

Non-Inferiority of Survival in Early Breast Cancer Patients with False-Negative Sentinel Nodes After Completing Axillary Lymph Node Dissection

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Abstract

Background Sentinel lymph node biopsy (SLNB) is standard approach of axillary region for early breast cancer patients with clinically negative node. The present study investigated patients with false-negative sentinel nodes (FNSN) of intraoperative frozen section.

Methods A case-control study with 1:3 ratio was conducted. FNSN was diagnosed as negative sentinel nodes (SNs) in frozen sections, but positive for metastasis in formalin-fixed paraffin-embedded (FFPE) blocks. Control was defined no metastasis of SNs in both frozen and FFPE sections.

Results Total 20 FNSN cases and 60 matched-controls were enrolled from 333 SLNB patients between April 1, 2005 and November 31, 2009. The demographics and intrinsic subtypes of breast cancer were similar between FNSN and controls. The FNSN patients had a larger tumor size in preoperative mammography and more lymphatic tumor emboli in core biopsy (P -value 0.033 and < 0.001 , respectively). Four FNSN patients had metastasis in the non-relevant SNs. Other 16 FNSN patients had results of benign lymphoid hyperplasia of SNs in frozen sections and metastasis in FFPE blocks. Micrometastasis (less than 2 mm) was detected in seven of 16 patients and metastases in non-sentinel nodes was recognized in two of 16 patients. All FNSN patients received second operation with axillary lymph node dissection (ALND). After a median follow-up of 143 months, no FNSN patients developed recurrence of breast cancer. The disease-free survival, disease-specific survival, and overall survival in FNSN were non-inferior than controls.

Conclusion Outcomes of FNSN patients after completing ALND was non-inferior to those without metastasis in SNs. ALND improved survival of patients with metastasis non-sentinel ALNs. However, omitting ALND had no effect for those have only micrometastasis in SNs.

Background

Breast cancer is the most common malignancy in women at Taiwan [1]. From cross-sectional study, the incidence of breast cancer in Taiwan women reaches a plateau around age 50 years. From longitudinal study, the prevalence of breast cancer is increased at all age groups in recent years [2]. The proportion of intrinsic subtypes in Asian patients with breast cancer is different from Western populations. Higher prevalence of hormone receptor-positive and human epidermal growth factor receptor 2 (HER2)-enriched subtypes is detected in Taiwan and Chinese [3, 4]. Further analysis of epidemiological data, the younger patients have a higher rates of hormone receptor-positive breast cancer than older patients [4, 5]. The different proportion of intrinsic subtypes between Taiwan and Western provides a reasonable theory that the epidemiology and pathophysiology of breast cancer in Taiwan and Western is not entirely the same. Treatment of breast cancer patients should be finely adjusted in different population and studying patients in Taiwan is necessary.

Lymphatic spreading of breast cancer is common and standardized operation with radical mastectomy and axillary lymph node dissection (ALND) is established by Dr. Halsted in 1894 [6]. With development of

adjuvant therapy, numerous patients survive after radical resection. However, these survivors suffer from severe lymphedema over ipsilateral upper limb after ALND [7]. Lower rate of ipsilateral axillary recurrence after ALND is reported. Therefore, the absolute benefit is low and the surgical morbidity is high [8]. The technique of sentinel lymph node biopsy (SLNB) is developed to eliminate number of resected lymph nodes in clinically nodal negative patients [9]. The practice guidelines of SLNB are established and standardized for patients with early-stage breast cancer [10, 11]. The clinical trial, ACOSOG Z0011, is conducted in 889 patients with cT1-2N0 breast cancers and 1 to 2 positive sentinel nodes (SNs) treated by breast-conserving therapy (BCT) with SLNB or ALND. The results show no difference of nodal recurrence, axillary failure, and patients' survival. ALND can be avoided in early breast cancer patients with 1 to 2 positive SNs receiving BCT and replaced by adjuvant whole breast irradiation [12, 13]. The ACOSOG Z0011 trial doesn't enroll patients with large tumor or those received total mastectomy. Furthermore, the indication of ALND in patients undergoing total mastectomy should be considered.

The sensitivity and specificity of SLNB is the first consideration. Localization of SNs by two kinds of indicators is standard procedure. However, the intraoperative evaluation of SNs is diverse. Some institutes use cytological analysis of touch imprint [9] and others accept frozen section of SNs [14]. Intraoperative evaluation of SNs detects metastasis and the operation changes to ALND immediately. The patients can complete surgery in a single stage [14]. Intraoperative frozen section of SNs has a sensitivity of 87% and a specificity of 100%. Estimated 3% patients are recalled for ALND due to a false negative result of frozen section during the first operation [15]. Number of SNs is also a confounding factor of false negative results of SLNB and harvesting only one sentinel node contributes higher rate of axillary recurrence [16]. Otherwise, the long-term clinical outcomes of patients with false-negative sentinel nodes is the second consideration. Kim et al report an increased rate of axillary recurrence with inadequate sampling of SNs. For patients who have micrometastasis (metastatic size greater than 0.2 mm and equal or less than 2 mm) in SNs, the hazard ratio of regional recurrence is 4.39 for those undergoing only SLNB comparing with those receiving ALND [17]. Some researches conduct a clinical study with large number of patients and conclude that the incidence of regional recurrence is very low in breast cancer patient with micrometastasis in SNs [18]. The disease-free survivals in patients with one or more micrometastatic SNs are similar with ALND or no ALND [19]. For patients who have macrometastasis (metastatic size greater than 2 mm) in SNs, the metastatic size predicts metastasis in non-sentinel axillary nodes (ALNs). Avoiding the ALND in the patients with false-negative sentinel nodes will result in underestimation of lymph node status and misleading stage of cancer [20]. In the present study, we investigated the predisposing factor of false-negative sentinel nodes in the clinical node negative patients with breast cancer. The other aim of this study was to evaluate the long-term outcome of patients with false-negative sentinel nodes.

