Mathematical Modelling of Lymph Node Grossing Techniques

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ABSTRACT
The optimal histopathological handling of sentinel lymph nodes remains the subject of debate. There is surprisingly little evidence in support of the manner in which sentinel lymph nodes are grossed. Some authors recommend that a lymph node be sectioned parallel to the long axis of the node, while others recommend that the short axis be used. In either case, the manner in which a sentinel lymph node is grossed determines the cut surface area exposed for histological examination and, in turn, the amount of area examinable for the presence of metastases. In this article, mathematical models of each of these approaches are presented and compared from the perspective of optimization of cut surface area. Computer modelling and simulations are also performed in order to assess how variously sized lymph nodes should be optimally grossed and to assess how frequently an optimal approach improves theoretical tumour focus detection. The results favour the use of the long-axis approach.

RÉSUMÉ
Le débat fait toujours rage à propos de la préparation histopathologique optimale du ganglion sentinel. Étonnamment, peu de données probantes déterminent le mode de préparation de ce ganglion. Des auteurs recommandent de le trancher en parallèle à son axe long, tandis que d’autres privilégient la coupe perpendiculaire à cet axe. Quoi qu’il en soit, l’angle de coupe détermine la surface exposée qui fera l’objet de l’examen histologique et, de ce fait, la taille de la zone examinée pour détecter la présence de métastases. L’article présente des modèles mathématiques des deux méthodes et les compare sur le plan de l’optimisation de la surface de la coupe sectionnée. La modélisation et les simulations informatiques permettent d’évaluer la préparation optimale de ganglions sentinelles de différentes tailles et de déterminer à quelle fréquence la méthode optimale améliore la détection théorique de cellules tumorales. Les résultats penchent pour la coupe parallèle à l’axe long.
The microscopic examination of lymph nodes is often an exercise in detection of tumour metastases. As outlined in the TNM staging system, lymph node metastasis, as part of the overall staging, is an indicator of tumour burden and outcome.\(^1\) The effort devoted to the detection of small tumour foci has increased because the standard of care in many instances now requires sensitivity to isolated tumour cells.\(^2\) Although the prognostic utility of a positive diagnosis of isolated tumour cells in a lymph node generally, and a sentinel lymph node specifically, remains debatable,\(^3\) a recent meta-analysis has noted that the identification of low-volume tumour metastasis (i.e., isolated tumour cells or micrometastases) in a sentinel lymph node in breast cancer cases is associated with non-sentinel lymph node disease in 10–15% of cases. It is logical, therefore, from both a patient care and completeness perspective, that the identification of any metastases, whether in the form of isolated tumour cells, micrometastases, or macroscopic foci, should be the ultimate goal of the pathological examination of a sentinel lymph node. As the literature indicates, however, maximizing the probability that this goal is achieved, and in the greatest number of instances, is both heuristically difficult and open to debate on the basis of merit.\(^3\)–\(^7\)

As Weaver aptly notes in his review, a pathologist can never be absolutely confident that a lymph node is negative for disease, especially if he or she equates the presence of isolated tumour cells with “positive” for metastasis.\(^4\) It follows that a certain level of error must be accepted as unavoidable, with the understanding that steps be taken to mitigate this error whenever possible. It is this mitigation of error that has sparked much research into the optimal histopathological evaluation of a sentinel lymph node. As long ago as 1948, it was recognized that a combination of deeper sectioning and retrospective review of slides could reduce the false-negative rate of lymph node evaluation.\(^8\) More recently, immunohistochemistry has become important in detecting otherwise “occult” lymph node metastases. Weaver et al. noted that a combination of deeper sectioning and immunohistochemistry could improve the sensitivity of lymph node evaluation, with 75% of false-negative sentinel lymph nodes in their series containing isolated tumour metastases.\(^5\) As much as deeper sections and immunohistochemical stains have been shown to reduce false-negative rates, the literature looking at the effect of the specific grossing techniques employed on the detection of nodal metastases is sparse.

Some sources have recommended that a lymph node be grossed by means of serial sections parallel to the long axis of the node; such a technique should maximize the histological visualization of the lymphatic channels serving the node and minimize the number of necessary tissue blocks.\(^3\)–\(^9\) Although intuitively logical, these arguments are incomplete. First, although serial sections through the long axis of a node may capture a number of lymphatic channels in a single section, there is insufficient evidence to say that multiple sections cut perpendicular to the long axis (and submitted in the same cassette moreover) would not do the same job as effectively. Furthermore, although the natural desire to reduce the total number of tissue blocks submitted for histological analysis is beneficial from a resource management perspective, the orientation of grossing (i.e., whether cuts are made parallel or perpendicular to the long axis) does not necessarily dictate the number of blocks since multiple smaller slices submitted when cutting perpendicular to the long axis may be placed in the same cassette. Moreover, a single slice taken parallel to the long axis may be so large as to necessitate multiple blocks. Finally, the localization and identification of lymphatic channels does not equate to the localization and identification of a tumour deposit since the very biology of a lymph node sees it acting as a sieve through which a tumour cell may trace out a path before taking root and forming a tumour focus. This latter point is exemplified by the seemingly random scattering of nevus cells that may periodically be observed in lymph nodes.

