ORIGINAL ARTICLE

Evaluation of Tumour-Associated Macrophages and Colony-Stimulating Factor-1 Expression in Invasive Breast Carcinoma and Their Association with Prognostic Parameters

Sediqi MF[a], Ahmad Affandi K[a], Muhammad N[a], A. Talib N[a], Che Al Hadi S[b], Abdullah S[c]

[a]Department of Pathology and Laboratory Medicine, Kulliyyah of Medicine, International Islamic University Malaysia, Pahang, Malaysia.
[b]Department of Surgery, Kulliyyah of Medicine, International Islamic University Malaysia, Pahang, Malaysia.
[c]Department of Pathology, Hospital Tengku Ampuan Afzan, Pahang, Malaysia.

ABSTRACT

INTRODUCTION: Recent breast cancer research has focused on tumour microenvironment (TME). Tumour-associated macrophages (TAMs) are the key players in TME as they provide pro-tumorigenic milieu for tumour progression and metastasis. These macrophages are primarily regulated by colony-stimulating factor-1 (CSF-1) secreted by breast cancer cells. This study investigated the association of localization of TAMs infiltration within breast carcinoma and CSF-1 expression by cancer cells with the pathological prognostic parameters. **MATERIALS AND METHODS:** TAMs were assessed in 128 cases of invasive breast carcinoma by CD163 immunohistochemical expression. The median TAM density in both the tumour nest and tumour stroma was utilized to classify TAMs into categories of low and high infiltration. The cancer cells were immunostained with anti-CSF-1 antibody and the staining intensity was evaluated as low or high expression. **RESULTS:** High nest and stromal TAMs were associated with higher tumour grades (p=0.005 and p=0.0001, respectively) whereas only high stromal TAMs showed significant association with negative oestrogen and progesterone receptors status (p=0.001 and 0.001, respectively); and triple-negative subtype (p=0.002). High CSF-1 expression was significantly associated with high stromal TAMs (p=0.031). High CSF-1 expression was associated with tumour grade and positive HER2 status (p=0.008 and 0.007, respectively). **CONCLUSION:** TAMs in tumour nest and stroma showed varying degrees of association with the clinicopathological parameters. High CSF-1 expression was associated with unfavourable prognostic parameters. Therefore, the evaluation of TAMs and CSF-1 expressions could potentially serve as prognostic markers and cellular targets for novel treatment modality in invasive breast cancers.

Keywords
breast cancer, colony-stimulating factor-1, tumour-associated macrophages, tumour microenvironment

Corresponding Author
Asst. Prof. Dr. Khairunisa Ahmad Affandi
Department of Pathology and Laboratory Medicine, Kulliyyah of Medicine, International Islamic University Malaysia, Kuantan, Pahang.
E-mail: khairunisa@iium.edu.my

Received: 22th November 2023; Accepted: 11st July 2024

Doi: https://doi.org/10.31436/imjm.v23i04

INTRODUCTION

Breast cancer is the most frequently diagnosed cancer in females and is also a leading cause of death in most countries.[1] In Malaysia, breast cancer accounted for 34.1% of all cancers among women.[2] Breast cancer is a clinical and pathological heterogeneous disease. Vast evidence has suggested that breast cancers exhibit distinct behaviours and different treatment responses regardless of the histological subtypes.[3] Despite current recommendations regarding prognostic and predictive factors, breast cancer is difficult to treat, and additional parameters are required to further stratify patients for personalized and ideal treatment. Recent cancer research has partly shifted focus to tumour heterogeneity, particularly the molecular and cellular mechanisms of cancer cells as well as the tumour microenvironment (TME). The TME is the non-cancerous cells surrounding the tumour and encompasses heterogeneous populations of stromal cells and different types of immune cells.[4] Tumour cells recruit these supporting cells into the TME, which in turn promotes cancer cell growth and metastasis.[5] Macrophages are the major immune cells within the microenvironment, and in tumours, they are referred to as tumour-associated

macrophages (TAMs). Generally, TAMs can be classified into classically activated (M1) or alternatively activated (M2) subtypes depending on the specific provoking factors involved. In early-stage tumours, TAMs are predominantly of M1 phenotype which exerts pro-inflammatory effects. As the tumour advances, the macrophages polarized to M2 phenotype essential for tumour progression.[6]

