ORIGINAL ARTICLES

# Evaluation of the circulating fraction of the HER-2/*neu* oncogene in patients with cervical cancer

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**Summary**

*Objective*: To evaluate circulating HER-2/neu in cervical cancer patients prior to and following treatment.

*Methods*: Controls, and patients with either cervical dysplasia or cancer taken from an active gynecologic oncology service were evaluated for the expression of HER-2/neu in serum by ELISA before and following surgery, radiation, chemotherapy or combinations thereof. The resulant data was then evaluated for significance by either ANOVA or non-parametric testing.

*Results*: Mean differences were noted for patients with cervical cancer compared to controls. Patients with a good response to the chemotherapy indicated an increase in the serum oncogene, while those not responding either had no marked change or decreased the level of serum HER-2/neu.

*Conclusions*: As serum HER-2/neu is a membrane bound portion of the intact molecule, these results suggest that due to the induction of cell death and breakdown, the liberation of this fraction (increased levels in the serum), is a viable indicator of response to treatment in some patients. A more detailed examination of this possibility along with expanded correlation with tissue expression is required.

*Key words:* HER-2/*neu*; Cervical cancer; Serum.

## Introduction

The HER-2/neu (c-erbB-2) oncogene codes for a 185kD transmembrane tyrosine kinase. Its presence has been reported in numerous tumors of various organ sites, including the cervix. It has been reported that expression is pronounced in some patients with Stage III and IV cervical disease [1]. Tissue levels have been examined by immunohistochemical means (IHC), utilizing the same techniques as for breast cancer. It has been reported that there was no survival difference between positive and negative expression, nor was it of any value as a predictor of recurrence [2]. Yet these authors state that 22% of the patients expressed the oncogene, but that it is a rare occurrence in cervical cancer; this is almost one in four patients. Another technique of evaluating tissue HER-2/neu is fluorescent in situ hybridization (FISH). Utilizing this technique, it was found that 8.7% of invasive cervical cancer tumors expressed the oncogene. This suggested to these authors that this technique was sensitive and feasible to evaluate these patients [3]. Another study found that this type of technique was more sensitive than southern blot analysis, 37% positive as compared to 14% [4]. When this type of evaluation was expanded to early (Stage I and II disease), only 12 of 136 patients were positive, suggesting that amplification is minimal in early cervical cancer [5].

As the tissue expression can only be realistically evaluated once, at the time of surgery, attempts to monitor this oncogene in serum were initiated. Serum levels were found to be elevated in advanced breast, lung, ovarian, prostate and gastric cancer, suggesting a possible use of the serum expression of this oncogene as a tumor marker [6]. This was further supported by expanded studies into gynecologic cancers [7]. Likewise in receptor positive breast cancer patients undergoing hormonal therapy, it was noted that survival was shorter in the patients with elevated HER-2/neu, with a lessened response to treatment [8]. When this study was expanded to 211 advanced breast cancer patients undergoing chemotherapy, it was found that the serum oncogene expression was a good predictive indicator of disease-free survival, but not overall survival. Further, it was a better predictor of recurrence risk than nodal involvement or receptor status [9]. An early study attempted to compare tissue levels to serum levels in ovarian carcinoma patients. Of these, 17 of 45 patients had overexpression of tissue levels by IHC, and five of these patients had elevated serum levels as well. This led to a specificity calculation of 93% correlation [10]. Another similar study found that from 57 ovarian cancer patients, eight overexpressed the oncogene, and four had elevated serum levels, 50%. This suggested that circulating HER-2/neu might act as a diagnostic tool in ovarian cancer [11].

The significance of the circulating form of HER-2/neu relates to its domains. This intact oncogene, (p185), has three specific domains. Intracellular, transmembrane, and the extracellular p105 fraction. It is this p105 fraction that is shed into the circulation and is the basis of the serum assay [12]. Elevated p105 correlated to a poor prognosis in terms of the patient's survival, especially in advanced

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disease. This suggested elevated serum p105 in ovarian cancer patients had a poor clinical outcome [13]. In a recent study [14], serum levels were elevated in 5.5% of benign ovarian masses, 16.7% of borderline tumors, and 38% of malignant ovarian tumors, but serum HER-2/neu was not useful even with corresponding CA125 to differentiate between benign and actual tumor patients. It has been suggested that HER-2/neu oncogenicity in epithelial tumors is related to its ability to act as a receptor for the stromal derived growth factors [15]. In those patients being followed after second-line chemotherapy, no significant response in serum HER-2/neu was found in either survival or treatment response [16]. In tissue of ovarian cancer patients following platin based chemotherapy, expression of the oncogene indicated a significant prognostic value for response to chemotherapy and survival. This suggested that HER-2/neu had a prognostic value after treatment, and low tissue expression in ovarian cancer correlated with a good outcome at second-look [17].

