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Prognostic Value of HER2 in Metastatic Colorectal Cancer: A Single Institutional Experience

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ABSTRACT

Background: HER2 (Human epidermal growth factor receptor 2) activation has been associated with poor prognosis in a number of tumours as breast, gastric and lung cancers, but the prognostic role of HER2 in colorectal cancer (CRC) remains unclear.

Objective: The aim of the current work was to detect the incidence and prognostic impact of HER2 overexpression in metastatic CRC patients in relation to clinico-pathologic features and outcome.

Patients and Methods: Data of metastatic CRC patients treated from January 2012 to end of December 2016 in a tertiary referral university hospital were collected. Eligible patients had their paraffin block tested for HER2.

Results: Clinico-pathologic features of 70 patients were available for analysis. Age ranged 20-73 years, at a median of 39.5 years. Fifty (71.4%) of these cases were left sided. Male to female ratio was 3:4. Mucinous variant was present in 27.1 %(19 cases). Synchronous metastasis constituted 61.4%. HER2 incidence was found in 8.57% (6 cases). Her2 positivity was significantly associated with a shorter time to progression on both first line of therapy, PFS1 (mPFS1 3 vs. 6 months, p=0.045) and PFS2 (mPFS2 4 vs.6 months, p=0.036). No significant relation to clinico-pathological characteristics or OS were detected.

Conclusion: HER2 positivity was not associated with clinico-pathologic features but was related to outcome with a shorter PFS but not OS in metastatic CRC. Further prospective data sets are required to confirm its prognostic role.

Keywords: HER2, Metastatic colorectal cancer, Prognostic factor.

INTRODUCTION

Colorectal cancer (CRC) is the third most frequently diagnosed cancer and the second leading cause of cancer death. Colorectal cancer incidence rates are about 4-fold higher in transitioned versus transitioning countries [(1)].

Various genomic landscapes contribute to the heterogeneity of CRC and have led to different diagnostic, prognostic and predictive approaches to characterize the disease [(2)].

The epidermal growth factor receptors (EGFRs) family is composed of four members of which the185-kDa transmembrane tyrosine kinase (TK) receptor HER2 protein (HER2/neu, ERBB2) is a member [(3)]. HER2 acts as an oncogene, amplification of the gene induces protein overexpression in the cellular membrane, cell growth, proliferation, and tumourigenesis [(4)].

HER2 overexpression and/or amplification has been implicated in numerous cancers. Invasive breast HER2 positive cancers constitute 13%–20% and are associated with a poor prognosis and inferior outcomes [(5)]. It has also been observed in gastric and lung amongst other malignancies [(6, 7)].

HER2's role as a prognostic biomarker in CRC remains uncertain, unlike its use as a therapeutic target that seems promising [(8)]. Additionally harboring this anomaly offers itself as a mechanism of resistance to EGFR-targeted therapies such as cetuximab and panitumumab [(9)].

The mere definition of HER2 presence was a subject extensively researched [(10)], in order to substantiate the use of targeted therapies effectively. HER2 Amplification for Colorectal Cancer Enhanced Stratification (HERACLES) trial, a proof-of-concept, multicenter, open-label, phase 2 trial, in which eight of 27 patients with HER2-amplified/overexpressing, KRAS wild-type metastatic colon cancer (30%) had objective responses to dual HER2 blockage with trastuzumab plus lapatinib [(11)]. Even further DESTINY-CRC01 also a phase 2 study on progressing CRC patients also reported an objective response of 45·3% with trastuzumab deruxtecan [(12)].

These encouraging data and personalized therapeutic approach urged us to explore further HER2 alterations in our area with all its implications, as regional incidence rates in HER2-CRC are lacking.

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PATIENTS AND METHODS

Patients with metastatic CRC (either synchronous or metachronous) presented at the Department of Clinical Oncology, Ain Shams University Hospitals, from January 2012 to December 2016 were selected. A total of 70 eligible patients had their paraffin block collected and tested for HER2 by immunohistochemistry (IHC). All available data (including patient, tumor, progression free survival, overall survival and response to treatment) were extracted from patients' files. Six months from initial diagnosis was set as the cut-off between defining the metastases as synchronous or metachronous. We excluded patients with second malignancy or younger than 18 years.