Methods

The present study was designed as a case-control study with case-to-control ratio of 1:3. The patients was diagnosed with invasive ductal carcinoma of breast and recruited between April 1, 2005 and November 31, 2009. All these patients were clinically negative in axillary or internal mammary lymph

node. The surgical planning included SLNB. The operative method for primary breast cancer was decided by the attending surgeon and patients after thorough discussion. The patients with ductal carcinoma in situ, lobular carcinoma in situ, stage T4 or metastatic disease, clinically node-positive disease, inflammatory breast cancer, other variants of carcinoma (phyllodes, sarcoma, or lymphoma), and who had received neoadjuvant chemotherapy, previous axillary surgery, or radiation were excluded. The pathological stage was classified according to the criteria defined by the American Joint Committee on Cancer (AJCC) Staging Manual, 7th edition. The study was reviewed and monitored by Institutional Review Board of National Cheng Kung University Hospital (A-ER-105-233).

All clinical information and pathological report were obtained by retrospective chart review. Dual methods were used to identify SNs. All patients received peritumoral subcutaneous injection with 1 mCi Technetium-99m phytate in 0.4 mL saline (Fujifilm RI Pharma, Chiba, Japan). Series of lymphoscintigraphy was performed in the first 10 min and 60 min after injection. The identified SNs were marked on skin. The area with greatest radioactive signal was identified by a handheld probe of Navigator system (RMD, Watertown, Massachusetts, USA). After anesthesia, total 2 mL methyl blue was injected at 8–10 points around the tumor in subcutaneous layer. Gentle massage was performed from tumor to ipsilateral axillary region to facilitate transmission of methyl blue along lymphatic ducts. Relevant SNs were defined as blue-stained nodes or nodes with *ex vivo* radioactive counts of at least 10% *in situ* counts. If a node without blue-stained nor high radioactive counts, it was defined as non-relevant SNs. The number of relevant SNs sent for frozen section was decided by the attending surgeon.

Detailed procedures of intraoperative frozen section were standardized as following. The SNs more than 4 mm were bisected along their long axis. Half of the nodes was embedded in Optimal Cutting Temperature Compound (OCT) and frozen in liquid nitrogen. Small sentinel lymph nodes (equal or less than 4 mm) were totally embedded in OCT and frozen in liquid nitrogen directly. Four mm-thick sections were cut and stained with hematoxylin and eosin for frozen examination. After preliminary frozen diagnosis, the OCT-embedded tissue of all nodes and the other half of large nodes were fixed in formalin and then embedded in paraffin for permanent sections. Three levels of permanent sections were taken on the formalin-fixed paraffin-embedded (FFPE) tissue blocks. Final diagnosis of sentinel lymph nodes was based on the permanent sections together with the findings of frozen sections.

Case subjects included patients who were diagnosed as negative of lymph node metastasis in frozen examination and turned to be positive of metastasis in permanent sections. The cases were named as those with false-negative frozen section (FNSN). Potential case subjects were identified from registry list at Cancer Center in National Cheng Kung University Hospital. Two chart reviewers examined the registry information. The first reviewer verified the frozen and permanent diagnosis of sentinel lymph nodes. The second reviewer completed all the details of clinical information, imaging studies, pathological reports, and surgical methods.

Three control subjects per case subject was selected from the registry list at Cancer Center in National Cheng Kung University Hospital and matched with the case subjects according to age (within 2 years),

date of operation (within one year), tumor stage, and intrinsic subtypes. For some case subjects, fewer than three controls who met all the matching criteria were chosen. Substitute controls matched with age and date of operation were found, and conformed either tumor stage or intrinsic subtypes.

All patients received preoperative examinations with sonography or mammography. The characteristics of breast tumor was recorded and the report was categorized according to the Breast Imaging Reporting and Data System (BI-RADs) [21]. Preoperative diagnosis of breast cancer was made by cytological study of fine needle aspiration (FNA) or pathological examination of core needle biopsy (CNB) in breast tumor. Some patients received CNB in other hospital and the original pathological report can't be accessed. Those data were coded as missing data.

Difference between case and control groups were compared using a Chi-square test or Fisher's exact test for categorical variables. The continuous variables were analyzed by non-parametric Mann-Whitney test. Survival curves were drawn by Kaplan-Meier method and group difference in survival time was calculated by a log-rank test. The definition of breast cancer-related disease-free survival (DFS) was the time from the date of first operation to the date of first recurrence of breast cancer, or death from any other cause. The patients with breast cancer recurrence were defined as events and those died due to any other cause were clarified as censored. Overall survival (OS) was the time from the date of first operation to the date of death from any cause. Breast cancer-specific survival (BCSS) was the time from the date of first operation to the date of death from breast cancer. The patients died due to other causes were defined as censored while calculating BCSS. The Cox proportional hazard model was applied for hazard ratio and 95% confidence interval (CI). All statistical tests were conducted by SPSS version 17.0 (IBM, SPSS Inc., Chicago, Illinois, USA) and the *P*-values less than 0.05 was defined as statistical significance.

