ORIGINAL ARTICLE

Serum HER-2 concentration in patients with primary breast cancer

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Objective: To evaluate whether serum HER-2/neu (HER-2) concentration is a valid index of HER-2 status in women with primary breast cancer, and to establish a normal reference range for serum HER-2 concentration in Korean women.

Methods: Serum HER-2 concentration was measured and immunohistochemistry (IHC) and fluorescence in situ hybridisation (FISH) carried out on tissue samples from 86 consecutive female patients. The results of the three datasets were compared. The cut off value of HER-2 concentration was determined from the mean +2SD values derived from the serum of 93 healthy volunteers.

Results: The IHC and FISH data were significantly correlated ($p < 0.01$), but neither showed significant correlation with the serum HER-2 data. The cut off value of serum HER-2 was 10.2 $\mu\text{g/l}$, and the serum HER-2 concentration of patients with primary breast cancer ranged from 5.0 to 17.5 $\mu\text{g/l}$. Only five patients had a serum HER-2 value above the cut off value.

Conclusions: Serum HER-2 concentration cannot be substituted for IHC or FISH to evaluate HER-2 status, nor can it be used as a diagnostic tumour marker in primary breast cancer, considering the low prevalence of serum HER-2 elevation.

The HER-2/neu (HER-2) proto-oncogene encodes a transmembrane glycoprotein with tyrosine kinase activity that mediates proliferation and differentiation in normal epithelial cells.¹ Approximately 15–30% of invasive breast cancers overexpress HER-2 protein, usually as a result of HER-2 gene amplification.² Amplification or overexpression of HER-2 is associated with a poor prognosis, and its serum concentration may have prognostic value.^{3–5} When a humanised monoclonal anti-HER-2 antibody (trastuzumab) became available as a treatment for patients with metastatic breast cancer,⁶ accurate evaluation of HER-2 status became important not only for its prognostic significance but also for its role in the decision about whether to treat with trastuzumab.

Two methods are used to determine a patient's HER-2 status—immunohistochemistry (IHC), which measures protein expression, and fluorescence in situ hybridisation (FISH), which detects gene amplification. Both show low specificity, and FISH is a costly procedure.^{7–8} Additionally, both methods require high quality tissue samples, which are not always available. We therefore sought an alternative method for evaluating HER-2 status in breast cancer patients.

Here we investigated whether measuring serum HER-2 levels, which have been studied primarily in metastatic breast cancer,^{9–11} would provide such an alternative. We determined HER-2 status by three methods—IHC, FISH, and serum HER-2 measurement—in the same patients, and we also established a normal reference range for serum HER-2 concentration in Korean women.

METHODS

Patients and samples

This study included 86 consecutive patients with primary breast cancer who attended the National Cancer Centre, Korea, from July 2002 to September 2002. None of them had distant metastases at the time of diagnosis. We evaluated clinicopathological features (histology, oestrogen and progesterone receptor status, number of axillary lymph nodes involved, grade, tumour size, and stage according to the

American Joint Committee on Cancer staging system).¹² We obtained specimens for IHC and FISH analysis surgically or by core needle biopsy, and serum samples from blood drawn before surgical treatment. We also collected blood samples from 93 healthy female Korean volunteers to determine normal serum HER-2 levels.

All participants gave informed consent for the use of their tissues in this study, which was approved by institutional review board.

Immunohistochemistry

Paraffin embedded tissue was cut into 4 μm sections and mounted on glass slides. The sections were deparaffinised for 10 minutes in xylene and rehydrated through alcohols to distilled water. Sections were incubated for 15 minutes in 10 mmol/l citrate buffer, pH 6.0, at 98°C. Polyclonal rabbit anti-human c-erbB-2 oncoprotein (code A0485; Dako Corporation, Carpinteria, California, USA) was applied at 1:100 dilutions. The slides were stained in an ES automatic stainer (Ventana Medical Systems), and the degree of staining was scored as recommended for the Dako HercepTest.¹³

Fluorescence in situ hybridisation

Tissue sections from each formalin fixed, paraffin embedded block were baked at 56°C overnight on positively charged slides and deparaffinised. We undertook FISH analysis using a PathVysion HER-2 DNA probe kit (Vysis, Downers Grove, Illinois, USA) according to the manufacturer's recommendations. Briefly, the tissues were treated with a pretreatment solution followed by protease digestion. A hybridisation solution containing directly labelled probes of both SpectrumGreen for the chromosome 17 centromere (CEP) and SpectrumOrange for the HER-2 gene locus was applied, and the denaturation and hybridisation procedures were carried out in a HYBrite system (Vysis). The slides were washed in buffer at 72°C for two minutes and counterstained