Still other authors have recommended that a sentinel lymph node be grossed by means of sections cut parallel to the short axis. In his recent review, Shidham noted that sentinel lymph nodes in cases of melanoma need to be grossed parallel to the short axis in order to maximize the delineable circumference on histological sections.\(^10\) It is stated that, since melanoma tends to form subcapsular tumour foci, an extensive examination of the nodal circumference is warranted, and Shidham provides a mathematical derivation in support of his technique. Unfortunately, both of these arguments can be easily refuted. First, the argument
that visualization of the nodal circumference should take precedence is faulted by the reality that tumour foci are not linear but, rather, three dimensional; maximizing the visibility of a uni-dimensional aspect of a tumour is therefore illogical when histological slides present tumour foci in two dimensions. Secondly, the mathematical derivations presented in his article are incomplete, and the summative effect of the circumference of multiple sections is not adequately accounted for. It would seem, therefore, that greater evidence in support of either of the grossing techniques described above is needed.

Since a lymph node cannot be examined in its entirety, it seems logical to try to maximize the cut surface area of the lymph node upon grossing to maximize the area that may be visible on a slide (and on subsequent deeper sections if needed). Thus, it may be surmised that the optimal detection of lymph node metastases may be improved if the grossing technique maximizes the cut surface area of a lymph node. From first principles there are three general methods in which a lymph node may be sectioned: with slices parallel to the long, short or intermediate axis. The intermediate axis approach is generally not chosen since, very simply, each slice taken in this manner will undercut the maximal surface area and will fail to produce the maximal number of slices. With respect to the other two methods, one (the “long axis” approach; Figure 1) will maximize the surface area of a given slice while sacrificing the total number of slices; the other (the “short axis” approach; Figure 2) will do the opposite. These two techniques are the extremes that are compared in this article. In either case, based on the College of American Pathologists’ guidelines, sectioning begins with a central cut followed by further cuts moving outwards from the centre, with each tissue section taken at a thickness of 2 mm.\textsuperscript{3,4,9}

Methods

A lymph node can be modelled mathematically by the ellipsoid centred at the Cartesian origin with semi-axes $a$, $b$, and $c$ according to the following:

\[
\frac{x^2}{a^2} + \frac{y^2}{b^2} + \frac{z^2}{c^2} = 1,
\]

$0 < a \leq b < c$
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This translates to a lymph node with long, short, and intermediate axis lengths, respectively, 2c, 2a, and 2b. This model need not take into account the perfect sphere since in this case the node would be grossed identically from any perspective. Other shapes such as the oval or the kidney-bean shape are not rigorously subsumed within this model since their equations are more complicated and cannot be easily described in Cartesian coordinates. In real life, however, the oval or bean-shaped lymph nodes can be approximated as ellipsoids since they usually have some amount of surrounding fat; in these cases the long, short, and intermediate axes can be measured and the shape approximated as an ellipsoid.

When sectioning the lymph node parallel to the short axis, surfaces with traces parallel to the x,y-plane are obtained; likewise, when sectioning the lymph node perpendicular to the long axis, surfaces parallel to the y,z-plane are obtained (see Appendix 1 at http://cap-acp.org/).

For each section in the short axis approach, the area of the trace ellipse is given by equation (6) of Appendix 1; the converse long axis approach is given by equation (7). When the cut surface areas exposed upon sectioning the entire node are summed, the following are obtained from equations (9) and (10):

Total Cut Surface Area, Short Axis Approach =

\[2 \pi b\left(\frac{c}{2}\right) + 1 - \frac{4}{6c^2}\left(\frac{c}{2}\right) + 1\left(\frac{2}{c}\right) + 1\]

Total Cut Surface Area, Long Axis Approach =

\[2 \pi b\left(\frac{a}{2}\right) + 1 - \frac{4}{6a^2}\left(\frac{a}{2}\right) + 1\left(\frac{2}{a}\right) + 1\]

(The angled brackets here refer to the floor function; see Appendix 1 at http://cap-acp.org/ for details.)

By comparing the values of the two equations for an individual node, the theoretical optimal surface area for each grossing technique can be determined. The specific technique producing the larger theoretical surface area should be chosen to maximize the optimal cut surface area available for histological evaluation.

To further analyze the optimal surface areas for each technique for nodes of various sizes, a computer analysis was performed to assess the optimal cut surface area of a hypothetical lymph node using either of the grossing techniques. In most laboratories, grossing is performed with a metric precision of 1 mm (i.e., the smallest gradations of grossing room rulers can be assumed to be 1 mm). Furthermore, most histopathology tissue cassettes can contain a maximal linear length of approximately 4 cm. Thus, using a range of axis lengths from 1 mm to 40 mm in increments of 1 mm, a reasonable approximation of possible lymph node sizes was generated. This was performed with the aid of the mathematical software MATLAB (see Appendix 2 at http://cap-acp.org/ for the code script).

