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Correlation between the intrinsic subtype and diagnostic methods of axillary lymph node metastasis in primary breast cancer

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Summary

Objectives: To discover the relationship between intrinsic subtypes and axillary lymph nodes metastatic proportion, to compare the accuracy of clinical examination, ultrasound-guided fine-needle aspiration cytology of suspicious axillary nodes, and to evaluate the best method of axillary lymph nodes staging in patients with breast cancer. *Materials and Methods:* From May 2010 to March 2015, patients presenting with primary breast cancer had clinical examination and ultrasonography for axillary lymph node staging. The pathological results of US-guided needle biopsy and clinical data of these patients were collected and analyzed retrospectively. *Results:* A total of 292 (41.8%) of 698 patients confirmed the presence of axillary lymph nodes metastases. Luminal B (HER2-) and HER2 positive tumors had a higher node positivity rate than other intrinsic types. Axillary lymph nodes metastases rate indicates significant difference in each intrinsic subtype. Sixty-eight (11.5%) node-positive patients were identified by ultrasound-fine needle aspiration cytology (BUS–FNAC) and spared unnecessary sentinel lymph node biopsy (SLNB). Fifteen (14.2%) patients who had palpable lymph nodes had negative results in both FNAC and SLNB, and spared unnecessary complete axillary node clearance. Clinical examination had an accuracy of 69.1%, and EBUS-FNAC had 79.4%. *Conclusion:* The intrinsic subtypes are related with axillary lymph node metastasis in primary breast cancer. In comparing with clinical examination, ultrasonography evaluation for axilla status, combined with ultrasound-guided needle biopsy of suspicious axillary node is more accurate, and it is valuable prior to SLNB in primary breast cancer treatment.

Key words: Breast cancer; Intrinsic subtypes; Lymph node; Fine-needle aspiration cytology; prognosis; treatment

Introduction

Breast cancer is increasingly considered a heterogeneous disease. Subtypes can be defined by genetic array testing [1], but they are clinically classified with immunohistochemistry (IHC) [2]. These subtypes have different epidemiological risk factors [3], natural histories [4], and responses to systemic and local therapies [5]. The axillary node stage of patients is another important prognosis factor to primary breast cancer.

Sentinel lymph node biopsy (SLNB) is now the gold standard for axillary node staging of patients with primary breast cancer [6]. Clinical examination is often used for initial evaluation of axillary nodes. However, clinical examination has been found to be of limited use, in determining axillary lymph node status were 23% to 33% margin of error [7]. Axillary ultrasonography has determined axillary lymph node status by alternant axillary lymph node imaging structural, filtered out the abnormal lymph nodes. The accuracy of ultrasonography is higher than clinical examination [8]. Axillary ultrasonography combined with fine-needle aspiration cytology (FNAC) of suspicious nodes has shown promising results [9], and has become the screening method prior to SLNB. This study searched for the relationship between intrinsic subtypes and node positivity rate, and evaluated the role of ultrasound-guided fine-needle aspiration of ultrasonography abnormal axillary lymph nodes in patients with breast cancer.

Materials and Methods

Between May 2010 to March 2015, 698 patients presenting with primary breast cancer had clinical examination and ultrasonography to stage the axilla before treatment, including 696 women and two men, of median age of 51 (range 21–92) years old. All of those patients' stage are $T_{0\text{-}2}N_{0\text{-}1}M_0$ by clinical examination.

Standard of sonographically abnormal lymph nodes: ultrasound machine (high-frequency ultrasound probe, frequency 10-12 MHz) was used for imaging of breasts and axillary nodes. Morphological features used to classify nodes as suspicious included focal eccentric cortical thickening, especially more than 3 mm, and absence of a central fatty hilum. Sonographically abnormal lymph nodes were required to undergo ultrasound-guided needle biopsy via fine-needle aspiration. The FNAC procedure was US-guided with a 20-G needle attached to a 10-ml syringe. Multiple passages of the needle tip through the nodal cortex were made under direct ultrasound vision to sample as much of the nodal cortex as possible. Cytological specimens were processed using Thin-Cytologic Test (TCT). All slides were prepared using the ThinPrep 3000 processor according to the manufacturer's instructions, and were Pap stained. The Bethesda System 2001 terminology was used for all reports. FNAC results were categorized according to

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Table 1. — *Baseline patient and tumor characteristics.*

	N. of patients (n=698)
Age (years)	51 (21-92)
Sex ratio (F:M)	696:2
Axillary lymph nodes	
Clinical examination *	
cN0	592 (84.8%)
cN1	106 (15.2%)
EBUS	
Normal image	487 (69.8%)
Abnormal image	211 (30.2%)
EBUS-FNAC	
pN1	148 (21.2%)
Others**	550 (78.8%)
Final pathology results	
pN0	406 (58.2%)
pN1	292 (41.8%)
Her-2	
HER2 positive	106 (15.2%)
HER2 negative	592 (84.8%)
Intrinsic subtypes	
'Luminal A-like'	269 (38.5%)
'Luminal B-like (HER2 -)'	210 (30.1%)
'Luminal B-like (HER2 +)'	53 (7.6%)
'HER2 positive (non-luminal)'	52 (7.4%)
'Triple negative (ductal)'	114 (16.3%)