CD68 and CD163 are glycoproteins expressed in monocytes and tissue macrophages and are widely used markers to detect TAMs in several cancer types. CD68 is relatively non-specific as it recognizes both M1 an M2 macrophages and is also expressed by a wide range of cells including fibroblasts, granulocytes, dendritic cells, endothelial cells, and some lymphoid subsets.[7] On the other hand, CD163 is a highly specific monocyte/ macrophage marker for M2-polarized macrophages.

High TAMs infiltration was associated with aggressive biological behaviours such as larger tumour size, higher tumour grade, lymphovascular invasion, and hormone receptor negative breast cancers.[7] However, there are conflicting data regarding association of TAMs and breast cancer prognosis. Some studies have found that there were no association between high TAMs infiltration with positive lymph node status, vascular invasion, and HER-2 expression.[8,9] These discrepancies may be due to the different methodologies used for histological assessment of TAMs, different cut-off values for definition of TAMs density, and different detection markers used.

Several studies have analysed association between total TAMs in tumour and poor prognosis without taking TAMs localization into account.[10,11] Meanwhile in other studies, they focused on the importance of TAMs localization in breast cancer tissue. One study found that increased CD163-positive TAMs in tumour stroma was correlated with unfavourable clinicopathological factors and overall survival (OS) of cancer patients; however, they did not find any statistical significance with TAMs in tumour nest.[12] On the contrary, another study showed that higher number of CD163-positive TAMs infiltration in tumour nest was correlated with unfavourable OS.[13] These conflicting findings warrants further investigations.

The crosstalk between tumour cells and the TME is initiated by various cytokines, chemokines and growth factors; and the main link between tumour cells and TAMs is colony-stimulating factor 1 (CSF-1). CSF-1, also known as macrophage colony-stimulating factor, is an important growth factor involved in cell differentiation, proliferation and activation via binding to the CSF-1 receptor (CSF-1R) expressed on macrophages.[11] CSF-1 is secreted by various types of cells such as monocytes, fibroblasts, endothelial cells and tumour cells. The paracrine signalling between breast cancer cells and TAMs is important for tumour progression and metastasis. Tumour cells secrete CSF-1, which is received by the CSF-1R on macrophages. In turn, TAMs upregulate the secretion of epidermal growth factor (EGF) and subsequently bind to the EGF receptor on the tumour cells.[14] EGF promotes the expression of CSF-1 by tumour cells, thereby generating a positive feedback loop. The EGF/CSF-1 positive feedback loop enhances the survival and proliferation of tumour cells and facilitates tumour cells to metastasize to secondary organs.

Studies have shown that breast cancer cells with high CSF-1 expression are associated with poor outcomes in both metastatic and non-metastatic breast cancers.[15,16] High CSF -1 expression is significantly correlated with poor clinicopathologic prognostic parameters such as larger tumour size, higher tumour grade, negative hormone receptor status, and HER2 positivity.[17] Hence, the detection of CSF-1 expression provides prognostic information in breast cancers. Moreover, new cancer treatments targeting CSF-1 and TAMs are emerging through reducing the number of TAMs in the TME and re-programming TAMs to anti-tumour phenotype.[18] LY3022855 is an example of monoclonal antibody directed against CSF-1R on macrophages by inhibiting the binding of CSF-1 on the receptor. Although a phase 1 study of LY3022855 in advanced refractory breast and prostate cancers showed limited clinical response of the subjects, there were evidence of immune modulation of TAMs in the tumour cells after therapy which warrants further evaluation.[19] In view of their prominent roles in breast cancer progression, more studies on TAMs and CSF-1 expression in breast cancer specimens as potential prognostic markers and their clinical application are required to stratify patients for targeted immunotherapies.