In order to test the potential improvement that the optimal grossing strategies noted above might have on tumour focus detection, a series of computer simulations were performed to test each grossing technique relative to the other (see code script from Appendix 3 at http://cap-acp.org/). It was surmised that the optimal grossing method should more frequently permit transection of a hypothetical tumour focus, thus closely approximating the likelihood the tumour focus will be detected on histological examination. In each simulation, 1,000 mathematically modelled lymph nodes were created with random measurements obtained using MATLAB’s built-in random number generator. This number of lymph nodes per simulation, by the law of averages, should adequately account for most possible lymph node sizes as set forth in the matrix of lymph node sizes above. In each lymph node, a focus of metastasis was simulated and assigned a random location (excluding the lymph node’s surface) and random size (<0.2 mm to limit the discussion to tumour foci smaller than micrometastases). The program actively excluded a node that was exactly spherical since, in such a case, the computer would be unable to discern an optimal grossing technique. The lymph node then underwent simulated grossing with cuts parallel either to the long axis or short axis starting from the centre and moving out. The program then determined which of the two methods was optimal based on the above calculations, calculated whether a grossing cut using either method would transect the tumour focus, and tallied
whether the optimal method, the non-optimal method, or either method worked best. The simulations were repeated a total of 1,000 times to permit the generation of statistical data.

**Results**

By comparing the total optimal surface area for each of the short axis and long axis approaches, a $40 \times 40$ diagonally symmetric matrix was generated. As Figure 3 indicates, of the 820 unique lymph node sizes, the long axis approach (pink) was optimal in 802 cases (98%). The remaining cases were either equivalent by either approach (grey) or showed optimal surface area using the short axis approach (blue). Furthermore, a specific pattern of repetition was noted in the few cases of short axis preference: the short axis technique was preferred when the long and short axes had measurements $4n + 1$ and $4n$, respectively, or $4n+2$ and $4n$, respectively (for $n \geq 1$).

One thousand simulations, with 1,000 simulated lymph nodes each, were conducted with each node bearing a randomly positioned and sized tumour deposit (smaller than a microfocus). An average of 87.7 lymph node tumour foci showed grossing transection using the optimal approach; an average of 86.7 lymph node tumour foci showed grossing transection using the non-optimal approach. The two-tailed $t$-test showed this to be a statistically significant difference of means ($p = .010$). Furthermore, on average 13.7 lymph node tumours were found to be equivocal to either technique. Notably, the number of lymph nodes whose tumour focus was not transected by either grossing technique was quite high, averaging at 811.9 (81%). This latter result is in keeping with the previous studies in which the frequency of missed tumour foci increased when the size cutoff was reduced.$^5$

**Discussion**

Although debate persists as to the extent to which efforts to identify low-volume tumour foci in sentinel and non-sentinel lymph nodes should proceed, on a case-by-case basis, most pathologists will be loath to miss a metastasis, however small. Research into the best-practice handling of lymph nodes persists, and the focus of this research tends to be centred on maximizing the efficiency of histological and ancillary techniques at detecting metastatic foci, with the caveat that, from a practical perspective, a lymph node cannot be fully and completely examined microscopically. Little research has examined the manner in which a lymph node is grossed, however, and its potential impact on maximizing the detection of metastases.

The present study uses a mathematical model of a lymph node to calculate the maximum optimal cut surface area available for histological examination when the grossing technique is varied from either parallel to the long axis or parallel to the short axis. A comparison of these two approaches reveals that the optimal grossing technique is the long axis approach in 98% of simulated lymph nodes of varying sizes. In the great majority of cases, therefore, the approach described as optimal by Weaver and others is sound. A small number of predictable cases, however, do seem to show optimal grossing using the short axis approach, as described above. Nonetheless, when a simulation of mathematically modelled lymph nodes is performed to test the optimal and non-optimal grossing techniques head to head, only a marginal improvement in theoretical detection is noted, although this is statistically significant.

![Figure 3. A $40 \times 40$ matrix of lymph node measurements; pink indicates optimal long axis approach, blue indicates optimal short axis approach, and grey indicates no difference in taking either approach.](image)
A “real-life” study to verify the above conclusions would be ideal but is highly unlikely. The resources required to adequately study even one lymph node in its entirety are a significant impediment. Furthermore, as pointed out in Weaver’s application of the Heisenberg uncertainty principle, real-life limitations make the impact of an individual lymph node’s questionable diagnosis of benignity impossible to assess; simulation, therefore, is the only plausible mode of study in this vein. The current study appears to be the strongest evidence thus far supporting the lymph node grossing practices described by Weaver et al. 5

References