Abbreviations: FISH, fluorescence in situ hybridisation; IHC, immunohistochemistry

Table 1 Clinicopathological characteristics and HER-2 status of 86 patients with primary breast cancer

Characteristic	No of patients	HER-2 amplified*	HER-2 non-amplified*	p Value†
Histology				
Ductal	77	19 (86)	58 (91)	0.35
Other	9	3 (14)	6 (9)	
Grade				
1	22	4 (18)	18 (28)	0.72
2	31	8 (36)	23 (36)	
3	25	7 (32)	18 (28)	
Tumour size (mm)				
1–20	53	15 (16)	38 (44)	0.45
21–50	29	7 (8)	22 (26)	
>50	3	0 (0)	4 (5)	
No of axillary nodes involved				
0	43	9 (41)	35 (55)	0.22
1–3	28	9 (41)	19 (30)	
4–9	9	2 (9)	7 (11)	
≥10	5	2 (9)	3 (5)	
Stage				
I	29	6 (27)	22 (34)	0.78
II	43	13 (59)	30 (47)	
III	14	3 (14)	11 (17)	
Oestrogen receptor status				
Negative	31	14 (64)	17 (26.6)	0.002
Positive	54	8 (36)	46 (71.9)	
Progesterone receptor status				
Negative	38	14 (64)	24 (37.5)	0.04
Positive	46	8 (36)	38 (59.4)	

*Values are n (%).

†Calculated by χ^2 test; stage was defined according to American Joint Committee on Cancer staging system.

with DAPI. Two observers assessed each slide using a Nikon Eclipse E600 fluorescence microscope equipped with narrow band pass filters. For each specimen, they scored at least 60 cells for HER-2 and CEP signals. We defined HER-2 gene amplification as a ratio of HER-2 to CEP greater than 2.0.

Serum HER-2 assay

Serum samples were stored at -20°C until used. To measure the serum HER-2 concentration, we used a chemiluminescence immunoassay (ADVIA Centaur system, Bayer Healthcare, Chicago, Illinois, USA). This is a two site sandwich immunoassay using direct chemiluminescent technology. The Lite reagent is composed of the monoclonal mouse antibody TA-1 labelled with acridinium ester. The fluorescein conjugate reagent is composed of the monoclonal mouse antibody NB-3 labelled with fluorescein. These two monoclonal antibodies are specific for unique epitopes on the extracellular domain of HER-2. The solid phase is composed of purified monoclonal mouse capture antibody (antifluorescein) covalently coupled to paramagnetic particles, which binds the immunocomplex. The reaction is initiated and the measured chemiluminescence is directly proportional to the quantity of HER-2 antigen in the sample. Detection sensitivity was $0.5 \mu\text{g/l}$, and the interassay coefficient of variation was 5.9%.

Statistical analysis

For statistical analysis, we used SPSS 10.0 software. We used the χ^2 test and logistic regression analysis to evaluate the relation between HER-2 status and other clinicopathological

Figure 1 Distribution of serum HER-2 concentration in primary breast cancer patients with FISH positive ($n=64$) or FISH negative ($n=22$) tissue, and in controls ($n=93$). The means (SD) were as follows: controls, $7.8 (1.2) \mu\text{g/l}$; FISH negative group, $7.7 (1.9) \mu\text{g/l}$; FISH positive group, $8.1 (2.5) \mu\text{g/l}$. There was no significant difference between the groups ($p=0.64$).

Table 2 Clinicopathological characteristics of five patients with raised serum HER-2 concentrations

Patient No	Age (y)	Serum HER-2 ($\mu\text{g/l}$)	Tumour size (mm)	Grade	Axillary LN (n)	Vascular invasion	Lymphatic invasion	IHC	FISH	OR	PR
1	50	11.5	10	1	1	N	N	2	NA	P	P
2	62	12.2	17	2	1	P	P	2	NA	P	P
3	35	12.7	10	1	0	N	N	1	NA	P	P
4	44	16.7	19	2	22	N	N	1	NA	P	P
5	57	17.5	25	2	4	N	P	2	A	N	N

A, amplification; FISH, fluorescence in situ hybridisation; IHC, immunohistochemistry; LN, lymph node; N, negative; NA, no amplification; OR, oestrogen receptor; P, positive; PR, progesterone receptor; y, years.

variables. We established the normal reference range of serum HER-2 as the mean +2 SD, and we evaluated the association of serum HER-2 concentration with age by correlation analysis. We used the χ^2 test to assess the concordance between FISH, IHC, and serum HER-2 level. We used analysis of variance (ANOVA) to investigate the serum HER-2 level in each group, including the control, the FISH amplified (FISH+), and the FISH non-amplified (FISH-) groups.

