The Detection and Analysis of Estrogen and Progesterone Receptors in Breast Cancer (Report of 1,393 Patients)

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OBJECTIVE To explore the distribution of estrogen receptors (ER) and progesterone receptors (PR) in patients with breast cancer and to compare the results with clinical parameters.

METHODS Breast cancer specimens of 1393 cases were stained for the ER and PR by a SP Two-Step method, and analyzed with respect to age, menstrual status, histopathology and metastasis of axillary lymph nodes.

RESULTS The correlation coefficients between ER and PR were positive (P<0.0001). The negative expression of ER in patients 39 years or less was the highest with a statistical significance (P<0.0001). There was no relationship between the patient's age and positive expression of ER, PR and negative expression of PR (P>0.05). There were significantly higher positive rates of ER and lower positive rates of PR in post-menopausal patients than in pre-menopausal cases(P<0.0001). There was no relationship between the status of ER, PR and the corresponding histopathology (P>0.05). The patients with no metastasis in the axillary lymph nodes had higher simultaneous positive rates of ER and PR (P <0.0001), and those with axillary lymph node metastasis had significantly higher rates of negative expression of ER and PR (P<0.0001).

CONCLUSION The positive and negative distributions of ER and PR have some regular patterns which may be used as a reference to choose combined therapy and to predict the prognosis for breast cancer patients.

KEYWORDS: breast cancer, estrogen receptor, progesterone receptor, lymph node metastasis.

The distribution of estrogen and progesterone receptors in patients with breast cancer has been important in choosing the endocrine therapy and predicting the prognosis.[1] It was reported that 60% of patients who were ER positive had a response to endocrine therapy, while, in those patients who were both ER and PR positive, the response increased to 70%~75%.[2] Thus, it is important to explore the relationship between the distribution of endocrine receptors and several clinical parameters. In this study, 1,393 specimens of breast cancer were stained for ER and PR, and compared with age, menstrual status, histopathology and metastasis of axillary lymph nodes in breast cancer patients.

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MATERIALS AND METHODS

Clinical data

All of the 1,393 breast cancer patients were female, whose ages ranged from 19 to 72 years(mean, 52.7). Radical mastectomies were performed in 687 cases and modified radical mastectomies in 583 cases. Mastectomies and resections of axillary lymph nodes were conducted in 115 cases. Quadrantectomy was performed in 8 cases. The clinical data are shown in Table 1.

Table 1. The clinical data for breast cancers

Clinical parameter	Cases	Per cent (%)
Age(years)		
≤39	173	12.4
40~49	578	41.5
50~59	381	27.4
≥60	261	18.7
Menstrual status		
premenopause	535	38.4
postmenopause	575	41.3
unknown*	281	20.3
Histologic type of carcinoma**		
invasive ductal	1,095	78.6
invasive lobular	64	4.6
medullary	96	6.9
bursa hyperplasia carcinoma	50	3.6
mucoid adenocarcinoma	31	2.2
special types***	57	4.1
Status of axillary lymph nodes		
metastasis	612	43.9
non~ metastasis	781	56.1

* Whose uterus was resected before menopause.
** sorted by WHO
*** Including apocrine cancer, squamous cell cancer, sarcoma,carcinoid and scirrhus, etc.

Methods

The samples of breast cancers were fixed in formalin, embedded with paraffin, and sectioned.

In this study, the SP(streptomycin anti-biotin protein -peroxidase linking method) two-step method was used. Main steps: 1) paraffin sections were dewaxed ; 2) treated with 3% H_2O_2 for 10~30 minutes; 3) further treatment (such as trypsin treatment or repair) based on the antibody; 4) added antibodies (ER and PR antibody, concentrations were 1:80), 37℃ or room temperature for 0.5~1 hour, then irrigated with PBS;5) added general IgG antibody (Fab fragment)-HRP polymers, 37℃ or room temperature for 15 minutes, then irrigated with PBS; 6) DAB substrated; 7) irrigated with distilled water, repeated staining, dehydrated and sectioned.

