Expression of mesenchymal–epithelial transition factor in estrogen receptor-negative breast carcinoma

Dalia M. Abouelfadl[a], Noha N. Yassen[a], Marwa E. Shabana[a], Heba A. Abdelal[b], Hebat Allah A. Amin[c]

[a]Department of Pathology, National Research Centre, [c]Department of Pathology, Faculty of Medicine, Helwan University, Cairo, Egypt, [b]Department of Pathology and Laboratory Medicine, Yale University Hospital, New Haven, Connecticut, USA

Correspondence to Noha N. Yassen, MD, Department of Pathology, Medical Division, National Research Institute, Cairo, Postal Code: 11571, Egypt. Tel: 0228476482, Mob: 01005041171; E-mail: noha.nazeeh@gmail.com

Received: 8 December 2021
Revised: 20 January 2022
Accepted: 7 February 2022
Published: 4 July 2022

Journal of The Arab Society for Medical Research 2022, 17:59–67

Background/aim
Breast carcinoma is a worldwide, heterogeneous disease that affects even the young patient population. The receptor tyrosine kinase mesenchymal–epithelial transition factor (c-Met) is suggested to be associated with reduced survival in cases of breast cancer. Few studies have specifically addressed the association between the c-Met and molecular subtype of breast cancer. This study aimed to evaluate the c-Met expression in estrogen receptor (ER)-negative breast cancers with different subtypes and its relation to the standard prognostic indicators.
Patients and methods
We examined the expression of c-Met in triple-negative and human epidermal growth factor receptor 2 enriched (i.e. ER-negative) breast cancers. Sixty formalin-fixed paraffin-embedded breast cancer surgical specimens were studied by immunohistochemistry. Prognostic indicators were analyzed with Cox models adjusted for clinical and pathological factors.
Results
Sixty-nine percent of cases were positive for Met. The reported mean Remmele score was 7.80±4.32. A significant positive correlation was observed between the tumor type, nodal status, multicentricity, and ductal carcinoma in situ ($P<0.05$). However, correlation with the Remmele score was borderline as regards the grade ($P=0.065$) and lymphovascular invasion ($P=0.059$).
Conclusions
Most of ER-negative breast carcinomas showed median to maximum Met expression and were associated with worse prognostic factors. So it can be used as a prognostic marker.

Keywords:
breast cancer, mesenchymal–epithelial transition factor receptor, targeted therapy, triple-negative breast cancer

J Arab Soc Med Res 17:59–67

1687-4293

Introduction

Worldwide, breast cancer is considered the most common cancer among females. According to the estimates of the GLOBOCAN 2018, breast cancer account for 11.6% of the reported malignancies. The number of newly reported cases in 2018 was 2.1 million. It is a leading cause of death with a death rate of 626 679 (6.6%) [1]. According to the International Agency for Research on Cancer (IARC) in 2019, 35.1% (23 081) new breast cancer cases were reported among Egyptian females[2].

Breast cancer is considered to be a heterogeneous disease and thus tumors with similar morphology and clinical stage may have diverse molecular changes imparting diverse prognostic outcomes. In breast cancer patients, the disease stage is the main prognostic factor [3].

Other important prognostic factors include cancer histological type, tumor grade, steroid receptor expression, and human epidermal growth factor receptor 2 (HER-2) expression [4]. Breast cancer immunohistochemical and molecular subtypes should be identified as they convey different clinical, biologic, and therapeutic implications [3].

Testing selected immunohistochemical and molecular markers and their association with the standard prognostic indicators may impart an aid to reclassify subgroups of breast cancers. This may also help to introduce new treatment strategies to improve the clinical outcome of estrogen receptor (ER)-negative patients [5].

A potential candidate biomarker is the tyrosine kinase receptor mesenchymal–epithelial transition factor

(c-Met), which has a key role in cell oncogenesis and angiogenesis. Previous studies documented its role in tumorigenesis [6]. Amplification of the c-Met gene is documented in the early stages of breast cancer as well as in metastatic disease [7,8].

Currently, c-Met is under investigation as a potential targeted therapy for several human cancers, as a result of its key function in tumorigenesis [9–12]. However, these are limited in breast cancer [13].

In their meta-analysis, Yan *et al*. [6] suggested that c-Met overexpression has a significant correlation with poor relapse-free survival and overall survival in Western breast cancer patients. This correlation was insignificant in Asian patients. However, studies were deficient in Africa [6].

The purpose of this study was to determine c-Met expression levels and their correlation with the ER-negative subset of breast carcinomas in Egypt. We aim to delineate the immunohistochemical and clinical associations including its prognostic significance in ER-negative tumors. We also aim to shed light on the possible role of subsequent cytogenetic studies in the development of c-Met inhibitors, targeted therapy in this subset of patients.

Patients and methods

Cases

The present study obtained 60 paraffin blocks retrospectively with their clinical history including the age of patient and operative findings. Pathological findings include the size of the tumor, its histologic subtype, tumor grade according to Nottingham modification of the Bloom–Richardson system presence of ductal carcinoma in situ (DCIS) and specification of its type and its percentage from the tumor bulk, axillary lymph node status, and remarkable lymphoplasmacytic reaction [14].

