprise and confusion. Not infrequently, salivary gland and thyroid fragments are found in cervical lymph nodes and melanocytic nevus cells in axillary and inguinal nodes [4]. Anatomical pathologists occasionally encounter ectopic breast tissue, embryologic remnants of the milk line, in the axillary fat as well as in the capsule and parenchyma of axillary lymph nodes [5–9]. It stands to reason that these ectopic epithelial elements will be recognized by intraoperative PCR and hence interpreted as metastasis. Indeed, one recent prospective study comparing intraoperative PCR and imprint cytology on alternating slices of axillary nodes identified one case where a positive PCR signal could be matched to ectopic breast tissue and not to a metastasis [9]. Unfortunately, once performed as the sole technique—outside of comparative studies—there will be no possibility to verify the malignant nature of the intraoperative PCR signal, because the lymph node is homogenized for the technique and hence no longer available for microscopic analysis.

For this reason, we thought that it would be important to know how frequently these ectopic epithelial inclusions are to be found in axillary nodes, hence how often patients have to expect being falsely diagnosed with metastatic disease by PCR technology. While ectopic epithelial inclusions have been described in case reports or a few mini-series [5–7], not a single study has ever addressed the incidence of such inclusions in a systematic fashion. Therefore, we have set out to quantify the presence of epithelial inclusions in 3,904 axillary lymph nodes removed during post-mortem examinations of patients without breast carcinoma.

Methods

This is an autopsy study that has examined the presence of ectopic epithelial inclusions in axillary lymph nodes. The study was performed on 80 patients (34 female and 46 male) who underwent consecutive medical post-mortem examination in the Department of Pathology at the Johannes Gutenberg University of Mainz, Medical school, during the period of 3/2002–6/2003. The mean age was 67.4 ± 14.2 and 63.9 ± 14.2 years for female and male patients, respectively, and the body mass index was 23.0 ± 3.6 and 26.9 ± 3.6. Patients had died of cerebro- and cardiovascular diseases (23 female and 27 male patients), followed by sepsis (6 female and 10 male patients), and the immediate consequence of malignant tumors (5 female and 8 male patients). 17 female and 18 male patients had malignant tumors, but which were not the immediate cause of death. None of the patients had undergone axillary dissection for breast carcinoma or for other reasons.

The study was performed in accordance to existing institutional guidelines. The use of tissue obtained during medical autopsies for research was specifically mentioned in the general covenant that patients signed upon entry into the hospital (http://www.unimedizin-mainz.de/index.php?id=1741#c36971) and also specifically stipulated in the document that family members signed as part of the consent to autopsy procedure. The study was performed entirely within the institute of pathology. No patient identifiers were used. No modification of the standard medical autopsy procedure was necessary as bilateral axillary lymph nodes could be accessed through the standard ventral Y-incision of throrax and abdomen.

Following instruction and under supervision by a board-certified gynaecological surgeon (PD Dr. Marcus Schmidt), the axillary fat with the lymph nodes was removed according to standard procedures, following well-established anatomical landmarks, thus separating the levels 1, 2, and 3. The fat removed from each level was weighed, fixed for 24 h in buffered formaldehyde (4%), and finally dissected for the isolation of individual lymph nodes. Holding the lymph nodes gently between two fingers, slices of 0.2 cm were placed perpendicular to the smallest diameter, and embedded in one histocassette, if possible. Smaller lymph nodes were embedded with other lymph nodes up to a maximum of ten lymph nodes per histocassette. Three 4 μm sections were cut every 100 μm across the entire lymph node and mounted on either normal glass slides for later hematoxylin-eosin (H&E) stains according to standard protocol, or on poly L-lysine-pretreated glass slides for immunohistochemistry. Immunohistochemistry was performed on lymph nodes (all levels) of ten male and ten female patients, chosen at random prior to the examination of H&E sections. For that purpose, we applied a

pan-cytokeratin antibody (clone MNF116, DAKO, Carpinteria, CA; dilution 1:100), which covers a wide spectrum of cytokeratins (cytokeratins 5, 6, 8, 17, 19).

The widest diameters (length and width) of each lymph node were measured on the H&E slide. Lymph nodes were then examined using a standard diagnostic microscope and all abnormalities were recorded, placing particular emphasis on the presence of ectopic epithelial inclusions.

