ORIGINAL ARTICLE

Clinical relevance of intra-operative sentinel lymph node examination in breast cancer management

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Objectives To determine the sensitivity, accuracy, and clinical relevance of intra-operative examination of sentinel lymph nodes in breast cancer management.

Design Retrospective study.

Setting Private anatomical pathology practice.

Participants Three hundred breast cancer patients who underwent axillary sentinel lymph node biopsies for intra-operative assessment between January 2004 and March 2006.

Main outcome measures Correct identification of metastatic carcinoma in sentinel lymph nodes during intra-operative examination, sparing patient a second surgery.

Results The intra-operative diagnosis of sentinel lymph node biopsies from 300 consecutive patients were compared with the final pathological diagnoses. The final pathology results showed metastatic carcinoma in 74 patients, of whom 63 (85%) were correctly diagnosed during frozen section examination. There was no false-positive case (positive predictive value 100%). The sensitivity for detecting macro- and micro-metastases were 95% and 50%, respectively ($P<0.01$). The sensitivity for detecting metastases in T1 and T2/above tumours were 72% and 90%, respectively ($P<0.05$).

Conclusions Intra-operative examination is a reliable and sensitive method for the detection of sentinel lymph node metastasis. Eighty-five percent of the patients with metastatic disease were correctly diagnosed and spared a second operation for axillary dissection. The disadvantage of missing a positive lymph node is by far outweighed by the advantage of a single stage operation in case of a positive diagnosis. The benefit of intra-operative examination was slightly less in patients with smaller tumours.

Introduction

The status of axillary lymph nodes is one of the most important factors in predicting long-term survival and in determining the need for adjuvant therapy in breast cancer.[1] Axillary lymph node dissection (ALND) is the traditional procedure to detect lymph node metastasis, and is also potentially therapeutic for the regional control of axillary metastases. However, ALND is associated with significant long-term morbidity. Sentinel lymph node (SLN) biopsy is an alternative method for the assessment of lymph node status in patients with clinically node–negative breast cancer, with morbidity markedly less than ALND. Numerous studies have confirmed the reliability of this method, which has a high detection rate and overall accuracy.[2-4] If SLNs are histologically negative, no further axillary surgery would be performed and consequently, unnecessary morbidity caused by ALND can be avoided.

The value of routine intra-operative SLN frozen section (FS) in this setting is controversial, with wide variation in results.[5-12] If positive, FS has the obvious advantage of allowing an immediate ALND and thereby avoiding re-operation. On the other hand, FS is costly, time-consuming for the pathologist and surgeon, and subject to false-negative results. We report our own experience with this procedure and examine its benefits in terms of patients spared of a second operation, and to identify the factors associated with false-negative results.

Key words
Axilla; Breast neoplasms; Frozen sections; Lymph node excision

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Methods

Between January 2004 and March 2006, 300 consecutive patients who had SLN biopsies by one surgeon were sent to Diagnostic Pathology Laboratories for intra-operative examination, with synchronous ALND offered to those with a positive diagnosis. The results of intra-operative examination were compared with the final pathology in each SLN specimen. The sensitivity, specificity, and positive and negative predictive values were determined.

Intra-operative assessment

Sentinel lymph nodes were identified by radioisotope method with injection of technetium-99m labelled colloid particles. Intra-operative pathology examination was performed by one of three pathologists from our laboratory. During the examination of each SLN received, the surrounding fatty tissue was first carefully trimmed off. All lymph nodes larger than 3 mm in maximum dimension were then bisected or dissected into slices 3 mm thick, and two touch imprints were made by pressing each cut half of the specimen onto a glass slide. The imprints were stained with Diff Quik (Medion Diagnostics, Dudingen, Switzerland) and toluidine blue stains. Half of each lymph node was submitted for standard FS, which included four sections (two haematoxylin and eosin [H&E] and two toluidine blue). Lymph nodes smaller than 3 mm were entirely submitted for FS. The frozen tissue and the remaining half of the lymph node were later embedded into paraffin blocks. The final pathological diagnosis was based on the examination of three H&E-stained paraffin sections at 50 μm interval, and for morphologically negative cases, two cytokeratin (pan-cytokeratin antibody MNF-116, Dako, Denmark) immunostained sections also at 50 μm interval from all lymph nodes. The maximum dimensions of the metastatic deposit in all SLNs were measured by an ocular micrometer. Macrometastasis was defined as a tumour deposit of more than 2 mm in size, micrometastasis as a tumour deposit of 0.2 to 2 mm in size, and isolated tumour cells as single or clusters of tumour cells of less than 0.2 mm in aggregate size.[13] The benefit of intra-operative examination was defined as the proportion of patients in whom metastatic carcinoma was identified, thereby allowing immediate ALND and avoiding re-operation in this reason. The sensitivity, false-negative rate (FNR), and positive predictive value were defined in their usual sense.