Results

Between April 1, 2005 and November 31, 2009, total 1,810 patients were diagnosed as breast cancer in our hospital. There were 1,525 patients receiving surgical intervention. 333 subjects underwent SLNB. There were 20 patients with invasive ductal carcinoma and false negative results of frozen section for SLNB and those were defined as FNSN group. Matched controls were selected in those with invasive ductal carcinoma and undergoing SLNB with true-negative results of frozen section. Table 1 shows the clinical demographics and tumor characteristics of 20 cases and 60 controls. The diagnosed age, operative methods, size and stage of primary breast cancer, nuclear grade of differentiation, extensive intraductal components, and immunohistochemical staining of hormone receptors and Her2 receptors were similar between FNSN and controls (Table 1). The patients with FNSN had a higher proportion of fascia, skin, or nipple invasion from breast cancer; however, the few numbers of patients could not suppose any assumption.

Table 1

Demographic and tumor characteristics of the subjects with false-negative sentinel nodes (FNSN), and of the controls.

	Control (n = 60)	FNSN (n = 20)	P-value
Age	48 (35–73)	48 (37–71)	0.772
Operative method for breast cancer			0.599
Total mastectomy	22 (37%)	9 (45%)	
Partial mastectomy	38 (63%)	11 (55%)	
Tumor size	1.7 (0.3–3.2)	1.8 (1.0–4.5)	0.331
Tumor stage			> 0.999
T1	43 (72%)	14 (70%)	
T2	17 (28%)	6 (30%)	
Nuclear grade			0.418
Grade I	18 (30%)	3 (15%)	
Grade II	20 (33%)	8 (40%)	
Grade III	22 (37%)	9 (45%)	
Extensive intraductal component	21 (35%)	5 (25%)	0.582
Fascia invasion	0	2 (10%)	0.060
Skin invasion	0	1 (5%)	0.250
Nipple invasion	0	2 (20%)	0.103
Estrogen receptor			0.750
Negative	12 (20%)	3 (15%)	
Positive	48 (80%)	17 (85%)	
Progesterone receptor			0.433
Negative	25 (42%)	6 (30%)	
Positive	35 (58%)	14 (70%)	
HER2/Neu			> 0.999
Negative	52 (87%)	18 (90%)	
Positive	8 (13%)	2 (10%)	

	Control (n = 60)	FNSN (n = 20)	<i>P</i>-value
Intrinsic subtypes			0.761
HR-positive, HER2-negative	43 (72%)	16 (80%)	
HER2-enriched	8 (13%)	2 (10%)	
TNBC	9 (15%)	2 (10%)	
Abbreviations: FNSN, false-negative sentinel nodes; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; IHC, immunohistochemistry; TNBC, triple negative breast cancer.			

Our hospital is a tertiary referral center at South Taiwan. Some patients received preoperative examinations in other hospital and was transferred for further treatment. Information of preoperative evaluation was missing in some referral patients. There were 61 patients receiving sonography and 78 patients getting a diagnostic mammography in our hospital. Tumor characteristics and sizes of primary breast cancer were similar between FNSN and controls (Fig. 1a,b). Sonography failed to detect the tumor in one patient in controls. The reports of sonography and mammography were conformed to the BI-RADs classification system from American College of Radiology [21]. The results of sonography were not different between FNSN and controls (Fig. 1c). The FNSN patients had a larger size of breast tumor in mammography (Fig. 1d). The median of tumor size in FNSN was 2.5 cm with a range of 1.4 to 4.0 cm. The median of tumor size in controls was 2.0 cm with a range of 0.6 to 3.2 cm. The difference of tumor size in two groups was significant with *P*-value 0.033. There were 80% of FNSN and 71% of controls had mass or mass with microcalcifications in preoperative mammography. Microcalcifications were the only abnormal finding in 5% of FNSN and 7% of controls. Suspicious findings with architectural distortion was detected in 7% of controls. Diagnostic mammography was unable to detect abnormalities in 15% of FNSN and 16% of controls (Fig. 1e). The categorical results of BI-RADs were similar in these two groups (Fig. 1f).

There were 36 patients in control and 13 patients in FNSN received FNA of primary breast tumor. The results of FNA revealed benign breast proliferation in three patients and unsatisfactory amount for diagnosis in three patients (Fig. 2a). Gold standard of preoperative diagnosis for breast cancer is pathological examination of CNB. In 2005, there were some surgeons in our hospital performed FNA of primary breast tumor in outpatient clinics. Excisional biopsy was performed under general anesthesia and the specimen was sent for frozen section. If the result proved to be carcinoma, the total mastectomy was performed as planning. Three patients (two in control and one in FNSN) in present study only received FNA preoperatively, then underwent excisional biopsy and total mastectomy because the results of frozen section showed malignancy. Other 77 patients received CNB preoperatively. 16 patients in control and six in FNSN accepted CNB at other hospital and we can't obtain formal pathological report. 42 patients in control and 13 in FNSN received CNB in our hospital. The accumulative size of samples in CNB was equal between two groups (Fig. 2b). The accuracy of CNB was also similar between two groups

(Fig. 2c). Three patients in control and one in FNSN was diagnosed as ductal carcinoma in situ with microinvasion in CNB. Two patients in control was diagnosed as invasive carcinoma without specific types and three as invasive ductal carcinoma with other features. All these patients were confirmed to be invasive ductal carcinoma in the final pathological examinations (Fig. 2c).