Tissues

Sixty formalin-fixed paraffin-embedded breast cancer surgical specimens were selected based on their negativity for ER. The samples were retrieved from the National Research Center and private practice archives. The submitted specimens were either modified radical mastectomies or conservative breast surgeries. Serial sections of 5 μm thickness were prepared from each paraffin block; one of them was mounted on a glass slide and stained by hematoxylin and eosin for histological evaluation. Cases of infiltrating breast carcinoma were stained for ER; ER-negative cases were selected for our study and were further stained with the c-Met receptor, progesterone receptor (PR), and HER-2neu.

Ethical approval

The present study was conducted with the Code of Ethics of the World Medical Association, according to the principles expressed in the Declaration of Helsinki. This study has been approved by the local Ethics Committee of National Research Centre under approval number 18/134. The used materials were derived from archived tissue samples embedded in paraffin blocks. The blocks and required data were collected from the patient reports after approval of the head of the laboratory and were anonymized.

Immunohistochemistry

Histologic sections were studied by immunohistochemistry with ER, PR, HER-2, and c-Met using a standard avidin–biotin–peroxidase system.

The sections were deparaffinized in xylene, and then were hydrated through a series of graded alcohols (95–70%), distilled water, and phosphate-buffered saline (at pH 7.6). The slides were then retrieved by immersion in protein kinase.

Then the endogenous peroxidase activity was inhibited by incubation in 3% hydrogen peroxide (H_2O_2) for 5 min. After washing with Tris-buffered saline, the sections were incubated with the primary antibody for 1 h at room temperature.

The antibody clones that were used were: those for the ER (ID5 Biogenex, USA Office 48810 Kato Road, Suite 200E, Fremont, CA, USA), the PR (PR 88 Biogenex), and HER-2 (EP1045Y Biogenex). The antibody clones that were used were c-Met (Novus bio NBP2-44306SS). The sections were washed in Tris-buffer and incubated with the avidin–biotin–peroxidase system (DAKO, Produktionsvej 42, 2600 Glostrup, Denmark) for 30 min. The peroxidase reaction was detected by the addition of diaminobenzidine tetrahydrochloride.

The PR score was based on the proportions and the intensities of the stained nuclei [5,6]. The HER-2 scores were based on the intensities and proportions of cells that showed membrane staining. Strong HER-2 staining of 3+ is reported when uniform and intense membrane staining of more than 30% of invasive tumor cells is noticed. A negative HER-2/neu result is reported for immunohistochemical staining of 0 or 1+ [5,7].

c-Met expression was recorded as regards the percentage of expression and intensity of expression. Immunoreactivity score for c-Met was performed using the Remmele score (0–12), that is by multiplying the intensity score by the percentage of staining score:

(1) Intensity: a scale from 0 (negative) to 3 (maximum), (0=negative, 1=minimum, 2=median, and 3=maximum). c-MET was scored as positive if any minimum or maximum cytoplasmic and/or membranous carcinoma cell staining was observed [8].
(2) Percentage: a scale from 1 to 4, (10%=1; <40%=2; <70%=3; >70%=4).
(3) Remmele score=intensity score x percentage [15].

Statistical analysis

Data were coded using SPSS (Statistical Package for the Social Sciences, Chicago, Illinois, USA), version 25. Data was recorded using mean, SD, median, minimum, and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data.

Comparisons between quantitative variables were done using the nonparametric Mann–Whitney tests test for independent samples for comparing two groups and Kruskal–Wallis for comparing more than two groups [16]. *P* values less than 0.05 were considered as statistically significant.

Results

Patient characteristics and clinicopathological parameters

A total of 60 ER-negative breast cancer surgical specimens of infiltrating breast carcinoma were included. Description of the tumor characteristics is presented in Table 1. The age of the patients ranged between 32 and 70 with the mean age being 48.02±9.25 years and a median of 50 years. Of the patients 49 underwent modified radical mastectomies (81.7%).

The mean tumor size was 4.29 cm (T2). Infiltrating ductal carcinoma was the predominant histopathological type reported in 41 (68.3%) cases, while the mucinous type was only reported in two (3.3%) cases. Grade II tumors constituted 76.7% of the cases and 23.3% were of grade III. None of the reported cases was of grade I.

The histopathological evaluation showed a large proportion of patients (50 cases) with desmoplastic reaction, while DCIS was noticed in 36 (60.0%) patients. On the other hand, a minority of cases showed multicentricity in 20% of patients (12 of 60 patients), lymphoplasmacytic infiltrate was noted in 28 (46.7%) cases while lymphovascular invasion was reported in 13 (21.7%) of the 60 cases.

Table 1 Clinicopathological parameters of estrogen receptor-negative breast carcinoma

	Count	Column (%)
Operation		
CBS	11	18.3
MRM	49	81.7
Tumor type		
IDC	41	68.3
ILC	12	20.0
Medullary	5	8.3
Mucinous	2	3.3
Tumor size		
T1	15	25.0
T2	23	38.3
T3	22	36.7
N stage		
N0	20	33.3
N1	5	8.3
N2	14	23.3
N3	21	35.0
Grade		
II	46	76.7
III	14	23.3
Multicentricity		
Yes	12	20
No	48	80
Inflammatory cells		
Yes	28	46.7
No	32	53.3
Lymphovascular invasion		
Yes	13	21.7
No	47	78.3
Desmoplasia		
Yes	50	83.3
No	10	16.7
DCIS		
Yes	36	60.0
No	24	40.0
PR		
+ve	23	38.3
–ve	37	61.7
HER-2		
+ve	30	50.0
–ve	30	50.0
c-Met intensity		
–ve	7	11.7
Minimum	11	18.3
Median	17	28.3
Maximum	25	41.7

DCIS, ductal carcinoma in situ; HER-2, human epidermal growth factor receptor 2; IDC, infiltrating duct carcinoma; ILC, infiltrating lobular carcinoma; Met, mesenchymal–epithelial transition factor; PR, progesterone receptor.