Therefore, this study aimed to evaluate the degree and histological localization of CD163-positive TAMs in invasive breast carcinoma, the proportion of CSF-1 expression and its association with the degree and histological localization of CD163-positive TAMs. We also aimed to investigate the association between degree of TAMs infiltration and CSF-1 expression with the pathological prognostic factors in invasive breast carcinoma at Sultan Ahmad Shah Medical Centre International Islamic University Malaysia (SASMEC@IIUM) and Hospital Tengku Ampuan Afzan (HTAA), Kuantan, Pahang, Malaysia.

MATERIALS AND METHODS

Sample Collection

This cross-sectional study involved 128 mastectomy specimens diagnosed as invasive breast carcinoma of no special type (NST) from January 2017 to December 2020 at SASMEC@IIUM and HTAA. Ethical approval from the IIUM Research Ethics Committee (IREC 2020-159) and National Medical Research Registry (NMRR-21-3749-38944 (IIR)) were obtained. The slides of these cases were reviewed by an experienced histopathologist to select the representative tumour tissue blocks containing the tumour nest and stroma. For each case, a representative formalin-fixed paraffin-embedded tumour tissue block including tumour stroma was retrieved. Clinicopathological data – patient age, gender, race, tumour size, histological grade, lymph node involvement, the status of oestrogen and progesterone receptors, and human epidermal growth factor receptor 2 (HER2) expression – were retrieved from pathology reports.

Immunohistochemical Staining Method

Tissue sections (3-µm thickness) were prepared on pre-coated slides and were heated in an oven for 20 min at 67° C. Two primary antibodies were used in this study: rabbit recombinant monoclonal CD163 antibody (Code EPR19518, Abcam, Cambridge, UK) and rabbit recombinant monoclonal CSF-1 antibody (Code SP211, Abcam, Cambridge, UK). Normal spleen and tonsil tissues were used as positive controls for CD163 and CSF-1, respectively. Immunohistochemical staining was performed using the VENTANA Immunohistochemistry Auto Stainer Benchmark ULTRA (Ventana Medical Systems, Inc., Oro Valley, AZ, USA). The tissue sections were baked for 16 min at 60°C and de-paraffinized in Ventana EZ Prep solution. Endogenous peroxidase blocking with ULTRA-View Universal DAB Inhibitor 3% was used for antigen retrieval and the slides were incubated in primary antibodies CD163 and CSF-1 at dilution 1:500 and 1:70, respectively for 60 min. Then, the slides were incubated in ULTRA-View HRP multimer, ULTRA-View Universal DAB H2O2, chromogen and copper. Finally, the slides were counterstained with haematoxylin 2 and bluing reagent.

Immunohistochemical Staining Analysis

Evaluation of TAMs

All CD163-stained slides were examined for quantification of TAMs. Positive cells expressed moderate to strong cytoplasmic staining. The areas with the highest density of CD163-positive macrophages (hot spots) were identified under low (100×) magnification. These areas included hot spots within the tumour nest and tumour stroma. Tumour nest TAMs is defined as macrophages in contact with tumour cells, whereas stromal TAMs are macrophages that reside at the tumour–stroma borders.[9] Large areas of necrosis were not included in the evaluation. Three hot spots for both tumour nest and tumour stroma were identified; and positive cells were manually counted using the plug-in cell counter in the ImageJ software in high-power fields (400× magnification). The mean of the three counts was calculated, and the median value of TAMs in both tumour nest and stroma was used as a cut-off point to categorize the patients into low- and high-TAM infiltration (Figure 1).

