



Immunohistochemical Expression of “c-MET” in Breast Carcinomas

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Abstract

Edited by: Sinisa Stojanoski
Citation: Elleithy NAY, Nakhla GA, Elsheikh SA, Abdelmagid MSE. Immunohistochemical Expression of “c-MET” in Breast Carcinomas. Open Access Maced J Med Sci. 2020 Sep 24; 8(A):824-832. <https://doi.org/10.3889/oamjms.2020.5070>
Keywords: c-MET; Immunohistochemistry; Breast cancer; Clinicopathological variables
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Received: 11-Jun-2020
Revised: 10-Sep-2020
Accepted: 14-Sep-2020
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Funding: This research did not receive any financial support
Competing Interests: The authors have declared that no competing interests exist
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BACKGROUND: Targeted therapies achieved great success in managing breast cancer, however, triple negative breast cancers (TNBCs) lack the expression of traditional therapeutic targets, and other subtypes develop resistance to current therapies. The c-MET receptor emerged as a potential target with well documented pro-proliferative promotility downstream signals and wide network of crosstalk with other effectors. Some reports describe a preferential expression of c-MET in TNBCs and promising results in early anti-c-MET clinical trials. However, the main cause of failure of these trials was attributed to patient selection.

AIM: The objectives of the study were to assessment of c-MET in subtypes of breast cancer and its association with other clinicopathological variables that may predict its expression and possibly establish other rationales to refine patient selection for clinical trials.

MATERIALS AND METHODS: Retrospective immunohistochemical study assessing c-MET (clone SP44) in 55 cases of breast carcinoma. The expression of c-MET > 5% was considered positive.

RESULTS: c-MET was detected in 42% of cases. A statistically significant association of c-MET with extremes of age, advanced prognostic stage, carcinoma with medullary features, and high tumor infiltrating lymphocytes (TILs) was observed for the first time in our study. Grade III, hormone receptor negativity and TNBCs were also significantly associated with c-MET. Only negative progesterone receptor (PR) and high TILs were independently associated with c-MET in a multivariate analysis ($p < 0.05$). No significant association between c-MET and multifocality, size, node status, anatomic stage, lymphovascular or perineural invasion, and Ki-67 expression.

CONCLUSION: PR negativity and high TILs might be useful c-MET predictors and selection tools for clinical trials but further studies are needed to validate the unprecedented findings which may not only aid in patient selection but may also inspire new paradigms in future studies.

Introduction

Breast cancer is the second most common cancer in general and the most common female cancer accounting for a major cause of death among women [1].

Although great advances in targeted anti-human epidermal growth factor-2 (HER-2) receptor and hormonal therapies have ameliorated the general outcome for many types of breast cancer, various resistance mechanisms were later discovered [2].

Moreover, triple negative breast cancer (TNBC) is a subtype of breast cancer that essentially by definition lacks the expression of traditional therapeutic targets – namely, estrogen receptor (ER), progesterone receptor (PR), and HER-2 – and exhibits aggressive clinical course and the poorest survival of all breast cancers [3].

The above challenges called for investigating more therapeutic targets and the c-MET (mesenchymal epithelial transition) transmembrane surface growth factor receptor stood out as a potential target with a well-documented downstream signaling cascade triggering cell cycle progression, cell proliferation,

motility, invasion, and survival culminating in an overall “invasive growth pattern” [4].

In the breast, c-MET is differentially overexpressed in carcinomatous cells as compared with benign tissues, making it a very attractive candidate for targeted therapy [5].

In addition, the c-MET receptor has a rich network of crosstalk with other growth factor receptors, especially the epidermal growth factor receptor family of receptors (including HER-2) and c-MET signaling was shown to contribute to resistance to many growth factor receptor-blocking therapies. Thus, c-MET antagonists may improve outcomes when used in combination with other growth factor inhibitors in targeted therapy strategies [6].

Regarding TNBCs, while some reports demonstrated a preferentially higher rate of c-MET expression in TNBCs [7], conflicting data were also presented [8]. Nevertheless, the most pronounced clinical benefit of c-MET blocking agents in breast cancers in early clinical trials was present in TNBC patients [9].

