The Prognostic Value of HER-2/neu Oncogene in Cervical Cancer*

BONIFACE NDUBISI, M.D.,† SARA SANZ, M.D.,‡ LEO LU, M.D.,‡ EDWARD PODCZASKI, M.D.,§ GUY BENRUBI, M.D.,† and SHAHLA MASOOD, M.D.‡

†Department of Obstetrics and Gynecology, §Department of Pathology, University of Florida Health Science Center, Jacksonville, FL 32209 and
§Department of Obstetrics and Gynecology, Pennsylvania State University School of Medicine, Hershey, PA 17033

ABSTRACT

Background
Expression of the HER-2/neu oncogene has been suggested to confer added virulence or aggressive behavior in gynecologic malignancies. The aim of this study is to determine the frequency of HER-2/neu expression in invasive cervical cancer and its impact on survival in women with cervical cancer.

Design
Archival tissue from 150 patients with cervical carcinoma was evaluated immunohistochemically for HER-2/neu oncoprotein expression. Survival information was retrieved retrospectively from patients' medical records.

Results
The HER-2/neu expression was observed in 34 out of 150 tumors (22%). The HER-2/neu positive tumors exhibited considerable heterogeneity in the distribution of immunoreactive tumor cells. Tumor grade and histology did not influence the pattern or intensity of HER-2/neu expression. There was no statistically significant difference in survival of patients with HER-2/neu positive and those with HER-2/neu negative tumors (P = 0.50). Tumor stage at diagnosis was the only covariate with prognostic significance in patient survival (P < 0.001).

Conclusion
Expression of HER-2/neu oncogene is a rare event in cervical cancer. Immunohistochemical detection of HER-2/neu expression is neither a predictor of survival of patients with cervical cancer nor does it identify subgroups of patients at higher risk for recurrence of disease.

* Send reprint requests to: Boniface U. Ndubisi, M.D., Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, University of Florida Health Science Center, 653-1 West Eighth Street, Jacksonville, FL 32209.
THE PROGNOSTIC VALUE OF HER-2/NEU ONCOGENE IN CERVICAL CANCER

Introduction

It has been demonstrated that oncogene amplification or overexpression and inactivation of tumor suppressor genes are frequently observed in human malignancies. The HER-2/neu oncogene is among those documented in cancers of the female genital tract. Several studies have suggested that amplification of the HER-2/neu oncogene in some female genital tract cancers may confer added virulence or aggressiveness in these tumors. Evidence exists that overexpression of HER-2/neu in breast and ovarian cancers may be associated with an adverse survival.1,2,3,4 These studies suggest that tumors with such overexpression behave more aggressively than tumors of similar histologic grade.

The purpose of this retrospective study was twofold: firstly, to determine the frequency of HER-2/neu amplification in invasive cervical cancers treated in our Institution; and, secondly, to determine the impact of this oncogene amplification on patient survival.

Materials and Methods

CLINICAL MATERIAL

Archival surgical specimens of 150 patients with invasive cervical carcinoma treated at the University of Florida Health Science Center/Jacksonville from 1986 to 1992 were retrieved. Tumor stage was assigned at the time of initial diagnosis according to the FIGO Staging System.5 The World Health Organization histological typing was used for histologic classification.6

The specimens include 125 (83.3%) tumors of squamous cell histology, 21 (14%) adenocarcinoma, and 4 (2.7%) adenosquamous carcinomas. There were 82 (54.7%) Stage I, 30 (20%) Stage II, 29 (19.3%) Stage III, and 9 (6%) Stage IV tumors.

Patients' medical records were reviewed retrospectively to obtain pertinent treatment, follow-up, and survival information. Survival was calculated from the date of treatment to the date of demise or last follow-up.

LABORATORY MATERIAL

Immohistochemical staining utilizing monoclonal antibody HER-2/neu oncoprotein was performed on sections of formalin-fixed paraffin-embedded cervical carcinoma specimens from 150 patients. Tissue blocks were selected, and each case included one tissue from the primary cervical tumor and an additional block from metastatic site when available.

Standard avidin-biotin enhanced immunoperoxidase technique was used for the immohistochemical staining. After deparaffinization and rehydration, 4 to 5 micron cut sections were incubated with primary antibody HER-2/neu (mouse monoclonal)* for two hours at room temperature (25°C on the Code-On automated stainer).