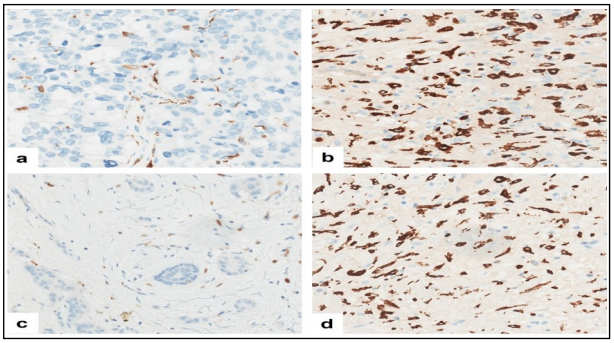

Figure 1: CD163-positive tumour-associated macrophages (TAMs) in tumour nest and tumour stroma. Examples of tissues with (a) low and (b) high TAMs infiltration in tumour nests; and (c) low and (d) high TAMs infiltration in tumour stroma (400× magnification).

Evaluation of CSF-1 Expression

The expression of CSF-1 by tumour cells was evaluated semi-quantitatively by the presence of diffuse brown cytoplasmic staining. The staining intensity of CSF-1 in tumour cells was scored as 0 (no staining), 1 (weak), 2 (moderate) or 3 (strong) as described in the study by Richardsen et al.[16] Score of 1 to 3 was considered positive staining. The CSF-1 staining intensity was further categorized into low expression (Score 0 and 1) and high expression (Score 2 and 3). The stained slides were assessed by two qualified histopathologists who were blinded to the clinicopathological data of the patients.

Statistical Analyses

Statistical analyses were performed using SPSS software version 25.0 (IBM Corp., Armonk, NY, USA). Continuous data were expressed as a mean with standard deviation. Categorical variables were presented as frequencies and percentages. The association between TAM infiltration and CSF-1 expression, and between TAMs infiltration and CSF-1 expression with clinicopathological parameters were calculated using Pearson's chi-square test. Fisher's exact test was used when appropriate. A p value of less than 0.05 was considered statistically significant.

RESULTS

Socio-Demographic and Clinicopathological Characteristics

The socio-demographic and clinicopathologic characteristics of the study subjects are illustrated in Table 1. The majority of the subjects were Malays (78.9%), while Chinese and Indians constituted 14.8% and 6.3% of the study participants, respectively. Eighty-three cases (64.8%) had tumour size between 2 – 5 cm, 32 cases (25%) had tumour size of more than 5 cm and only 13 cases (10.2%) had tumour size of less than 2 cm. Histologically, 73 cases (57%) were histological grade 2, 40 cases (31.3%) were grade 3 and 15 cases (11.7%) were grade 1. Positive lymph node metastasis was detected in 79 cases (61.7%). Cases with positive oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) status were 82 (64.1%), 74 (57.8%), and 46 (35.9%) cases, respectively. Twenty-one cases (16.4%) were triple-negative breast cancers.

Table I: Socio-demographic and clinicopathological characteristics of the 128 invasive breast carcinoma cases.

Characteristic	Categories	Frequency	Percentage
Age (Mean ± SD)	55.29 ± 11.875		
Age group (years)	≤50 years	40	31.3
	>50 years	88	68.8
Gender	Female	128	100
Race	Malay	101	78.9
	Chinese	19	14.8
	Indian	8	6.3
Tumour size	≤2 cm	13	10.2
	2 – 5 cm	83	64.8
	>5 cm	32	25
Histological grade	Grade 1	15	11.7
	Grade 2	73	57
	Grade 3	40	31.3
Lymph node metastasis	Absent	49	38.3
	Present	79	61.7
ER status	Negative	46	35.9
	Positive	82	64.1
PR status	Negative	54	42.2
	Positive	74	57.8
HER2	Negative	82	64.1
	Positive	46	35.9
Triple-negative subtype	No	107	83.6
	Yes	21	16.4

SD, standard deviation; ER, oestrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

Degree of TAMs Infiltration and Their Histological Localization within Breast Cancer Tissue

The degree of TAMs infiltration was variable. The median numbers of TAMs per high-power field was 30.5 (interquartile range: 21 - 42). High CD163-positive TAMs infiltration was more common in tumour stroma (55%) as compared to tumour nest (40%).

