Pathological Evaluation of Sentinel Lymph Nodes for Breast Cancer

Takuya Moriya,[1] Shin Usami,[1,2] Hiroshi Tada,[2] Atsuko Kasajima,[1] Kazuyuki Ishida,[1,2] Yoshiyuki Kariya,[1] Noriaki Ohuchi[2] and Hironobu Sasano,[1] Departments of [1]Pathology and [2]Surgery, Tohoku University Hospital, Sendai, Japan.

Recently, sentinel lymph node (SLN) biopsy has been employed to avoid unnecessary lymph node dissection, because SLN negativity for carcinoma metastases may imply an extremely low possibility of non-SLN involvement. Pathological evaluation is essential, but standardized procedures have not yet been determined. Intraoperative consultation, either by frozen section (multiple slices are desirable) or touch imprint cytology, are usually very useful. Their accuracy, however, is variable and depends on the procedures used, but specificity is characteristically 100%, and the missed metastatic focus is always quite minute. After fixation, multiple sections, immunohistochemistry, and their combination will be able to detect small metastatic foci more frequently. The clinical significance of small or submicro- or occult metastases have not yet been clarified, and further investigations are needed. If the SLN is positive for carcinoma metastases, both the procedure for detection and the size of the metastatic focus should be clarified on the pathological reports. [*Asian J Surg* 2004;27(4):256–61]

Introduction

The occurrence of breast carcinoma has increased in Japan,[1] and currently, about one in 30 Japanese women will suffer from breast cancer during her life. This increase is mainly due to changes in lifestyle, especially in eating habits, and to the development of mass screening programmes for breast cancer. The detection of early-stage cancers by screening mammography has led to an increasing incidence of non- or early invasive carcinomas. Additionally, recent advances in breast cancer treatment enable us to perform breast-conserving surgery for early-stage cancers.

Pathological analysis of regional lymph nodes is crucial for tumour staging, which is a prognostic indicator.[2] However, total removal of axillary lymph nodes may cause significant morbidity, including limb oedema, loss of sensation and disturbances in limb motion. Sentinel lymph node biopsy (SLNB) is a new trend in breast-conserving surgery. If the sentinel lymph node (SLN) is negative for carcinoma, additional dissection may be avoided, because SLNB is considered a sensitive and specific procedure for predicting regional lymph node status.

General features of lymph node metastases

In Osaka Prefecture, 32% of breast carcinomas were positive for lymph node metastasis in 1996–1998,[3] which is about average in Japan. The proportion of node-positive cancers decreased from 46% in 1975–1977. As nodal involvement is more frequent in larger tumours,[4] the increasing incidence of early-stage cancer may lead to a decrease in the incidence of positive nodes. Many cancers may be truly node negative, but some may be positive with metastatic foci too small to find in routine practice. Multiple sectioning of the lymph nodes for histopathological analysis may improve the detection rate for small metastases.[5] Details of methods for multiple sections or other alternative procedures, as well as their clinical significance, will be discussed later.

Address correspondence and reprint requests to Dr. Takuya Moriya, Department of Pathology, Tohoku University Hospital, 1-1 Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan.
E-mail: moriya@patholo2.med.tohoku.ac.jp • Date of acceptance: 3 November, 2003

Lymph node metastases mostly occur via lymphatic vessels and, rarely, blood vessels. Small foci of metastatic tumour cells are frequently seen in the subcapsular (marginal) sinus, and these are thought to be the initial evidence of node metastasis. They are either floating within the sinus or attached to the capsule,[6] and may extend to the sinusoids and invade the node parenchyma. Therefore, pathologists must seek the area just beneath the capsule in routine practice if extensive metastases are not seen in low-power view. Additionally, one study suggests a higher probability of metastatic breast carcinoma at the inflow junction of the afferent lymphatic vessels.[7]

Usually, small foci of metastasis do not enlarge the lymph node. However, it is well known that a significant proportion (approximately 20–25%) of clinically node-negative patients will have metastatic foci pathologically.[8] Additionally, negative nodes may show extensive enlargement, caused by accompanying reactive processes such as sinus histiocytosis or reactive hyperplasia of the lymph follicles.