Estimation standard

The cancer cells with brown-yellow granules in the cytoplasm and/or in the nuclus were ER or PR positive cells(Fig1~4). Those tissues whose number of positive cells were ≤ 10% were recorded as negative. In this study, those tissues whose number of positive cells were >10% were scored as positive asing the following designation: 10%~25% (+); 25%~50% (++); >50% (+++).

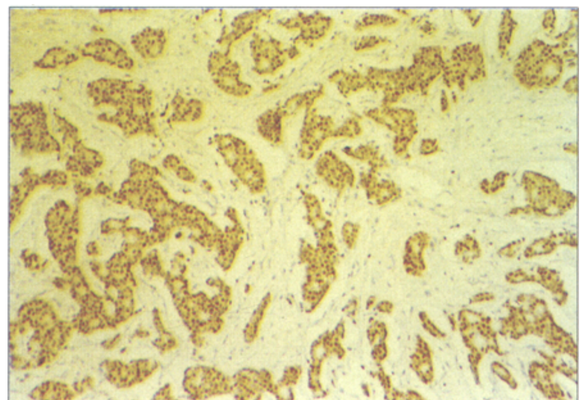

Fig. 1. ER positive breast cancer(+++) SP two step-method × 100.

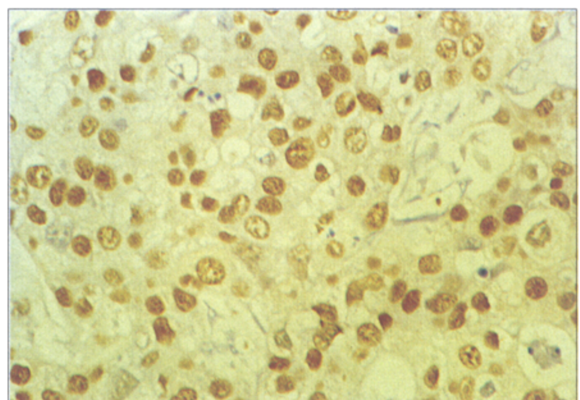

Fig. 2. ER positive breast cancer(+++) SP two-step method × 400.

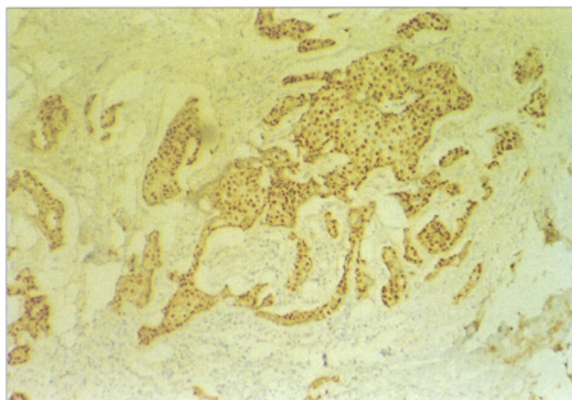

Fig. 3. PR positive breast cancer (+++) SP two-step method× 100.

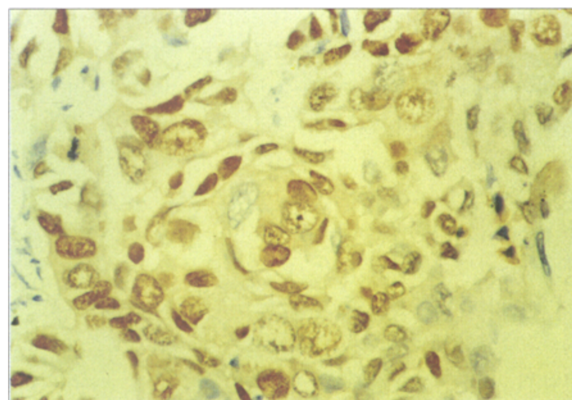

Fig. 4. PR positive breast cancer(+++) SP two-step method× 400.