The main issue behind the failure of these trials has been related to patient selection, that is, the

identification of effective pathological features and biomarkers to select those patients who are likely to derive most benefit from targeted c-MET inhibition allowing for the optimization of outcomes and minimizing unnecessary toxicity exposure [10].

In this study, we explored the expression of c-MET in immunohistochemical surrogates of molecular subtypes of breast cancer and its association with other clinicopathological variables that may predict its expression and possibly establish other rationales to refine patient selection for clinical trials.

Materials and Methods

Retrieval of cases

The material of this cross-sectional study was collected as 55 formalin-fixed paraffin embedded breast carcinoma tissue sections from archives of Pathology Department, Kasr AL-Ainy (Cairo University hospital) and private laboratories in the period between January 2015 and June 2017. The authors obtained the approval of the Ethical Committee in the Faculty of Medicine, Cairo University.

Inclusion criteria included cases of primary breast carcinomas that underwent a modified radical mastectomy or conservative breast surgery with or without axillary clearance. Exclusion criteria included cases with missing data (age of patients at presentation, tumor multifocality, tumor size (T), hormone receptor status, and HER-2 status), exhausted, poorly fixed or unavailable tissue blocks, and tru-cut biopsies.

Histopathological examination

Histopathological examination of H and E stained slides was performed for the confirmation of diagnosis of tumor histotype according to the 2012 WHO Classification of Tumors of the Breast [11].

Tumor histological grading for invasive duct carcinoma was confirmed according to the Nottingham Grading System [12]. The grading of other types was based mainly on the nuclear pleomorphism and mitotic activity [13].

The minimum criteria for perineural invasion diagnosis were the observation of cytologically malignant cells in the perineural space of nerves and in equivocal cases, the observation of total or near-total circumferential involvement was considered [14].

Lymphovascular invasion was defined as the presence of emboli of tumor cells within an endothelial lined space (lymphatic and/or blood vessel) outside the border of the tumor and/or presence of an artery or vein accompanying the involved vessel, and ensuring that

the outline of the tumor cell aggregate does not exactly conform to the shape of the space in which it lies [15], [16].

Regarding the tumor infiltrating lymphocytes (TILs), they were scored following the recommendations of the International TILs Working Group 2014 [17]. We scored stromal TILs subjectively in 10% increments and tumors were defined as High-TILs ($\geq 30\%$) or Low-TILs ($< 30\%$) [18], [19].

Staging and molecular subtyping basis

Tumors staging was performed using the TNM staging system. The cases were further divided into anatomic stages and prognostic stage groups according to the latest edition of the American Joint Committee for Cancer (AJCC) staging manual [20].

ER, PR, HER-2, and Ki-67 status were retrieved from immunohistochemistry reports. ER, and PR-positive nuclear staining in $\geq 1\%$ of tumor cells were considered positive according to the American Society of Clinical Oncology/College of American Pathologists guidelines recommendations [21]. HER-2 status was assessed according to Wolff *et al.*, 2014 criteria [22]. Ki-67 status was retrieved for 29 cases only and a proliferation index ≥ 20 was considered high [23].

Regarding the molecular subtyping, the tumors were classified according to the St. Gallen International Expert Consensus 2013 recommendations and St. Gallen International Expert Consensus 2017 recommendations [24], [25].

C-MET immunohistochemical staining and evaluation

Immunohistochemical staining for c-MET (clone SP44, Ventana Medical Systems) was carried out using rabbit monoclonal antibody directed against c-MET membranous and/or cytoplasmic epitope. Fully automated staining was carried out on the BenchMark XT platform from Ventana utilizing the *VIEW* DAB detection kit using a section of non-small cell lung carcinoma as positive control (Figure 1).

