Overexpression of HER2/neu Receptor – Prognostic Factor in Endometrial Cancer

Ilyés L[1], Rădulescu Carmen[1], Grama O[1], Gârbovan O[1], Ádám A[1], Jung J[2], Rădulescu C[1]

[1] Clinic of Obstetrics and Gynecology II, Faculty of Medicine, University of Medicine and Pharmacy, Tîrgu Mureș, Romania
[2] Department of Pathology, University of Medicine and Pharmacy, Tîrgu Mureș, Romania

Objective: The purpose of the study is to assess the incidence of the HER2/neu transmembrane receptor in patients diagnosed with endometrial cancer, and to determine the association of HER2/neu with other negative prognostic factors.
Material and method: We followed the survival rates for 2 and 3 years depending of the presence of HER2/neu, correlated with other prognostic factors of endometrial cancer like the stage of the disease, the histological type and the grade of malignancy.
Results: Out of 42 patients treated, 72.42% were HER2/neu positive. In stage I 65%, in stage II 73.33%, in stage III 100% were positive for HER2/neu. The cases with endometrioid type were positive in 65.62%, with non-endometrioid type in 90%. In well differentiated forms 50%, in moderately differentiated forms 84.21% and in undifferentiated forms 77.77% were HER2/neu positive. The 2 year survival rate was 80% in HER2 positive cases, and 83.33% in negative cases. The 2 year survival rate was 85% in stage I, 80% in stage II, 66.66% in stage III and 92.85% in G1, 89.47% in G2 and 55.55% in G3 forms.
Conclusions: High expression of HER2/neu was present in advanced stages, in non-endometrioid types and in less differentiated forms of endometrial cancer. The stage of the disease and the degree of malignancy are the factors that can influence the long term survival. The presence of HER2 transmembrane receptor seems not to influence the survival rates. More important prognostic predictors are the stage of the disease, the histological type and the grade of malignancy.

Keywords: endometrial cancer, HER2/neu, prognostic factors, survival rates

Introduction

Endometrial cancer is a malignant tumor developing from epithelial and glandular elements of the mucosa. It is the most frequent malignant disease of the female genital tract, representing almost half of the gynecological cancers in the US and Western Europe. It is the 4th cause of death after breast, colon and pulmonary cancer and the 7th cause of death due to women's cancer [1].

Most risk factors of endometrial cancer are associated with long stimulation by estrogen action not antagonized by progesterone, such factors being nuliparity (50% of cancers), late menopause (2–3 times greater risk in women who enter menopause at the age of 53 compared with women who enter menopause at the age of 49), obesity by converting adrenal androstendion in estrone in adipose tissue (8 times higher risk compared with normal body weight levels), polycystic ovary syndrome or functional ovarian tumors and estrogen substitution in postmenopausal treatment.

High expression of the HER2/neu oncogene plays an important role in regulating cellular proliferation and differentiation [2]. The product of the HER2/neu gene (c-erbB2) similar with the EGF2, is a transmembrane receptor protein with a very important role of coordination in complex network erb B, in growth and cellular differentiation [3]. Several studies have shown that in ovarian and breast cancer the increased expression of the gene is an independent negative prognostic factor, with a high treatment resistance and significant decrease in survival, tumors having a great biological aggressiveness [4]. Abnormalities of the expression structure and protein activity synthesized by the protooncogene HER2/neu contribute to the development and maintenance of a malignant phenotype in papillary serous endometrial cancer [5]. It has been demonstrated that amplification of oncogene expression of HER2/neu in papillary serous endometrial cancer can explain the more severe prognosis of this type of cancer in the African-American population from the US [6].

An immunohistochemical avidin-biotin immune-peroxidase method with MAb anti-HER2/neu has shown an increased biological aggressiveness of papillary serous endometrial cancer in black women, but the same aggressiveness could be proved in the Caucasian population. There are studied treatments with Trastuzumab (Herceptin), a HER2/neu antibody, used already in breast and ovarian cancer, in papi-llary serous endometrial cancer and type II endometrial cancer [7].

