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The prognostic significance of pathological features in Her-2 overexpressing breast carcinomas: A single institution experience in southern Tunisia

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Abstract.
BACKGROUND: Breast cancer is the most frequent malignant neoplasm affecting Tunisian women. It represents 25 to 35% of all female cancers. There is no published study about the features of Her-2 overexpressing breast carcinomas in North African women.
OBJECTIVE: The aim of this study is to assess the prognostic significance of pathological features in a cohort of a Her-2 overexpressing breast carcinoma originating from the region of south Tunisia.
METHODS: This study investigated a series of 100 patients followed from January 2006 to December 2011 for a Her-2 positive invasive breast carcinoma. Pathological features included in this study were: histological type, histological grade, tumor size, vascular invasion, perineural invasion, mitotic index, lymph nodes stage, positive lymph node capsular effraction, inflammatory infiltrates, nipple involvement and hormone receptors status.
RESULTS: Multivariate analysis showed that pT stage, pN stage, capsular effraction, vascular invasion, perineural invasion and Nipple involvement were independent prognostic factors for overall survival and disease free survival in patients free from distant metastasis at diagnosis. For patients with synchronous metastasis, there is no independent pathologic prognostic factor for survival.
CONCLUSIONS: Our study demonstrates that pathological features are important prognostic factors for non metastatic Her-2 overexpressing breast carcinomas. This supports the idea that HER2-positive disease is a heterogeneous entity. We believe that these findings reinforce the need to identify molecular predictors of benefit and resistance to anti-Her-2 based therapies.

Keywords: Breast cancer, HER-2, pathology, prognosis, survival

1. Introduction

Breast cancer is the most frequent malignant neoplasm affecting Tunisian women. It represents 25 to 35% of all female cancers. The age standardized incidence varied from 28 to 31.8/100.000 inhabitants [22, 26]. In North Africa, breast carcinoma displays specific features including young age at diagnosis and a

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higher proportion of aggressive disease. In fact, inflammatory breast carcinoma and triple negative tumors in North African women are more prevalent than in western countries [5,9,12,17,20,21,29,30,34]. Although, some recent papers from Tunisia and Morocco investigated molecular breast cancer subtypes based on immunohistochemical studies, there is no published study about features of the human epidermal growth factor receptor 2 (Her-2) overexpressing breast carcinomas in North African women [3,10,31,32].

The aim of this study is to assess pathological features and their prognostic significance in a cohort of a Her-2 overexpressing breast carcinoma originating from the region of south Tunisia.

2. Patients and methods

2.1. Patients

This study investigated a series of 100 patients followed from January 2006 to December 2011 at the department of medical oncology and the department of radiotherapy of the university hospital HabibBourguiba (Sfax, Tunisia) for an invasive breast carcinoma with overexpression of Her-2. Selected patients represent 19% of patients treated for breast cancer in the same period. All patients were proposed for surgery followed by anthracyclines-taxanes based chemotherapy and Trastuzumab. Hormonotherapy with Tamoxifen or aromatase inhibitors was delivered for patients with positive hormone receptors.

Overall survival (OS) was defined as the interval between the date of the pathological diagnosis of invasive carcinoma and death or last follow-up. Disease free survival (DFS) was defined as the interval between the end of treatment and recurrences or last follow-up.

2.2. Pathological features

Pathological features included in this study were evaluated at the department of pathology of the university hospital Habib Bourguiba (Sfax, Tunisia). They include: histological type according to the 2012 WHO classification, histological grade according the modified Scarff-Bloom-Richardson (SBR) histological grading system, tumor size, vascular invasion, perineural involvement, mitotic index, lymph nodes stage, capsular effraction, tumoral stroma, nipple involvement, surgical margin, and hormone receptors status.