Subsequently, a biotinylated antimouse immunoglobulins was applied, followed by incubation with streptavidin-biotin-peroxidase complex.† The peroxidase reaction was developed using 3'3 diaminobenzidine‡. The slides were then counterstained lightly with hematoxylin. To assess the specificity of the reaction, positive (known HER-2/neu positive breast cancer tissue) and negative (primary antibody substituted by PBS) controls were used.

The slides were examined by three pathologists independently. A case was defined as positive when there was definite yellow-brown cell membrane staining (figures 1 and 2). Granular cytoplasmic staining was regarded negative in our study. A percentage of positive staining and intensity of staining reactions were examined on each slide to achieve a score ranging from 0 to 400. Percentage of positive staining range was from 1% to 100% and the intensity of positive staining range was from 1 plus to 4 plus. The product of the percentage of positive staining and intensity of staining was the score for each case assigned.

---

* Triton Biosciences Ab # 100102; Ciba Corning Diagnostics Corp., Alameda, CA.
† LSAB-2 kit, Dako Corp. Carpenteria, CA.
‡ DAB, BioGenex kit #HK153-5K; BioGenex, San Ramon.
FIGURE 1. HER-2/neu membrane staining in squamous cell cervical cancer.
Figure 2. HER-2/neu membrane staining in adenocarcinoma of the cervix.
Actuarial survival for patient groups was calculated using the Kaplan-Meier method.8 Prognostic factors contributing to survival (stage, age, histology, HER-2/neu expression) were assessed using the log-rank method.9,10

Results

The HER-2/neu staining was noted in 34 (22%) of 150 tumors. This membrane staining exhibited a heterogeneous pattern of distribution of positive cells in tumors. In the same tumor, the intensity of staining varied, with strongly positive cells adjacent to cells that were weakly positive.

Semiquantitative scoring of HER-2/neu expression among positive tumors did not show any correlation with stage, histology, grade, or patient age.

The overall five-year survival was 51% and 56% for patients with and without HER-2/neu expression, respectively. This was not statistically significant (P = 0.36), figure 3. The only covariate that was associated with survival was the stage of the disease at diagnosis (P < 0.001), figure 4, and table I.

In summary, expression of HER-2/neu oncogene is a rare event in cervical cancer. Immunohistochemical detection of HER-2/neu oncoprotein expression is neither a predictor of outcome of patients with cervical cancer nor does it identify subgroups of patients at higher risk of recurrence. Additionally, this
The Prognostic Value of HER-2/neu Oncogene in Cervical Cancer

TABLE I
Prognostic Factors Affecting Survival

<table>
<thead>
<tr>
<th>Covariates</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER-2/neu (presence or absence)</td>
<td>0.362</td>
</tr>
<tr>
<td>Stage of disease at diagnosis</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Histology</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Discussion

Immunohistochemistry as a method of analysis was used in our study because it allows direct evaluation of HER-2/neu expression in malignant cells and has been suggested to be the most reliable method of detecting overexpression of HER-2/neu. Berchuck and colleague evaluated 26 patients with squamous cell carcinoma of the cervix and found only light staining for HER-2/neu in 25 of 26 tumors. They concluded that HER-2/neu expression is a rare event in squamous cell carcinoma of the cervix. In our study, immunohistochemical analysis of invasive cervical cancer in 150 patients revealed expression of the HER-2/neu oncogene in 22% of the tumors. Furthermore, the HER-2/neu positive tumors exhibited considerable heterogeneity in the distribution of immunoreactive tumor cells. The reason for this is not clear, but may be attributable to tumor heterogeneity or cell cycle variations in HER-2/neu levels.

No association was demonstrated between HER-2/neu expression and the histomorphologic covariates: histologic type, grade of tumor differentiation, and the stage of the tumor at diagnosis. Similarly, actuarial survival was not related to the presence or absence of HER-2/neu expression. The overall five year survival was 51% for patients with HER-2/neu expression and 56% for those without HER-2/neu expression. This study suggests that assessment of overexpression of HER-2/neu oncogene in cervical cancer does not provide additional prognostic information.

Stage of the disease at diagnosis was the only parameter that influenced patient's survival (P < 0.001). This inverse association between the increasing stage of the disease and a decrement in survival has been consistently demonstrated in the literature.

Acknowledgements

Special thanks are extended to the office of the Dean for the educational/research grant, without which this manuscript would not have been possible.

References