Pathological staging for metastatic carcinoma

Small metastatic foci are currently divided into two categories:[9] isolated tumour cell(s), defined as single cells or small clusters of cells not greater than 0.2 mm in largest dimension, usually floating on the sinus, without any proliferation or stromal invasion and no evidence of malignant activity; and micrometastasis, found in tumour deposits of 0.2–2.0 mm in largest dimension, that may proliferate and destroy the stroma and may have malignant activity. If these small metastases are detected by procedures such as immunohistochemistry or reverse transcriptase-polymerase chain reaction (RT-PCR), they are recommended to note how to detect the metastatic focus.[9] Submicrometastases are metastases that can only be detected by immunohistochemistry and are usually isolated tumour cells.[10] Occult metastases are foci missed by initial screening and identified on subsequent screening, or metastases identified at additional evaluation using paraffin-embedded tissue blocks.[8] Occult metastases are not defined by size but are no larger than micrometastases.

Pathological examination of sentinel lymph nodes

Specimen handling for intraoperative diagnosis

Procedures and guidelines are available for pathological analysis.[4,10] It is strongly recommended that most pathology analysis for SLNs be performed intraoperatively, by frozen-section diagnosis, a combination of frozen section and cytology or supplementary immunohistochemistry, because these results will immediately affect further surgical procedures. Some authors consider that frozen-section diagnosis is not reliable,[11] but it may be effective if technicians and pathologists are well trained and experienced. Frozen-section diagnosis is a safe method, even if radioactive materials are used for SLN detection,[12] as pathologists and technicians suffer only minimal exposure.

In general, as the number of examined slides increases, the rate of detection of micrometastases increases. We believe that one haematoxylin and eosin (H&E)-stained section along the long axis of the node is not sufficient, especially if the node is large. However, serial or step sections across the whole node is time-consuming and costly, and should only be used in research.[13] In routine practice, pathologists should measure the size of the node and then cut it into almost 2 mm thick sections, then perform careful gross examination to detect focal lesions. Cutting along the long axis may be a standard, but it is also possible to cut along the short axis, according to the shape and size of the node. It is desirable that three levels of frozen sections are made for each slice.[10] After surgery, frozen sections should be fixed in formalin and permanent sections made to confirm the intraoperative diagnosis.

Cytology by touch preparation of the cut surface is another procedure for detecting micrometastases. It does not waste tissue by sacrificing for frozen sections and does not suffer from freezing artefacts. It is also easier and faster than frozen-section cutting. However, the evaluation of the specimen is not always easy, and may potentially lead to indeterminate or deferred diagnoses.[4] Pathologists require cytology training, including screening and cell interpretation. The advantages and disadvantages of cytology and frozen-section diagnosis are shown in Table 1.

Accuracy of intraoperative consultation

In SLN analysis, it is very important that pathologists detect metastatic carcinoma accurately. However, the procedure for treating removed node(s) has not been standardized. Therefore, it is not easy to compare different studies to assess the accuracy of each procedure.

The accuracy of frozen-section diagnosis is compared with that of paraffin sections in Table 2.[14–19] Both types of section were evaluated by H&E staining only. There is significant variation in the sensitivity (52–100%) and false-negative rate (0–48%) of frozen-section diagnosis. These discrepancies are probably due to differences in the handling process (including

Table 1. Advantages and disadvantages of frozen section and imprint cytology for intraoperative sentinel lymph node analysis

	Frozen section	Imprint cytology
Advantages	– Interpretation of nodal architecture available – More specific diagnosis possible – Size of metastatic focus measurable – Rapid immunostains available	– Simple/cheap/rapid – Interpretation of cytological/nuclear details available – Avoids tissue loss
Disadvantages	– Relatively time-consuming – More expensive – Freezing artefacts – Tissue loss (by sacrificing)	– Size and area of metastatic focus not detectable – Indeterminate/deferred diagnoses – Need special training to interpret – Sampling error may occur

Table 2. Studies of intraoperative frozen-section diagnosis for sentinel lymph nodes

Authors	No./interval of H&E sections	*N*	Accuracy, %	Sensitivity, %	Specificity, %	False-negative rate, %
Canavese et al (1998)[14]	3 (both sides)	96	96	86	100	14
Schneebaum et al (1998)[15]	Not described	47	98	91	100	9
Koller et al (1998)[16]	3 consecutive	107	83	64	100	36
Imoto et al (2000)[17]	Not described	52	96	89	100	11
Noguchi et al (2000)[18]	2	38	79	60	100	40
Noguchi et al (2000)[18]	> 3	45	93	85	100	15
Noguchi et al (2000)[18]	2 mm interval	26	100	100	100	0
Motomura et al (2000)[19]	1	101	88	52	100	48