2.3. Immunohistochemical and in situ hybridization studies

Serial 4-μm sections from paraffin-embeded tissue were used for immunohistochemical and in situ hybridization analyses. Estrogen receptor (ER) was tested by using clone 1D5 (Dilution: 1/40, *DAKO*). Progesteron receptor (PR) was tested by using clone PgR 636 (Dilution: 1/50, *DAKO*). Hormone receptors (ER and PR) were considered positive when more than 1% of infiltrating tumor cell nuclei were stained. Her-2 testing was tested by using HercepTest$^{\mathrm{TM}}$ (*DAKO*) and clone CB11 (Dilution: 1/40, *Novocastra*). Immunohistochemistry findings were scored on a 0–3 scale according to the ASCO/CAP Her-2 test guideline recommendations during the study period [2]. Tumors scored as 3+ were considered overexpressing Her-2. Tumors with a score 2+ were considered as equivocal and were tested with a Fluorescence (*HER2 FISH pharmDx*$^{\mathrm{TM}}$ *Kit, DAKO*) or chromogenic (HER2 *CISH pharmDx*$^{\mathrm{TM}}$ *Kit, DAKO)* in situ hybridization (ISH). ISH was considered positive if a HER2 gene copy number is $\geqslant$ 6.0 signals per cell for CISH technique and if FISH ratios (HER2/CEP17) were $\geqslant$ 2.2.

2.4. Statistical analysis

Statistical analysis was performed using SPSS 20.0 statistical software (SPSS Inc, Chicago, IL). The association between pathological features and survival parameters were analyzed by Kaplan Meier plot and compared with Log-rank test. A p value less than 0.05 was considered significant.

3. Results

3.1. Clinicopathological features of the study population (Table 1)

Patients' age ranged from 26 to 86 years (mean, 49.8 years). Only 9 patients were aged less than 35 years. Mean clinical tumor size was 52, 75 mm (0–140 mm). Two thirds of patients had axillary lymph nodes involvement. At diagnosis, 86 patients were free of distant metastasis and 14 had synchronous metastasis particularly in bone and liver.

Histological type was invasive carcinoma of no special type in 91 cases (91%), mixed carcinoma in 5 cases (5%), lobular carcinoma in 2 cases (2%) and metaplastic carcinoma in 2 cases (2%). Tumor multifocality was

Table 1
Clinicopathological features of the study population

Characteristics	
Median age at diagnosis	49.8 (26–86)
Median pathological tumor size	36.8 (0.2–140)
SBR Grading	
I and II	59 (64.8%)
III	32 (35.2%)
NA	9
Histological type	
Invasive carcinoma of non specific type	96 (96%)
Others	4
Vascular invasion	
Present	34 (45.3%)
Absent	41 (54.7)
NA	25
Perineural invasion	
Present	34 (46%)
Absent	40 (54%)
NA	26
Axillary lymph nodes status	
pN0	25 (26.3%)
pN1	36 (37.8%)
pN2	20 (21%)
pN3	14 (14.7%)
NA	5
M stage	
M0	86 (86%)
M+	14 (14%)
Inflammatory infiltrates	
Present	42 (42%)
Absent	58 (58%)
Mitotic index	
$<$ 10/10HPFs	22 (24.1%)
$>$ 10/10 HPFs	69 (75.9%)
NA	9
Lumph node capsular effraction	
Present	33 (47.1%)
Absent	37 (52.9%)
Nipple involvement	
Present	10 (12%)
Absent	73 (88%)
Hormone receptors status	
HR+	55 (55%)
HR-	45 (45%)

NA: not available.

noted in 21% of the cases. Carcinomas were classified as grade I in 4 cases (4%), grade II in 55 cases (55%) and grade III in 32 cases (32%). Tumor grading was not available in nine cases. Tumor size varied from 0.2 to 14 cm, with a mean size of 3.6 cm. Vascular and perineural invasion were noted in respectively 45% and 46% of cases. Mitotic index was more than 10 mitosis per 10 high power fields in 76% of cases. Inflammatory infiltrates was seen in 42% of the cases. A Paget disease was noted in 10.6% of all mastectomy specimens. Axillary lymph nodes involvement was seen in 73.8% of the cases; capsular effraction was noted in 47.8% of the cases. Surgical margin were clear in 82%

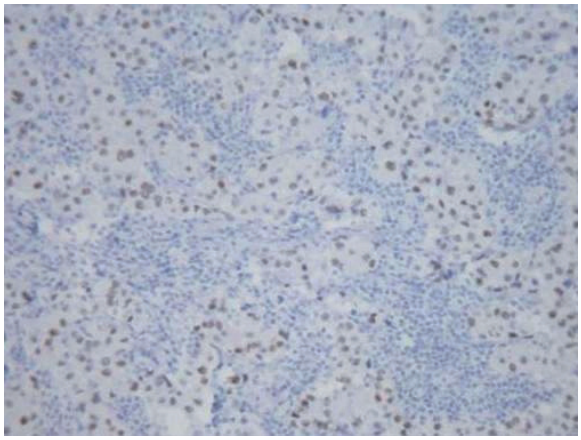

Fig. 1. Positive immunostaining of tumor cells for Estrogen receptor (x 400). (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/BD-150414)