RESULTS

Relative expression of ER and PR

In 1,393 breast cancer patients, there were 943 with both ER and PR(+), 145 with both ER and PR(-), 208 with ER(+) PR(-), and 97 with ER(-) PR(+). It was found that there ws a positive relationship between ER and PR(χ^2=185.07, P<0.0001).

Age, ER and PR

In this study, all patients were sorted into four groups according to age: ≤ 39,40~49,50~59 and ≥ 60. The largest number (41.5%) were in the 40~49 group. The tissues with greatest ER (-) were in the ≤ 39 group, 33.8% (χ^2=23.9705, P<0.000). There were no differences in all groups with ER(+), PR(+) and PR(-) cases (χ^2=3.6789, χ^2=1.3843, χ^2=3.1378, P>0.05), as shown in Table 2.

Table 2. Relationship of age and status of ER and PR

Age		Distribution of ER and PR(%)			
(Year)	Cases	ER(+)PR(+)	ER(-)PR(-)	ER(+)PR(-)	ER(-)PR(+)
≤39	173	106(61.3)	36(20.8)	12(6.9)	19(11.0)
40~49	578	410(70.9)	49(8.4)	66(11.4)	53(9.2)
50~59	381	258(67.7)	48(12.6)	62(16.3)	13(3.4)
≥60	261	169(64.5)	12(4.6)	68(26.0)	12(4.6)

χ^2=83.8714, P<0.0001

Menstrual status, and ER and PR

Patients in this series were divided into 2 groups: those before menopause as premenopausal, and those 2 years after menopause as postmenopausal. Those having a hysterectomy before menopause for various reasons (281 patients) were excluded from designating menstrual status. In the remaining 1,112 cases, premenopausal and postmenopausal patients were 535 (48.2%) and 577 (51.8%) respectively. The distinct difference in the distribution of ER and PR in the two groups is shown in Table 3.

Table 3. Menstrual status and the distribution of ER and PR in 1,112 patients

		Menstrual status		
ER, PR	Cases	Pre-menopausal	Post-menopausal	P value
ER, PR				
ER(+)PR(+)	299	139	160	
ER(-)PR(-)	324	169	155	
ER(+)PR(-)	242	68	174	
ER(-)PR(+)	247	159	88	<0.0001*
ER				
ER(+)	541	207	334	
ER(-)	571	328	243	<0.0001**
PR				
PR(+)	546	298	248	
PR(-)	566	237	329	<0.0001***

*χ^2=67.4283 **χ^2=40.9386 ***χ^2=17.9721

Histopathology,metastasis of axillary lymph nodes, and ER and PR

In this series, patients with invasive ductal and with invasive lobular cancer were 78.6% and 4.6% respectively with other types less than 7.0%. There were 612 patients with metastasis of axillary lymph nodes and 781 patients without metastasis. The results of this study are displayed in Table 4.

Table 4. Histopathology,metastasis of axillary lymph nodes and ER and PR

	Cases	Distribution of ER and PR				*P* value
		ER(+)PR(+)	ER(+)PR(+)	ER(+)PR(+)	ER(+)PR(+)	
Histopathology						
Invasive ductal	1095	783	109	125	78	
Invasive lobular	64	39	10	11	4	
Medullary	96	42	19	25	10	
Bursa hyperplasia carcinoma	50	42	2	4	2	
Mucoid adenocarcinoma	31	12	4	7	8	
Others	57	14	17	8	18	>0.05*
Status of axillary lymph node						
With metastasis	612	386	74	84	68	
Non-metastasis	781	557	71	124	29	<0.0001**

$^{*}\chi^2$=3.1213 $^{**}\chi^2$=34.4472

DISCUSSION

The breast is one of the target organs for female hormones, where the sex hormone receptors play a very important role.[3-5]

Many researches have shown [1,2,6,7] that the ER and PR are proteins in the cell nucleous or cytoplasm that have a strong affinity for estrogen and progesterone. The estrogen from the circulation combines with the ER, and is transported to the nucleus causing stimulation of protein synthesis and accelerating cell proliferation; however the PR protein might enhance the response of the ER to estrogen. The status of estrogen and progesterone has a very important effect not only on tumor progression but also on therapy and prognosis of breast cancer.[6,7] The detection of ER and PR in breast cancer helps to select the therapeutic regimen and to predict the patient's prognosis.

