Probabilities of Identifying a Micrometastasis in a Cervical Lymph Node from Laryngeal Cancer. A Stereometric Approach

ALEXANDER DELIDES¹, MICHAEL MANIADAKIS² and EUSTATHIOS STATHOPOULOS³

¹Second ENT Department, “Attikon” University Hospital, Athens, Greece; ²Research Programme KA 948, University of Crete Medical School, Crete, Greece; ³Department of Pathology, University Hospital of Heraklion, Crete, Greece

Abstract. Background: Lymph node micrometastases are important for prognosis and treatment. Their identification in neck dissection specimens with large numbers of nodes is a matter of chance unless serial sectioning or step sectioning at intervals of 200 μm is used, a method which is impractical. Materials and Methods: For a case of squamous cell laryngeal carcinoma where a micrometastasis was found in one of the lymph nodes, we performed a stereometric analysis of the probability of identifying the lesion. Results: One section at a depth of 100 μm from the surface of each bisected lymph node revealed a micrometastasis, with a range of conditional probability of 75-98%. Conclusion: Serial sectioning of all excised nodes is unrealistic for routine practice. Knowing the probability of actually identifying a micrometastasis could be important additional information for the clinician and the management of the patient. By performing three sections, the probability of missing a micrometastasis within a normally appearing node would be 2-25%.

The significant role of cervical node metastasis is well-established in squamous cell carcinomas, which comprise the vast majority of head and neck carcinomas, whereas the clinical significance of micrometastases is still under consideration. In breast carcinomas, the American Joint Commission on Cancer (AJCC) designates as micrometastasis, deposits greater than 0.2 mm and not greater than 2.0 mm in their greatest dimension while cases with lymph node (LN) micrometastasis are classified as “mi” in the TNM classification (1). Adoption of this definition of micrometastasis has been suggested for head and neck carcinomas since the most recent edition of the Cancer Staging Manual of the AJCC does not have a separate category of micrometastases for head and neck cancer. (2) Metastatic deposits with a diameter less than 0.2 mm are named “isolated tumor cells” (ITCs) and those with a diameter greater than 2.0 mm are characterized as macrometastases.

Detection of micrometastases by routine examination is rather a matter of chance and systematic sectioning of LN is necessary. In this article we analyze the probability of identifying a micrometastasis in a cervical LN from squamous cell carcinoma of the larynx.

Materials and Methods

Case report. A 59-year-old male with locally advanced laryngeal carcinoma underwent total laryngectomy and elective neck dissection on the left side. Histology revealed a moderate-to-well differentiated transglottic squamous cell carcinoma in the larynx; eight LNs were found in the dissection specimen. No metastases were identified in routine examination. Step sectioning of the nodes according to the protocol used for axillary LNs (3) revealed a micrometastasis located at the periphery in one of them. The LN had a spherical shape, 0.9 cm in diameter and the metastasis itself was 0.3 mm in its maximum diameter (Figure 1).

Analysis of probability; a stereometric approach. Wilkinson and Hause were the first to analyze the probability of identifying a metastasis in a LN (4). “A representative malignant node was considered spherical in shape with a spherical lesion. It was assumed that there was a random distribution of possible metastases locations. The center of a metastasis lies within a hypothetical sphere homocentric to the spherical node with a diameter equal to the diameter of the node minus the diameter of the lesion. Then the conditional probability (Pₓ) that the center of the lesion is located within a given part of the sphere is the proportion of the volume of that part of the sphere (DS) to the total volume of the sphere. For a slice taken out of the sphere this conditional probability was calculated by the equation:

\[ Pₓ = 1.5[(X₁-X₀)/Dₛ]–2[(X₁³–X₀³)/Dₛ³], \]

where \( Dₛ \) stands for the diameter of the hypothetical sphere and the variables \( X₁, X₀ \) represent the distances from the center of the node to the surfaces of the slice. \( X₁–X₀ \) equals the thickness of the slice (4).

Correspondence to: Alexander Delides, MD, Ph.D., Agion Saranta St. 25, Papagou, Athens 15669, Greece. Mob: +30 6944769769, Fax: +30 2106561982, e-mail: delidis@athens-orl.gr

Key Words: Micrometastasis, lymph node, laryngeal cancer, head and neck cancer, probability.
In our described spherical LN of 0.9 cm diameter, and with the lesion 0.3 mm in diameter located randomly within the node, if the LN is embedded in two halves (hemispheres), using the above equation the conditional probability would be 0 for one section taken through the middle of the hemisphere (\(D_S=4.5, X_1=2.25+0.15 \) and \(X_0=2.25-0.15\)).

The probability of identifying a micrometastasis in axillary LNs by different step and serial sectioning methods was also analyzed by Meyer (5) and later by Cserni (6). They both assumed that metastases were spherical and randomly distributed in the node, and only a single metastasis was hypothesized. Step sections taken 200 or 250 μm apart were considered ideal for revealing a micrometastasis.

Metastases tend not to be randomly distributed but rather to be located within the capsule or the subcapsular regions of LNs (5), as in the case described.

Hamakawa et al., step-sectioned 639 cervical lymph nodes obtained from 73 patients with oral cancer (7). Micrometastases were detected in 29 sites in 23 lymph nodes from 16 patients. Twenty-two micrometastatic foci (75.9%) were located in the marginal sinus.