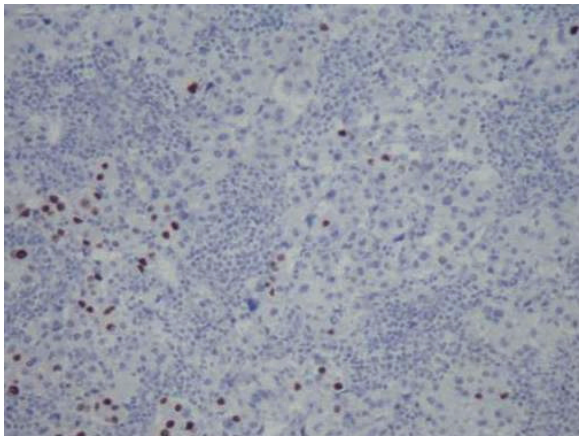

Fig. 2. Positive immunostaining of tumor cells for Estrogen receptor (x 400). (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/BD-150414)

of the cases. Immunohistochemical study showed reactivity for ER and/or PgR in 55% of the cases (Figs 1 and 2). Her-2 status was scored as 3+ in 88 cases and 2+ in 12 cases (Fig. 3). Hybridization in situ technique using chromogenic method (10 cases) and fluorescent method (2 cases) confirmed the amplification of the Her-2 gene.

3.2. Treatment details for non-metastatic patients

Eighty-five patients underwent surgery. It was preceded by neoadjuvant chemotherapy for 16 patients. Modified radical mastectomy with axillary lymphadenectomy was performed in 71 patients. The remaining patient had conservative surgery (lumpectomy and lympadenectomy).

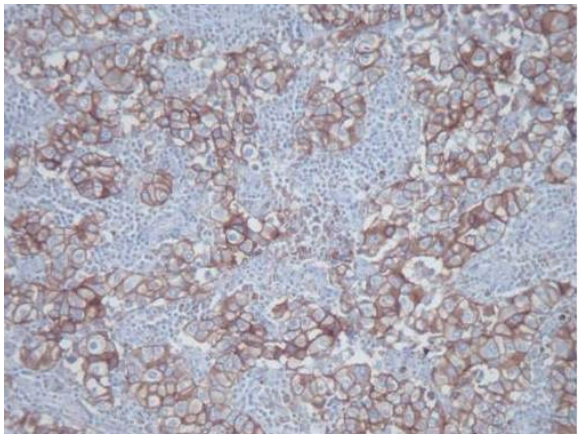

Fig. 3. Intense and complete membranous staining of tumor cells for Her-2 (score 3+) (x 400). (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/BD-150414)

Locoregional radiotherapy was delivered for 79 patients and adjuvant chemotherapy for 84 patients. Hormonotherapy consisted of Tamoxifen in 32 patients and aromatase inhibitors in 17 patients. Only 55 patients received adjuvant Trastuzumab.

3.3. Treatment details for metastatic patients

All patients received primary anthracyclines based chemotherapy. A modified radical mastectomy with axillary lymphadenectomy was performed in 10 patients. Two other patients had only palliative mastectomy. Locoregional and cerebral radiation therapy were performed for respectively 8 and 2 patients. Hormone therapy and Trastuzumab were done for respectively 5 and 4 patients. Zoledronic acid was proposed for 10 patients with bone metastasis

3.4. Survival data

Five-year overall and disease free survivals rates were respectively 70.5% and 66.3% for patients free of distant metastasis at diagnosis (Figs 4 and 5). Five-year overall survival rate was 32.1% for patients with synchronous metastasis.

3.5. Correlation between pathological features and survival parameters

The correlation between pathological features and survival data in univariate analysis was shown in Table 2.

Multivariate analysis showed that pT stage, pN stage, capsular effraction, vascular invasion, perineural invasion and nipple involvement were independent prognostic factors for overall survival and disease free survival in patients free from distant metastasis at diagnosis (Table 3). For patients with synchronous metastatic disease, there is no independent prognostic factor for overall survival (Table 4).

