Concentration of Serum HER-2/neu as a Prognostic Factor in Locally Advanced Breast Cancer (LABC) and Metastatic Breast Cancer (MBC)


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ABSTRACT

Aim: to determine whether serum HER-2/neu level could be used as a prognostic factor in locally advanced breast cancer (LABC) and metastatic breast cancer (MBC).

Methods: a prospective cohort study was done in LABC and MBC patients in dr. Sardjito Hospital Yogyakarta from April 2006 to March 2008. Serum concentration of HER-2/neu was measured by ELISA done before and after chemotherapy. HER-2/neu expression tissue examination was done by immunohistochemistry. The clinical responses on therapy, survival and progression were recorded.

Results: twenty seven cases were obtained. Average concentration of serum HER-2/neu was 21.02±7.1 ng/ml. The level of serum HER-2/neu in LABC was lower than MBC (17.21 ng/ml vs 28.64 ng/ml; p=0.32). Average concentration of serum HER-2/neu in partial responders was 13.20 ng/ml (95% CI 0.142 – 26.25), stable responders was 19.42 ng/ml (95% CI 0.255 – 39.09) and 29.35 ng/ml (95% CI 1.95 – 56.74) in progressors (p=0.468). Patients with better clinical response had a lower average HER-2/neu serum level (16.12 ng/ml vs 29.35 ng/ml; p=0.247). HER-2/neu over expression was found in 40.7% of the tissues, 44% of LABC and 33.3% of MBC tissues (p=0.692). Negative HER-2/neu tissue protein expression had better clinical response (75% vs 45.5%; p=0.224), and longer survival (p=0.08).

Conclusion: neither the expression of HER-2/neu in the tissue nor the level of serum HER-2/neu can be used as clinical prognosis factor on advanced stage breast cancer in our study population.

Key words: HER-2/neu, locally advanced breast cancer, metastatic breast cancer.

INTRODUCTION

Breast cancer is one of the most common types of cancer found in women visiting our Cancer Unit in Dr. Sardjito Hospital Yogyakarta, Indonesia. Usually patient is in a stadium that is still capable to endure operation (I-IIIA stadium) showing that the awareness towards health and early detection has become better throughout time. Nevertheless, it is occasionally still found in advanced stadium such as in locally advanced breast cancer (LABC) and metastatic breast cancer (MBC).

LABC is a breast cancer with the tumor size above 5 cm accompanied by skin or chest wall infiltration, or spreading of the tumor to the axillar lymph gland of the same side or to the mammaria interna lymph gland entering the stadium III breast cancer category. MBC is a stadium IV tumor with a long distance spreading.

Survival rate for breast cancer patients depends on many factors, i.e the disease’s stadium when diagnosed, hormonal status, histology type and the differentiation degree of the tumor. Therefore, determination of the prognostic factors is very important to be sought for deciding the best therapy approach for patients.

HER-2/neu is one of the most widely investigated gene that is diagnostically valuable for breast cancer prognostics. It is located on the 17q chromosome and decodes a transmembrane tyrosine growth receptor that produces protein receptor on the cell membrane with the molecular weight of 185KD. It is composed of an internal domain cytoplasm with a tyrosine kinase
activity, transmembrane domain, and an extracellular domain (ECD) that can be released from the surface of the breast cancer cells. It is a member of the epidermal growth factor receptor (EGFR) family that will form a heterodimer with the other member namely HER-1, HER-3 and HER-4. This heterodimer will result in the activation of the transduction signal from the membrane to the nucleus to maintain normal cell proliferation. Heterodimerization of HER-2/neu causes an HER-2 protein overexpression that will result in the increase of cell proliferation and an exceed growth in the cell. In breast cancer, HER-2 over expression is found in 30% of the cases and usually shows the fast and aggressive metastatic trait of the tumor.4-12 Various studies show that the released part of the ECD have the molecular weight between 97 and 115 KD and is mentioned as p105.13-15 The released part of the ECD is increased in the serum of metastatic patient.14

Tumor tissue examination to detect the presence of protein over expression (HER-2/neu) can be done on the gene level by Fluorescence In Situ Hybridization (FISH) or on the protein level with the immunohistochemistry (IHC). These two methods are laborious and demand a high skilled expert.16 Other method that can be used is Enzyme Linked Immunoassay (ELISA). This method has been used since 1991 to determine the circulating extracellular domain (ECD) from HER-2/neu in the serum and or plasma. The advantage of the ELISA method is of its non invasiveness that it can be used as a tool to monitor the disease and done serially.17 Data from later research showed that 18.5% from the 1,923 primary breast cancer patients (PBC) and 43% from the 4,622 MBC patients had a circulating HER-2/neu concentration ECD above normal levels.18

We aimed at investigating whether the concentration of the HER-2/neu serum in peripheral blood could be a prognostic factor in the locally advanced breast cancer (LABC) and metastatic breast cancer (MBC) in Indonesia. We also investigated the correlation between HER-2/neu serum concentration with HER-2/neu overexpression in the tissue. Such data have not been reported yet from our Indonesian population to the best of our knowledge.