At the Department of Pathology of the University Hospital of Heraklion, Crete, a systematic combination of serial and step sectioning of sentinel LNs of invasive ductal breast carcinoma, followed by meticulous histological and/or immunohistochemical evaluation, led to the conclusion that micrometastases (sized 0.2-2 mm) in 13 out of 14 cases (96%) were located at the level of the peripheral sinus (unpublished observations by senior author).

In order to calculate the probability for identifying a metastasis, a representative general scheme of the LN can be constructed by the following parametric equations:

\[
x = (1-n) \cdot \sin(t) \\
y = (a^2+b^2) \cdot \cos(t)-n \cdot \cos\{[(a/\beta)^k+1]t\} \\
z = \cos(x)+\sin(x),
\]

where \(x\) and \(y\) represent the values of \(x\) and \(y\) axis respectively, within the generated from the above equations chart. Values \(\alpha\) and \(\beta\) represent the largest dimensions of the LN for a two-dimensional shape, whereas \(n\) represent changes of the curvature; for a spherical LN, \(n=0\). We adopted a free parameter \(t\) and transformed \(a\) and \(b\) values as functions of \(t\).

The node analyzed here was spherical in shape, but this need not be the case. A LN may have an oblong shape, or be ellipsoid or lentoid.

Inserting the three parametric equations into a spreadsheet (Microsoft Excell®), the shape of the solid can be modified after setting \(\alpha\), \(\beta\) and \(n\) to different values. In this manner, we depicted three typical cases of cut sections of LNs as shown in Figure 2.

**Results**

In order to calculate the probability of identifying a lesion within a LN, one must construct the largest possible parallelepiped (a solid body of which each face is a parallelogram) that can fit within the given LN (if an ellipsoid or lentoid) and that will be formed after sectioning of the node; for a spherical LN the embedded parallelepiped is a cube. Taking into consideration that a micrometastasis is located at the level of the peripheral sinus, the lesion will be located in the volume of the node not included in the parallelepiped.

The probability is calculated from the above equations, whatever the shape of the node, depending on parameters \(\alpha\), \(\beta\) and \(n\). For example, assuming \(a\), \(b\) and \(k\) equal to 1 in different schemes (\(n\)) and with one section taken 100 μm below the surface of each half of a bisected node, the probability is provided in Table I.
Given a 0.96 probability of finding the micrometastasis in the periphery of the lymph node, the conditional probability is presented in the third column of Table I.

In the case of the spherical node illustrated (Figure 1; n=0), the probability equals 0.98025 and the conditional probability 0.94104.

In the article by Hamakawa et al., 76% of the lesions were found at the periphery (7). Assuming that this is the case, the probability of identifying a micrometastasis is reduced to 75% (74% for a spherical node).

### Discussion

In patients with head and neck cancer submitted to radical LN dissection without histologically detected metastases, a recurrence rate of 10% has been reported. It appears that metastases are present but not detected (8).

The clinical significance of micrometastases from squamous head and neck carcinoma in cervical LNs has been investigated in several studies. Different views are expressed regarding further treatment. It has been argued that “At this point in time, the proper clinical response to a finding of micrometastases remains to be defined” (8).

In many of the above studies immunohistochemical staining was applied (9). Nevertheless, regardless of the method of staining, the investigation is entirely based on the number of the sections examined.

Saphir and Amromin in 1948 were the first to demonstrate that routine histological examination of dissected nodes may be inadequate depending on the thoroughness of examination (3). In a study of 30 patients with breast carcinoma who were reported as being free of LN metastasis, they revealed that 10 of these patients had LN metastasis on serial sections of the originally examined nodes. They suggested that serial sectioning of all nodes with an average of 332 sections per node is necessary (9), a task not practical in everyday routine.

Step sectioning at intervals of 200 μm of the node analysed in the present report resulted in the microscopic examination of approximately 45 sections, whereas on the basis of the herein elaborated probabilistic argument and since the node was spherical, examination of two sections would be theoretically adequate for the detection of the lesion.

The actual diameter of a lesion detected with sections as described above may be different. For a spherical macro-metastasis, a section taken near the surface of the sphere may reveal a diameter in the range of micrometastasis, and in the case of micrometastasis, ITCs may be detected. If a lesion is identified, serial sectioning is necessary for the estimation of its size.

In conclusion, with the presented analysis, we demonstrate that one section from each bisected LN taken at a depth of 100 μm below the surface is theoretically adequate for revealing a micrometastasis, provided that it is located within the capsule or the sub-capsular regions, as it appears to be in the majority of cases. The limitations of the proposed method are that a number of lesions, with a range between 4-
25%, not located at the periphery, will be missed. Furthermore, the above calculations are absolutely theoretical and based on a single case. On the other hand, one should keep in mind that step sectioning of LNs, especially in radical neck dissection is not practical in routine work.

The proposed method facilitates the investigation with frozen sections so that decisions regarding treatment can be reached quickly during surgery.

Conflicts of Interest

The Authors have no conflicts of interest in regards to this study.

Acknowledgements

This study was supported by Research Programme KA 948 of the University of Crete. Dr Arapantoni Dadioti of Metaxas Cancer Hospital provided the case studied.

References