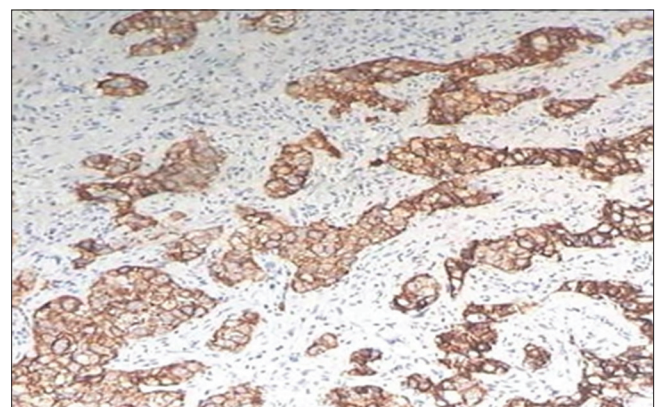


Figure 1: Non-small cell carcinoma of the lung positive control for c-MET expression ($\times 200$ original magnification)

Immunoreactivity for c-MET was observed as cytoplasmic and/or membrane staining in carcinoma cells using literature suggested criteria: A sample was considered c-MET positive if >5% of definitive tumor cells showed immunohistochemical reactivity on the cell cytoplasm [26], [27].

Statistical analysis

We have investigated the association of c-MET expression with the age of patients at presentation, tumor multifocality, tumor size (T), lymph node status (N), AJCC anatomic and prognostic stage groups, histotype, histological grade, TILs, lymphovascular and perineural invasion, hormone receptor status, HER-2 status, Ki-67 expression, and immunohistochemical surrogates of molecular subtypes. Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Sciences) version 25. Simple descriptive statistics (arithmetic mean and standard deviation) were used for quantitative data while frequency (count) and relative frequency (percentage) were used for categorical data. For comparing categorical data, Chi-square (χ^2) test was performed. Exact test was used instead when the expected frequency is <5. Logistic regression multivariate analysis was done to detect independent predictors of c-MET expression. $p < 0.05$ is considered statistically significant and $p < 0.01$ is considered statistically highly significant.

Results

This study included 55 cases of breast carcinoma. The age of them ranged from 22 to 86 years with a mean of 56.87 ± 12.76 years. Multifocal tumor masses were detected in $n = 11$, 20%. The majority of cases 74.55% were classified as T2, while T1 and T3 accounted for 12.73% of cases each. In about half of the selected cases (51%), the detected lymph nodes were free of tumor deposits and N1 was the most common among node-positive tumors accounting for 25.4% of studied cases. The anatomic stage main group distribution was as follows: 9.1% of cases were Stage I, 63.64% were Stage II, and 20% were Stage III. Cases with unverified lymph node status were not classified. According to the AJCC prognostic staging classification, 30.91% of cases were Stage I, 40% of cases were Stage II, and 21.89% of cases were Stage III while 7.27% were unclassified. A total of 24 cases were reclassified according to this prognostic approach, with 14 downstaged cases (25.45%) and ten upstaged cases (18.18%).

The tumor histological subtypes were as follows; invasive duct carcinoma of no special type (NST) ($n = 42$, 76%), and carcinoma with medullary features ($n = 9$, 16%), lobular carcinoma including a single case of

pleomorphic lobular carcinoma ($n = 2$, 4%), and invasive papillary carcinoma and cribriform carcinoma ($n = 1$, 2%) each (Figure 2).