Long term survival depends on the stage of the disease at the time of treatment, the histological type, the grade of malignancy and the treatment. Different studies show that 5 year survival, depending on the grade of malignancy, is 73–93% in stage I, 55–90% in stage II, 42–72% in stage III, and 18–35% in stage IV.

The purpose of this study is to assess the incidence of transmembrane receptor HER2/neu in patients with endometrial cancer, to determine the association of HER2/neu with other negative prognostic factors, and the relationship between the presence of the receptor and long term survival of patients.

Material and method

We conducted a prospective study, which included all the patients diagnosed and treated with endometrial cancer between January 1, 2004 and December 31, 2008 at the Cli-

Table I. Evaluation criteria for the anti-Her2 antibody reactions

Score	Her 2 expression characteristics
0	Reaction absent or present in <10% of the tumor cells
1+	Weak, interrupted membrane reaction in >10% of the tumor cells
2+	Weak-moderate, continuous membrane reaction, in >10% of the tumor cells intense, continuous membrane reaction in ≤30% of the tumor cells
3+	Intense, continuous membrane reaction in >30% of the tumor cells

nic of Obstetrics and Gynecology no II from Tîrgu Mureş, Romania. In order to follow-up the patients for several years, we created a database containing their personal identification data.

Selection criteria for our cases were the following:

- Patients with endometrial carcinoma, confirmed by clinical symptoms and histopathology results;
- HER2/neu growth factor data;
- Existing documentation at the Population Registration Department of IJP Mureş.

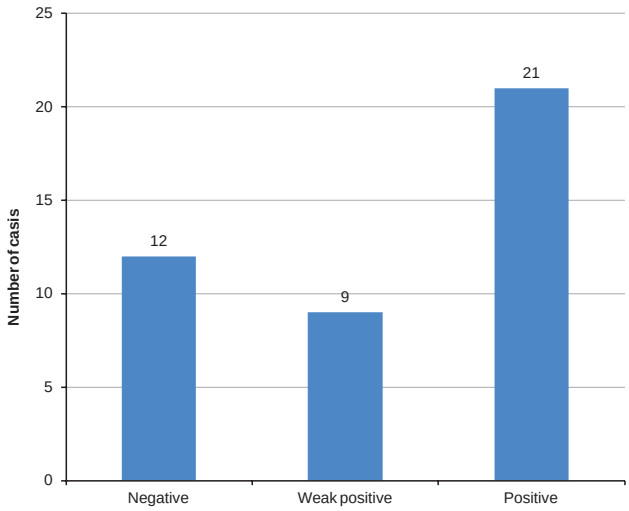

Fig. 1. Distribution of cases based on the presence of HER2/neu receptor

During this period 42 patients were diagnosed with endometrial cancer. Forty-one were operated allowing staging

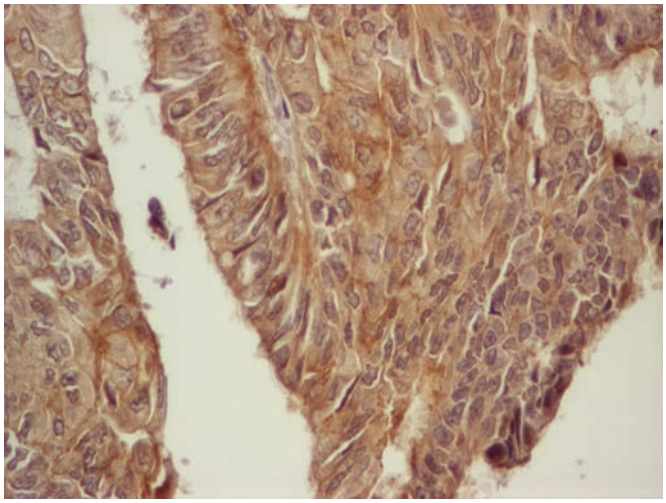

Fig. 2. Endometrial adenocarcinoma with intense HER2/neu expression (+++)