Staining of HER-2/neu protein was performed on 4 µm thick slides using the Ventana (4B5) mouse monoclonal antibody (Ventana medical systems, Tucson, AZ, USA), following deparaffinization, antigen retrieval and incubation with blocking agent the 4B5 monoclonal antibody directed against HER-2/neu was incubated using Ventana Bench Mark autostainer.

Interpretation of HER by IHC using a four-tired scoring system according to manufacturer's guidelines. Score 0 is defined as no staining or membranous staining in <10% of tumor cells. Score 1+ is defined as faint membranous staining >10% of tumor cells. Score 2+ defined as weak to moderate staining in >10% of tumor cells and a score 3+ is defined as strong staining of the entire membrane in >10% of tumor cells. Cytoplasmic staining may have been present but was not included in the determination of positivity. A score of 0 or 1+ve was considered negative while a score 3+ was considered positive. Score 2+ was considered an equivocal result that required confirmation by silver in situ hybridization (SISH) via the HER-2 probe kit VENTANA Inform HER2 dual-color on the BenchMark Ultra system (Inform HER2 DNA dual color assay—Roche Tissue Diagnostics, VENTANA Medical Systems, SA) and was deemed positive if a HER2/CEP17 ratio of ≥2.0 was found [10, 13].

Response evaluation was based on modified Response Evaluation Criteria in Solid Tumors (RECIST) 1.1. Progression-Free Survival (PFS) was defined as the time from date of presentation until objective recorded radiological tumour progression or death. PFS1 and 2 were also incorporated to assess for subsequent lines of therapy/care. Overall survival (OS) was defined as the time from date of presentation until date of last follow-up or death.

Ethical consent:

An approval for the study was obtained from Ain Shams University Academic and Ethical Committee. An informed written consent was obtained from participants. This work was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical methods

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 21 for Windows® (IBM SPSS Inc, Chicago, IL, USA). First descriptive analysis for the whole study population was done using count and percentage for categorical variable and mean ± SD or quantitative variables. Univariate frequency analysis was used performed using Chi-square test and Fisher exact test for categorical variables and independent and paired t-test for numerical valuables. Statistical significance was established at a p-value of less than 0.05.

RESULTS

Initial demographic characteristics of the included 70 eligible patients revealed a higher female presentation; 40 cases (57.1%), synchronous metastasis (n=43) and a median age 39.5 years at time of diagnosis (Table 1).

Table (1): Characteristics of the study group

Characteristic		Min.	Max.	Median	Mean	SD
Age at diagnosis (years)		20.00	73.00	39.5	44.11	14.34
		Number (70)		**%**		
Gender	**Female**	40		57.1		
	Male	30		42.9		
Clinical presentation	**Constipation**	15		21.4		
	Pain	18		25.7		
	IO	11		15.7		
	Bleeding	26		37.1		
ECOG Performance Status at diagnosis	**1**	58		82.9		
	2	11		15.7		
	3	1		1.4		
Family history	**No**	58		82.9		
	Yes	12		17.1		
Site of Primary	**Right**	20		28.6		
	Left	21		30		
	Rectum	29		41.4		
Type	**Mucinous variant**	19		27.1		
Grade (adenocarcinoma)	**1**	1		1.4		
	2	46		65.8		
	3	4		5.7		
Timing of metastasis	**Synchronous**	43		61.4		
	Metachronous	27		38.6		
Site of metastasis	**Liver**	15		21.5		
	Lung	2		2.8		
	Peritoneum	4		5.7		
	Bone	2		2.8		
	Non regional Lymph nodes	4		5.7		
	Multiple sites(including liver)	43		61.5		

IO: intestinal obstruction

In our study score 3+ was observed in 4 cases out of 70 cases(5.7%),score 2+ in 2 cases(2.9%) where further confirmation was sought by an amplified SISH, score 1+ in 3 cases(4.3%) and score 0 in 61 cases(87.1%) Thus HER2 was dichotomized as positive (2+, 3+) or negative (≤ 1+).HER 2 positivity was found in 8.57% (6 out of 70 cases). Figures 1 and 2 represent IHC staining for cases.