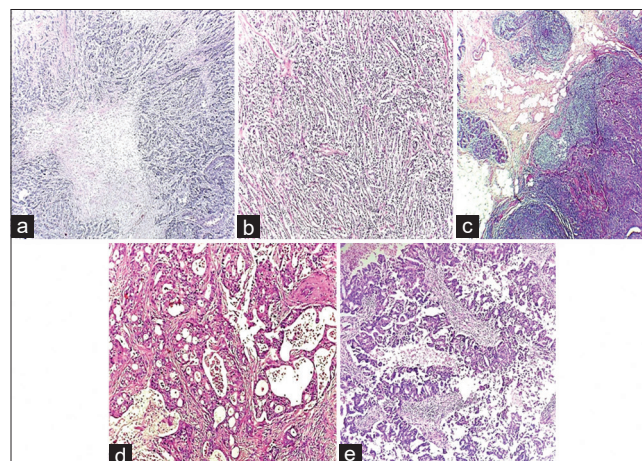


Figure 2: (a) Invasive duct carcinoma no special type, (H and E $\times 100$ original magnification). (b) Invasive lobular carcinoma (H and E $\times 100$ original magnification). (c) Carcinoma with medullary features (H and E $\times 40$ original magnification). (d) Cribriform carcinoma (H and E $\times 200$ original magnification). (e) Invasive papillary carcinoma no special type (H and E $\times 200$ original magnification)

Most of the studied cases were Grade II ($n = 43$, 78%) and the rest were Grade III ($n = 12$, 22%). A high rate of TILs was observed in ($n = 10$, 18%). Tumor-associated lymphovascular invasion was detected in $n = 15$, 27%. Evidence of perineural invasion was detected in $n = 5$, 9% (Figure 3).

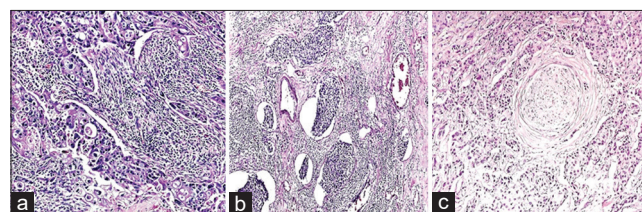


Figure 3: (a) Carcinoma with medullary features, high tumor infiltrating lymphocytes (H and E $\times 200$ original magnification). (b) Lymphovascular invasion (H and E $\times 100$ original magnification). (c) Perineural invasion (H and E $\times 400$ original magnification)

Positive ER expression was detected in $n = 24$, 43.64%, positive PR expression in $n = 20$, 36.36%, and positive HER-2 in $n = 1$, 1.8%. Ki-67 expression was retrieved for only 29 cases, 11 of which (37.93%) showed high Ki-67 expression. The TNBCs accounted for 54.6% of studied cases and luminal A was the most common subtype in the non-triple negative group accounting for 29%, luminal B HER-2 negative represented 14.55% of cases, and a single HER-2 positive case was encountered accounting for 1.8%.

The expression of c-MET was detected in 23 cases (42%) (Figure 4).

The TNBCs were associated with the highest rate of positive c-MET expression versus other molecular subtypes (luminal A and B HER2 negative), while the luminal A subtype showed the highest rate of negative c-MET expression versus other molecular subtypes (luminal B HER-2 negative and triple negative) and these

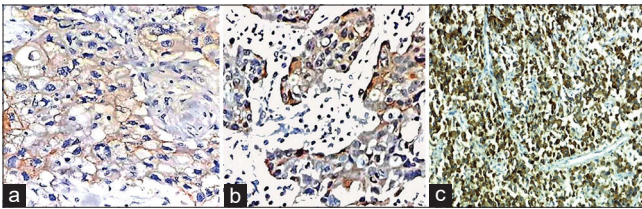


Figure 4: Positive c-MET expression with varying intensities (a) weak c-MET expression (×400 original magnification), (b) moderate c-MET expression (×400 original magnification), (c) strong c-MET expression (×100 original magnification)

two relations were statistically significant (p = 0.003) and (p = 0.026), respectively. All tumors at extremes of age at presentation (<30 and >80 years) were c-MET