Results

A total of 3,904 lymph nodes were isolated from the 160 axillary dissections (bilateral axillary dissections in each patient). We counted a median of 23 lymph nodes per axilla (mean ± SD: 24.6 ± 10.9). In the median, 15, 6, and 1 lymph nodes were counted in the levels 1, 2, and 3, respectively (mean ± SD: 16.1±7.5; 6.9±5.1; 1.6±2.1). There was no significant difference between the number of lymph nodes found in male and female patients, and between right-sided and left-sided axillas (not shown). The average maximal diameters of the lymph nodes was 0.60 ± 0.09 cm (length, mean ± SD) and 0.35 ± 0.05 cm (width, mean ± SD), with no difference between male and female patients and between left-sided and right-sided axillas (not shown). However, maximal lymph node size was larger in level 1 (0.68 ± 0.14 cm × 0.40 ± 0.09 cm) then in level 2 (0.48 ± 0.11 cm × 0.29 ± 0.07 cm) and in level 3 (0.36 ± 0.18 cm × 0.22 ± 0.08 cm). This difference was statistically significant ($P < 0.01$, Wilcoxon). Of the 3,904 lymph nodes, we obtained a median of eight sections at 100 μm distance, ranging from 3 to a maximum of 16. In this way, a total of 30,450 slides were examined by standard H&E and 8,825 slides by immunohistochemistry (corresponding to 1,102 lymph nodes of ten male and ten female patients).

Upon histological examination of the lymph nodes, the following abnormalities were noted, in decreasing order of frequency: sinus histiocytosis (57.4%), focal scarring (41.7%), hyaline fibrosis (33.9%), lipomatous transformation (31.0%), and fibrous thickening of the capsule (7.6%). 39 lymph nodes in four patients contained metastases of non-mammary origin: one metastatic neuroendocrine carcinoma of small intestinal origin, one primary bronchial carcinoma, one melanoma, and one Non-Hodgkin lymphoma. In all these cases, the association of the lymph node metastasis with the primary tumor was confirmed by standard histology or by immunohistochemistry. After histological examination of H&E stained sections of 3,904 lymph nodes, totalling 30,450 slides, we could not find a single focus of ectopic epithelial inclusion, neither within the lymph node parenchyma, nor in the capsule or in the peri-nodal adipose tissue. Neither did we identify any cytokeratin-positive epithelial cells in a total of 1,102 lymph nodes subjected to immunohistochemical examination on serial sections (8,825 slides).

Discussion

The principal finding of this study is that not a single focus of ectopic epithelial inclusion was found in almost 4,000 investigated lymph nodes. This finding was unexpected since the incidence of supra-numerary breast tissue in the axilla has been estimated at 5–6% [5, 10]. If these figures were correct, we should have found inclusions in at least four patients. Based on this assumption, the chance to not find a single ectopic epithelial inclusion in our population of 80 patients can be calculated at $P < 0.02$ by binomial distribution. We hence believe that our study is sufficiently powered to suggest that epithelial cell inclusions are much rarer than the 5% estimated in the existent literature.

Maiorano and coworkers [6] reported on seven foci of ectopic epithelial inclusions in sentinel axillary lymph nodes. Four of these cases had been in-house cases found among a total of 3,500 sentinel lymph nodes and three cases were consultations. Based on these numbers, a rough incidence of 0.1% can be estimated, which would still be far from the 5% reported in the literature. While it may be speculated that epithelial inclusions are somewhat more frequent in sentinel nodes because of their privileged position for a potential embryologic nodal entrapment, there currently exists no data to support this hypothesis. Based on the finding of our study and the data reported by Maiorano and coworkers, we believe that a figure of roughly 0.1% can safely be assumed as a reasonably correct figure when counselling patients about the risk of a false-positive PCR signal during intraoperative sentinel node examination.

The number of lymph nodes found in our autopsy series (median: 23 lymph nodes) corresponds rather well with data reported before for axillary dissections in alive patients: an average of around 19 lymph nodes per axilla were reported by Turner and coworkers [11], 27 lymph nodes by Viale and coworkers [12], and 23–26 lymph nodes by Cappello and coworkers [13]. In contrast to a previous publication by Misnik [14], we could not confirm that the number of lymph nodes per axilla increased with the age of the patients: we found an average of 25 lymph nodes in 38 patients under the age of 65 and 22 lymph nodes in 42 patients above the age of 65.

Conclusion

In conclusion, we have to acknowledge that not a single epithelial inclusion was found in this prospective series of

3,904 meticulously examined axillary lymph nodes in non-breast cancer patients. While we are very well aware that 3,904 axillary lymph nodes in 80 patients is not the same as 3,904 sentinel lymph nodes in 3,904 patients, we believe that our study is of relevance since it helps to better appreciate the frequency of epithelial inclusions in axillary nodes of breast cancer patients and also the risk of putative false-positive results in PCR-based intraoperative sentinel node examination, as a basis for treatment decisions and patient counselling.

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Conflict of interest The authors declare that they have no competing interests.

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