The nodal stage was classified as N0 in 20 (33.3%) cases, N1 in five (8.3%) cases, N2 in 14 (23.3%) cases, and N3 in 21 (35.0%) cases.

Immunohistochemical results

According to the Remmele score, the recorded maximum score was 12, mean score was 7.80±4.32 with the median being 8 (Fig. 1). All cases were confirmed to be ER-negative nuclear staining on restaining the tissue sections; 23 cases showed positive nuclear staining for PR (38.3% of the cases) as shown in Fig. 2.

Half of the cases showed positive membranous overexpression in HER-2neu (score +3) and 50% were HER-2neu negative (scores 0 and +1). None showed weak overexpression (score +2) (Fig. 2 and Table 2).

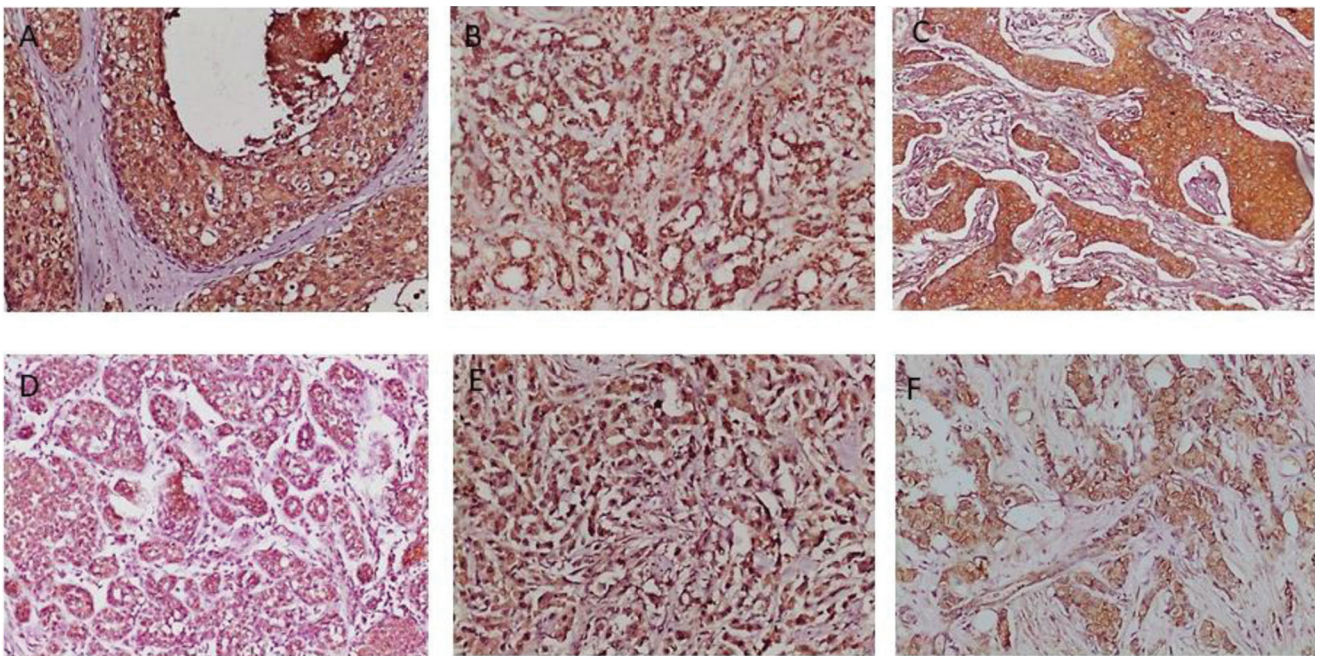
The membranous staining of c-Met was expressed in 88.3% of cases with gradual intensities minimum, median, and maximum as 18.3, 28.3, and 41.7%, respectively (Table 1). c-Met expression using Remmele score and c-Met expression percentage are represented in detail in Table 2 and Table 3, respectively.

The association between Remmele score of Met expression and the clinicopathological prognostic parameters is listed in Table 2, which showed that 88.3% of the examined ER-negative cases was c-Met positive. The correlation between Met expression with PR and HER-2 was nonsignificant (P=0.164 and 0.096, respectively). A significant positive correlation was observed between Remmele score and some of prognostic variables as the tumor type, nodal status, multicentricity, and DCIS being (P<0.001, P=0.025, P<0.001, and P=0.001, respectively). However, correlation with the Remmele score was borderline as regards the grade (P=0.065) and lymphovascular invasion (P=0.059).

On the other side, the majority of cases (70%) showed median to maximum reactions for c-Met. The intensity of c-Met expression was assessed. A significant correlation was noticed between the percentage of c-Met expression – in isolation from the intensity – in association with a grade (P=0.010) and lymphovascular invasion (P=0.022), as shown in Table 3.