H&E = haematoxylin and eosin.

the number and interval of slices, gross inspection and procedures for microscopic slide preparation), and procedures for final pathological evaluation. Other possible influences on the detection rate of metastatic foci are differences in the characters of primary tumours. The size of the primary cancer influences the results of frozen-section diagnosis.[20]

Most metastatic foci missed by frozen-section analysis are either micrometastases or isolated tumour cells. This argues for an awareness that small metastatic foci may be missed at routine intraoperative examination. It is interesting that the specificity of frozen-section diagnosis was 100% in all the studies listed. It is unlikely that trained pathologists will miss foci of carcinomas on microscopic examination. Thus, it is possible that the accuracy of frozen-section diagnosis may be improved either by multiple slices or step/serial sectioning, if the bias due to the skill of the pathologists is ignored. Veronesi and colleagues analysed SLNs by frozen sections every 100–500 μm, but the false-positive rates were 36% and 32% in two studies.[11,21] Therefore, they examined 15 levels of frozen sections at intervals of 50 μm using immunohistochemical analysis. The false-positive rate was reduced to 6%.[11] The accuracy of their last proposal was confirmed by Viale et al.[13] Although this procedure gives good results, it may be too complex and time-consuming for routine practice in most institutions. The significance of immunohistochemistry will be discussed later.

Imprint cytology is compared with permanent H&E-section diagnosis in Table 3. The procedure is quite simple and as accurate as tissue sections. Accuracy and sensitivity are good, and specificity was almost 100%, similar to frozen-section diagnosis.[19,22–26] It is unlikely that benign cells (i.e. histiocytes, lymphocytes) will erroneously be interpreted as carcinoma metastases in most cases. However, we have had some experience of atypical cells on the smear being tentatively described as carcinoma. In such cases, experience is necessary and, if the situation allows, the combination of both frozen section and imprint cytology will be useful.[22]

Multiple levels of H&E sections

The average diameter of ductal carcinoma cells is 20 μm. Theoretically, to detect tumour cell nests of 20–30 cells, it is necessary to make step sections at intervals of 250 μm.[27]

The results of multiple levels of H&E sections are summarized in Table 4. The rate of node-positive patients is increased (4–18% of patients upgraded) by various multiple-section procedures.[28–30] These methods are not always employed at the time of frozen-section diagnosis because they are time-consuming for technicians.

Immunohistochemical analysis of SLNs

As microscopic analysis is somewhat subjective, there are some limitations to detecting metastatic foci on routine staining, even by skillful pathologists. To make examinations more accurate, immunohistochemistry has been used as an adjunct to routine stains, both intra- and postoperatively (Table 5).[30–36] Moreover, if suspicious cells are found on H&E sections, additional immunohistochemistry will be a strong tool for confirmation. Rapid immunohistochemistry using imprint cytology has also been used.[37] Usually, detection of cytokeratin is used in both histology and cytology; 2–20% of patients are upgraded by this procedure. The combination of multiple H&E sections with either single immunohistochemistry or

Table 3. Studies of intraoperative imprint cytology for sentinel lymph node examination

Authors	No./interval of sections	*N*	Accuracy, %	Sensitivity, %	Specificity, %	False-negative rate, %
Moriya et al (1994)[22]	1	286	99	95	100	5
Rubio et al (1998)[23]	1	124	99	96	100	5
Ratanawichitrasin et al (1999)[24]	2	55	98	93	100	7
Motomura et al (2000)[19]	2 mm interval	101	96	91	99	9
Henry-Tillman et al (2002)[25]	> 1	479	99	94	100	6
Karamlou et al (2003)[26]	1	446	–	75	100	5

Results were compared with permanent haematoxylin and eosin sections of the same level; studies with immunohistochemical analysis were eliminated.