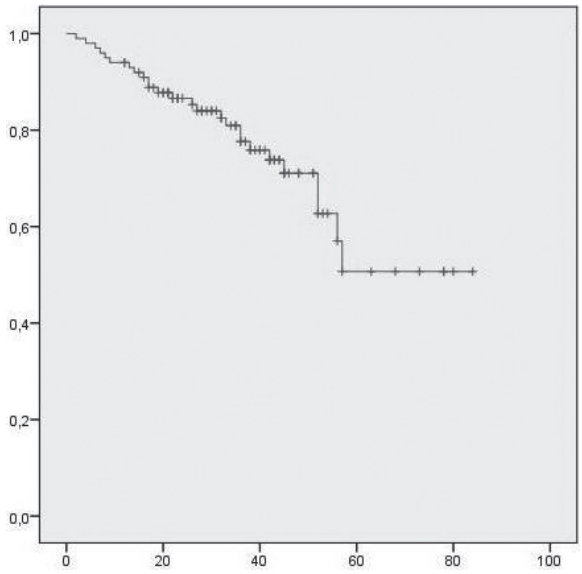

Fig. 4. Overall survival of 100 Her-2 overexpressing breast carcinoma patients free of distant metastasis at diagnosis.

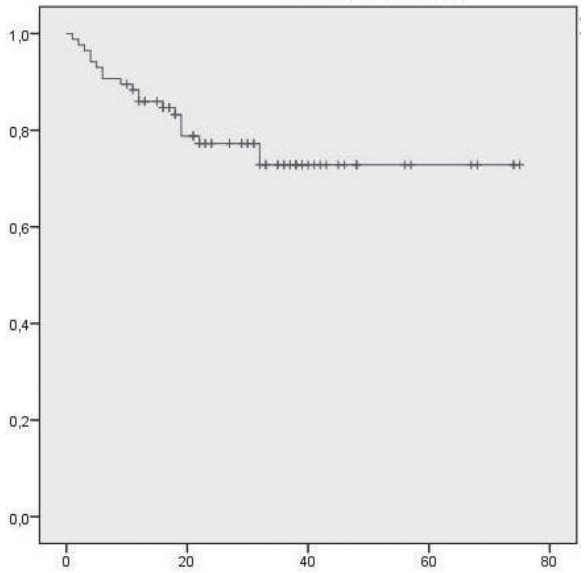

Fig. 5. Disease free survival of 100 Her-2 overexpressing breast carcinoma patients free of distant metastasis at diagnosis.

Table 2
Correlation between survival parameters and pathological features in univariate analysis for patients free of metastasis at diagnosis

	OS	P	DFS	p
pT stage				
pT1	80.9		82.4	
pT2	74.6	0.039	72.5	0.022
pT3	56.1		59.6	
pN stage:				
pN0	88.6		90.3	
pN1	84.8	0.021	70	0.018
pN2	64		51	
pN3	37		30.6	
Lymph node apsular effraction				
Absent	84.3	0.005	79.1	0.003
Present	41.6		57.1	
Histological grade				
I-II	80.3	0.123	70.8	0.215
III	55.1		67.1	
Mitotic index				
$<$ 10/10HPFs	76.5	0.838	78.6	0.907
$>$ 10/10HPFs	68.8		70.5	
Vascular invasion				
Present	63	0.013	64.4	0.010
Absent	93.8		65.5	
Perineural invasion				
Present	63.8	0.032	64	0.027
Absent	92.9		67.2	
Tumoral stroma				
Fibrous	71.1	0.965	68.6	0.981
Inflammatory	69.8		65.3	
Nipple involvement				
Absent	77.2	0.003	70.2	0.002
Present	38.1		41.7	
Hormone receptors status				
HR +	70.3	0.451	81.6	0.203
HR −	72.9		55.6	

4. Discussion

According to data from Tunisian cancer registry, breast cancer is the most frequent malignant neoplasm, affecting female patients with a standardized incidence of 28.5/100000 [24]. Although this incidence is 2 to 4 times lower than in France or USA, breast cancer represents nearly 30% of all female cancers in Tunisia [26].

Breast carcinomas in Tunisia and in North African countries are diagnosed at advanced stage compared to that reported from developed countries. That can be explained by the lack of screening programs of breast cancer in Tunisia and by the prevalence of two aggressive forms of breast cancer (inflammatory breast cancer and triple negative breast cancer) [1,20,21,27–29]. In these countries, breast cancer appears to be predominantly a disease of young women; 50% to 60% of patients are below 50 years of age. Moreover, the two aggressive forms of breast cancer (inflammatory breast cancer and triple negative breast cancer) are associated with young age [22].