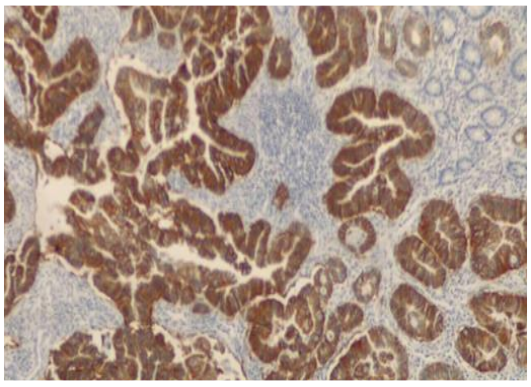

Figure (1): A case of colonic adenocarcinoma showing strong positive circumferential membranous staining for HER2.

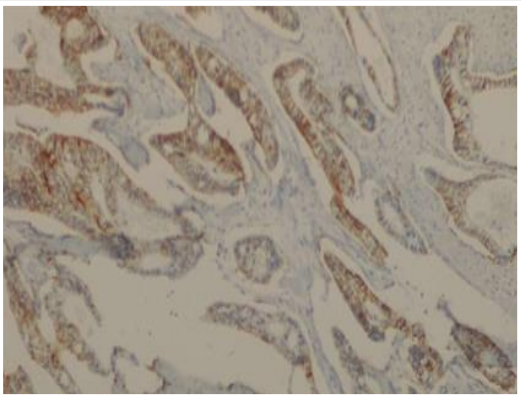

Figure (2): A case of colonic adenocarcinoma showing moderate circumferential membranous staining in more than 10% of neoplastic cells.

To investigate the clinical relevance of HER2 status, we evaluated the association between clinicopathological variables and HER2 status. Although statistically non-significant between both groups yet numerically greater, HER2 positive tumours tended to be more frequent in female patients (5 vs 1, p=0.23), in left sided CRC (5 vs 1, p=0.42) and more metachronous in metastatic presentation than synchronous (p=0.08) (Table 2).

Table (2): Relation between HER2 and tumor characteristics

		Her 2				X^{2*}	P value
		Positive (N=6)		Negative (N=64)			
		N	%	N	%		
Age group (years)	**20-40**	3	50	31	48.4	0.005	0.99
	41-60	2	33.3	22	34.4		
	>61	1	16.7	11	17.2		
Gender	**Female**	5	83.3	35	54.7	1.84	0.18
	Male	1	16.7	29	45.3		
Site of Primary	**Right**	1	16.7	19	29.7	1.72	0.42
	Left	1	16.7	20	31.2		
	Rectum	4	66.6	25	39.1		
Type	**Adenocarcinoma**	5	83.3	46	71.9	0.36	0.67
	Mucinous variant	1	16.7	18	28.1		
Grade	**1**	0	0	1	1.6	1.24	0.43
	2	4	66.6	42	65.6		
	3	1	16.7	3	4.7		
Timing of metastasis	**Synchronous**	2	33.3	41	64.1	3.66	0.08
	Metachronous	4	66.7	23	35.9		
Site of metastasis	**Liver**	1	16.7	14	21.9	5.42	0.37
	Lung	0	0	2	3.1		
	Peritoneum	0	0	4	6.2		
	Bone	1	16.7	1	1.6		
	Non regional Lymph nodes	0	0	4	6.2		
	Multiple sites (including liver)	4	66.6	39	60.9		

*Chi square test (Fisher Exact)

Analysis of different lines of chemotherapy received by the study population revealed that as first line most of the cases received was an oxaliplatin based combination; 51.4% (36 cases).Patients declined treatment or chose to forego it due to poor performance/ comorbidities in 14 cases (20%). Irinotecan based combination chemotherapy comprised 17.2% (12 patients).

As second line therapy 61.4% (43 cases) did not receive any chemotherapy due to intolerance or death. Irinotecan based treatment was administered for 32.9 % (23 cases) whilst oxaliplatin based was given in 5.7% (4 patients).