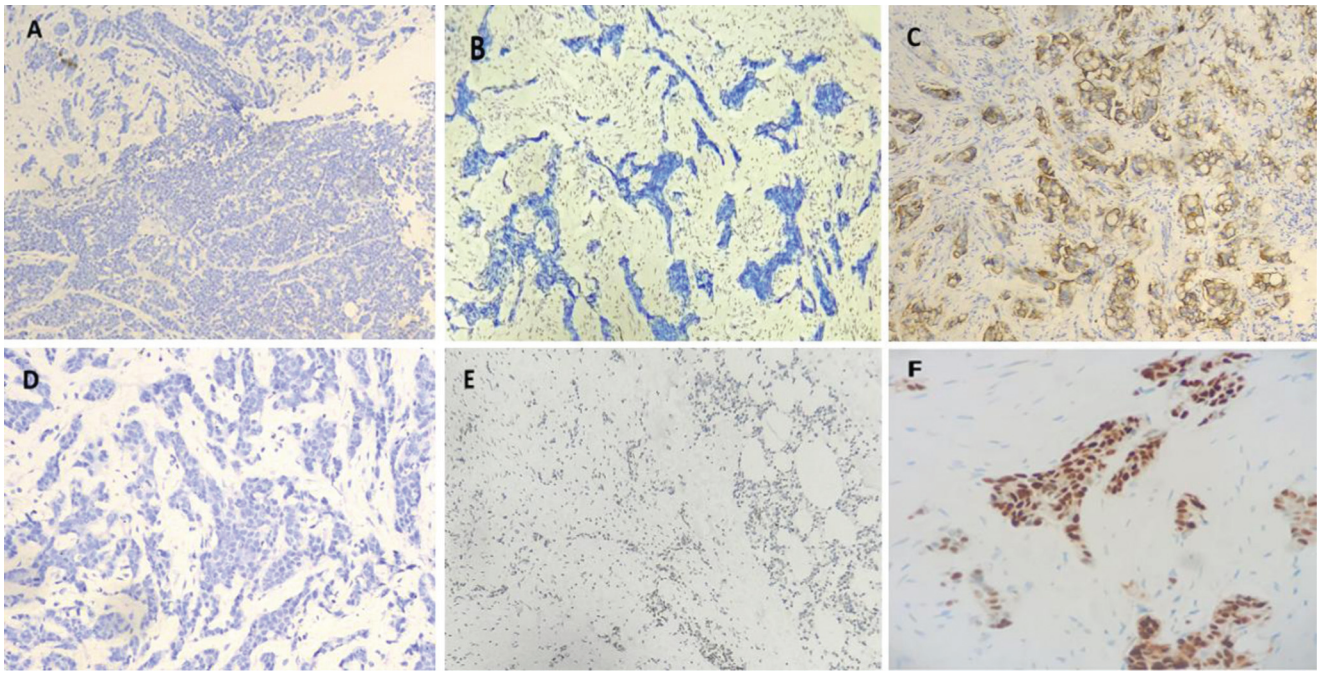
A significant positive correlation was observed between Remmele score and some of prognostic variables as the tumor type, multicentricity DCIS, and nodal stage. However, correlation with the Remmele score was borderline as regards the nuclear grade and tumor size (Fig. 3).

Figure 1



Photomicrography of c-Met membranous immunostaining with Remmele scoring: (a) DCIS, Remmele score 8; (b) tubular carcinoma, Remmele score 12; (c) infiltrating duct carcinoma NOS, Remmele score 8; (d) LCIS, Remmele score 6; (e) lobular carcinoma, Remmele score 12; (f) infiltrating duct carcinoma NOS, Remmele score 6 (Met immunohistochemical staining, ×100). DCIS, ductal carcinoma in situ; Met, mesenchymal–epithelial transition factor.

Figure 2



Photomicrography of ER, HER-2, PR expression: (a) ER negative, (b) HER-2 negative, (c) HER-2 positive; (d) PR negative; (e) PR medium positive; (f) PR positive (ER, HER-2, PR immunohistochemical staining, respectively, ×100). ER, estrogen receptor; HER-2, human epidermal growth factor receptor 2; PR, progesterone receptor.

Discussion

Breast cancer is a highly heterogeneous disease. Present clinical classification of breast cancers is based mainly on the expression of ER, PR, and HER-2. These are categorized into four groups, ER+/PR+/HER-2–, ER+/PR+/HER-2+, ER–/PR–/HER-2+, and ER–/PR–/HER-2– [triple-negative breast cancer (TNBC)]. Molecular subtypes are luminal A, luminal B, HER-2 enriched, triple-negative/basal-like, and normal-like. ER and HER-2 also serve as prognostic markers and direct therapeutic strategies [17,18]. Among these intrinsic subtypes, HER-2 overexpression and TNBC are of particular interest due to the aggressive clinical course they follow and the lack of standard targeted hormone therapy [19].

The tyrosine kinase c-Met fosters a complex invasive growth pathway promoting cell proliferation, survival, invasion, and angiogenesis. In the last decade, a significant number of research articles have described c-Met overexpression and its suggested pathway and role in the progression of breast cancer [6,20–29]. However, studies in Africa are still deficient.

In breast cancer, molecular studies highlighted the role of c-Met deregulation on carcinogenesis and the development of aggressive phenotypes, as suggested by the higher incidence of mammary-invasive carcinomas in mouse models harboring Met mutations [30]. Noteworthy, the discrepancies in the methodology and scoring systems used for the c-Met analysis hinder, to some extent, the comparative analysis among them [6].

c-Met shows mainly a membranous expression in the resting epithelial cells. Following hepatocyte growth factor stimulation the receptor is internalized with a predominantly cytoplasmic/perinuclear expression. Thus, c-Met overexpression is observed in the cytoplasm as well as the membranes of cancer cells and DCIS as in the majority of tumors derived from the epithelium [31].