The operative procedures for breast cancer were listed in Table 1. All these patients received localization of sentinel lymph nodes by dual localization with Technetium-99m phytate and methyl blue before the first operation. The attending surgeon decided which relevant SNs would be sent for frozen section and every surgeon had specific criteria to define the relevant SNs according to size or radioactivity counts of SNs. Other SNs were preserved in FFPE blocks and included in the final reports. Frozen section of relevant SNs from four patients in FNSN were negative for malignancy in final pathological report of FFPE blocks; however, metastatic foci were detected in the non-relevant sentinel nodes. Other 16 patients in FNSN had results of benign lymphoid hyperplasia in frozen section of SNs and the final pathological report detected metastatic carcinomas in FFPE blocks of SNs. The detailed pathological results of sentinel nodes were listed in Table 2. The correct evaluation of pathological stage was performed after second operation with ALND in FNSN patients. The number of relevant SNs sending for frozen sections or the number of hot spots in lymphoscintigraphy were similar between two groups. However, the patients in FNSN had a greater number of total SNs during first operation because the residual radioactivity over axillary region was higher than control (P -value = 0.072, Table 2). The number resected and positive lymph nodes was higher in FNSN than control because all the FNSN patients received second operation with ALND. The patients in FNSN also had a higher proportion of lymphatic tumor emboli in CNB, and an advanced nodal or AJCC tumor-node-metastasis stage in final reports (Table 2).

Table 2

Pathologic nodal findings from intraoperative and final diagnosis of the subjects with false-negative sentinel nodes (FNSN), and of the controls.

	Control (n = 60)	FNSN (n = 20)	<i>P</i> - value
Frozen section of lymph nodes			< 0.001
Frozen section for relevant SNs and metastasis in non-relevant SNs	0	4 (20%)	
False negative results	0	16 (80%)	
Micrometastasis in SNs		7	
Metastasis in SNs and non-sentinel ALNs		1	
Metastasis only in SNs		6	
Macrometastasis in SNs		9	
Metastasis in SNs and non-sentinel ALNs		1	
Metastasis only in SNs		8	
True negative results	60 (100%)	0	
Lymphatic tumor emboli	10 (17%)	18 (90%)	< 0.001
Number of relevant SNs for frozen section	1 (0–9)	2 (1–4)	0.881
Number of hot spots in lymphoscintigraphy	1 (1–5)	1 (1–4)	0.570
Total number of SNs during 1st operation	3 (1–13)	5.5 (1–17)	0.072
Lymph node invasion			< 0.001
Negative	60 (100%)	0	
Positive	0	20 (100%)	
Resected lymph nodes (including SLNB and ALND)	3 (1–13)	23 (9–39)	< 0.001
Positive lymph nodes (including SLNB and ALND)	0	2 (1–7)	< 0.001
Nodal stage			< 0.001
N0	60 (100%)	0	

	Control (n = 60)	FNSN (n = 20)	<i>P</i>- value
N1	0	17 (85%)	
N2	0	3 (15%)	
AJCC TNM stage			< 0.001
Stage IA	43 (72%)	0	
Stage IIA	17 (28%)	12 (60%)	
Stage IIB	0	5 (25%)	
Stage IIIA	0	3 (15%)	
Abbreviations: AJCC TNM stage, American Joint Committee on Cancer tumor, node, metastasis stage; ALN, axillary lymph node; ALND, axillary lymph node dissection; FNSN, false negative sentinel node; SN, sentinel node; SLNB, sentinel lymph node biopsy.			

The median follow-up time was 143 months for all patients with a range of 55 to 176 months. All patients received standardized adjuvant therapy designed by attending physicians. There were three patients in control developed recurrence during follow-up and all of them received salvage therapy with two breast cancer-related mortality (Table 3). No patients in FNSN developed recurrence. One patient in FNSN died due to lung cancer with multiple metastases. The other patient in FNSN lost follow-up and the cause of her death was unknown. No patients in FNSN groups had breast cancer-related deaths. One patient in control had neutropenic fever and died due to septic shock after adjuvant chemotherapy. Other two patients in control died due to exacerbated second malignancy (hepatocellular carcinoma and ovarian cancer, respectively).

Table 3

Disease-free survival events and number of deaths of the subjects with false-negative sentinel nodes (FNSN), and of the controls.

	Control (n = 60)	FNSN (n = 20)	P-value
Disease-free survival events*	54	18	> 0.999
Breast cancer events	3 (5%)	0	0.569
Lung	2 (3%)	0	> 0.999
Liver	2 (3%)	0	> 0.999
Bone	3 (5%)	0	0.569
Brain	1 (2%)	0	> 0.999
Regional lymph nodes (Axillary and/or internal mammary chains)	1 (2%)	0	> 0.999
Distant lymph nodes	2 (3%)	0	> 0.999
Local recurrence	1 (2%)	0	> 0.999
Non-breast cancer deaths	3 (5%)	2 (10%)	0.594
Other malignancy	2 (3%)	1 (5%)	> 0.999
Sepsis	1 (2%)	0	> 0.999
Unknown	0	1 (5%)	0.250
Breast cancer-related deaths	2 (3%)	0	> 0.999
* Excluding breast cancer-related and non-breast cancer-related events.			

The Kaplan-Meier survival analysis was showed in Fig. 3. The disease-free survival (Fig. 3a), breast cancer-specific survival (Fig. 3b), and overall survival (Fig. 3c) were similar between FNSN and controls. The patients in control tends to have a worse disease-specific and disease-free survival than those in FNSN; however, the difference was slight because of limited number of patients in present study.

Discussion

Standard treatment for the patients with early breast cancer and clinically negative node include total or partial mastectomy and SLNB. In present study, we studied the FNSN of frozen section during first operation. A case-matched-control study was designed with one-to-three-ratio. The patients with FNSN had a larger tumor size in preoperative mammography and increased ratio of lymphatic tumor emboli in core biopsy. The patients with FNSN had more number of SNs during first operation because of the

higher radioactivity over axillary region in Navigator system. Metastasis was detected in non-relevant SNs of 4 FNSN patients and in relevant SNs from FFPE sections of 16 patients which was not found during intraoperative frozen sections. Seven FNSN patients had micrometastasis in SNs. Two FNSN patients had metastasis in SNs and non-sentinel ALNs. All these 20 FNSN patients received secondary operation with ALND. Long-term outcomes of patients with FNSN or controls were similar. There was no obvious difference between two groups in disease-free survival, disease-specific survival, and overall survival. No breast cancer-associated events developed in patients with FNSN.