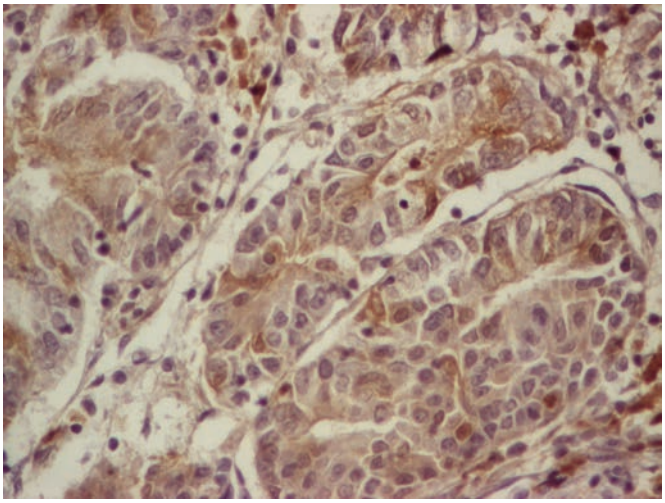

Fig. 3. Endometrial adenocarcinoma with moderate HER2/neu expression (++)

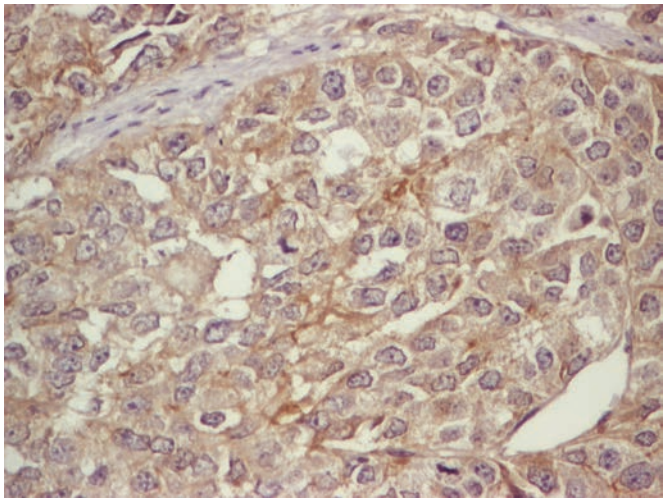

Fig. 4. Endometrial adenocarcinoma with weak HER2/neu expression (+)