Previous study has observed the expression of c-Met in both cancer and normal breast tissue. However, in the majority of histological specimens, the expression of the c-Met protein was more intense in cancer compared with the surrounding physiological mammary gland tissue [29].

According to one of some relevant studies, across all the subtypes of breast cancers, c-Met is overexpressed in 14–53.6% of the cases [6]. Also in a previous one that studied 924 invasive breast cancer cases and proved that Met was expressed in 41.8% [32].

Table 2 Correlation between mesenchymal–epithelial transition factor expression by Remmele score and prognostic variables

Prognostic variables	c-Met expression by Remmele score					
	Mean	SD	Median	Minimum	Maximum	*P* value
Tumor type						
IDC	6.93	4.29	8.00	0.00	12.00	<0.001*
ILC	12.00	0.00	12.00	12.00	12.00	
Medullary	8.00	0.00	8.00	8.00	8.00	
Mucinous	0.00	0.00	0.00	0.00	0.00	
Operation						
CBS	6.91	4.32	6.00	0.00	12.00	0.441
MRM	8.00	4.33	8.00	0.00	12.00	
Tumor size						
T1	6.40	5.19	4.00	0.00	12.00	0.525
T2	8.43	3.63	8.00	0.00	12.00	
T3	8.09	4.32	8.00	0.00	12.00	
N stage						
N0	5.35	4.50	4.00	0.00	12.00	0.025*
N1	7.60	4.98	8.00	0.00	12.00	
N2	9.43	2.41	8.00	6.00	12.00	
N3	9.10	4.17	12.00	0.00	12.00	
Grade						
II	8.39	4.01	8.00	0.00	12.00	0.065
III	5.86	4.87	8.00	0.00	12.00	
Multicentricity						
Yes	10.60	1.96	12.00	8.00	12.00	<0.001*
No	6.40	4.51	6.00	0.00	12.00	
Inflammatory cells						
Yes	7.75	4.11	8.00	0.00	12.00	0.792
No	7.84	4.56	10.00	0.00	12.00	
LVI						
Yes	9.54	4.48	12.00	0.00	12.00	0.059
No	7.32	4.19	8.00	0.00	12.00	
Desmoplasia						
Yes	7.88	4.10	8.00	0.00	12.00	0.917
No	7.40	5.50	10.00	0.00	12.00	
DCIS						
Yes	6.33	4.20	6.00	0.00	12.00	0.001*
No	10.00	3.54	12.00	0.00	12.00	
PR						
+ve	7.17	3.47	8.00	0.00	12.00	0.164
–ve	8.19	4.77	12.00	0.00	12.00	
HER-2						
+ve	8.90	3.39	8.00	3.00	12.00	0.096
–ve	6.70	4.89	8.00	0.00	12.00	

DCIS, ductal carcinoma in situ; HER-2, human epidermal growth factor receptor 2; Met, mesenchymal–epithelial transition factor; PR, progesterone receptor. *Significant difference at *P* value less than 0.05 using Kruskal–Wallis test.

In the present study, we measured c-Met intensity, percentage, and the Remmele score. On using the Remmele score, we identified more significant correlations between the cancer subtypes and among the other prognostic indicators (grade, nodal metastasis, and multicentricity).

In the current study, the immunoreactivity for c-Met protein was evaluated in 60 patients, seven (13.3%) cases of whom did not stain for c-Met protein. Our results were different from those achieved by Lengyel *et al*. [23], who used the same scoring system, Remmele score, evaluating the c-Met expression in a group of 40 patients with breast cancer and axillary lymph node involvement. They showed minimum immunoreactivity for this protein in 69% of patients and a maximum one in 31%. Another previous study also evaluated the percentage of cells, as well as the intensity of c-Met immunoreactivity; however, they used a scale other than Remmele score. Their results

Table 3 The correlation between percentage of mesenchymal–epithelial transition factor expression and the prognostic variables

Prognostic variables	c-Met expression %					*P* value
	Mean	SD	Median	Minimum	Maximum	
Tumor type						
IDC	66.83	31.66	80.00	0.00	100.00	0.030*
ILC	85.83	5.15	90.00	80.00	90.00	
Medullary	74.00	8.94	70.00	70.00	90.00	
Mucinous	0.00	0.00	0.00	0.00	0.00	
Operation						
CBS	67.27	26.11	70.00	0.00	90.00	0.443
MRM	69.39	31.32	80.00	0.00	100.00	
Tumor size						
T1	55.33	36.23	60.00	0.00	90.00	0.065
T2	71.74	24.24	80.00	0.00	100.00	
T3	75.45	29.88	90.00	0.00	100.00	
N stage						
N0	55.00	32.20	60.00	0.00	90.00	0.008*
N1	62.00	34.93	80.00	0.00	80.00	
N2	79.29	9.97	80.00	70.00	100.00	
N3	77.14	32.43	90.00	0.00	100.00	
Grade						
II	75.00	24.74	80.00	0.00	100.00	0.010*
III	49.29	38.52	70.00	0.00	90.00	
Multicentricity						
Yes	90.50	8.26	90.00	80.00	100.00	<0.001*
No	58.25	31.53	70.00	0.00	90.00	
Inflammatory cells						
Yes	69.64	33.83	80.00	0.00	100.00	0.374*
No	68.44	27.25	80.00	0.00	100.00	
LVI						
Yes	76.92	34.49	90.00	0.00	100.00	0.022*
No	66.81	28.98	80.00	0.00	100.00	
Desmoplasia						
Yes	71.40	27.70	80.00	0.00	100.00	0.386
No	57.00	40.29	80.00	0.00	90.00	
DCIS						
Yes	65.28	33.51	75.00	0.00	100.00	0.520
No	74.58	24.13	80.00	0.00	90.00	
PR						
+ve	69.57	22.05	70.00	0.00	100.00	0.225
–ve	68.65	34.65	80.00	0.00	100.00	
HER-2						
+ve	76.67	14.93	80.00	50.00	100.00	0.631
–ve	61.33	38.93	80.00	0.00	100.00	