RESULTS

The median age of the patients was 44 years (range 29 to 76). Table 1 shows their clinicopathological characteristics. Neither HER-2 overexpression nor raised serum HER-2 level was significantly associated with any of the clinicopathological variables, and HER-2 amplification was significantly associated only with the oestrogen receptor, which was more commonly negative in patients with HER-2 amplification ($p < 0.01$).

The median age of the 93 healthy volunteers was 34 years (range 21 to 66). Their serum HER-2 concentration ranged from 5.5 to 11.0 $\mu\text{g/l}$, and the cut off value was 10.2 $\mu\text{g/l}$. The serum concentration of healthy volunteers was not affected by age ($r = 0.14$). The preoperative serum HER-2 concentrations of the 86 breast cancer patients ranged from 5.0 to 17.5 $\mu\text{g/l}$ with a mean (SD) of 7.8 (2.1) $\mu\text{g/l}$, and the level was above the cut off value in five patients. The clinicopathological characteristics of the five patients with raised serum HER-2 are shown in table 2.

The association between the IHC and FISH results was statistically significant ($p < 0.01$) (table 3), but the serum HER-2 results were not concordant with either the IHC results ($p = 0.59$) or the FISH results ($p = 0.77$), and there was no significant difference between the control, the FISH+, and the FISH- groups ($p = 0.64$) (fig 1).

DISCUSSION

This study showed that IHC and FISH results were closely associated, which is consistent with previous reports.¹⁴⁻¹⁵ In contrast to previous studies, however, elevation of serum HER-2 level in this study was infrequent (6%). Previous studies report that the mean prevalence of increased serum HER-2 concentration in primary breast cancer patients is 18.5%.¹⁶

Table 3 Comparison of HER-2 status determined by fluorescence in situ hybridisation, immunohistochemistry, and serological evaluation

	FISH	
	Not amplified (n = 64)	Amplified (n = 22)
IHC		
0	6	0
1+	21	0
2+	37	7
3+	0	15
Serum HER-2 ($\mu\text{g/l}$)		
≤ 10.2	60	21
> 10.2	4	1

Values are numbers of patients.

The χ^2 test showed a significant association between HER-2 FISH and IHC ($p < 0.01$), but dichotomised serum HER-2 status showed no association with either FISH results ($p = 0.77$) or IHC results ($p = 0.59$).
FISH, fluorescence in situ hybridisation; IHC, immunohistochemistry.

A recent study on primary breast cancer showed a 13.4% positivity rate for serum HER-2 and a significant correlation between raised serum HER-2 and tumour grade by IHC.¹⁷ This is not consistent with our result, which showed no correlation with clinicopathological variables. This finding may be explained by the small number of positive cases of raised serum HER-2 in our study, which may have been insufficient to obtain statistical significance.

Because the serum HER-2 concentration did not show concordance with the IHC or FISH results in this study, it may not be a suitable substitute for IHC or FISH in evaluating a patient's HER-2 status, nor can it be used as a diagnostic tumour marker in primary breast cancer, considering the low prevalence of serum HER-2 elevation in our population. However, there still may be a role for the measurement in metastatic breast cancer because in that condition serum HER-2 concentration is concordant with IHC results and is strongly associated with HER-2 gene amplification.¹⁸

The cut off value of serum HER-2 in present study was lower than in other studies (15 $\mu\text{g/l}$),¹⁹⁻²⁰ which suggests that normal serum HER-2 levels vary between populations. Moreover, the calculated cut off value of the serum HER-2 assay used in our study might be appropriate for Asian women in general; to our knowledge, no value has been established for that population until now. It also suggests that cut off values need to be established for each population.

The strategy for evaluating HER-2 status in breast cancer patients varies among countries.²¹⁻²³ From the results of the present study in our population, we recommend initial testing of tissue samples by IHC, with 0/1+ being regarded as negative and 3+ as positive; subsequent FISH testing should be carried out only on IHC positive cases (2+ or more).²¹ The relation between HER-2 amplification and clinicopathological features showed that oestrogen receptor negative cases were prone to non-amplification, and this finding was consistent with a previous study.²⁴

In conclusion, although measurement of serum HER-2 is easy and relatively inexpensive, and samples are readily obtained, it may not be a useful index of HER-2 status in primary breast cancer patients owing to the lack of correlation with HER-2 gene amplification and HER-2 protein overexpression. The cut off value of serum HER-2 needs to be determined for each population because it can vary between them.

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