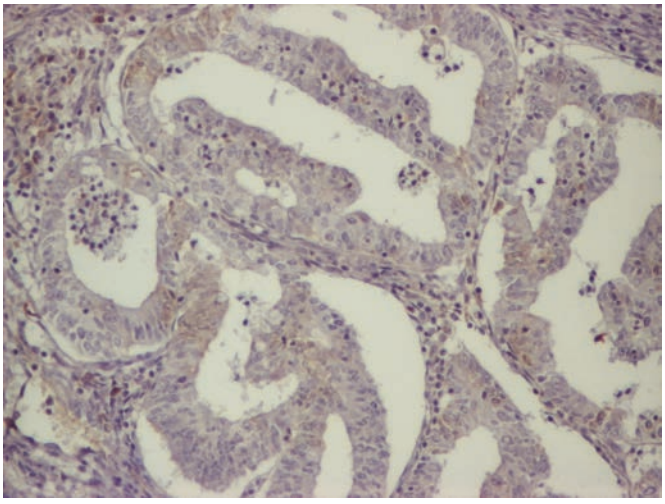

Fig. 5. Endometrial adenocarcinoma negative for HER2/neu expression

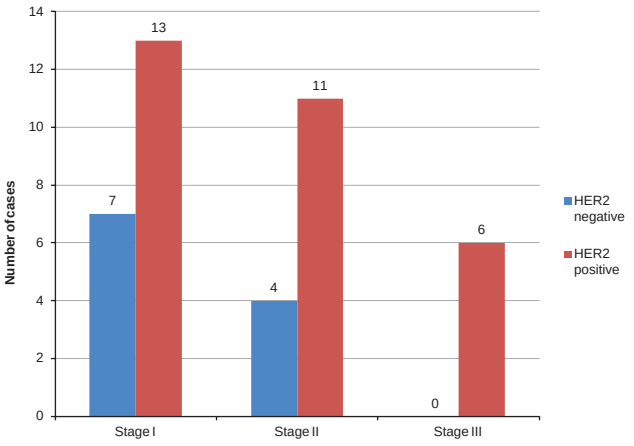

Fig. 6. Frequency of HER2/neu positive cases in different stages of the disease

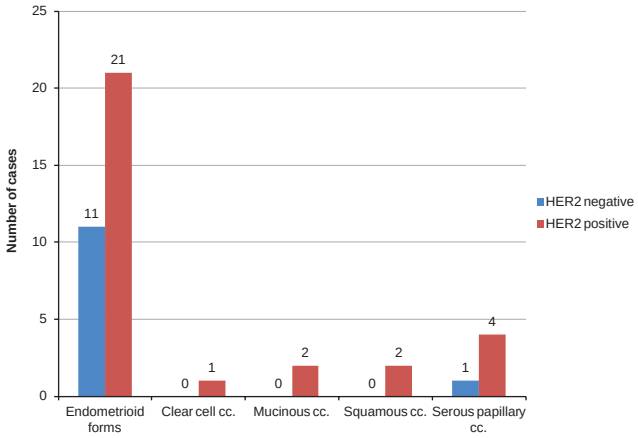

Fig. 7. Frequency of HER2/neu positive cases in different histological types

of the disease. One patient had absolute contraindication against surgery and has been treated with radiotherapy.

The tumor and biopsy materials of all patients underwent histopathogical examination, and immunohistochemistry for detection of HER2/neu. The follow-up lasted 2 and 3 years depending on the presence of HER2/neu, correlated with other prognostic factors of endometrial cancer like the stage of the disease, the histological type and the grade of malignancy.

Immunohistochemical (IHC) reactions have been performed at the Pathology Department of the Clinical County Emergency Hospital. HER2 monoclonal antibodies have been used (DAKO, Denmark), clone PN2A.

Description of the procedure

We used the Ultra Vision (LabVision) system on formalin fixed tissue samples embedded in paraffin. For immunohistochemistry, the sections have been dewaxed, incubated at 100°C in citrate solution at pH 6, and were washed in distilled water. After blocking the endogenous peroxidase the primary antibodies were applied for 60 minutes, then, following a washing cycle with TBS, the biotinylated antibody has been applied (Biotinylated Goat Anti-Polyvalent Solution) for 5 minutes. After another TBS wash, the sections were incubated with streptavidine-peroxidase for another 5 minutes. The reactions were visualized using a DAB (3,3' Diaminobenzidine Dihydrocloride) solution. The nuclei have been stained with Mayer hematoxylin.

Interpretation of IHC reactions

The anti-HER2/neu membrane expression has been quantified based on the criteria presented in Table I.

Evaluation of HER2/neu expression is performed under a microscope, and may be partially subjective. The TMA method (tumor microarray analysis) or proteomics analysis may have a higher accuracy in establishing the intensity of the HER2/neu expression. We considered positive all cases in which HER2/neu was detectable (1+, 2+, 3+), and negative the ones without antibody expression.

The results were analyzed using percent statistics, and we have calculated significance statistical indices like χ^2 (chi square), statistical p and Odds Ratio. A chi >3.84 and a p <0.05 were considered statistically significant.

Results

Out of the 42 patients treated in our hospital 12 (28.57%) were HER2/neu negative, 9 were weak positive (1+) and 21 were intensely positive (2+, 3+) (Fig. 1).