** According to clinical medical records*

*** Including two patients whose results were categorized that suspicious cells can be seen (because the results of SLNB were also negative, in the calculation of the data processing by negative).*

Table 2. — *Node positivity rate with Her-2 status.*

	Her-2 negative	Her-2 positive	Total
Lymph node metastases	243 (41%)	49 (46.2%)	292 (41.8%)
Non-lymph node metastases	349 (59.0%)	57 (53.8%)	406 (58.2%)

Chi-square test: $\chi^2 = 3.344$, $p = 0.067$

malignant cells, suspicious cells, or only lymph cells. Sonographically normal lymph nodes and TCT negative lymph nodes underwent SLNB. Final pathology positive result was defined as TCT and SLNB results categorized as malignant cells; the others results were defined as negative. ER, PR, HER-2, and Ki-67 analyses were performed using IHC staining techniques. Tumors with greater than 10% of total tumor cells staining for ER or PR of any intensity were considered positive. Tumors with more than 20% PR-positive cells were considered high PR [10]. The HER-2 staining intensity score was evaluated relative to the provided control slides from 0 to 3+, and specimens staining 3+ were coded as positive and 0 and 1+ were coded as negative. Specimens staining 2+ underwent fluorescent in situ hybridization (FISH) test. Definition and treatment strategies of breast cancer subtype according to the St. Gallen consensus in 2013 [11]. Luminal A: ER+ and PR+, HER2 negative, and Ki-67 low expression (< 20%); Luminal B: Luminal B (HER2-negative) ER+, HER2 negative, and Ki-67 high expression (≥ 20%) or PR negative or low; Luminal B (HER2 positive) ER+, HER2 over-expression or amplified, any Ki-67, any PR; HER2 positive (non-Luminal): ER and PR absent, HER2-over-expression or amplified; Triple-negative: ER and PR absent, HER2-negative. The investigation was conducted according to the Declaration of Helsinki principles, and all participating women provided written and informed consent.

Results

In all 698 patients, 292 patients had confirmed the presence of metastases by pathology before receiving treatments, 148 (50.7%) patients had confirmed metastases by FNAC, and 566 patients underwent SLNB; of these, 144 patients were confirmed the presence of metastases, including 21 patients who had negative results in FNA (Table 1).

As for Her-2 status, it seemed that Her-2 positive patients had higher axillary lymph nodes metastases ratio than that of Her-2 negative patients. However, the statistical difference was not significant (Table 2).

According to the 2013 St. Gallen Surrogate definitions of intrinsic subtypes of breast cancer, a significant difference existed among different intrinsic subtypes in this study according to the Chi-square test (Table 3). The authors observed that luminal B (HER2-) and HER2 positive tumors had a higher node positivity rate than others.

In comparing of the clinical examination and BUS-FNAC evaluation in this report, the sensitivity of clinical examination was only 31.2% and the sensitivity of BUS-FNAC was 50.7%. BUS-FNAC was more sensitive than clinical examination for axillary lymph nodes staging. The specificity of clinical examination was 96.3%. When BUS was combined with ultrasound-guided needle biopsy of suspicious axillary node, the specificity was 100%. The accuracy of the two methods were: clinical examination 69.1% and BUS-FNAC 79.4%. The statistical test results showed that comparing the two methods, the sensitivity, specificity, and accuracy of judgment for axillary lymph node status rates was statistically significant (Table 4).

Five hundred ninety-two patients had no palpable lymph node by clinical examination. Sixty-eight (11.5%) node-positive patients were identified by BUS-FNAC and spared unnecessary SLNB. One hundred six patients had palpable axillary lymph node. However, there were 14.2 % (15/106 patients) that had negative results in both FNAC and SLNB, who were spared unnecessary completion of axillary node clearance.

Discussion

According to the 2013 St. Gallen Surrogate definitions of intrinsic subtypes of breast cancer, treatment strategies are different to subtypes [10] (Table 5). The axillary lymph node staging is another important prognosis factor in primary breast cancer, because it could help determine treatment policy, especially for patients with luminal-like tumor.