Intraoperative evaluation of SNs is not recommended by several researches. Some investigators advise intraoperative assessment is reserved for patients with clinical node positive disease or those with aggressive disease after neoadjuvant chemotherapy [22]. Cost-benefit of frozen section is one of the reasons. Other researches still recommend intraoperative assessment of SNs. For clinical node negative breast cancer, intraoperative frozen section has a sensitivity of 87%, specificity of 100%, and a patient recall rate of 3% [15]. Risk factors of FNSN include tumor location, lymphovascular invasion, suspicious node in preoperative study, less than three SNs, invasive lobular carcinoma, and poorly-differentiated cancer [23, 24]. In present study, we excluded patients with invasive lobular carcinoma because the difficult diagnosis by H & E stain in these samples. There were 20 patients with FNSN and invasive ductal carcinoma in 333 SLNB patients. The proportion of FNSN is 6.0% in our hospital. Larger tumor size in preoperative mammography and lymphatic tumor emboli in samples of core biopsy were associated with increased risk of FNSN (Table 2). The patients with FNSN had more number of total SNs during first operation. For a patient with tumor larger than 2.5 cm in preoperative mammography and prolonged operation due to high residual radioactivity over axillary region detected in Navigator system, higher risk of FNSN should be informed to the patient and family (Fig. 1d). However, other risk factors could not be identified in present study because the minimal amount of patients' number (Table 1).

The clinical significance of underestimating nodal staging is discussed in several manuscripts. Anderson *et al* re-evaluate FFPE tissue blocks of SNs and undiagnosed metastases of SNs are discovered in 18% patients with metastasis at ALND or subsequent axillary recurrence. In the patients without any axillary recurrence, 11% patients have undiagnosed metastases in SNs during re-evaluation [25]. Underestimation of lymph node staging is possible in SLNB. In present study, four patients had metastasis in non-relevant SNs but negative in relevant SNs. Failure to identify SNs by lymphoscintigraphy and methyl blue stain was possible. Other 16 FNSN patients had results of benign lymphoid hyperplasia in frozen section of relevant SNs and the final pathological report detected metastatic carcinomas in FFPE blocks of the same nodes. The proportion of micrometastasis in SNs was 44%. The survival rates of FNSN patients undergoing ALND and those without lymph node metastasis were similar (Fig. 3). The long-term recurrence rates were also the same (Table 3). Our results were consistent with other studies. The breast cancer patients with micrometastasis in SNs have similar survival as others without metastasis [12, 19]. For the patients with early breast cancer and 1 or 2 SNs containing metastasis, ten-year overall survival in those treating with SLNB was noninferior to ALND [13]. Axillary radiotherapy is another choice of treatment instead of axillary lymph node dissection [26].

The present study collected patients from 2005 to 2009, before the publication of results in ACOSOG Z0011 trial [12]. This was the reason that ALND was performed for our FNSN patients. The two-step operation with delayed ALND has similar long-term morbidity but with a longer operative time [27, 28]. The number of lymph nodes identified is slightly reduced in delayed ALND patients without clinical significance and the risk of lymphedema is similar between delayed and immediate ALND [29, 30]. The major risk from delayed ALND for FNSN patients comes from perioperative and anesthesia-related distress, especially in elderly patients [31, 32]. In present study, two patients had metastasis in SNs and non-sentinel ALNs. These two patients got benefit from delayed ALND with a good survival. From the results of present study, delayed ALND could be held form the patients with micrometastasis in SNs. However, delayed ALND should be considered for those with macrometastasis in SNs after evaluating risks of secondary operation.

Conclusion

SLNB is standard approach of axillary region for the patients with early breast cancer and clinically negative node. We conducted a one-to-three ratio of case-matched control study. The incidence of false-negative sentinel nodes (FNSN) is 6% in patients undergoing SLNB. Risk factors included a larger tumor size in preoperative mammography and increased ratio of lymphatic tumor emboli in core biopsy. All there 20 FNSN patients received secondary operation with ALND. Our results demonstrated that decreased sensitivity of intraoperative frozen section in micrometastasis of SNs. Four of 20 FNSN patients have metastasis in non-relevant SNs and other two patients have metastasis in non-sentinel ALNs. Long-term survival of patients with FNSN after completing ALND was noninferior to those without metastasis in SNs.

Abbreviation

95% C.I., 95% confidence interval; AJCC TNM stage, American Joint Committee on Cancer tumor, node, metastasis stage; ALN, axillary lymph node; ALND, axillary lymph node dissection; BCSS, breast cancer-specific survival; BI-RADs, breast imaging, reporting and data system; CNB, cord needle biopsy; DCIS, ductal carcinoma in situ; DFS, disease-free survival; FFPE, formalin-fixed paraffin-embedded; FNA, fine needle aspiration; FNSN, false negative sentinel node; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; IDC, invasive ductal carcinoma; IHC, immunohistochemistry; OCT, Optimal Cutting Temperature; OS, overall survival; SN, sentinel node; SLNB, sentinel lymph node biopsy; TNBC, triple negative breast cancer

Declarations

Ethics approval and consent to participate

The study was reviewed and monitored by Institutional Review Board of National Cheng Kung University Hospital (A-ER-105-233).