Table 1: Relation of c-MET expression with clinicopathological variables

Pathological characteristics	c-MET positive (%)	c-MET negative (%)	p-value
Age			
Extremes (<30 and >80 years)	4 (100)	0 (0)	0.026*
Other cases	19 (37.3)	32 (62.7)	
Multifocality			
Present	2 (18.2)	9 (81.8)	0.097
Absent	21 (47.7)	23 (52.3)	
T status			
T1	2 (28.6)	5 (71.4)	0.901
T2	18 (43.9)	23 (56.1)	
T3	3 (42.9)	4 (57.1)	
Node metastasis			
Absent	11 (39.3)	17 (60.7)	0.54
Present	11 (47.8)	12 (52.2)	
N status			
N0	11 (39.3)	17 (60.7)	0.522
N1	8 (57.1)	6 (42.9)	
N2	1 (20)	4 (80)	
N3	2 (50)	2 (50)	
Main anatomic stage groups			
I	1 (20)	4 (80)	0.612
II	16 (45.7)	19 (54.3)	
III	5 (45.5)	6 (54.5)	
Main prognostic stage groups			
I	1 (5.9)	16 (94.1)	0.001*
II	14 (63.3)	8 (36.4)	
III	7 (58.3)	5 (41.7)	
Histotype			
Carcinoma with medullary features	7 (77.8)	2 (22.2)	0.026*
Other histotypes	16 (34.8)	30 (65.2)	
Invasive duct, NST	16 (38.1)	26 (61.9)	0.314
Other histotypes	7 (53.8)	6 (46.2)	
Histological grade			
I	0 (0)	0 (0)	<0.001*
II	12 (28.6)	30 (71.4)	
III	11 (84.6)	2 (15.4)	
TILs			
High	9 (90)	1 (10)	0.001*
Low	14 (31.1)	31 (68.9)	
Perineural invasion			
Present	1 (20)	4 (80)	0.387
Absent	22 (44)	28 (56)	
Lymphovascular invasion			
Present	6 (40)	9 (60)	0.867
Absent	17 (42.5)	23 (57.5)	
ER			
Positive	4 (16.7)	20 (83.3)	0.001*
Negative	19 (61.3)	12 (38.7)	
PR			
Positive	2 (10)	18 (20)	<0.001*
Negative	21 (60)	14 (40)	
HER-2			
Positive	1 (100)	0 (0)	0.418
Negative	22 (40.7)	32 (59.3)	
Ki-67			
High	4 (36.4)	7 (63.6)	0.717
Low	8 (44.4)	10 (55.6)	
Luminal A			
Present	3 (18.8)	13 (81.3)	0.026*
Absent	20 (51.3)	19 (48.7)	
Luminal B HER-2 negative			
Present	1 (12.5)	7 (87.5)	0.120
Absent	22 (46.8)	25 (53.2)	
Triple negative			
Present	18 (60)	12 (40)	0.003*
Absent	5 (20)	20 (80)	

*Statistically significant. TIL: Tumor infiltrating lymphocyte, PR: Progesterone receptor, ER: Estrogen receptor, NST: No special type.

positive and this relation was statistically significant (p = 0.026).

There was also a higher rate of statistically significant positive c-MET expression in tumors with advanced prognostic stage groups (p = 0.001) and despite that higher rates of positive c-MET expression were also found in advanced anatomic stage tumors, the later relation failed to demonstrate statistical significance (p = 0.612). A higher rate of positive c-MET expression was detected in carcinoma with medullary features versus other tumor histotypes and this difference was statistically significant (p = 0.026). A highly statistically significant association between positive c-MET expression and Grade III tumors (p < 0.001), high TILs (p = 0.001), ER negative status (p = 0.001), and PR negative status (p < 0.001) was also noticed.

There were no significant differences in c-MET expression regarding tumor multifocality (p = 0.097), T status (p = 0.901), lymph node status N (p = 0.522), perineural invasion (p = 0.387), lymphovascular emboli (p = 0.867), HER-2 status (p = 0.418), Ki 67 (p = 0.717), and luminal B HER-2 negative subtype (p = 0.120).

Details of the relation of c-MET expression with clinicopathological variables of studied cases are summarized in Table 1.

Multivariate analysis

Logistic regression was done to detect independent predictors of c-MET and exclude possible confounders, we found that the only independent predictors of c-MET expression were high TILs and PR negative status and all other variables were excluded by forward conditional regression (Table 2).