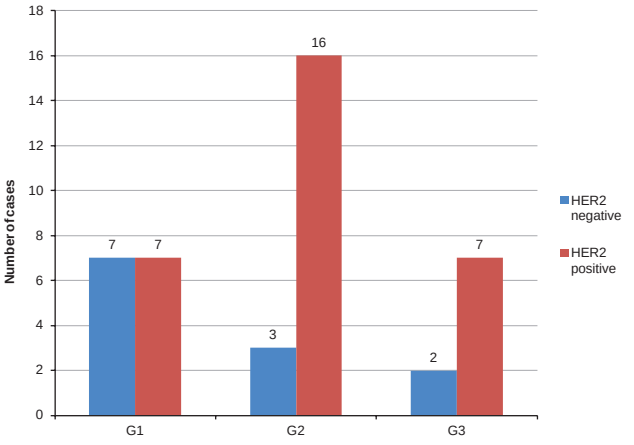

Fig. 8. Frequency of HER2/neu positive cases in different stages of differentiation

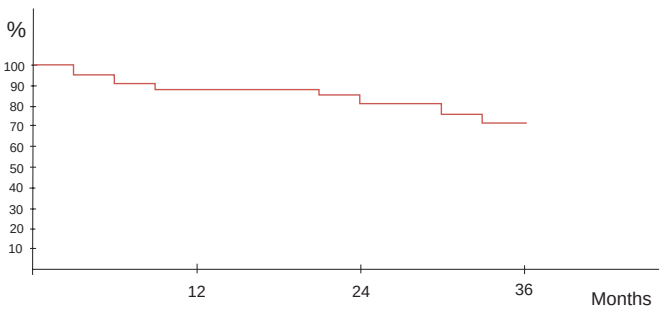

Fig. 9. Global survival of patients

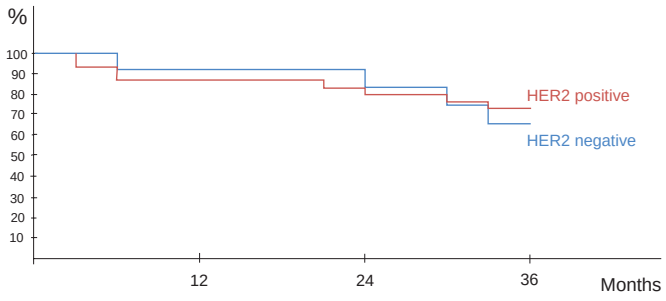

Fig. 10. Patient survival in relation to the presence of HER2/neu

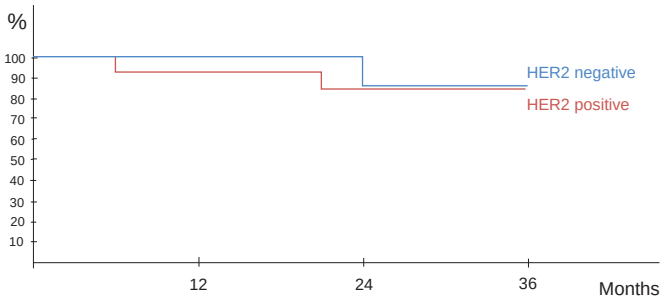

Fig. 11. Survival rates in stage I

Out of the 21 patients with intense HER2/neu positivity, only 5 had well differentiated cancer, the rest being moderately differentiated and non-differentiated endometrioid type, and non-endometrioid cancer.

Figures 2, 3, 4 and 5 show microscopic images of different expression degrees of the HER2/neu growth factor.

The relationship between the stage of disease and the presence of HER2/neu is as follows (Fig. 6):

- in stage I there were 20 patients from which 7 (35%) were HER2/neu negative and 13 (65%) were positive (2 weakly positive and 11 intensely positive cases);
- in stage II there were 15 patients, 4 HER2 negative (26.66%) and 11 positive (73.33%) (4 weakly positive and 7 intensely positive cases);
- all the patients in stage III were positive for HER2, 3 of those being intensely positive;
- there were no stage IV patients in the studied period;
- for the patient that do not underwent surgery the stage of the disease could not be established, but the immunohistochemistry has been performed on biopsy material, and it was positive for HER2.