There have been reports on the diversity of the expression of ER and PR in breast cancer. The distribution of ER and PR in 100 breast cancer patients was reported by Yan et al.[7] as follows: ER(+), PR(+) 66%; ER(-), PR(-) 14%; ER(+), PR(-) 6%; ER(-), PR (+) 14%. In our study, 1,393 cases were examined showing: ER(+), PR(+) 67.7%; ER(+), PR(-) 14.9%; ER (-), PR (-) 10.4%; ER (-), PR (+) 7.0%. The distribution of ER and PR showed markedly positive significance (*P*<0.0001), and positive rates of PR increased with the increase of positive ER.

Some reports have shown that there was no distinct difference between ER and PR based on different age groups.[8-10] Liu et al.[11] reported that the rate of ER(+), PR(+) in a ≤ 39 year group was much higher than in a 40~49 year group, however, there were no differences in other groups. Our results showed obvious differences in all groups (P<0.0001, Table 2). The rate of ER(+), PR(+) in the 40~49 group were higher than in other groups, however, the rates of ER(-), PR(-) or ER(-), PR(+) in the ≤ 39 year group and ER(+), PR(-) in ≥ 60 year group were much higher. The rates of ER (+), PR(+) and PR(-) in all groups showed no distinct differences, however, ER (-) in the ≤ 39 year group was higher than in other groups (*P*<0.0001). It was indicated that the level of ER (-) was higher in young patients, and with increasing age, ER (+) increased gradually, but ER(-) decreased.

Wittliff [6] reported, the positive rate of ER in premenopausal women was 45% (222/488), while in postmenopausal 63% (523/826). Before menopause the estrogen in the cytoplasm might prevent the combination with the site of the ER.

Saez found that the positive rate of ER in female before climacteric was lower than that in postmenopausal women in contrast to the PR. In our 1, 112 patients, the distribution of ER, PR before and after menopause was different. The patients who were ER (-) and PR (-) and ER (-), PR (+) in the pre-climacteric group were more than those who were postmenopausal, however, ER(+), PR(+) and ER(+), PR (-) after menopause were much more in number (*P*<0.0001, Table 3). For rates of ER or PR, ER(+) after menopause was more than premenopausal (*P*<0.0001, Table 3), and the rate of PR(+) was less as reported in the liferatare.[12] But up to now, the reason for the variation of ER and PR in breast cancer before or after menopause was not clear.[13]

In this study invasive ductal cancer was one of the most frequent histologic types of carcinoma.

Statisticly, there were no marked differences among the histologic types of carcinoma and ER, PR ($P>0.05$, Table 4). Both Wittliff [6] and Helin [14] reported that metastasis of lymph nodes was not related to ER, PR ($P>0.05$, Table 4). But Christopher [15] pointed out that alcohol use appears to be more strongly associated with risk of lobular carcinomas and hormone receptor-positive tumors than it is with other types of breast cancer. Liu et al.[11] reported the rate of ER(+), PR (+) in a metastasis group was 43.2% and that the number without metastasis was 37.5%, with no distinct difference. This result showed that the patients without metastasis had a rate of ER(+), PR(+) more than those with metastasis($P<0.0001$, Table 4). The rate of ER(-) and PR (-) of patients with anxillary lymph node matastsis was high, which indicated better prognosis in the early clinical stage. The prognosis of patients with ER(-), PR(-) was worse.

The distribution of ER and PR falls into certain regular patterns, which contributes to the determination of the therapeutic regimen and for the prediction of the prognosis for breast cancer patients.

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