Table 2: Independent predictors of c-MET expression

c-MET	B	S.E.	Df	p-value	OR	95%CI	
						Lower	Upper
Lymphocytic infiltrate	2.197	1.130	1	0.05*	9.000	0.982	82.496
PR status	-2.773-	1.109	1	0.012*	0.063	0.007	0.549

B: Unstandardized coefficient, SE: Standard error, df: Degree of freedom, OR: Odds ratio, CI: Confidence interval, PR: Progesterone receptor.

Discussion

In this study, we investigated c-MET expression in various breast carcinoma immunohistochemical profiles and the association of c-MET expression with the other pathological variables. Expression of c-MET was detected in 42% of the cases in this study. Similar percent (41.8%) was found using the same clone of monoclonal antibody (SP44, Ventana Systems) [28]. Lower rates as much as 3% and 12% were found by using the same clone with a higher positive cutoff value (>50% tumor area) to assess marker "over" expression [8], [9]. A higher rate (about 69%) was detected using a polyclonal antibody and a low cutoff value (5% of tumor cells) to detect c-MET expression [27].

Of note, the wide range of c-MET positivity across different studies can be attributed to the different study populations as well as the significant variability in c-MET assessment and sampling techniques, usage of polyclonal versus monoclonal antibodies, different antibody clones, and variable scoring systems with variable cutoff values. The use of a monoclonal antibody, in our study, aimed to achieve better reproducibility. The choice of (SP44) clone against the intracellular domain of the receptor, stemmed from previous studies reporting that overexpression of this domain is far more common than the extracellular domain in breast cancer [29]. On a molecular level, results obtained with antibody SP44 were relatively consistent with quantitative real-time PCR and Western blot analysis data [28]. The use of full-face sections rather than tru-cut biopsies stemmed from the previous reports of heterogeneous c-MET expression [30]. The relatively low cutoff (5% of tumor area) was according to previously suggested criteria and in view of the discordance between c-MET expression in tissue biopsy specimens using high immunohistochemical cutoff values and its expression in circulating tumor cells by flow cytometry in some studies [9], [26], [27].

The c-MET expression variation among different age groups (10-year intervals) was statistically significant, with the highest expression in extremes of age of our cohort (<30 and above 80 years). Patients younger than 40 years were previously found to exhibit c-MET overexpression at higher proportions, though the difference was not significant ($p = 0.099$) [3]. These findings can be partially explained and are in line with the emerging evidence that young age breast carcinoma (YABC) is rather a biologically distinct entity with possibly specific molecular profiles that may include c-MET expression [31]. YABC is more likely to exhibit poor differentiation/high grade, advanced stages, and hormone receptor negativity, all of which may confound its association with c-MET [32]. Elderly patients generally have a higher genetic damage burden due to aging and thus may have a greater chance of incurring c-MET aberrations and may also have other confounding variables such as advanced stage or high grade which was truly the case in our study. In contrast, many studies found no significant correlation with patient age at presentation and c-MET expression. These studies either had a narrower patient age range that did not include our extreme age groups 37-69 years or did not stratify patients into age groups in relation to c-MET expression [33], [34]. Similarly, studies that broadly stratified patients into broad dual categories did not show a significant association of c-MET with age which might miss out on the subtle variation among the finer 10 years interval age groups in our study [8], [35], [36].

The percentage of c-MET positive cases was greater in unifocal tumors; however, the relationship between c-MET expression and tumor multifocality was statistically insignificant. To the best of our knowledge, the relationship between tumor multifocality and c-MET

expression in breast cancers has not been thoroughly investigated and our results are in contrast to the association of tumor multifocality and c-MET expression observed in other organs [37], [38], [39], [40]. Thus, larger-scale studies investigating multifocality and c-MET genetic aberrations, protein expression, and associated biomarker expression in breast carcinoma might add or dismiss similar insights.