The increasing expression of the HER2/neu has also showed variations depending on the histological types. In our study, endometrioid forms of endometrial cancer were present in 32 patients (76.19% of cases), 65.62% of them (21 cases) were HER2 positive (14 intensely positive cases). Of the remaining patients with non-endometrioid cancer (10 cases), 9 were positive for HER2/neu (8 intensely positive cases). Non-endometrioid histological types found in our study were: 1 case of clear cell adenocarcinoma, which was positive for HER2/neu, each of 2 cases of squamous cell carcinoma and 2 cases of mucinous carcinoma, were HER2/neu positive and 5 cases of serous papillary carcinoma which 4 (80%) were positive for HER2/neu (Fig. 7).

Concerning the relationship between the degree of malignancy and the presence of HER2/neu we found that (Fig. 8):

- out of 14 cases with well differentiated forms of endometrial cancer, HER2/neu was positive in 7 cases (50%, 5 intensely positive cases);
- nineteen cases had moderate differentiated forms HER2/neu being positive in 16 of the cases (84.21%, 11 intensely positive cases);
- undifferentiated forms were present in 9 cases, 7 cases being positive for HER2/neu (77.77%, 5 intensely positive cases).

Overall survival of our patients with endometrial cancer was 80.95% after 2 years and 71.42% after 3 years (Fig. 9).

In terms of HER2/neu negative patients (12 cases) the survival after 2 years was 83.33% (10 cases) and after 3 years was 66.66% (8 cases). Those 30 HER2 positive cases had a survival of 80% (24 cases) after 2 years and 73.33% after 3 years (22 cases) (Fig. 10).

The 2 and 3 year survival differences between the two patient groups are statistically not significant (at 2 years: $\chi^2 = 0.06$, p = 0.42, OR = 1.25; at 3 years: $\chi^2 = 0.18$, p = 0.33, OR = 0.72).

In the first stage of the disease the survival after 2 and 3 years was 85%. From 7 cases HER2/neu negative, 6 survived 2 or 3 years (85.71%) and from 13 HER2/neu positive patients 11 survived 2–3 years that means 84.6% (Fig. 11).

The 2 and 3 year survival differences between the two patient groups are statistically not significant as well (at 2