METHODS

A prospective cohort study was done at Dr. Sardjito Central Hospital from April 2006 to March 2008. The research population are the LABC and MBC patients who are enduring standard therapy.

Inclusion criteria consisted of: 1) patient approval by informed consent; 2) women aged >17 years and <70 years old; 3) diagnosed as LABC and MBC that cannot be managed with surgery or curative radiotherapy; 4) diagnosis conducted histologically; 5) have not been in chemotherapy before; 6) the disease can be measured with the RECIST criteria; 7) a good performance status with WHO ≤2; 8) laboratorium resulted in Hb ≥10 g/dl, ANC >1.5 x 10^9/L, AT >100.000, have an adequate heart function, bilirubin no more than the upper limit normal proportion, SGOT/SGPT no more than 5 x upper limit normal proportion. Exclusion criteria: 1) pregnant; 2) comorbid condition that does not allow the patient to go through chemotherapy; 3) suffer from other cancer.

HER-2/neu expression tissue is examined with mouse monoclonal antibody NCL-13E-356 (Novocastra) by Streptavidin-Biotin method. HER-2/neu level in the serum was examined by EL502 (Oncogene Science/Bayer Health Care LLC) by using Solid Phase Sandwich ELISA according to manufacturer instruction.

Independent variable is a HER-2/neu concentrate serum, dependent variable is a response towards therapy (complete response, partial response, stable disease, progression) and survival (overall survival and progression free survival for 2 years).

Clinical staging by chest rontgenogram and abdominal ultrasound, a routine blood, and blood chemical examination were conducted in LABC and MBC patients who fit the inclusion and exclusion criteria. Clinical evaluation was conducted when the therapy was complete, and followed up every 6 months. The degree of HER-2/neu was correlated with clinical responses and survival. The qualitative degree of HER-2/neu tissue overexpression was compared with the quantitative level of serum HER-2/neu.

Ethical clearance was obtained from Faculty of Medicine Gadjah Mada University. The demographic data are shown descriptively. The difference of the average was analyzed using t-test. The difference of proportion between the two groups was analyzed using chi square test.

RESULTS

Baseline Characteristics

Twenty seven patients met the inclusion criteria. The characteristics of the patients as shown on Table 1 had an average age of 48.85±7.27 years old (38-65 years old). Locally advanced stadium (LABC) was 18 (66.7%) and MBC was 9 (33.3%). The most frequent histology type was ductal infiltrative.
Immunohistochemistry and serology examinations were done in all patients. Twenty six patients (96.29%) received chemotherapy and one patient deceased before chemotherapy was commenced. One patient dropped out (3.7%), median follow up was 9.5 months. Three patients died during this observation study (11.1%) and 24 patients are still alive (88.9%). Three of the patients died of disease progression. The response towards chemotherapy was as follows: progression in 10 (37%) patients, stable disease in 8 patients (29.6%) and partial response in 9 patients (33.3%).

The average HER-2/neu concentration in all patients was 21.02 ng/ml. The average of HER-2/neu level in LABC was lower compared to the MBC (17.21 ng/ml ± 5.9 vs 28.64 ± 11.1 ng/ml; p=0.32). Based on a cut-off point of 15 ng/ml as positive HER-2/neu serum, the proportion of positive HER-2/neu in MBC patients was 44.4% MBC while in LABC was 22.2% (p=0.37).

Survival curve analysis showed no difference in the overall survival (OS) rate between patients with high and low level of HER-2/neu serum, as seen in Figure 3.

**HER-2/neu Tissue Protein Expression by Immunohistochemistry**

HER-2/neu protein expression examination by IHC was conducted by 2 examiners. The first examiner found 11 positive samples (40.7%) and 16 samples (59.3%) negative samples. Second examiner found 9 (36%) positive results and 16 (64%) negative results. Two samples were excluded due to insufficient amount of tissues. The agreement reached by the two examiners was 0.834. Positive HER-2 was found in 8 LABC patients and in 3 MBC patients (Figure 4). There was
no HER-2/neu tissue expression difference based on cancer stadium (p=0.692). When assessed to therapy response, negative HER-2/neu patients showed a better clinical response compared to the positive ones (75% vs 45.5%; p=0.224).