There has been no attempt as of yet to evaluate serum HER-2/neu in those patients with cervical cancer prior to, during, and following treatment, much less comparing tissue and serum levels. To this end, the following study was undertaken.

## Patients and methods.

All patients with either cervical dysplasia (n = 17) or cervical cancer (n = 12), participating in this study were seen at the Southwest Cancer Center of Texas Tech Health Sciences Center, Division of Gynecologic Oncology. Control patient serum (n = 68), was obtained from the Gynecology Clinic from premenopausal women not taking oral contraceptives. All volunteer blood samples were collected following signed informed consent; this is an Institutional Review Board for Human Subject (IRB) approved study. The patients with either dysplasia or cancer all had a biopsy to pathologically verify the type and stage of cervical disease. The 17 patients with dysplasia had a serum sample collected just prior to a cone or LEEP procedure. The 12 patients with verified cervical cancer had a serum sample collected just prior to initiation of treatment, during various time spans during treatment, and at the conclusion of treatment. Five patients with "early" cervical cancer (Stage I) had only a hysterectomy. Treatment of the seven patients with advanced cervical cancer, Stage III or IV, or those with early cervical cancer with associated spread beyond the cervix consisted of standard chemotherapy plus radiation. Chemotherapy was platin-based, 40 mg/M$^2$ given weekly for six cycles (Platinol, Bristol Pharm.). Pelvic radiation consisted of 180 cGy daily for 28 fractions followed by brachytherapy.

*Analysis.* Archival cervical cancer tissue was examined by immunohistochemistry (IHC), for the gene expression of the p185 fraction of the HER-2/neu molecule. The levels of serum HER-2/neu (p105) were determined by ELISA utilizing a kit obtained from Oncogene Science Diagnostics, Cambridge, MA.

*Statistics.* Data was analyzed by several methodologies. When comparing dysplasia, or cervical cancer patients to the controls, the non-parametric Mann-Whitney t-test was used. Patients with cervical cancer were compared before, during and following treatment, which was accomplished utilizing the repeated measures ANOVA. All significance was at the p = 0.05 or greater for two-tailed tests.

## Results

Evaluation of tissue expression of HER-2/neu, either by FISH or IHC, indicated that no patient in this study with cervical cancer had overexpression of this oncogene. However, the serum levels obtained at the same time (just prior to surgery) from these patients were significantly elevated over controls (p = 0.003), with the mean being 9.9 ± 2.2 vs 8.4 ± 1.4 ng/ml. Also, there was no correlation between the tissue and serum levels.

The levels of serum HER-2/neu in the study patients can be seen in Table 1.

Table 1. — *Serum HER-2/neu in controls or patients with either cervical dysplasia or cancer. Mean ± SD, ng/ml.*

| | Controls | Dysplasia | Cervical Cancer |
|---|---|---|---|
| n | 68 | 17 | 36* |
| HER-2/neu | 8.4 | 8.6 | 9.9 |
| | ± | ± | ± |
| | 1.4 | 1.7 | 2.3 |
| p= | | | 0.0001 |

* All samples from patients regardless of time span from treatment include pretreatment, during and after treatment for all 12 patients.

The levels of HER-2/neu in the patients with cervical cancer when compared to controls before, during and after treatment, either surgery or chemoradiation, can be seen in Table 2, where significant differences were noted. However, when the patients were compared by repeated measures of ANOVA against the time span of treatment, no difference was observed (p = 0.2).

Table 2. — *Serum HER-2/neu in patients with cervical cancer prior to surgery or chemoradiation, during treatment (4 weeks) and and following treatment (8 weeks). Mean ± SD, ng/ml.*

| | Controls | Pretreatment | During treatment | Post-treatment |
|---|---|---|---|---|
| n | 68 | 12 | 7 | 7 |
| HER-2/neu | 8.4 | 9.2 | 8.5 | 10.2 |
| | ± | ± | ± | ± |
| | 1.4 | 1.9 | 2.3 | 2.8 |
| p= | | 0.04 | | 0.005 |

When patients with early cervical cancer, Stage I (n = 5, prior to treatment), were compared to the controls, a significant difference was noted (10.6 vs 8.4 ng/ml, p = 0.001). When the pretreatment serum HER-2/neu levels in those patients with Stage III or IV or tumor spread (n = 7) were compared to the controls, no significant difference was as noted (8.3 vs 8.4 ng/ml). Although there was a great deal of variability between these patients' levels (range 6.1-11.2 ng/ml), when the levels of HER-2/neu in the early cervical cancer patients were compared to the

advanced, this difference in the means was significant ($p = 0.04$, 10.6 vs 8.3 ng/ml).