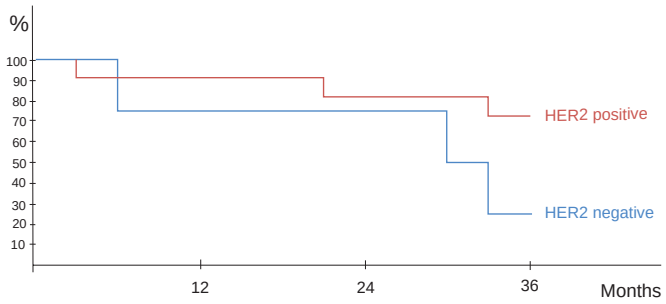

Fig. 12. Survival rates in stage II

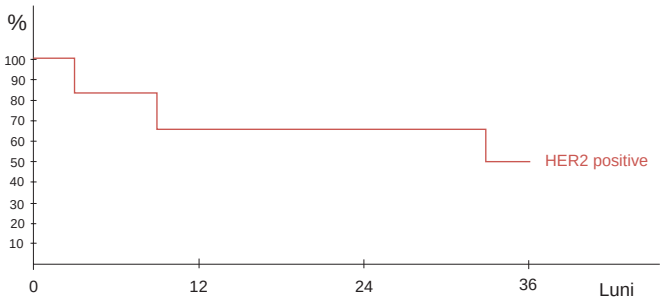

Fig. 13. Survival rates in stage III

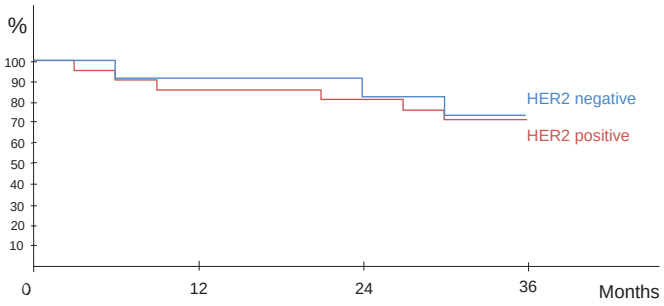

Fig. 14. Survival rates in patients with endometroid type endometrial cancer

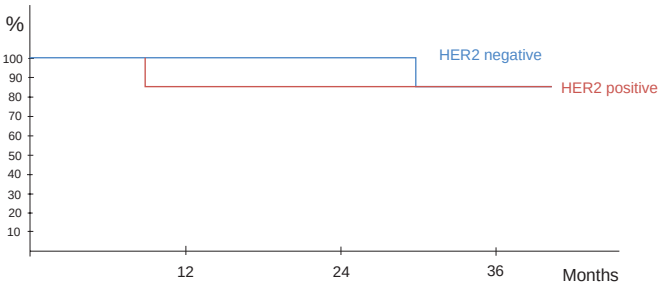

Fig. 15. Survival rates of G1 patients

years: $\chi^2 = 0.004$, p = 0.49, OR = 0.9).

In the second stage of the disease 80% of the patients survived for 2 years, 60% for 3 years. Four patients were HER2 negative, 3 survived for 2 years (75%) and just only one survived more than 3 years (25%). For HER2/neu positive patients (11 cases) the survival was 81.81% (9 cases) at 2 years, and 72.77% (8 cases) at 3 years. (Fig. 12).

The lower survival rates in HER2/neu negative patients with stage II disease may be explained by the small number of cases in this group.

All the patients (6 cases) with stage III endometrial carcinoma were HER2/neu positive and only 4 survived at 2 years (66.66%) and 3 at 3 years (50%) (Fig. 13).

The patient who could not be operated received radiotherapy and she was still alive after 4 years.

The survival rate of patients with endometrial cancer of the endometrioid type and with positive HER2/neu was 80.95% at 2 years and 71.42% at 3 years, and that of the patients with negative HER2/neu was 81.81% at 2 years and 72.72% at 3 years (Fig. 14).

There is no statistically significant difference in the survival rate between HER2/neu positive and negative cases both at 2 and at 3 years (p >0.05).

The survival at 2 and 3 years depending on the grade of malignancy is as follows: from all the patients with well differentiated forms (G1) the survival after 2 years was 92.85% and after 3 years was 85.71%. In case of the HER2/neu negative cases (7 cases) the survival at 2 years was 100%, at 3 years 85.71% (6 cases). For HER2/neu positive patients (7 cases) the survival at 2 and 3 years was 85.71% (6 cases) (Fig. 15).

In well differentiated tumors also, there is no statistically significant difference in the survival rates between HER2/neu positive and negative cases at 2 and 3 years (p >0.05).

In the moderately differentiated forms (G2) the survival was 89.47% at 2 years and 78.94% at 3 years. HER2/neu negative patients (3 cases) survived for 2 and 3 years in 66.66% (2 cases) and the HER2/neu positive patients (16 cases) the percentage was 93.75% at 2 years (15 cases) and 81.25% at 3 years (13 cases) (Fig.16).

In the undifferentiated forms of endometrial cancer (G3) 55.55% of patients survived at 2 years, 33.33% at 3 years. Only 2 patients were HER2/neu negative, one survived for 2 years and none after 3 years. 7 cases were HER2/neu positive, 42.85% of them survived 2 and 3 years (3 cases) (Fig. 17).