The HER-2 gene is amplified in 20% to 25% of human breast cancers [11]. Overexpression of Her-2 promotes receptor activation, increased signaling, and excessive cellular division. Her-2 positive tumor cells therefore have increased proliferation and survival characteristics that typically result in aggressive tumors [8,15,16,23]. As adverse pathologic prognostic factors (higher grade, larger size, lymph node involvement, increased proliferative index) are also associated with Her-2 status, this further emphasizes Her-2 status as a key molecular marker, which drives both the biology of the tumor as well as the clinical course for the disease. HER2 amplification and overexpression are recognized as important markers for aggressive disease and are the molecular targets for specific therapies, such as Trastuzumab and Lapatinib [11]. Only Trastuzumab have been approved by the Tunisian social security system for the treatment of non-metastatic HER-2-positive breast cancer.

In Tunisia, the determination of the Her-2 status and the introduction of anti-Her-2 based therapy in routine practice started respectively ten and sixyears ago. The prevalence of Her-2 positive breast carcinoma varied in North African population from 29% to 30.9% [31,32]. In a previous publication from our institute, we reported in a series of 155 cases of invasive breast carcinoma collected from January 2000 toDecember 2004 that Her-2 overexpression was noted in 18.1% of cases [19]. In this previous study, Her-2 overexpression was not associated with patient age [19]. The present study showed similar proportion (19%) to that previously reported. Our results are similar to those reported in western countries and seemto be different from others North African studies. We think that following updated recommendations for HER2 testing in breast cancer could explain, in part this variation.

In this report, we investigated the prognostic significance of pathological features of Her-2 positive breast carcinoma. We found that in non metastatic Her-2 breast carcinomas many pathological features are independent prognostic factors for OS and DFS. As previously reported [4,33], we demonstrated also that lymph nodes status and tumor size are two strong independent prognostic factors in Her-2 positive breast carcinomas.Moreover, as Traina A et al, we found a correlation between vascular invasion and poor outcome [6]. Tumor grade and lymphoid infiltration were not associated with prognosis as shown in our series [33]. In our study, we found that perineural invasion, lymph node

Table 3
Correlation between survival parameters and pathological features in multivariate analysis for patients free of distant metastasis at diagnosis

	OS		DFS	
	RR hazard ratio	P	RR hazard ratio	p
pT Stage	1.97 (0.88–4.37)	0.095	2.11 (0.98–4.53)	0.05
pN Stage	1.98 (1.19–3.32)	0.009	1.63 (1.03–2.57)	0.03
Lumph node capsular effraction	0.26 (0.09–0.72)	0.010	0.26 (0.10–0.67)	0.006
Vascular invasion	0.11 (0.01–0.90)	0.04	0.17 (0.03–0.78)	0.02
Perineural invasion	0.14 (0.01–1.13)	0.065	0.21 (0.04–0.97)	0.04
Nipple involvement	0.226 (0.077–0.664)	0.007	0.22 (0.08–0.63)	0.005

Table 4
Correlation between overall survival parameters and pathological features in multivariate analysis for patients with distant metastasis at diagnosis

	OS (at 3 years)	P
pN Stage		
pN1	33.3	0.139
pN2+pN3	25	
Histological grade		
I-II	50	0.449
III	45	
Hormone receptors status		
RH+	60	0.570
RH-	50	

capsular effraction and nipple involvement were also independent prognostic factors. These findings were not previously reported.

Hormone receptors status had little influence on OS and DFS for patients with Her-2 positive breast carcinomas [6,33]. Our study showed the same results. Vaz-Luis I et al found that the patients with HR-positive tumors were more likely to achieve a long-term clinical benefit to a first-line trastuzumab based regimen [13].

In metastatic Her-2 positive breast carcinomas, hormone receptors status is the principal pathological feature included in studies. The effect of Hormone receptors in response to trastuzumab is less clear. Trastuzumab given either alone or in combination with chemotherapy in the first-, second-, or third-line treatment for advanced HER2-positive breast cancer have shown similar magnitude of benefit regardless of Hormone receptors [7,14,18,25]. In our study, we didn't find a significant statistical correlation between hormone receptors status and outcome.

5. Conclusion

Our study, demonstrates that pathological features are important prognostic factors for non metastatic Her-2 overexpressing breast carcinomas. Pathologists play a central role in this process through the determination of the Her-2 status and through the assessment of the prognostic pathological features. We believe that these findings reinforce the need to identify molecular predictors of benefit and resistance to anti-Her-2 based therapies in the near future.

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