Table 4. Studies on multiple levels of haematoxylin and eosin (H&E) sections for sentinel lymph node examination

Authors	*N*	Study design	Patients positive by standard methods, *n* (%)	Patients upgraded by alternative methods, *n* (%)
Turner et al (1999)[28]	52	2 H&Es at 40 μm interval vs additional 2 H&Es at 160 μm interval	10 (19)	2 (5)
Nahrig et al (2000)[29]	40	1 H&E vs 4 additional H&Es at 150 μm intervals	18 (45)	4 (18)
Torrenga et al (2001)[30]	250	1 H&E vs additional 4 H&Es at 250 μm intervals	69 (28)	8 (4)

Table 5. Studies of immunohistochemical staining (IHC) for sentinel lymph node examination

Authors	*N*	Study design	Patients positive by standard methods, *n* (%)	Patients upgraded by IHC, *n* (%)
Czerniecki et al (1999)[31]	41	1 H&E vs 4 levels of IHC	12 (29)	3 (7)
Noguchi et al (1999)[32]	62	1 H&E vs IHC	24 (39)	1 (2)
Pendas et al (1999)[33]	478	1 H&E vs IHC	93 (19)	41 (9)
Kowolik et al (2000)[34]	33	2 H&Es vs IHC	8 (24)	4 (12)
Mann et al (2000)[35]	51	1 H&E vs IHC	10 (20)	10 (20)
Wong et al (2001)[36]	973	1 H&E vs 2 levels of IHC	104 (11)	58 (6)
Torrenga et al (2001)[30]	250	1 H&E vs IHC	69 (28)	5 (2)
Torrenga et al (2001)[30]	250	1 H&E vs 4 levels of IHC	69 (28)	17 (7)

H&E = haematoxylin and eosin.

additional multiple levels of immunohistochemistry leads to significant upgrading. For example, Freneaux et al reported upgrading in 47% of examined cases using four H&E sections and six additional levels of cytokeratin immunohistochemistry at intervals of 0.15 mm.[38]

Most metastatic foci detected only by immunohistochemistry will be either micrometastases or isolated tumour cells. There is a small possibility that cells other than metastatic carcinomas may be positive (false-positive staining), such as some macrophages. Benign transport of breast epithelium and pseudometastasis from noninvasive carcinomas have been reported.[39,40] To avoid pseudometastasis and to detect only clinically significant metastases, it is recommended that the number of immunohistochemistry-positive cells be quantified,[10] e.g. less than 10 cells, 10–100 cells, and more than 100 cells, as represented in two dimensions on a slide.

Molecular analysis

RT-PCR has been used for molecular analysis of SLNs. It is more sensitive than immunohistochemistry, but specific markers are lacking. The results of upgrading are still variable, and the procedure is not feasible in all pathology laboratories. At least currently, it is only used for research.[4]

Assessment of metastases detected in SLNs

The clinical significance of carcinoma metastases in SLNs is important because almost half of SLN-positive cases may have further metastases in non-sentinel nodes.[41] Extranodal invasion from the SLN, the size of the metastatic focus in the SLN, the number of positive SLN nodes, and the size and lymphovascular invasion of the primary tumour are correlated with non-SLN metastases.[41,42] Conversely, small primary tumours (i.e. T1a) and micrometastases are unlikely to have further metastases in non-SLNs.[20,42]

The negative predictive value of SLNs is considered good,[43] but the probabilities are significantly changed according to pathological procedures. Turner and colleagues analysed 1,087 non-sentinel nodes from 60 patients who were SLN-negative by H&E and immunohistochemistry. Only one node (one case) was positive for carcinoma, and the lesion was only detected by additional immunohistochemistry.[44] Thus, the probability of non-SLN metastasis will be less than 0.1% if SLN negativity is confirmed by both H&E and immunohistochemistry. In other words, isolated tumour cells in SLNs are unlikely to be associated with non-SLN involvement.

Finally, the clinical significance of micrometastases and/or isolated carcinoma cells has not been well elucidated. There are some studies,[4,27,45,46] but the real prognostic significance of micrometastases (i.e. detected by immunohistochemistry only) will only be clarified by future additional studies.