Figure 1 illustrates the changes occurring in a patient with Stage IIIB cervical cancer being treated with chemoradiation. As can be seen, the levels were all elevated compared to the baseline sample, with the highest values at the conclusion of the treatment period. This is even more pronounced in the patient illustrated in Figure 2, who was a Stage IIB also treated with chemoradiation, and followed for an extended period.

Two patients with Stage IB cervical cancer with metastatic involvement to the vagina (Figure 3) and the other with positive lymph nodes (Figure 4), showed a decrease in HER-2/neu following treatment. The patient illustrated in Figure 4 was treated with two cycles of neoadjuvant chemotherapy with no response. She was then surgically evaluated revealing positive lymph nodes and started on chemoradiation. Within one hour of treatment changes were noted in her serum HER-2/neu.

## Discussion

A review of the literature found that tissue overexpression of the HER-2/neu oncogene has occurred in gynecologic adenocarcinomas of the ovary, endometrium, fallopian tubes, and cervix. This overexpression was a poor prognostic factor for survival for advanced gynecologic cancer [7]. This ovarian relationship has been suggested to be of prognostic value in response and survival following chemotherapy, with a low tissue level correlating with a favorable second-look outcome [17]. The presence of this oncogene on the cell membrane of cervical carcinoma cells was noted and its positivity was 42% of the total number of patients, which increased significantly with stage, and this also reflected a worse survival rate for those patients who expressed the oncogene. It was indicated that the survival differences for the patients who received only radiation were related to local recurrence as opposed to distant metastasis [18].

Few papers have addressed the possible role or function of the p105 fraction of HER-2/neu in the blood of patients with gynecologic cancer. Cheung *et al.* [14] described elevated serum levels in 38% of patients with ovarian cancer, which is similar to the expression rate seen in malignant ovarian tissue. They did not however follow these patients after treatment. Nevertheless, serial blood samples were followed after hormonal

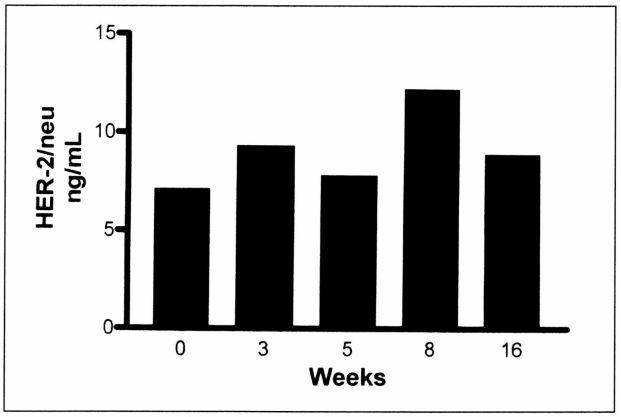


Figure 1. — Serum HER-2/neu in a patient with Stage IIIB cervical cancer prior to and following chemoradiation.

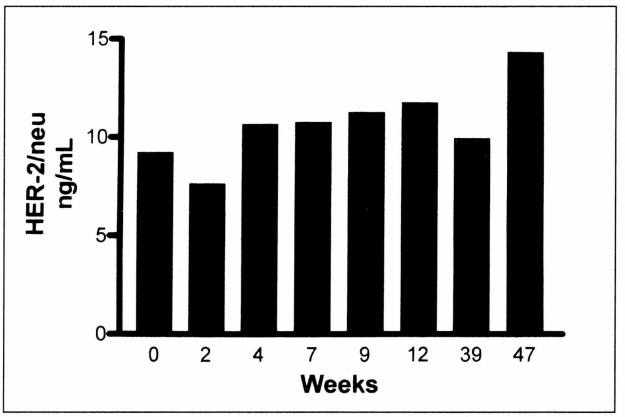


Figure 2. — Serum HER-2/neu in a patient with Stage IIB cervical cancer prior to, during and following chemoradiation.