Results

Among the 300 patients, 38 had a final diagnosis of ductal carcinoma in-situ and 262 had a final diagnosis of invasive mammary carcinoma. A total of 1172 SLNs were examined intra-operatively (mean, 3.9 nodes; range, 1-10 nodes examined per case). Multiple SLNs were sampled in most cases since studies have shown that sampling three or more SLNs was associated with a significantly lower FNR, compared to single node sampling only.[14] Once a positive SLN was identified, the remaining nodes would not be examined intra-operatively. There were no false-positive cases, giving a positive predictive value of 100%. All incorrect results were false negatives.

The overall results of intra-operative examination indicated that in general, both the SLN positive rate and FS sensitivity increased with tumour size (Table 1). The benefit was thus more notable in patients with larger tumours. Overall, 74 (25%) of the 300 cases had SLN metastases in the final pathology. In 11 of these cases, the FS diagnoses were negative (FNR, 15%). Of the 63

術中前哨淋巴結檢驗對乳癌治療的臨床意義

目的 確定在乳癌手術中進行前哨淋巴結檢驗的敏感度、準確度和臨床相關程度。

設計 回顧研究。

安排 在私營化驗所進行解剖病理學檢驗。

參與者 2004年1月至2006年3月，300位乳癌患者接受腋窩前哨淋巴結活檢的術中評估。

主要結果測量 通過術中檢驗確定是否有前哨淋巴結轉移性癌，使患者不需要接受另一次手術。

結果 將連續300位患者術中前哨淋巴結檢驗的結果，與最後的病理學檢驗結果比較。最後的病理學檢驗結果顯示，74人出現轉移性癌，冷凍切片檢查正確診斷當中63例（85%）。本研究並無發現假陽性病例（陽性預測值為100%）。偵測大規模轉移和微轉移的敏感度分別為95%和50%（P<0.01）。偵測TI型和TII/以上型腫瘤中的轉移敏感度分別為72%和90%（P<0.05）。

結論 術中檢驗能有效地偵測前哨淋巴結轉移性癌，正確診斷出本研究中的85%轉移性癌病例，免去了要施行另一次腋窩清掃手術。對檢驗結果為陽性的病例來說，單一次手術的優點遠大於檢驗不到淋巴結呈陽性反應的缺點。術中檢驗對腫瘤比較小的病人作用略少。

TABLE 1. Summary of intra-operative sentinel lymph node (SLN) frozen section (FS) examination findings

Tumour size	Patients (n)	SLN+ (%)	Sensitivity FS+/SLN+ (%)	Benefit FS+/Total (%)
Tis	38	0	N/A*	0/38 (0)
Tmic-1a	19	1 (5)	1/1 (100)	1/19 (5)
T1b	26	3 (12)	2/3 (67)	2/26 (8)
T1c	91	18 (20)	13/18 (72)	13/91 (14)
T2	120	47 (39)	43/47 (91)	43/120 (36)
T3	6	5 (83)	4/5 (80)	4/6 (67)
Total	**300**	**74 (25)**	**63/74 (85)**	**63/300 (21)**

* N/A denotes not available

TABLE 2. Relationship between metastatic tumour size in sentinel lymph nodes and the sensitivity of frozen section (FS) examination

FS diagnosis	n	Size range (mm)	Median size (mm)	Mean size (mm)
False-negatives	11	0.48-6.0	0.6	1.6
True-positives	63	0.40-50.0	5.0	10.6
P value (*t*-test)	-	-	-	>0.05

TABLE 3. Correlation between sentinel lymph node (SLN) metastasis and lymphovascular invasions (LVI)*

Status of LVI	No. of cases	No. of cases with SLN+
Absent	136	13 (10%)
Focal†	86	29 (34%)
Multifocal‡	29	19 (66%)
Diffuse§	11	10 (91%)
Total number with LVI (focal+multifocal+ diffuse)	126	68 (54%)

* P<0.01, χ^2 test

† Focal: limit to 1 quadrant, or 2 contiguous but not opposite quadrants of the circumference around tumour