Higher rates of c-MET expression were detected in larger sized tumors (T2, T3), though the relationship between tumor size (T status) and c-MET expression was statistically insignificant. Most studies similarly showed no significant association with c-MET expression [3], [8], [41]. However, a significant association between c-MET expression and large-sized tumors was reported in a large sample size study ($n = 924$) and in a meta-analysis reviewing many immunohistochemical and non-immunohistochemical technique based studies [28], [42]. These apparently divergent results were reported comparing c-MET scores (high vs. low expression) rather than the incidence of expression *per se* and the high c-MET scores associated with smaller tumor sizes lead to a postulation that c-MET might have a more significant role in the early progression of smaller tumors [32].

The rate of positive c-MET expression was slightly higher in positive lymph node tumors, but the relationship between c-MET expression and lymph node status was statistically insignificant. A similar insignificant association was observed in the majority of encountered studies [3], [8], [27], [41]. However, a significant correlation with node involvement was found in a larger sized sample study using slightly higher cutoff value for c-MET expression [28]. On the contrary, Ho-Yen *et al.*, 2014, by assessing scores rather than rates of expression found a significant relationship between high c-MET and node-negative tumors. The latter findings were explained by the high c-MET expressing basal-like tumors included in that study which have a tendency to metastasize more frequently through the hematogenous route and thus a higher incidence of node negativity [32].

The relationship between c-MET expression and anatomic stage (TNM) groups was statistically insignificant ($p = 0.91$). Similar findings were encountered [27], [42]. However, a significant association between c-MET expression and advanced stage was reported in studies having a large sample size or considering correlation with high score levels [28], [35]. In our study, highest rate of c-MET expression was detected in the most advanced prognostic Stage III; moreover, most of the prognostic Stage I tumors lacked c-MET expression and the relationship between c-MET expression and the prognostic stage groups was statistically highly significant. None of the compared studies assessing c-MET in breast cancer staged their cases according to the most recently published AJCC prognostic staging system. In our study, only the

associations of c-MET expression with the prognostic stages, incorporating the more important grade and hormone status, were statistically significant. In light of this result, the prognostic stage might provide a more reasonable predictor for expression and a helpful tool in patient selection for further studies.

As regard the tumor histotype, no significant association with the invasive duct carcinoma, NST histotype, and similar results were found in the many encountered studies [3], [8], [27], [28]. However, the rate of positive c-MET expression was significantly higher among the carcinoma with medullary features ($p = 0.026$). Yet, Ho-Yen *et al.*, 2014, found that there is no correlation between c-MET scores and carcinoma with medullary features. This apparently divergent result might be due to their larger sample size and correlation with high c-MET scores [32]. Of note, most studies have not adequately addressed specifically the relationship between c-MET and the various histological subtypes, and comparison of c-MET expression was compared in invasive duct carcinoma NST versus minimally represented or an unspecified group of other histotypes [3], [8], [27], [28], [41].

The rate of c-MET positive expression was greater in the Grade III tumors versus Grade II tumors and this relation was statistically highly significant ($p < 0.001$). Similar results were approved in a meta-analysis [42]. Other studies showed no statistically significant relationship between tumor grade and c-MET. Wang *et al.*, 2018, study had only six cases to represent low-grade tumors (Grades I and II) (6% of total cases) which might have affected the statistical association with c-MET [8]. Constantinou *et al.*, 2018, compared c-MET patients with well-differentiated tumors (Grade I) and moderately to poorly differentiated tumors (Grades II-III) [43]. Both Ren *et al.*, 2016, and Constantinou *et al.*, 2018, showed no significant association with tumor grade in exclusively sampled TNBC study population [3], [43]. The loss of a significant impact of the tumor grade status on c-MET expression in a certain group (triple negative cancers) in the later studies might indicate that the association with tumor grade is not in itself predictive of c-MET expression but rather it might be confounded by other variables (such as the receptor status) as will be further demonstrated by multivariate analysis in our study.

The rate of c-MET expression was significantly higher in tumors with high TILs. The relationship between c-MET expression and TILs in breast carcinomas has not been addressed in the previous studies; however, our results can be interpreted in view of the evidence in the literature for a role for c-MET signaling in promoting T cell recruitment by inducing an autocrine chemokine loop [44]. This is further backed up by emerging data describing a TIL pro-survival effect of c-MET signaling by suppressing Programmed Death Ligand-1; an immunomodulatory molecule that triggers apoptosis of TILs, and dual inhibition of both molecules

in experimental models synergistically enhances the tumor suppressing abilities [45].