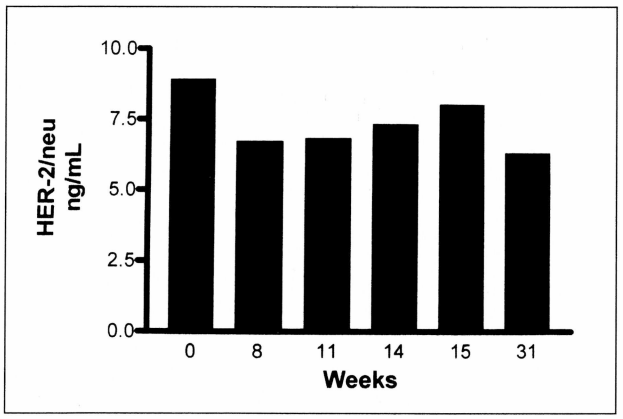


Figure 3. — Serum HER-2/neu in a patient with Stage I cervical cancer with vaginal metastasis receiving chemoradiation.

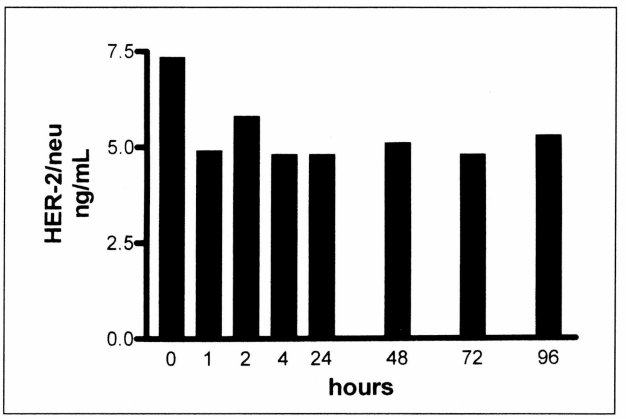


Figure 4. — Serum HER-2/neu a patient with Stage 1 cervical cancer with regional lymph node metastasis during the early time course of chemoradiation.

treatment of patients with breast carcinoma [19]. Of these women, 19% had elevated serum levels prior to treatment, and had varied responses during or after treatment. Of these, 58% were reported to correlate with the clinical outcome. This would tend to suggest a relationship between treatment and serum levels of HER-2/neu, even using only hormonal therapy and not a cytotoxin and/or radiation, which should have a much more pronounced effect not only on the tumor cell, but also on the circulating p105 HER-2/neu.

When we evaluated our patients with cervical cancer, regardless of pre- or post-treatment, we saw a significant increase in the levels of serum HER-2/neu compared to the controls (Table 1). When the cervical cancer patients were evaluated prior to treatment, nine of the 12 had serum HER-2/neu levels greater than the mean of the controls (75%). None of these patients expressed a positive HER-2/neu presence in the tumor tissue by IHC. When we examined the effects of treatment on the expression of serum HER-2/neu, a significant increase was observed over the control values. This would be a favorable response as the p105 fraction being measured in the serum is membrane bound and liberation of this fraction into the blood would suggest disruption of membrane integrity of the tumor cell. We saw no significance between pre- and post-treatment in the seven patients with sampling during and following treatment. This is probably reflective of the small number of patients and the variation between patients (the range was 5.6 to 13.2 ng/ml). However, all patients except for three had a favorable increase in the levels of HER-2/neu after treatment. Of these three, two had metastatic disease and one was a Stage Ia1 who only underwent a hysterectomy. This last case would suggest that apoptosis was not occurring, possibly due to the lack of cytotoxin-induced cell death.

The potential value of monitoring serum HER-2/neu during and after treatment is best illustrated by examining individual patient response as exemplified in figures 1-4. Even though both patients in the first two figures had advanced cervical cancer, their levels of the oncogene increased following treatment, which would indicate apoptosis of the tumor cells following treatment with resulting liberation of the membrane bound p105 fraction. These patients are still in remission of the disease after nine and 13 months respectively. The patients with metastatic disease (Figures 3 and 4), suggest that the response to treatment in terms of HER-2/neu is not as favorable. This would suggest that the treatment modality is either not reaching all of the cancer cells, or that the tumor burden is such that the cells are not being readily destroyed. It also should be pointed out that not all patients express HER-2/neu. From the literature, only 30 to 40% do so, and none of the patients in this study expressed tissue HER-2/neu. It is also interesting that the patient in Figure 4 showed such a rapid decrease in HER-2/neu during treatment, starting within one hour. This overall decrease during treatment is also noted in Table 2.

## Conclusion

The preliminary data from this study suggests that evaluating serum HER-2/neu during and after treatment of patients with cervical cancer might be indicative of those patients who respond to treatment and those who do not. The data suggests that the induction of tumor cell death by chemoradiation liberates the membrane-bound portion of the HER-2/neu oncogene into the circulation and may serve as a marker of response in those patients whose tumor expresses HER-2/neu. The relationship to long-term survival still needs to be validated. In addition, as this was a preliminary examination into a new area of investigation, more patients need to be studied.

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