Rechallenge with oxaliplatin or irinotecan based combinations was the main modality in 4 and 5 patients respectively as a third line option with 5 patients receiving other therapies.

The mPFS for first line chemotherapy was 6 months (range: 3-30 months), while it was also 6 months (range:1-6 months) for second line chemotherapy/care.

Interestingly, by analysis of this data statistical significance appeared regarding response to different lines of chemotherapy/ or care, as HER2 positive cases showed shorter PFS1 (4 vs.6 months, p=0.036) to first line mostly oxaliplatin- based therapy and second line care in which almost a third received irinotecan based treatment (PFS2 3 vs.6 months, p=0.045).

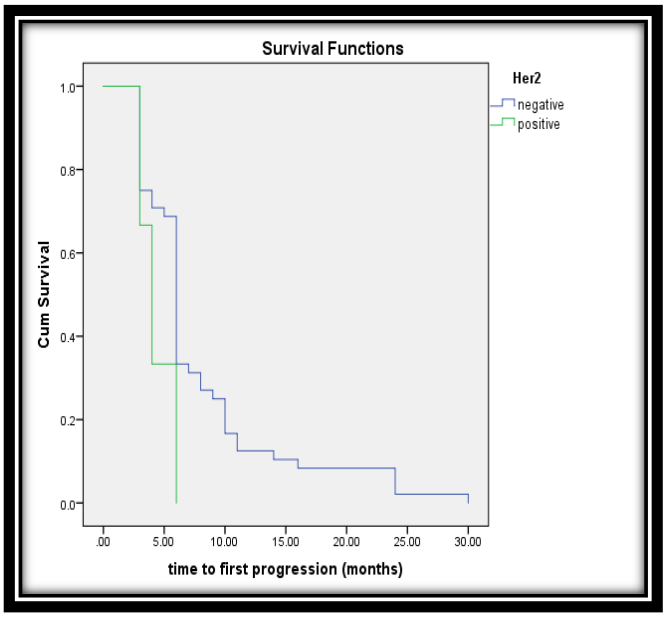

Figure (3): Kaplan–Meier curve of time to first progression-PFS1.

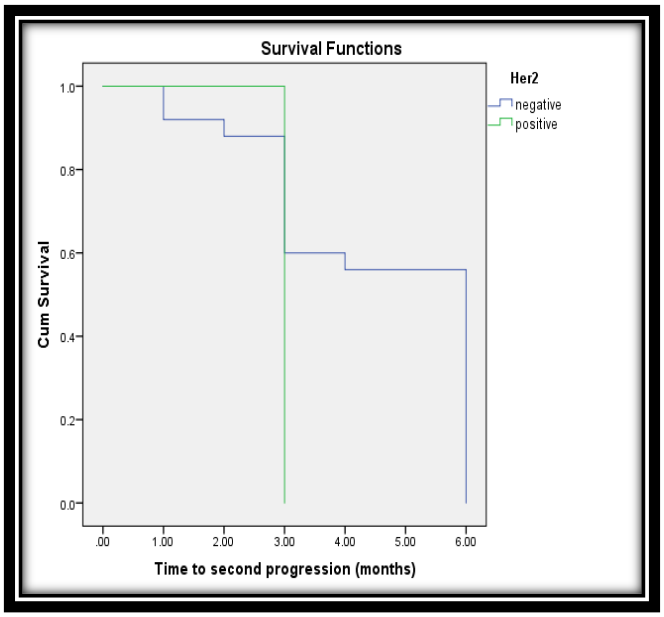

Figure (4): Kaplan–Meier curve of time to second progression-PFS2.

After a mean follow up time of 26.27 months (range 4-60 months) no statistically significant association (p=1) regarding OS was found, despite achieving a numerical difference in median survival time in HER2 positive at 30 months (95% Cl 27.006-32.994; S.E=1.528) and 38 months (95% Cl 31.42-41.26; S.E=3.015) for HER2 negative cases as displayed in figure 5.