References

1. The Research Group of Population-based Cancer Registration in Japan. Cancer incidence and incidence rates in Japan in 1997. Estimates based on data from 12 population-based cancer registries. *Jpn J Clin Oncol* 2002;32:318–22.
2. Silverberg SG, Chitale AR. Assessment of significance of proportions of intraductal and infiltrating tumor growth in ductal carcinoma of the breast. *Cancer* 1973;32:830–7.
3. Osaka Cancer Registry (OCR), 65th issue. Osaka Prefecture, Japan: Osaka Cancer Registry, 2002. [In Japanese]
4. Cserni G, Amendoeira I, Apostolikas N, et al. Pathological work-up of sentinel lymph nodes in breast cancer. Review of current data to be considered for the formulation of guidelines. *Eur J Cancer* 2003; 39:1654–67.
5. Pikren JW. Significance of occult metastases. *Cancer* 1961;14:1266–71.
6. Carr I, Murari PJ, Pettigrew NM. Lymph nodes and spleen. In: Silverberg SG, ed. *Principles and Practice of Surgical Pathology*, 2nd edition. New York: Churchill-Livingstone, 1989:375–430.
7. Diaz LK, Hunt K, Ames F, et al. Histologic localization of sentinel lymph node metastases in breast cancer. *Am J Surg Pathol* 2003;27: 385–9.
8. Weaver DL. Sentinel lymph nodes and breast carcinoma. Which micrometastases are clinically significant? *Am J Surg Pathol* 2003;27: 842–5.
9. Greene FL, Page DL, Fleming ID, et al, eds. *AJCC Cancer Staging Manual*, 6th edition. New York: Springer, 2002:223–40.
10. Schwartz GF, Giuliano AE, Veronesi U, for the Consensus Conference Committee. Proceedings of the Consensus Conference on the Role of Sentinel Lymph Node Biopsy in Carcinoma of the Breast, April 19–22, 2001, Philadelphia, Pennsylvania. *Hum Pathol* 2002;33: 579–89.
11. Veronesi U, Paganelli G, Galimberti V, et al. Sentinel-node biopsy to avoid axillary dissection in breast cancer with clinically negative lymph-nodes. *Lancet* 1997;349:1864–7.
12. Strattmann SL, McCarthy TM, Kuhn J. Radiation safety with breast sentinel node biopsy. *Am J Surg* 1999;178:454–7.
13. Viale G, Bosari S, Mazzarol G, et al. Intraoperative examination of axillary sentinel lymph nodes in breast carcinoma patients. *Cancer* 1999;85:2433–8.
14. Canavese G, Gipponi M, Catturich A, et al. Sentinel lymph node mapping opens a new perspective in the surgical management of early-stage breast cancer: a combined approach with vital blue dye lymphatic mapping and radioguided surgery. *Semin Surg Oncol* 1998; 15:272–7.
15. Schneebaum S, Standler J, Cohen M, et al. Gamma probe-guided sentinel node biopsy – optimal timing for injection. *Eur J Surg Oncol* 1998:24:515–9.
16. Koller M, Barsuk D, Zippel D, et al. Sentinel lymph node involve-