Association between Degree of TAMs Infiltration in Tumour Nest and Tumour Stroma with Pathological Prognostic Factors in Invasive Breast Carcinoma

High degree of TAMs infiltration was associated with poor prognostic parameters. High TAMs infiltration in both tumour nest and tumour stroma were significantly associated with higher histological grades. High nest TAMs was detected in 23 (57%) grade 3 tumour (p=0.006) whereas high stromal TAMs was detected in 33 (82.5%) grade 3 tumours (p=0.001). High TAMs infiltration in tumour stroma were also significantly associated with negative hormone receptor status. Thirty-seven (80.4%) ER-negative cases, 39 (72.2%) PR-negative cases, and 19 (90.5%) of triple-negative cases displayed high TAMs infiltration in tumour stroma (p=0.001, 0.001, and 0.001, respectively). The association between TAMs infiltration

Table II: Association between pathological prognostic factors and TAMs status in tumour nest and infiltration in stroma.

Factors			TAMs in Tumour Nest			TAMs in Tumour Stroma		
		N	Low n (%)	High n (%)	p	Low n (%)	High n (%)	p
Age	≤50	40	25 (62.5)	15 (37.5)	0.715	18 (45)	22 (55)	0.943
	>50	88	52 (59.1)	36 (40.9)		39 (44.3)	49 (55.7)	
Size	≤2 cm	13	9 (69.2)	4 (30.8)		7 (53.8)	6 (46.2)	
	2 – 5 cm	83	52 (62.7)	31 (37.3)	0.361	38 (45.8)	45 (54.2)	0.563
	>5 cm	32	16 (50)	16 (50)		12 (37.5)	20 (62.5)	
Grade	Grade 1	15	13 (86.7)	2 (13.3)		13 (86.7)	2 (13.3)	
	Grade 2	73	47 (64.4)	26 (35.6)	0.006	37 (50.7)	36 (49.3)	0.001
	Grade 3	40	17 (42.5)	23 (57.5)		7 (17.5)	33 (82.5)	
Lymph node metastasis	Negative	49	26 (53.1)	23 (46.9)	0.197	19 (38.8)	30 (61.2)	0.302
	Positive	79	51 (64.6)	28 (35.4)		38 (48.1)	41 (51.9)	
ER	Negative	46	25 (54.3)	21 (45.7)	0.315	9 (19.6)	37 (80.4)	0.001
	Positive	82	52 (63.4)	30 (36.6)		48 (58.5)	34 (41.5)	
PR	Negative	54	30 (55.6)	24 (44.4)	0.364	15 (27.8)	39 (72.2)	0.001
	Positive	74	47 (63.5)	27 (36.5)		42 (56.8)	32 (43.2)	
HER2	Negative	82	51 (62.2)	31 (37.8)	0.529	37 (45.1)	45 (54.9)	0.858
	Positive	46	26 (56.5)	20 (43.5)		20 (43.5)	26 (56.5)	
Triple-negative subtype	No	107	66 (61.7)	41 (38.3)	0.426	55 (51.4)	52 (48.6)	0.001
	Yes	21	11 (52.4)	10 (47.6)		2 (9.5)	19 (90.5)	

in tumour nest and tumour stroma with the clinicopathologic parameters are summarized in Table II.

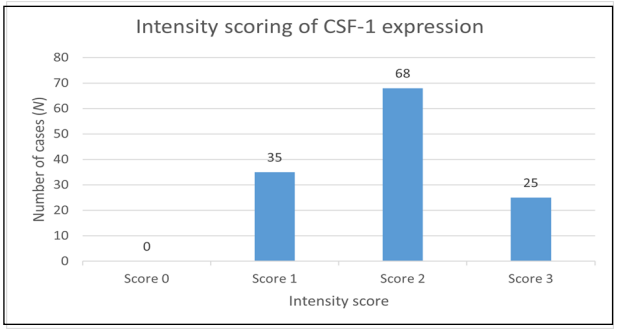

Figure 2: Intensity score of CSF-1 expression in breast cancer cells.