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

Conception and design: H.P.H. Patient care: K.T.L., Y.P.C. and Y.L.K. Writing first draft of manuscript: Z.J.L. and H.P.H. Editing manuscript: W.P.C. and Y.T.H. Review and revision of the manuscript: H.P.H. and C.C.H. All of the authors have read and approved the final submitted manuscript and they participated in writing and reviewing the manuscript.

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References

1. Cancer Registry Annual Report. 2016, Taiwan. Health Promotion Administration, Ministry of Health and Welfare, Taiwan [accessed 2 Feb, 2020].
2. Sung H, Rosenberg PS, Chen WQ, Hartman M, Lim WY, Chia KS, et al. Female breast cancer incidence among Asian and Western populations: more similar than expected. *J Natl Cancer Inst.* 2015;107:djv107.
3. Su Y, Zheng Y, Zheng W, Gu K, Chen Z, Li G, et al. Distinct distribution and prognostic significance of molecular subtypes of breast cancer in Chinese women: a population-based cohort study. *BMC Cancer.* 2011;11:292.
4. Lin CH, Cheng YC, Chiang CJ, Lu YS, Kuo KT, Huang CS, et al. The emerging epidemic of estrogen-related cancers in young women in a developing Asian country. *Int J Cancer.* 2011;130:2629–37.

5. Lin CH, Liao JY, Lu YS, Huang CS, Lee WC, Kuo KT, et al. Molecular subtypes of breast cancer emerging in young women in Taiwan: evidence for more than just westernization as a reason for the disease in Asia. *Cancer Epidemiol Biomarkers Prev.* 2009;18:1807–14.
6. Ghossain A, Ghossain MA. History of mastectomy before and after Halsted. *J Med Liban.* 2009;57:65–71.
7. Cormier JN, Askew RL, Mungovan KS, Xing Y, Ross MI, Armer JM. Lymphedema beyond breast cancer: a systemic review of cancer-related secondary lymphedema. *Cancer.* 2010;116:5138–49.
8. Sanghani M, Balk EM, Cady B. Impact of axillary lymph node dissection on breast cancer outcome in clinically node negative patients: a systematic review and meta-analysis. *Cancer.* 2009;115:1613–20.
9. Johnson MT, Guidroz JA, Smith BJ, Graham MM, Scott-Conner CE, Sugg SL, et al. A single institutional experience of factors affecting successful identification of sentinel lymph node in breast cancer patients. *Surgery.* 2009;146:671–7.
10. Lyman GH, Temin S, Edge SB, Perkins CL, Weaver DL, Giuliano AE. Sentinel lymph node biopsy for patients with early-stage breast cancer: American Society of Clinical Oncology clinical practice guideline update. *J Clin Oncol.* 2014;32:1365–83.
11. Lyman GH, Somerfield MR, Bosserman LD, Perkins CL, Weaver DL, Giuliano AE. Sentinel lymph node biopsy for patients with early-stage breast cancer: American Society of Clinical Oncology clinical practice guideline update. *J Clin Oncol.* 2017;35:561–4.
12. Morrow M, Van Zee KJ, Patil S, Petruolo O, Mamtani A, Barrio AV, et al. Axillary dissection and nodal irradiation can be avoided for most node-positive Z0011-eligible breast cancers: a prospective validation study of 793 patients. *Ann Surg.* 2017;266:457–62.
13. Giuliano AE, Ballman KV, McCall L, Beitsch PD, Brennan MB, Kelemen PR, et al. Effect of axillary dissection vs. no axillary dissection on 10-year overall survival among women with invasive breast cancer and sentinel node metastasis: the ACOSOG Z0011 (Alliance) randomized clinical trial. *JAMA.* 2017;318:918–26.
14. Ali R, Hanly AM, Naughton P, Castineira CF, Landers R, Cahill RA, et al. Intraoperative frozen section assessment of sentinel lymph nodes in the operative management of women with symptomatic breast cancer. *World J Surg Oncol.* 2008;6:69.
15. Lu Q, Tan EY, Ho B, Teo C, Seah MD, Chen JJ, et al. Achieving breast cancer surgery in a single setting with intraoperative frozen section analysis of the sentinel lymph node. *Clin Breast Cancer.* 2013;13:140–5.
16. Kim MK, Park HS, Kim JY, Kim S, Nam S, Park S, et al. The clinical implication of the number of lymph nodes harvested during sentinel lymph node biopsy and its effects on survival outcome in patients with node-negative breast cancer. *Am J Surg.* 2017;214:726–32.
17. Pepels MJ, de Boer M, Bult P, van Dijck JA, van Deurzen CH, Menke-Pluymers MB, et al. Regional recurrence in breast cancer patients with sentinel node micrometastases and isolated tumor cells. *Ann Surg.* 2012;255:116–21.