**Equivalence of HER-2/neu Serum and HER-2/neu Tissue Examination**

Seven patients with positive HER-2/neu serum (>15ng/ml) were also positive on the tissue examination with the IHC method. Fifteen negative HER-2/neu serum were also negative for HER-2/neu tissue. The equivalence between HER-2/neu serum with that of tissue was 0.59 (p=0.001).

**DISCUSSION**

This study confirmed previous research that monoclonal antibody with ELISA sandwich is capable to detect the HER-2/neu ECD protooncogen on 20-40% patients with advanced and metastatic breast

| Table 2. The equivalence between HER-2/Neu serum and tissue |
|-----------------|-----------------|----------------|
| Positive Elisa | Negative IHC | IHC +++ (%)  | Negative IHC (%) |
| >15 ng/ml       |                 | 7 (63.6)      | 1 (6.3)          | Kappa 0.59     |
| Negative        |                 | 4 (36.4)      | 15 (93.8)        | p = 0.001      |
| Total patients  |                 | 27            |                  |                |
cancer stadium. Several studies showed HER-2/neu (ECD) serum that can be used as a predictive factor and a disease prognostic factor. Positive HER-2/neu on primary breast cancer is often linked with proliferation at a fast rate so it does not respond to chemotherapy.\textsuperscript{16,20} Our study also showed a tendency of worse therapy response in patients with higher level of HER-2/neu (ECD) serum concentration.

Our data also showed that there was one patient with HER-2/neu serum positive (>15 ng/ml) but negative on tissue (IHC) examination. This point is also appropriate with the study from the Ardavanis et al in 2008 including 95 advanced stadium breast cancer patients with negative tissue HER-2/neu who failed in enduring two chemotherapy line including anthracycline and taxane. After that, those 95 patients had undergone HER-2/neu serologic examination and 22 (23.15\%) among them were positive. Fifteen patients had the docetaxel and trastuzumab and 7 others received the pacitaxel and trastuzumab with the result of 23\% partial response and 50\% stable disease (73\% clinical profit). The research showed that there was a good biochemical and clinical response towards the combined therapy of trastuzumab and taxane on patients with negative HER-2/neu IHC but with positive serum HER-2/neu. This study reported the application of the anti HER-2/neu therapy on advanced breast cancer which showed negative tissue HER-2/neu but positive HER-2/neu ECD.\textsuperscript{21} Based on this study, we recommend the examination of HER-2/neu in the serum for the patient with tissue HER-2/neu negative who failed to endure first line chemotherapy.

We were unable to find the relevancy between HER-2/neu serum concentration with sensitivity towards chemotherapy. This is in accordance with the study by Hayes et al in 2001 that showed HER-2/neu (ECD) to be high in 35-40\% (cut off point >10.5 ng/ml) in MBC patients; however, such high level can not become the predictive marker for hormonal therapy.\textsuperscript{19}

There are a few possibilities for the reason why HER-2/neu serum cannot be a predictive factor for breast cancer LABC and MBC stage. The first possibility is that the positive HER-2/neu cells still have relative sensitivity towards anthracycline so the response rate on the group with HER-2/neu overexpression showed no difference with the non overexpression group. However, the survival of patients with positive HER-2/neu (ECD) serum was worse than the patients with normal HER-2/neu serum receiving anthracycline. This point is concordant with previous studies.\textsuperscript{22-25} The second possibility is that an advanced stadium and metastatic breast cancer might have a different behavior from early stadium cancer in terms of sensitivity towards anthracyclin based on HER-2/neu expression. The correlation between HER-2/neu serum and the use of anthracyclin regimen on metastatic stadium needs to be further investigated.\textsuperscript{19}

CONCLUSION
HER-2/neu (ECD) protooncogene in the serum was found in 29.6\% advanced stage and metastatic breast cancer. The average concentration of HER-2/neu serum was not different between LABC and MBC. The concentrations of HER-2/neu (ECD) serum was not related with the response to anthracyclin based therapy in LABC and MBC. The concentration of HER-2/neu serum only has equivalence with the IHC of 0.59. HER-2/neu ECD examination in the serum cannot yet be recommended as a predictive factor in LABC and MBC.

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