Association between CSF-1 Expression with The Degrees and Histological Localization of TAMs Infiltration in Breast Cancer Tissue

CSF-1 immunoreactive staining of tumour cells were observed in all cases with variable proportion and staining intensity (Figure 2). The CSF-1 expression scoring is illustrated in Figure 3. Cases that scored 0 and 1 were subcategorized into low CSF-1 expression whereas cases with scores 2 and 3 were considered as high expression. Ninety-three cases had high CSF-1 expression. Expression of CSF-1 by tumour cells was significantly associated with the degree and histological localization of TAM infiltration in breast cancer tissue (Table III). High CSF-1 expression was seen in 57 (61.3%) cases with high TAM infiltration in tumour stroma (p=0.031) as compared to only in 41 (44.1%) cases with high nest TAMs (p = 0.110).

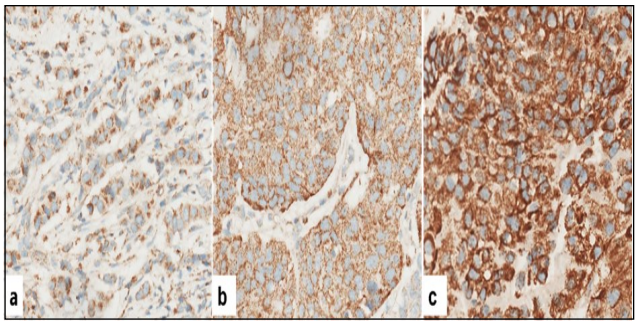

Figure 3: Scoring for CSF-1 expression in breast cancer cells. Tumour cells showed diffuse brown cytoplasmic staining with variable intensity scoring: (a) Score 1, (b) Score 2 and (c) Score 3 (400× magnification).

Table III: Association between CSF-1 expression with low and high TAM infiltration in tumour nest and tumour stroma.

CSF-1 Expression	TAMs in Tumour Nest			TAMs in Tumour Stroma		
	Low n (%)	High n (%)	p	Low n (%)	High n (%)	p
Low	25 (71.4)	10 (28.6)	0.110	21 (60.0)	14 (40.0)	0.031
High	52 (55.9)	41 (44.1)		36 (38.7)	57 (61.3)	

CSF-1, colony stimulating factor-1; TAMs, tumour-associated macrophages.

Association Between CSF-1 Expression with Pathological Prognostic Factors in Invasive Breast Carcinoma

CSF-1 expression was significantly associated with tumour grade and HER-2 cases, as illustrated in Table IV. High CSF-1 expression was significantly associated with higher histological grade (p=0.008). Furthermore, 40 HER-2-positive cases (87%) had a high expression of CSF-1 in breast cancer (p=0.007).

Table IV: Association between CSF-1 expression and pathological prognostic factors.

Factors		CSF-1 Expression		p
		Low n (%)	High n (%)	
Age	≤ 50 years	8 (20.0)	32 (80.0)	0.209
	> 50 years	27 (30.7)	61 (69.3)	
Tumour size	≤ 2 cm	3 (23.1)	10 (76.9)	0.856
	2–5 cm	24 (28.9)	59 (71.1)	
	> 5 cm	8 (25.0)	24 (75.0)	
Histological grade	Grade 1	9 (60.0)	6 (40.0)	0.008
	Grade 2	15 (20.5)	58 (79.5)	
	Grade 3	11 (27.5)	29 (72.5)	
Lymph node metastasis	Negative	9 (18.4)	40 (81.6)	0.073
	Positive	26 (32.9)	53 (67.1)	
ER status	Negative	8 (17.4)	38 (82.6)	0.058
	Positive	27 (32.9)	55 (67.1)	
PR status	Negative	12 (22.2)	42 (77.8)	0.267
	Positive	23 (31.1)	51 (68.9)	
HER2	Negative	29 (35.4)	53 (64.6)	0.007
	Positive	6 (13.0)	40 (87.0)	
Triple negative	Negative	30 (28.0)	77 (72.0)	0.691
	Positive	5 (23.8)	16 (76.2)	