Table 3. — *Node positivity rate of different intrinsic subtypes.*

	Luminal A	Luminal B (HER2 -)	Luminal B (HER2 +)	HER2 positive	Triple negative
Lymph node metastases	103	102	22	25	40
Lymph node negative	166	108	31	27	74
Node positivity rate	38.3%	48.6%	41.8%	48.1%	35.1%

Chi-square test: $\chi^2 = 27.132$, $p < 0.0001$

Table 4. — *Outcomes of clinical examination and BUS-FNAC.*

		Lymph nodes metastases		Sensitivity	Specificity	PPV	NPV	Accuracy
		+	-					
Clinical examination	+	91	15	31.2%	96.3%	85.8%	66.0%	69.1%
	-	201	391					
EBUS-FNAC	+	148	0	50.7%	100.0%	100.0%	73.8%	79.4%
	-	144	406					
McNemar test *p*-value*				< 0.001	**< 0.001			< 0.001

* $p < 0.05$ *was considered statistically significant*

** *The specificity of BUS-FNAC is 100%, using the methods of weighting, the weight given 0.01.*

Table 5. — *Definition and treatment strategies of breast cancer subtypes according to the St. Gallen consensus in 2013.*

Subtypes	Definition	Treatment strategies
Luminal A-like	All of: ER and PgR positive HER2 negative Ki-67 <20%	Endocrine therapy is the most critical intervention and is often used alone.
Luminal B-like (HER2 -)	ER positive HER2 negative and at least one of: Ki-67 ≥20% PgR 'negative or low'	Endocrine therapy for all patients, cytotoxic therapy for most.
Luminal B-like (HER2 +)	ER positive HER2 over-expressed or amplified Any Ki-67 Any PgR	Cytotoxics + anti-HER2 + endocrine therapy
HER2 positive (non-luminal)	HER2 over-expressed or amplified ER and PgR absent	Cytotoxics + anti-HER2
Triple negative (ductal)	ER and PgR absent HER2 negative	Cytotoxics

HER2 is a receptor tyrosine kinase that regulates cell growth and differentiation signaling pathways and is significantly overexpressed (up to 100-fold) in ~20% of breast cancers when compared to normal tissues; HER2 expression is highly correlated with amplification of the human HER2-encoding gene ERBB2 [11, 12]. HER2 over-expression is associated with strong activation of the phosphate idylinositol 3-kinase (PI3K) pathway, a major signal transduction conduit that is turned on in many cancer types and stimulates the cell division cycle by activating the protein kinase AKT and downregulating the cyclin-dependent kinase (CDK) inhibitor p27 [13]. HER2 can also activate other pathways such as the mitogen-activated protein kinase (MAPK) pathway via interaction with SHC and GRB2 adaptor proteins [14]. Furthermore, clinical data demonstrate that ERBB2 amplification in breast cancer is associated with worse overall survival compared to patients whose tumors lack ERBB2 amplification [15]. However, in this study, comparing axillary lymph nodes metastases rate, there is not statistical difference with HER-2 status. Lymph node metastases rate indicates significant difference in intrinsic subtypes. Luminal B (HER2-) and HER2 posi-

tive tumors had a higher node positivity rate than others.

SLNB is now the standard method for axillary node staging of patients with primary breast cancer [6]. According to the NCCN Breast cancer practice guidelines 2013 [16], patients should undergo SLNB if lymph nodes are clinically negative. In addition, according to American Joint Committee on Cancer staging manual [17], "clinically apparent" is defined as detected by clinical examination or by imaging studies. However, physical examination is inadequate, which is related to physicians' experience, therefore the accuracy of physical examination is liable, affected by human factors. In this study, clinical examination had a margin of error of 30.9%. These results are similar to those of Mills *et al.*, who reported a margin of error of axillary lymph node status by clinical examination in 23% to 33% [18].

Ultrasound is the most advantageous technique and has been extensively used to characterize lymph nodes and detect axillary lymph node metastases. Ultrasound's result is closely related with physicians' experience, concept, and multiple factor. The result is affected by the resolution and blood flow sensitivity of the machine [19]. Therefore, in this study, nodes' cortex thickness ≥ 3 mm and morphologic criteria was used to formulate the standard of ultrasound diagnosis, which is simple to operate and minimizing the effect of human factors. Ultrasound was combined with FNAC for results that are more accurate. In this study, TCT was performed which could increase the accuracy of cytology.

In the present study, overall 11.5% of patients with breast cancer were spared unnecessary SLNB. These results are similar to those reported by Van Rijk *et al.* [20-23], that unnecessary SLNB was avoided in 8% of patients. Moreover, 14.2% of clinical positive patients were spared unnecessary axillary node clearance, whose complication of axillary node clearance was avoided, and gained better quality of life.

EBUS-FNAC has higher specificity, which signifies that it will have a lower false-positive rate than ultrasound alone. FNAC of axillary lymph nodes can markedly improve the specificity and accuracy of clinical examination and ultrasound alone in detecting metastatic lymph nodes. Compared with clinical examination, ultrasonographic examination of axilla combined with ultrasound-guided needle biopsy of suspicious axillary node is more accurate.

Conclusion

The intrinsic subtypes are related with axillary lymph node metastasis in primary breast cancer. The axillary lymph node metastasis proportion is higher in Luminal B (HER2-) and HER2 positive subtype than others. Compared with clinical examination, ultrasonographic evaluation of axilla, combined with ultrasound-guided needle biopsy for suspicious axillary node is more accurate, and is valuable prior to SLNB in primary breast cancer treatment.

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