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HER2 Amplification Has no Prognostic Value in Sporadic and Hereditary Ovarian Tumours

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Abstract

Whereas *HER2* amplification is a well-known phenomenon in breast tumours, its frequency and clinical importance in ovarian cancer have not been established. The aim of the study was to compare the frequency of *HER2* amplification in hereditary (*BRCA*-positive) and sporadic (*BRCA*-negative) ovarian tumours and to estimate the association of this gene alteration on clinical outcome in ovarian cancer patients. We analysed *HER2* amplification in 53 ovarian tumours: 20 from mutation carriers (18 in *BRCA1* and 2 in *BRCA2* gene) and 33 from non-carriers. Fluorescence *in situ* hybridization for *HER2* was performed on 'touch' slides from frozen tumour samples or formalin-fixed, paraffin-embedded tissue. Our results indicate that high amplification (*HER2*: centromere ratio>5) is an infrequent phenomenon in ovarian tumours (6/53 cases). It occurs in both hereditary (4/20) and sporadic (2/33) tumours and no difference in the frequency of *HER2* amplification exists between these groups. There is no significant difference in the clinical outcome of patients with *HER2* amplified and non- amplified tumours (p=0.3). Our results suggest a different biological role of *HER2* amplification in ovarian and breast cancer.

Introduction

HER2 amplification is a well-known phenomenon in breast tumours and its clinical importance is established in these malignancies. It was observed that patients with *HER2*-amplification in breast cancer tissue showed shorter disease-free time intervals and they benefit from therapy with the anti-Her-2/neu antibody, Herceptin [6, 19, 25].

It was suggested that the presence of amplification of *HER2* is associated with the advanced clinical stage of ovarian cancer and does not occur in borderline and early stage disease [10, 29]. Some studies reported a significant difference in overall survival between women with *HER2* negative and positive tumours [2, 7, 22] in contrast to later studies which demonstrated that *HER2* over-expression had no relationship with prognosis [3, 5, 17, 21]. At present, there are few published reports of the clinical significance of *HER2* amplification, demonstrated by the FISH techniques, in ovarian tumours, and the predictive value of *HER2* assessment has not been demonstrated.

There is evidence that germline mutations in the *BRCA1/2* genes confers increased susceptibility to breast and ovarian cancer. It is suggested that a germline mutation in the *BRCA1* gene is associated with a significantly lower level of *HER2* amplification in breast tumours [1, 9, 19, 25]. Similarly, it was noticed that

BRCA-linked ovarian carcinomas and serous carcinoma of the peritoneum seemed to develop through a unique pathway of tumorigenesis that does not involve mutation in *K-RAS* or *HER2* and *C-MYC* amplification [20, 24]. Some authors, however, did not notice differences in *HER2* expression between *BRCA*-linked ovarian cancer carriers and ovarian cancer as control [15].

The aim of the study was to compare the frequency of *HER2* amplification in hereditary and sporadic ovarian tumours and to estimate the influence of this gene alteration on clinical outcome in cancer patients.

Methods

In the present study we analysed 53 ovarian tumour tissue sections: 20 from *BRCA* mutation carriers and 33 from non-carriers. In the group of carriers there were 18 women with *BRCA1* and two with *BRCA2* germline mutation. All patients were diagnosed and operated on at the Department of Gynaecology, Institute of Obstetrics and Gynaecology, Medical University of Gdańsk. All cases represented a histological type of serous adenocarcinoma. There were four patients diagnosed in FIGO stage I, three in II, 35 in III and 11 in stage IV.

Fluorescence in situ hybridization (FISH) for *HER2* was performed on 'touch' slides from frozen tumour samples or formalin-fixed, paraffin-embedded tissue slides.

HER2/NEU/Alphasatellite17 cocktail dual-color probe (Q-Biogene) was used for FISH analysis. The HER2/NEU probe labelled with Rhodamine contains DNA sequences specific for the HER2/NEU human gene locus and hybridized to human chromosome 17q12 and chromosome 17 enumeration probe labelled with Fluorescein contains α-satellite DNA that is targeted to the centromeric region of chromosome 17 (CEP17). Deparaffinization, in situ hybridization and staining were performed as per the manufacturer's protocols (Q--Biogene). Fluorescent signals were scored using a Zeiss Axioplan microscope with an x100 planar objective, using a triple band-pass filter that permits simultaneous blue, green and red colours.

In each sample, an average of 100 non-overlapping interphase nuclei were scored. Because an increase in number of chromosome 17 homologues is a frequent phenomenon in ovarian tumours, the CEP17 probe was used as an internal control in dual hybridization with the HER2/NEU probe. Tumours with a HER2/NEU: CEP17 signal ratio of <2 were considered to be non-amplified, whereas those with a ratio of 2 or greater were considered to have low amplification (2-3), moderate amplification (3.1-5), or high amplification (>5).

The Kaplan-Meier method and the log-rank test were used to identify predictors for outcome. Demographic and disease characteristics were compared between BRCA-associated and sporadic ovarian cancer patients and between *HER2*-amplified and non-amplified cases using the χ^2 square test.