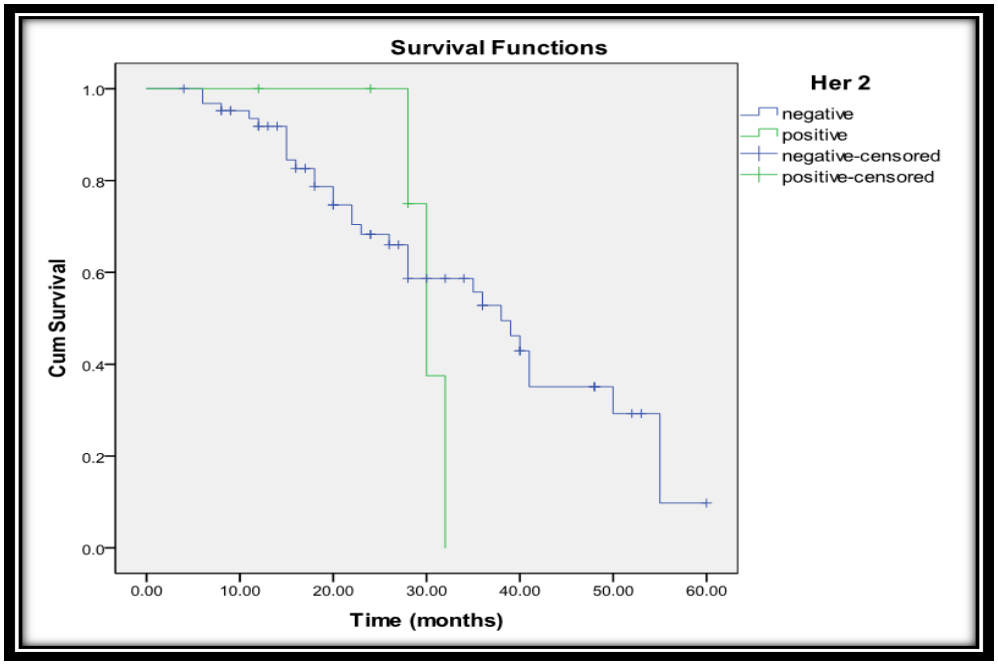

Figure (5): Kaplan–Meier curve illustrating OS between HER2 positive and negative cases.

DISCUSSION

Management of advanced colorectal cancer has witnessed drastic changes in the past few years with the advent of the molecular age and personalized approach underscoring the necessity of finding an effective biomarker that prognosticates and predicts response to therapy. This study found HER2 positivity present in 8.57% of metastatic CRC patients (IHC HER2 score 2+ / 3+) mostly left sided (83.3%) and synchronous (61.4%) in metastatic presentation. In this small subset it was not associated with clinico-pathological features or OS yet some significance was displayed with PFS with first line chemotherapy (mPFS 3 vs. 6 months, p=0.045) and PFS2 (mPFS 4 vs.6 months, p=0.036).

In line with world literature CRC HER2 incidence varies from 2 to 11% but capped off at 5 % for stage IV KRAS exon 2 wild-type tumors [14, 15, 16]. This variation may mainly be a reflection of the lack of a unified scoring system for HER2 testing, small population sizes

in studies with heterogeneous clinicopathologic features [15].

Patients with HER2-positive, KRAS wild type mCRC were selected in the phase II HERACLES[11] trial of HER2-targeted therapy. The HERACLES Diagnostic Criteria were established as a result of this work and are more rigorous in defining HER2 positivity than breast and gastric tumors. A HER2 3+ score in > 50% of cells by IHC, or HER2 2+ score and HER2: CEP17 ratio>2 in >50% of cells by FISH was set to define positivity [11, 16]. The current study did pursue equivocal cases with SISH and set >10% as positive thus possibly explaining the higher rate of HER2 positive cases in the current cohort.

Although we observed HER2 to be more positive numerically only in female patients, left sided CRC and a metachronous metastatic presentation the small sample size prohibits drawing any conclusions.

The PETACC-3 adjuvant chemotherapy trial found distal (60/127, 47%) than in proximal (23/72; 32%) carcinomas displayed amplification of chromosomal regions hosting receptor tyrosine kinases including the ERBB family members HER2 and EGFR (16/127 versus 1/72, Fisher's test $P < 0.001$) [17]. Similar incidence was observed by other studies [18, 19]. Others have found no correlation between HER2 expression and primary sidedness [20, 21].