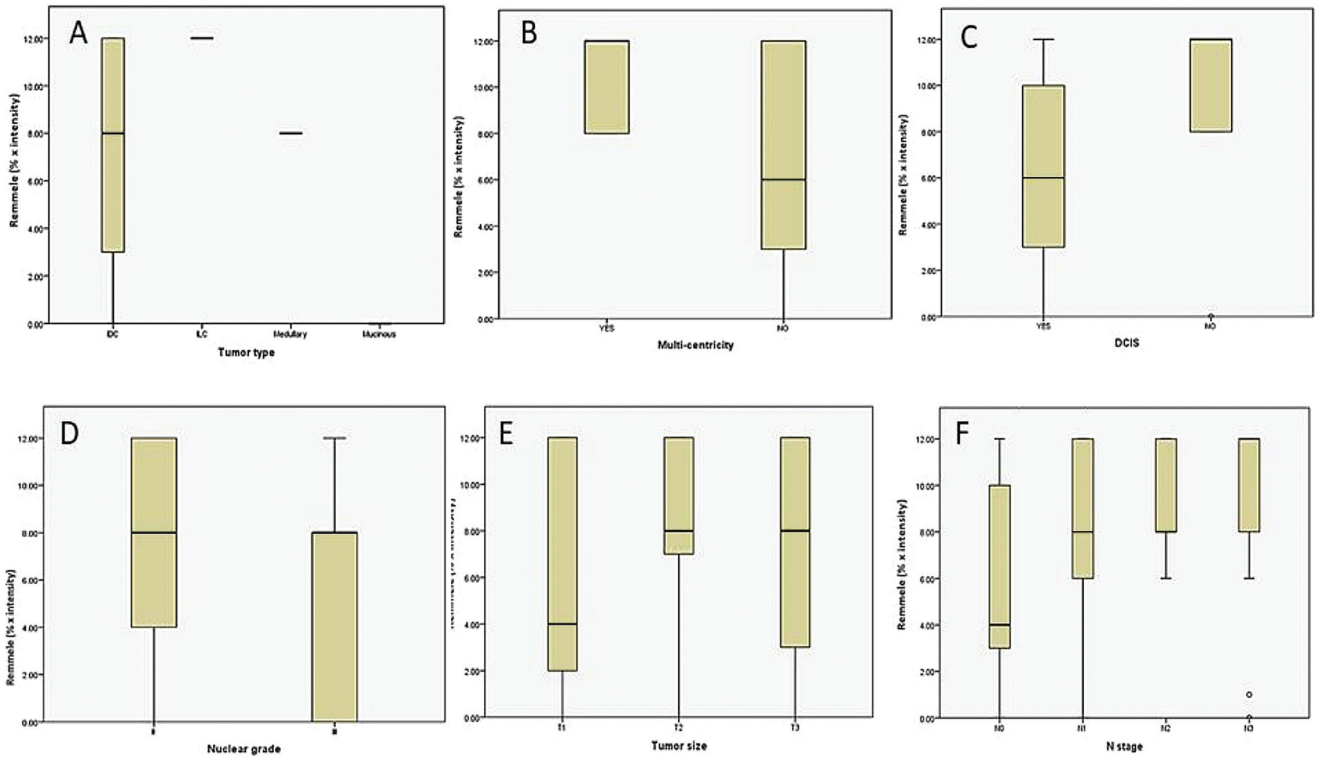
DCIS, ductal carcinoma in situ; HER-2, human epidermal growth factor receptor 2; Met, mesenchymal–epithelial transition factor; PR, progesterone receptor. *Significant difference at *P* value less than 0.05 using Kruskal–Wallis test.

were contradictory to the present study as 25 and 75% of breast cancer tissues showed maximum and weak immunoreactivity for c-Met protein, respectively [33].

Other studies describing the pattern of c-Met expression in breast cancer tissue, depended on the percentage of cells with positive immunohistochemical staining for c-Met, and multifaceted cutoff limits were used [24]. Ocal *et al.*'s [27] study used the image analysis system (area percentage) to evaluate the point of maximal c-Met expression.