The lower survival rates of HER2/neu negative patients with moderately differentiated and undifferentiated tumors may also be explained by the small number of cases (3 and 2 patients, respectively).

Discussions

Significant overexpression of the HER2/neu growth factor is rare in endometrial cancer. Increased levels of HER2/neu are present in aggressive phenotype of endometrial cancers, with enhanced proliferation, and in patients with decreased survival [8].

The HER2/neu growth factor has an important role in the regulation of cell proliferation and differentiation [9]. Multiple studies have shown that increased HER2/neu expression in ovarian and breast cancer is an independent factor predicting a poor prognosis. In papillary-serous endometrial cancer amplification of the HER2/neu oncogene has been demonstrated [10], especially in advanced stages [11], explaining the poorer prognosis of this type of cancer.

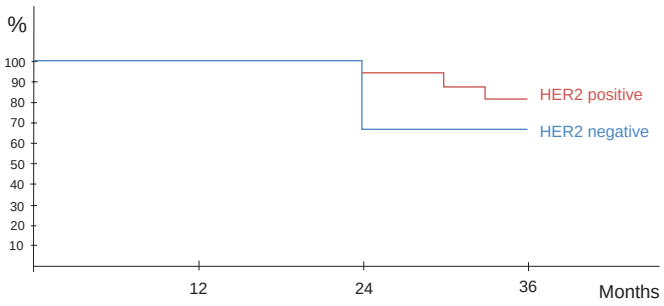

Fig. 16. Survival rates of G2 patients

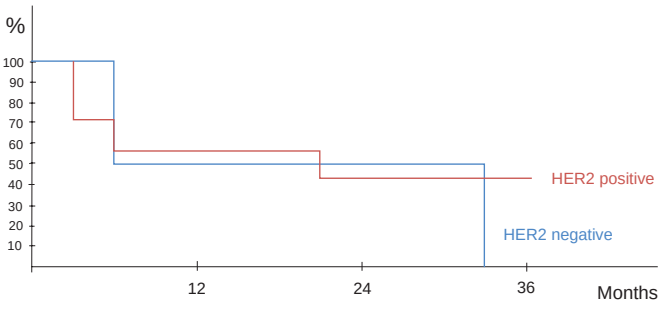

Fig. 17. Survival rates of G3 patients

The papillary-serous variant of endometrial cancer is rare (under 2% of all endometrial cancers), and only 16.7% of them displays HER2/neu amplification [12]. In our study we found an increased HER2/neu expression in 80% of the papillary-serous endometrial cancer.

Multiple studies, analyzing the presence of the HER2/neu growth factor, have concluded that its overexpression would have a negative impact on long term survival [13].

Determining the amplification of HER2/neu may aid in selecting the patients with type II ACE who might benefit from targeted therapeutic strategies for HER2/neu [14].

While in breast cancer there are well established protocols, i.e. all invasive cancers are tested for HER2/neu regardless of the type, grade or the stage of the disease, in patients with endometrial cancer there are no clear protocols. Patients with endometrial cancer that should be tested for HER2/neu are those with undifferentiated cancers, regardless of the stage of the disease, and all patients staged over III A. In these patients the HER2/neu expression could predict in almost 50% of the cases a poor prognosis [15].

Conclusions

The results of this study demonstrate the existence of a frequent association of the HER2/neu receptor with other negative prognostic factors of the endometrial cancer. The presence of HER2/neu expression increased with the stage of the disease, significant differences being found between stages I and III. The expression of HER2/neu was lower in differentiated forms (G1) than in moderately differentiated (G2) or undifferentiated forms (G3), significant differences being found between G1 and G2 ($p < 0.05$). The large majority of cancers with increased expression of the HER2/neu growth factor (2+, 3+) were moderately differentiated and undifferentiated endometrioid cancers, and non-endometrioid cancers.

The presence of HER2/neu transmembrane receptor seems not to influence the survival rates. More important prognostic factors seem to be the stage of the disease, the histological type and the grade of the malignancy.

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