18. Pernas S, Gil M, Beni´tez A, Bajen MT, Climent F, Pla MJ, et al. Avoiding axillary treatment in sentinel lymph node micrometastases of breast cancer: a prospective analysis of axillary or distant recurrence. *Ann Surg Oncol*. 2010;17:772–7.
19. Galimberti V, Cole BF, Viale G, Veronesi P, Vicini E, Intra M, et al. Axillary dissection versus no axillary dissection in patients with breast cancer and sentinel-node micrometastases (IBCSG 23 – 01): 10-year follow-up of a randomised, controlled, phase 3 trial. *Lancet Oncol*. 2018;19:1385–93.
20. Mittendorf EA, Hunt KK, Boughey JC, Bassett R, Degnim AC, Harrell R, et al. Incorporation of sentinel lymph node metastasis size into a nomogram predicting nonsentinel lymph node involvement in breast cancer patients with a positive sentinel lymph node. *Ann Surg*. 2012;255:109–15.
21. American College of Radiology. ACR BI-RADS atlas: breast imaging reporting and data system, (5th ed.), Reston, Virginia. (2013).
22. van der Noordaa MEM, Vrancken Peeters MTFD, Rutgers EJT. The intraoperative assessment of sentinel nodes - Standards and controversies. *Breast*. 2017;34:64–9.
23. Wei S, Bleiweiss IJ, Nagi C, Jaffer S. Characteristics of breast carcinoma cases with false-negative sentinel lymph nodes. *Clin Breast Cancer*. 2014;14:280–4.
24. Lee SH, Lee HM, Lee HW, Yang BS, Park JT, Ahn SG, et al. Risk factors for a false-negative result of sentinel node biopsy in patients with clinically node-negative breast cancer. *Cancer Res Treat*. 2018;50:625–33.
25. Andersson Y, Frisell J, Sylvan M, de Boniface J, Bergkvist L. Causes of false-negative sentinel node biopsy in patients with breast cancer. *Br J Surg*. 2013;100:775–83.
26. Donker M, vanTienhoven G, Straver ME, Meijnen P, van de Velde CJ, Mansel RE, et al. Radiotherapy or surgery of the axilla after a positive sentinel node in breast cancer (EORTC 10981–22023 AMAROS): a randomised, multicentre, open-label, phase 3 non-inferiority trial. *Lancet*. 2014;15:1303–10.
27. Olson JA, McCall LM, Beitsch P, Whitworth PW, Reintgen DS, Blumencranz PW, et al. Impact of immediate versus delayed axillary node dissection on surgical outcomes in breast cancer patients with positive sentinel nodes: results from American College of Surgeons Oncology group trials Z0010 and Z0011. *J Clin Oncol*. 2008;26:3530–5.
28. Goyal A, Newcombe RG, Chhabra A, Mansel RE. Morbidity in breast cancer patients with sentinel node metastases undergoing delayed axillary lymph node dissection (ALND) compared with immediate ALND. *Ann Surg Oncol*. 2008;15:262–7.
29. Ballal H, Hunt C, Bharat C, Murray K, Kamyab R, Saunders C. Arm morbidity of axillary dissection with sentinel node biopsy versus delayed axillary dissection. *ANZ J Surg*. 2018;88:917–21.
30. Damgaard OE, Jensen MB, Kroman N, Tvedskov TF. Quantifying the number of lymph nodes identified in one-stage versus two-stage axillary dissection in breast cancer. *Breast*. 2013;22:44–6.
31. Braghiroli KS, Braz JRC, Rocha B, El Dib R, Corrente JE, Braz MG, et al. Perioperative and anesthesia-related cardiac arrests in geriatric patients: a systematic review using meta-regression analysis. *Sci Rep*. 2017;7:2622.