We think the selected method of scoring and analysis for c-Met in breast cancer tissue by assessing both the percentage of stained cells and the intensity of expression following the Remmele scoring system is precise. It also provides more detailed information about the pattern of immunoreactivity. However, it limits the possibility of comparing our results with

Figure 3



Correlation between c-Met expression by Remmele score and different prognostic parameters using Kruskal–Wallis test summary: (a) significant correlation with tumor type, (b) significant correlation with multicentricity, (c) significant correlation with DCIS, (d) nonsignificant with nuclear grade, (e) nonsignificant correlation with tumor size, (f) significant correlation with lymph node stage. DCIS, ductal carcinoma in situ; Met, mesenchymal–epithelial transition factor.

those yielded by other authors, as few evaluated both the percentage of stained cells and the intensity of expression.

The analysis of c-Met expression among different clinical subtypes of breast cancer shows a significant correlation between the levels of c-Met expression. Mucinous carcinoma showed a negative Met expression. There is a significant correlation between c-Met expression and histological type of carcinoma.

However, the correlation is nonsignificant among breast cancer subtypes on considering the percentage in isolation from the intensity. This explains the contradictory results concerning c-Met expression in the various studies, as the methodology in analyzing the c-Met expression differs from one study to another [22,30,33,34].

In our study, there is a significant correlation between c-Met expression and histological type of carcinoma, and lymph node status, which came with a previous study that claimed that lymph node status is the best prognostic indicator of breast cancer and reported higher c-Met expression in metastatic lymph nodes than the primary tumor [23].

In the examined ER-negative cases, 88.3% were c-Met positive. However, on using Remmele score the correlation with HER-2/neu is insignificant. On analyzing the intensity of c-Met with HER-2/neu, the correlation is borderline. Lindemann *et al.* [21] also did not observe the correlation between c-Met and HER-2/neu expression. Other researchers in their meta-analysis reported that in HER-2/neu-positive breast carcinomas, c-Met might not be associated with prognosis [6].

A previous study declared that c-Met showed high expression in ER-negative and ER-negative/ HER-2 negative cancer [24]. The c-Met overexpression was reported to be observed in a wide range from 3.8% in a cohort of 78 ER and HER-2-positive invasive breast cancer to 70.4% in a study of 257 patients with invasive breast cancer regardless of the hormone receptor status or the HER-2 status [35,36].

The result of Xixi Z. *et al.* [20] confirmed that c-Met overexpression was independent of the hormone receptor status. However, another study established that there was no significant difference of c-Met expression between the TNBC and the non-TNBC group, which indicated that c-Met could be a target for

breast cancer regardless of the hormone status [37]. Also, it was proved that c-Met might act at the early stage of breast cancer, and its expression on postoperative pathology to predict prognosis and guide postoperative treatment [6].

Conclusion

There is a variable relationship between c-Met and established prognostic factors, possibly reflecting the complexity of HGF/c-Met signaling. Crucially, we have shown an association between c-Met and ER-negative breast cancer that is independent of other prognostic factors and biomarkers associated with this aggressive subtype of breast cancer. So, it can be used as a prognostic marker for breast carcinoma.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Bray F, Jacques Ferlay ME, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Cancer J Clin 2018; 68:398–404.

2 The International Agency for Research on Cancer :WHO Cancer fact sheets worldwide 2019.

3 Sinn HP, Kreipe H. A brief overview of WHO classification of breast tumor, 4th edition, focusing on issue and updates from 3rd edition. Breast Care 2013; 8:149–154.

4 Lee H, Jung SY, Ro JY, Kwon Y, Sohn JH, Park IH, *et al.* Metaplastic breast cancer, clinicopathological features and its prognosis. J Clin Pathol 2012; 65:441–446.

5 Stopeck AT, Swart R, Downey L. Breast cancer risk factors. E Medicine from webmed. Updates Sep. 26, 2012.

6 Yan S, Jiao X, Zou H, Li K. Prognostic significance of c-Met in breast cancer: a meta-analysis of 6010 cases. Diagn Pathol 2015; 10:62.

7 Iqbal BM, Buch A. Hormone receptor (ER, PR, HER2/neu) status and proliferation index marker (Ki-67) in breast cancers: their onco-pathological correlation, shortcomings and future trends. Med J DY Patil Univ 2016; 9:674–679.

8 Zhang Y, Xia M, Jin K, Wang S, Wei H, Fan C, *et al.* Function of the c-Met receptor tyrosine kinase in carcinogenesis and associated therapeutic opportunities. Mol Cancer 2018; 17:45

9 Mo HN, Liu P. Targeting MET in cancer therapy. Chronic Dis Transl Med 2017; 3:148–153.

10 Drilon A, Cappuzzo F, Ou SI, Camidge DR. Targeting MET in lung cancer: will expectations finally be MET? J Thorac Oncol 2017; 12:15–26.

11 Zhang J, Jiang X, Jiang Y. Recent advances in the development of dual VEGFR and c-Met small molecule inhibitors as anticancer drugs. Eur J Med Chem 2016; 108: 495–504.

12 Anestis A, Zoi I, Karamouzis MV. Current advances of targeting HGF/c-Met pathway in gastric cancer. Ann Transl Med 2018; 6:247–256.

13 Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, Pietenpol JA. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest 2011; 121:2750–2767.

14 Rosai J. Invasive carcinoma. In: Rosai J. Surgical Pathology. Ninth edition. St. Louis, MO, USA: Published by Elsevier Mosby; 2011; 2:1802–1803. Ackerman, Lauren Vedder ISBN 10: 8181474406/ISBN 13: 9788181474407.