Whilst a few studies associated aggressive features such as higher grade, stage or lymph node affection to the biomarker, we failed to recognize this link. In fact, a meta-analysis of 30 studies with over four thousand cases of CRC found higher HER2 positivity when comparing Dukes C/D to Dukes A/B (OR = 0.335, 95% CI = 0.198-0.568, P < 0.001) and lymph node metastasis (OR = 1.987, 95%CI = 1.209-3.265, P = 0.007) [22].

Additionally some have conferred positivity of HER2 to anti-EGFR therapy resistance, which is deployed in RAS wild type advanced CRC [20, 23]. We were not able to test at the time of this work for RAS, and although testing has recently become sponsored yet reimbursement policies and finances restrict usage of these therapies on a wide scale.

HER2 positivity had statistical significance for shorter PFS on both first and second progressions. For first line therapy which consisted of mainly oxaliplatin based chemotherapy (p=0.036) this may be assumed, however in the second PFS (p=0.045) irinotecan based chemotherapy was given to a third of the cases adding to the complexity of resistance interpretation as therapy was not unanimous. An extensive review by **Hammond and colleagues** [24] addressed the various mechanisms of resistance in CRC when pertaining to HER2 and focus was on EGFR antagonists solely.

Our study also demonstrated that HER2 positivity had no statistically significant impact on patients' overall survival, but HER2 positive tumours displayed a tendency to poorer courses with shorter estimated median survival time (30 months vs.38 months). The PETACC-8 trial[9] of stage III colon cancer patients, ERBB2 alterations were related to earlier recurrence (HR: 1.55, p = 0.04) and shorter OS (HR: 1.57, p =0.05) significantly. Contrastingly, an analysis of 3256 patients enrolled in the QUASAR, FOCUS and PICCOLO CRC trials[14] a non-significant trend with HER2 expression and recurrence was observed, and no association to PFS or OS thus adding to the perplexity of the categorization of HER2 as a prognostic biomarker.

Of the shortcomings of this work, the limited sample with the added bias of retrospective data collection must be mentioned; nevertheless the percentage expressing the marker correlates well to international rates. Second, full analysis of CRC markers such as RAS were not done to enable further data interpretation on a wider scale neither were targeted therapies given to this cohort due to financial issues. Lastly, SISH confirmation was done for the two cases expressing HER2 2+ by IHC to ascertain their positive status but FISH is commonly deployed in this setting, adding to the complexity of interpretation of this marker in CRC, highlighting the importance of a unified scoring system. However, **Valtora *et al.*** [16] reported complete concordance of 100% between SISH and FISH in a validation study. Pertaining to this final point recent interest has sparked in further sub classifying HER2 to low and high. Preliminary data, again on limited samples due to the rarity of this marker in CRC, deemed HER2 positive as as IHC3+ or IHC2+/FISH positive, HER2-low as IHC 2+/FISH negative or IHC 1+, and HER2 negative as IHC 0. Better prognosis with low HER2 expression was observed compared to HER2 positive [25].

To the best of our knowledge this is the first study of HER2 metastatic CRC as a prognostic marker in our region with relevance to clinico-pathologic features and outcome. This is valuable as it contributes to the growing body of international literature by providing a more varied contribution from different ethnic populations worldwide paving the way for further analysis of disease trends and hopefully a much needed personalized molecularly- oriented approach to therapy in this unique subset of CRC patients.

CONCLUSION

HER2 incidence in metastatic CRC was 8.57% in this series and its prognostic value remains vague. It could be concluded that HER2 positivity is significantly associated with a shorter PFS and displayed a non-significant tendency for a shorter OS. Further studies will elucidate its role to unleash full therapeutic potential in management of this subset of patients.

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Author contribution: MMEB and MYM were responsible for the conception and design of this study. ME, RAY and MMEM performed the study selection

and data extraction, and contributed to the writing of the manuscript. RAY and ME collected statistical output and were major contributors in writing the manuscript. All authors contributed to the article and approved the submitted version.

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