Results

FISH analysis demonstrated *HER2* gene amplification in 10 (19%) cases (Table 1). Our results indicate that high amplification (HER2:centromere ratio>5) is an infrequent phenomenon in ovarian tumours. High amplification was observed in six (11%) out of 53 cases (two sporadic and four hereditary), whereas moderate and low amplification were observed in one (2%) and three (6%) tumours respectively.

No significant differences in the frequency of *HER2* amplification were identified in hereditary (BRCA-positive) and sporadic (BRCA-negative) tumours ($p=0.2$).

No association was observed between *HER2* amplification and age of onset, serum Ca-125 level before therapy, FIGO stage, tumour grade and histological type. There was also no association between HER--positive and negative cases in outcome (overall

Table 1. Distribution of HER2 amplification results in ovarian tumours from BRCA1 and BRCA2 carriers and non-carriers

HER2 amplification status	BRCA1	BRCA2	Non-carriers
	n (%)	n (%)	n (%)
high amplification	2 (11)	2 (100)	2 (6)
low and moderate amplification	2 (11)	0	2 (6)
total amplified	4 (22)	2 (100)	4 (12)
no amplification	14 (78)	0	29 (88)
total	18	2	33

survival and response to chemotherapy) (Fig. 1). Narrowing the examined group to FIGO III/IV patients (46 cases), we also did not notice any influence of *HER2* status on outcome.

Discussion

Previous studies indicated that a high level of *HER2* oncogene amplification did not occur or was not frequent in breast and ovarian tumours of *BRCA* mutation carriers. In contrast, *HER2* was relatively highly amplified in sporadic breast or ovarian tumours [1, 9, 19, 20]. At present, there are few published papers on the frequency of *HER2* amplification in hereditary and sporadic ovarian tumours. Our study did not reveal any difference in the frequency of *HER2* amplification between *BRCA*-linked or sporadic ovarian cancers. Similar results were presented by Lakhani et al. [15] on the basis of immunochemical analysis of 178 *BRCA1* and 29 *BRCA2* mutation carriers and 235 controls.

The frequency of *HER2* amplified breast and ovarian tumours varies depending on applied methods. There are two basic techniques applied for the examination of *HER2* amplification: immunohistochemical (IHC) and fluorescence in situ hybridization (FISH). High concordance is observed between FISH and IHC results, especially in positive cases scored in IHC as 3+ and in negative cases. However, a substantial number of cases showed discrepant results from these two methods [13, 16, 23]. *HER2* amplification/over-expression has been reported in 20-30% of human breast cancers and in 7-50% of ovarian cancers [3, 15, 18, 21, 26]. We decided to apply the FISH technique as it is a precise and reliable method. FISH offers a clear advantage over IHC, as it is reproducible and less susceptible to variations in tissue fixation and processing. It allows us to estimate the degree of amplification, gathering cases in groups depending on how many signals occur in the cellular nuclei. On the basis of published studies, we used the HER2/NEU:centromere 17 fluorescence ratio of 2 as the cutoff [8, 9, 17, 23].

On the basis of immunohistochemical analysis, it was observed that *HER2* over-expression increased the risk of mortality in ovarian cancer patients [18]. Similar studies based on the chromogenic in situ hybridization (CISH) method revealed that amplification (>5 copies per cell) has a significant influence on survival [16]. Moreover, further results showed that *HER2* over-expression has a prognostic value both in univariate and multivariate survival analysis [12, 28, 4, 11]. Some authors, however, did not observe a correlation between *HER2* expression and survival or observed such a correlation only in selected groups of patients. [5, 14, 21, 26, 27]. The multi-centre study provided on 361 Caucasian patients indicates that *HER2* over-expression is a predictive factor for the response to first-line chemotherapy but has no significant influence on overall and disease-free survival independent of FIGO stage and tumour grade [21]. Our study, based on the FISH method, found neither an association between *HER2* amplification and outcome nor any influence on response to chemotherapy. This study demonstrated that *HER2* amplification in ovarian tumours was not a prognostic risk factor, suggesting a different biological role of *HER2* from that previously demonstrated in breast cancer.

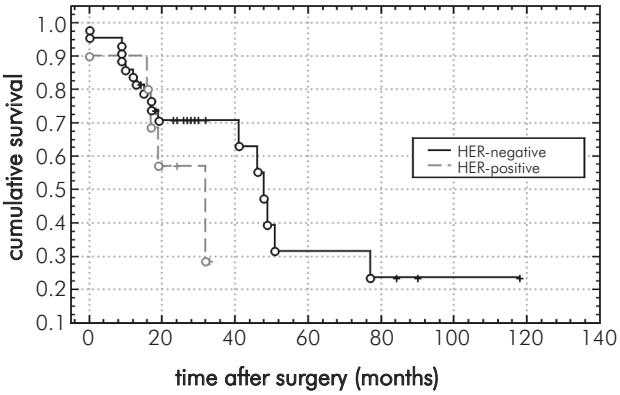

Fig. 1. Kaplan-Meier survival curves for 53 patients with ovarian cancer with HER2 amplification in the tumour tissue (group 1, n=10) versus patients demonstrating no HER2 amplification (group 2, n=43). The difference was not significant by the log-rank test (p=0.33)

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