Figures

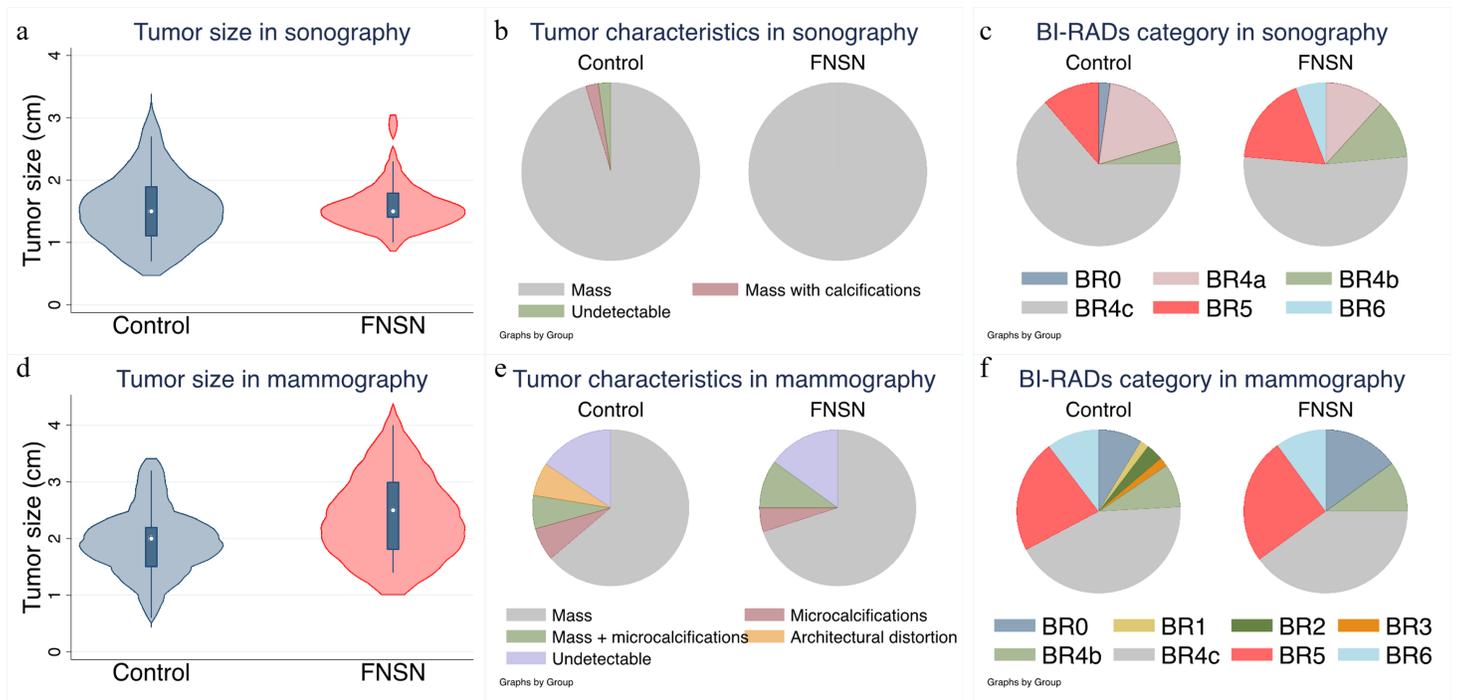


Figure 1

Preoperative imaging studies of the subjects with false-negative sentinel nodes (FNSN), and of the controls. Note: (a) Size of detectable tumors in sonography. (b) Tumor characteristics in sonography. (c) Results of sonography were categorized by Breast Imaging Reporting and Data System (BI-RADs). The tumor failed to identify by sonography and the patients who didn't receive sonography in our hospital were excluded from these three analyses. (d) Size of detectable masses in mammography. Area of microcalcifications and architectural distortion was not included in measurement of tumor size. (e) Characteristics of breast cancer in mammography. (f) Results of mammography were categorized by BI-RADs. The tumor failed to identify by mammography and the patients who didn't receive mammography in our hospital were excluded from these three analyses.

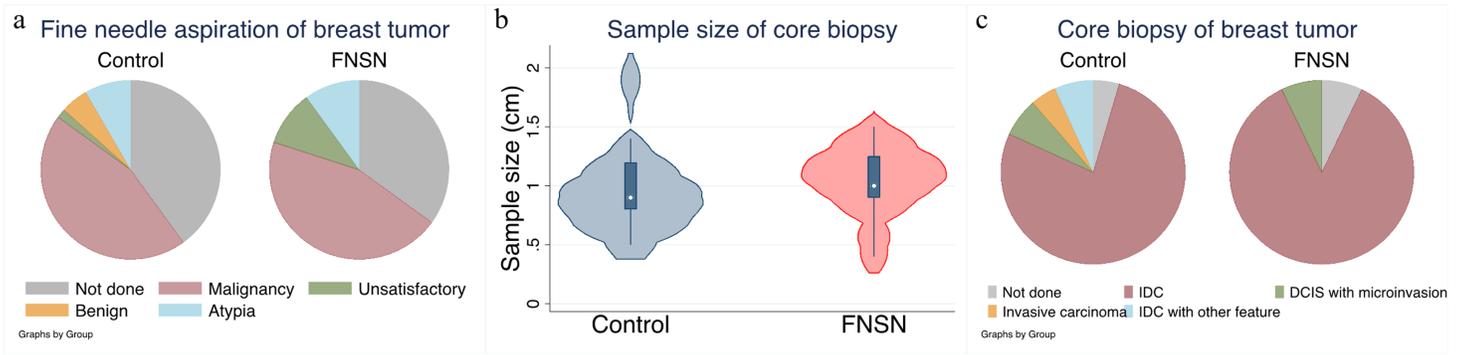


Figure 2

Preoperative diagnosis of the subjects with false-negative sentinel nodes (FNSN), and of the controls. Note: (a) Cytology results of fine needle aspiration from primary breast tumor. (b) Long-axis length of samples from core needle biopsy. (c) Histological results of core needle biopsy from primary breast tumor. The patients who didn't receive tissue-proved examinations in our hospital were excluded from these analyses.

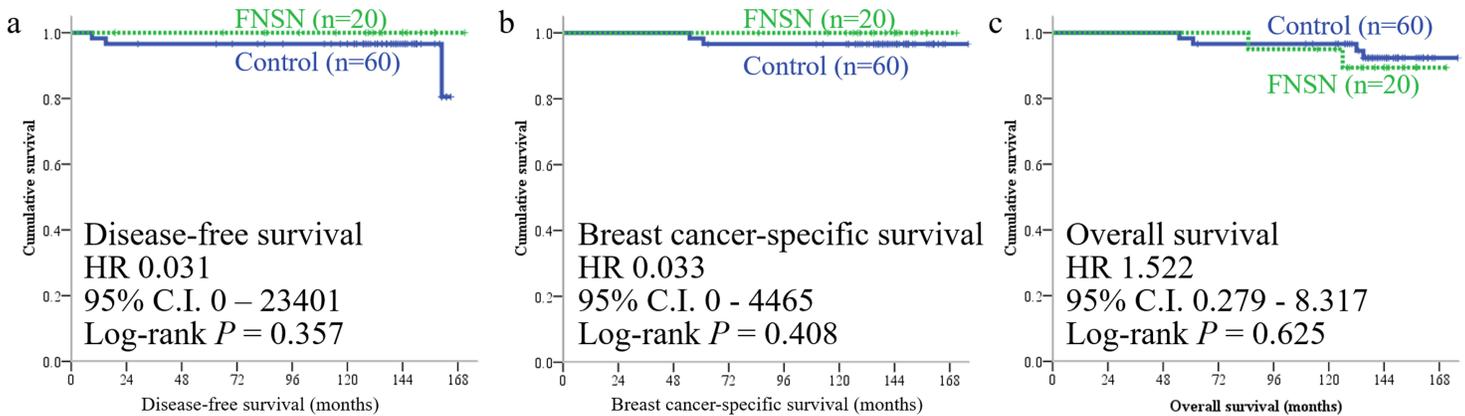


Figure 3

Kaplan-Meier survival analysis of the subjects with false-negative sentinel nodes (FNSN), and of the controls.