‡ Multifocal: present in 2 opposite quadrants or more than 2 quadrants of the circumference around tumour

§ Diffuse: as in multifocal, plus extension of more than 1 cm beyond border of tumour

TABLE 4. Comparison of sentinel lymph node (SLN) characteristics between T1 and T2/larger tumours in positive cases

Tumour size	Proportion of micrometastasis*	Mean size of largest tumour deposits (mm)	Mean number of SLN+
T1	13.0%	8.4	1.8
T2/larger	19.2%	9.6	1.9
P value†	>0.05	>0.05	>0.05

* % of SLN with micrometastasis only/total number of SLN+

† Chi squared test

positive cases, 27 went on to have additional positive nodes in the axillary dissection. Of the 11 cases missed during intra-operative examination, one of six patients who opted for follow-up axillary dissection showed additional positive lymph nodes. Five patients did not opt for second surgery. Metastatic lymph node involvement was only seen in invasive carcinoma cases, but not in in-situ tumours. On a lymph node basis, there were a total of 104 positive SLNs from these 74 patients. The sensitivity for detection of macrometastasis (95%) is much higher than that for the detection of micrometastasis (50%, P<0.01). Moreover, as shown in Table 2, the mean maximum dimension of SLN tumour deposits in missed cases ranged from 0.48-6.0 mm (median=0.6 mm) and that for the correctly diagnosed cases ranged from 0.4-50.0 mm (median=5.0 mm) [P<0.01]. The sensitivity of intra-operative examination in patients with smaller tumours (T1) was 72% and that for patients with larger tumours (T2 or above) was 90% (P<0.05). In addition to tumour size, the presence and extent of lymphovascular invasion was found to be a highly significant predictor of SLN metastasis (Table 3). A higher proportion of micrometastasis was not found in patients with smaller tumours (T1); nor was there a significant difference in the size of metastatic tumour deposits between T1 and T2/larger tumours (Table 4), neither were there more positive SLNs per case associated with larger tumours.

Discussion

The sentinel node theory postulates that SLNs are the most likely sites of lymph node metastasis. Accordingly, a more detailed pathological work-up of SLNs is more likely to reveal metastases undetected by conventional histology of other lymph nodes. Routine intra-operative examination of SLNs can detect metastatic disease, allowing immediate axillary dissection, if necessary, and avoiding the need for re-operation. A review of the literature showed that the FNR for this procedure varied between 14 and 43%,[5-12] as a result of heterogeneity in the methods used both intra-operatively and postoperatively. Enhanced intra-operative analysis of the SLN, such as additional serial sections, touch smear cytology, and even immunohistochemical stains will increase the apparent sensitivity of intra-operative examination, whereas enhanced postoperative analysis of the paraffin sections with serial sections and immunohistochemical stains at multiple levels will decrease the apparent sensitivity of intra-operative examination. In our study, we examined four serial sections and two touch smears intra-operatively, which was more exhaustive than most other studies. Our examination of three H&E levels and cytokeratin immunostains at two levels from each half of the lymph node (a total thickness of approximately 0.5 mm) also represented a relatively more diligent postoperative effort. A common observation from previous studies was that the FNR is higher among patients with small tumours. This was usually due to the fact that a higher proportion of micrometastasis occurred in patients with small tumours. We made the same observation, but unlike the others, we did not find a higher proportion of micrometastasis in patients with smaller tumours (T1).

A recently published large series of more than 2300 patients from the Austrian sentinel node study group reported an overall FNR of 15.6%,[15] which is similar to ours. The benefits of intra-operative examination of SLNs far outweighed its drawbacks, because if intra-operative examination were not performed, the cost of a re-operation—which would have been necessary in 28% of our patients with invasive ductal carcinoma—far exceeded the cost of intra-operative SLN examination for all patients. A common observation from previous studies was that the FNR is higher among patients with small tumours. This was reportedly due to a higher proportion

of micrometastasis in such patients.[16] Furthermore, not all SLN-positive patients require axillary dissection and it is of no benefit if the rest of the axilla is negative. Predictive models have been developed to define subgroups of SLN-positive patients with disease limited to the SLN,[17-20] and micrometastasis in SLNs seems to be associated with a smaller risk of having other non-SLNs positive.[21]

Conclusions

The sensitivity of intra-operative examination of SLN in breast carcinoma is tumour size dependent, and false-negative results were largely due to failure to detect micrometastasis. The benefit of FS in avoiding re-operation ranged from 5% in T1a tumour to 67% in a T3 tumour.

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