The relationship between perineural invasion and c-MET expression was statistically insignificant; however, our results are in line with findings in other organ neoplasms as colorectal carcinoma and melanomas [46], [47]. This relation was not tackled in breast carcinoma, and in view of the few cases with perineural invasion in our study ($n = 5$), this relation merits further investigation.

The relationship between lymphovascular invasion and c-MET expression was statistically insignificant ($p = 0.867$). Similarly, there was no significant association between lymphovascular invasion with c-MET expression or its levels in many studies [8], [32], [43].

The rate of c-MET expression was significantly higher in ER-negative and PR-negative tumors and the relationship between hormone receptor status and c-MET expression was statistically highly significant ($p = 0.001$). Similar results were obtained in a large sample study [28]. However, other studies showed no significant association with the hormone receptor status by investigating a different c-MET antibody clone and patients receiving neoadjuvant therapy before c-MET assessment or by considering score levels of c-MET (high/low) expression [27], [35].

The relationship between Ki-67 and c-MET expression was statistically insignificant ($p = 0.717$). A similar insignificant association was reported using Ki-67 cutoff value (14%) close to ours (20%) [3]. Significant association of c-MET with Ki-67 behavior using cutoff values for Ki-67 higher than our study (>30%) was also described [43].

There was a significant variation among molecular subtypes in c-MET expression with significantly higher rate c-MET negativity in Luminal A subtype versus other subtypes and a higher rate of c-MET positivity among the triple negative subtype. Similar findings were found by Kim *et al.*, 2014, and are in keeping with the Ho-Yen *et al.*, 2015, review which showed a significant association of basal subgroup of triple negative tumors with c-MET expression [28], [41]. In contrast, Koh *et al.*, 2014, found that c-MET positive tumors were less likely to have the triple negative subtype than c-MET negative ones. The latter study used a different antibody clone to assess c-MET, had a fewer number ($n = 23$) as well as a smaller proportion (18%) of triple negative cases than our study and the patients were subjected to neoadjuvant therapy before c-MET evaluation which may alter its assessment [27].

The only independent predictors of c-MET were high TILs and negative PR status and all other variables were excluded by forward conditioned regression. Similarly, Carracedo *et al.*, 2009, in a fluorescence *in situ* hybridization/immunohistochemical study showed that negativity for PR significantly correlated with c-MET expression in a multivariate analysis [48].

Conclusion

Tight clinicopathological correlation is needed in future clinical trials to identify clinically relevant score cutoff values for c-MET expression that can be translated into beneficial clinical responses. When considering patients for anti-c-MET clinical trials, patients at extremes of age (<30 and above 80 years), and prognostic stage rather than the anatomic stage groups, Grade III tumors, carcinoma with medullary features, tumors with high TILs, and TNBCs are more likely to express c-MET and as such, they are suitable candidates. By excluding confounding factors, high TILs and PR negative status were especially important and independently significant in a multivariate analysis.

Tumor multifocality, tumor size, lymph node status, anatomic stage, lymphovascular or perineural invasion, and Ki-67 expression are less likely to reflect c-MET status. Many factors that failed to demonstrate a significant relation with c-MET, in our study, were either poorly represented (such as HER-2 positive tumors) or have been shown to significantly correlate with c-MET in other studies or in other organ cancers and as such merit further investigation in large scale studies.

To the best of our knowledge, tumors at extremes of age, carcinoma with medullary features, and prognostic stage were not previously recognized in the literature as significant predictors of c-MET expression. Moreover, high TILs relation with c-MET expression in breast carcinoma has never been addressed. The latter relation retains its weight in multivariate analysis and merits special consideration in the light of emerging back up data depicting a pro-survival effect of c-MET on TILs, as such, its utility may surpass patient selection to inspire a whole new paradigm in future studies and clinical trials.

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