15 Gistereka I, Lataa E, Halonb A, Matkowskia R, Szelachowskaa J, Biecekc P, Kornafel J. Prognostic role of c-Met expression in breast cancer patients. Rep Pract Oncol Radiother 2011; 16:173–177.

16 Chan YH. Biostatistics102: quantitative data – parametric & non-parametric tests. Singapore Med J 2003; 44:391–396.

17 Perou C, Sorlie T, Eisen MB. Molecular portraits of human breast tumors. Nature 2000; 206:747–752.

18 Guedj M, Marisa L, de Reynies A, Orsetti B, Schiappa R, Bibeau F, *et al.* A refined molecular taxonomy of breast cancer. Oncogene 2012; 31:1196–1206.

19 Abouelfadl DM, Amin HAA, Yassen NN, Shabana ME, Salem AMH. Androgen receptor expression in hormone-negative breast cancers and its prognostic significance. J Arab Soc Med Res 2018; 13:113–118.

20 Xixi Z, Qu J, Hui Y, Zhang H, Sun Y, Liu X, *et al.* Clinicopathological and prognostic significance of c-Met overexpression in breast cancer. Oncotarget 2017; 8:56758–56767.

21 Lindemann K, Resau J, Nährig J, Kort E, Leeser B, Annecke K, *et al.* Differential expression of c-Met, its ligand HGF/SF and HER2/neu in DCIS and adjacent normal breast tissue. Histopathology 2007; 51:54–62.

22 Garcia S, Dalès JP, Charafe-Jauffret E, Carpentier-Meunier S, Andrac-Meyer L, Jacquemier J, *et al.* Poor prognosis in breast carcinomas correlates with increased expression of targetable CD146 and c-Met and with proteomic basal-like phenotype. Hum Pathol 2007; 38:830–841.

23 Lengyel E, Prechtel D, Resau JH, Gquger K, Welk A, Lindemann K, *et al.* c-Met overexpression in node-positive breast cancer identifies patients with poor prognosis independent of Her2/neu. Int J Cancer 2005; 113:678–682.

24 Carracedo A, Egervari K, Salido M. FISH and immunohistochemical status of the hepatocyte growth factor receptor (c-Met) in 184 invasive breast tumors. Breast Cancer Res 2009; 11:R402–R403.

25 Masuya D, Huang C, Liu D, Nakashima T, Kameyama K, Haba R, *et al.* The tumour-stromal interaction between intra tumoral c-Met and stromal hepatocyte growth factor associated with tumour growth and prognosis in non-small-cell lung cancer patients. Br J Cancer 2004; 90:1555–1562.

26 Gisterek I, Matkowski R, Suder E, Hałoń A, Szelachowska J, Łata E, *et al.* Correlation between hepatocyte growth factor receptor and vascular endothelial growth factor-A in breast carcinoma. Folia Histochem Cytobiol 2010; 48:78–83.

27 Ocal IT, Dolled-Filhart M, D'Aquila TG, Camp RL, Rimm DL. Tissue microarray-based studies of patients with lymph node negative breast carcinoma show that met expression associated with worse outcome but is not correlated with epidermal growth factor family receptors. Cancer 2003; 97:1841–1848.

28 Parr C, Watkins G, Mansel RE, Jiang WG. The hepatocyte growth factor regulatory factors in human breast cancer. Clin Cancer Res 2004; 10:202–211.

29 Edakuni G, Sasatomi E, Satoh T, Tokunaga O, Miyazaki K. Expression of the hepatocyte growth factor/c-Met pathway is increased at the cancer front in breast carcinoma. Pathol Int 2001; 51:172–178.

30 Graveel CR, DeGroot JD, Su Y, Koeman J, Dykema K, Leung S, *et al.* Met induces diverse mammary carcinomas in mice and is associated with human basal breast cancer. Proc Natl Acad Sci U S A 2009; 106:12909–12914.

31 Ho-Yen C, Jones JL, Kermorgant S. The clinical and functional significance of c-Met in breast cancer: a review. Breast Cancer Res 2015; 17:52.

32 Jin KY, Jong-Sun C, Kyungsoo J, Ensei O, Jinwon S. MET is a potential target for use in combination therapy with EGFR inhibition in triple-negative/ basal-like breast cancer. Int J Cancer 2014; 134:2424–2436.

33 Camp RL, Rimm EB, Rimm DL. Met expression is associated with poor outcome in patients with axillary lymph node negative breast carcinoma. Cancer 1999; 86:2259–2265.

34 Charafe-Jauffret E, Ginestier C, Monville F, Finetti P, Adélaïde J, Cervera N, *et al.* Gene expression profiling of breast cell lines identifies potential new basal markers. Oncogene 2006; 25:2273–2284.

35 Zagouri F, Brandstetter A, Moussiolis D, Chrysikos D, Dimitrakakis C, Tsigginou A, *et al.* Low protein expression of MET in ER positive and Her2 positive breast cancer. Anticancer Res 2014; 34:1227–1231.

36 Raghav KP, Wang W, Liu S, Chavez-MacGregor M, Meng X, Hortobagyi GN, *et al.* C-Met and phospho-c met protein level in breast cancer and survival outcome. Clin Cancer Res 2012; 18:2269–2277.

37 Zhao W, Jia L, Yang X, Tian W, Gou S, Huang W. Increased expression of c-Met is associated with chemotherapy-resistant breast cancer and poor clinical outcome. Med Sci Monit 2018; 24:8239–8249.