ment – a predictor for axillary node status with breast cancer – has the time come? *Eur J Surg Oncol* 1998;24:166–8.
17. Imoto S, Fukukita H, Murakami K, et al. Pilot study on sentinel node biopsy in breast cancer. *J Surg Oncol* 2000;73:130–3.
18. Noguchi M, Tsugawa K, Miwa K, et al. Sentinel lymph node biopsy in breast cancer using blue dye with or without isotope localization. *Breast Cancer* 2000;7:287–96.
19. Motomura K, Inaji H, Komoike Y, et al. Intraoperative sentinel lymph node examination by imprint cytology and frozen sectioning during breast surgery. *Br J Surg* 2000;87:597–601.
20. Weiser MR, Montgomery LL, Susnik B, et al. Is routine intraoperative frozen-section examination of sentinel lymph nodes in breast carcinoma worthwhile? *Ann Surg Oncol* 2000;7:651–5.
21. Veronesi U, Paganelli G, Viale G, et al. Sentinel lymph node biopsy and axillary dissection in breast cancer: results in a large series. *J Natl Cancer Inst* 1999;91:368–73.
22. Moriya T, Manabe T, Irei I, et al. Significance of imprint/scrape cytology in intraoperative consultation of breast diseases. *Pathol Clin Med* 1994;12:719–23. [In Japanese]
23. Rubio IT, Korourian S, Cowan C, et al. Use of touch preps for intraoperative diagnosis of sentinel lymph node metastases in breast cancer. *Ann Surg Oncol* 1998;5:689–94.
24. Ratanawichitrasin A, Biscotti CV, Levy L, et al. Touch imprint cytological analysis of sentinel lymph nodes for detecting axillary metastases in patients with breast cancer. *Br J Surg* 1999;86:1346–8.
25. Henry-Tillman RS, Korourian S, Rubio IT, et al. Intraoperative touch preparation for sentinel lymph node biopsy: a 4-year experience. *Ann Surg Oncol* 2002;9:333–9.
26. Karamlou T, Johnson NM, Chan B, et al. Accuracy of intraoperative touch imprint cytologic analysis of sentinel lymph nodes in breast cancer. *Am J Surg* 2003;185:425–8.
27. Dowlatshahi K, Fan M, Bloom KJ, et al. Occult metastases in the sentinel lymph nodes of patients with early stage breast carcinoma: A preliminary study. *Cancer* 1999;86:990–6.
28. Turner RR, Ollila DW, Stern S, et al. Optimal histopathologic examination of the sentinel lymph node for breast carcinoma staging. *Am J Surg Pathol* 1999;23:263–7.
29. Nahrig J, Richter T, Kowolik J, et al. Comparison of different histopathological methods for the examination of sentinel lymph nodes in breast cancer. *Anticancer Res* 2000;20:2209–12.
30. Torrenga H, Rahusen FD, Meijer S, et al. Sentinel node investigation in breast cancer: detailed analysis of the yield from step sectioning and immunohistochemistry. *J Clin Pathol* 2001;54:550–2.
31. Czerniecki BJ, Scheff AM, Callans LS, et al. Immunohistochemistry with pancytokeratins improves the sensitivity of sentinel lymph node biopsy in patients with breast carcinoma. *Cancer* 1999;85: 1098–103.
32. Noguchi M, Bando E, Tsugawa K, et al. Staging efficacy of breast cancer with sentinel lymphadenectomy. *Breast Cancer Res Treat* 1999; 57:221–9.
33. Pendas S, Dauway E, Cox CE, et al. Sentinel node biopsy and cytokeratin staining for the accurate staging of 478 breast cancer patients. *Am Surg* 1999;65:500–5.
34. Kowolik JH, Kuhn W, Nahrig J, et al. Detection of micrometastases in sentinel lymph nodes of the breast applying monoclonal antibodies AE1/AE3 to pancytokeratins. *Oncol Rep* 2000;7:745–9.
35. Mann BG, Buchanan M, Collins PJ, et al. High incidence of micrometastases in breast cancer sentinel nodes. *Aust NZ J Surg* 2000;70: 786–90.
36. Wong SL, Chao C, Edwards MJ, et al, for the University of Louisville Breast Cancer Study Group. The use of cytokeratin staining in sentinel lymph node biopsy for breast cancer. *Am J Surg* 2001;182: 330–4.
37. Salem AA, Douglas-Jones AG, Sweetland HM, et al. Intraoperative evaluation of axillary sentinel lymph nodes using touch imprint cytology and immunohistochemistry. I. Protocol of rapid immunostaining of touch imprints. *Eur J Surg Oncol* 2003;29:25–8.
38. Freneaux P, Nos C, Vincent-Salomon A, et al. Histological detection of minimal metastatic involvement in axillary sentinel nodes. A rational basis for a sensitive methodology usable in daily practice. *Mod Pathol* 2002;15:641–6.
39. Carter BA, Jensen RA, Simpson JF, et al. Benign transport of breast epithelium into axillary lymph nodes after biopsy. *Am J Clin Pathol* 2000;113:259–65.
40. Page DL, Carter BA. Sentinel lymph nodes, breast cancer, and pseudometastases. *Breast Dis* 2002;12:362–3.
41. Turner RR, Chu KU, Qi K, et al. Pathologic features associated with nonsentinel lymph node metastases in patients with metastatic breast carcinoma in a sentinel lymph node. *Cancer* 2000;89:574–81.
42. Chu KU, Turner RR, Hansen NM, et al. Do all patients with sentinel node metastasis from breast carcinoma need complete axillary node dissection? *Ann Surg* 1999;229:536–41.
43. Liu GJ, Fan ZM, Tang Q, et al. Lymphatic mapping and sentinel lymph node biopsy in the patients with breast cancer. *Chin J Cancer Res* 2000;12:293–5.
44. Turner RR, Ollila DW, Krasne DL, et al. Histopathologic validation of the sentinel lymph node hypothesis for breast carcinoma. *Ann Surg* 1997;226:271–6.
45. Millis RR, Springall R, Lee AH, et al. Occult axillary lymph node metastases are of no prognostic significance in breast cancer. *Br J Cancer* 2002;86:396–401.
46. Lagios MD. Clinical significance of immunohistochemically detectable epithelial cells in sentinel lymph node and bone marrow in breast cancer. *J Surg Oncol* 2003;83:1–4.