CSF-1, colony stimulating factor-1; ER, oestrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

DISCUSSION

Tumour-associated macrophages (TAMs), predominantly the M2 subtype are known to secrete various cytokines, chemokines, and proteolytic enzymes; which promote immunosuppressive activity, tumour proliferation, and tumour angiogenesis.[20] TAMs also play an important role in tumour metastasis by facilitating tumour cell invasion, migration, and tumour seedling to distant sites.[21,22] first part of this study, we investigated the degree of TAMs infiltration in invasive breast carcinoma and its association with the pathological prognostic factors of breast cancers.

In this study, we have demonstrated that high CD163-positive TAMs infiltration is more common in tumour stroma as compared to tumour nest. In breast tumours, TAMs are numerous within the stroma at the margins of breast cancer and becoming lesser towards the centre of the tumour.[14] These macrophages are also abundant at areas of tumour necrosis and preferentially associated with blood vessels. TAMs in various tumour locations had diverse phenotypes and functions. It was proposed that tumour stromal TAMs influence tubular architecture and, eventually, tumour grade.[23] During development, trophic macrophages are recruited to the growing breast ductal structures and play a role in tissue patterning and branching morphogenesis.[14] It is found that TAMs share similar properties to these trophic macrophages in tumour growth. Stromal TAMs also promote cell division by producing growth factors, cytokines and chemokines including transforming growth factor-β (TGF- β), basic fibroblast growth factor-2 (bFGF-2), platelet derived growth factor (PDGF), interleukin-10 (IL-10), and chemokine receptor type (CXC) ligand.[24] Stromal TAMs are associated with high expression epithelial-mesenchymal transition (EMT) markers contributes to rapid tumour progression and metastasis in these morphological variants.[25]

On the other hand, tumour nest TAMs is associated with hypoxia-induced angiogenesis and responses.[23] In poorly vascularised tumour especially intratumoural regions, nest TAMs upregulate hypoxia-inducible factor-1α (HIF-1α) and HIF-2 α which in turn stimulate the production of pro-angiogenic factors such as vascular endothelial growth factor (VEGF), bFGF, PDGF, and EGF to facilitate angiogenesis.[26] Hypoxia also augment nest TAMs with immunosuppressive features and further promote tumour growth and metastasis.

Our research findings showed there were significant associations between nest TAMs and stromal TAMs with pathological prognostic markers. We demonstrated a significant association between high TAMs infiltration in tumour nest and tumour stroma with higher tumour grades. Our study conforms with earlier research which demonstrated high numbers of CD163-positive TAMs in both tumour stroma and tumour nests were associated with higher histological grades.[13] In another study, high TAMs infiltration in tumour nest was significantly associated with high tumour grade but not with stromal TAMs.[9] It was postulated that increased density of CD163 -positive TAMs within high-grade tumours may be contributed by higher cytokines release by tumour cells to recruit TAMs such as CSF-1, IL-10, and TGF- β.[27] High stromal TAMs is recruited in tumours with solid architecture, hence, higher grade tumour as compared to tubular structure.[28] It was also suggested that TAMs in tumour stroma had more important roles that TAMs in the tumour nest in the aggressive behaviours of breast cancers.[23]

With regards to hormone receptor status, our study demonstrated a significant association between the high TAMs infiltration in tumour stroma and the ER and PR hormonal status. Most of the ER-negative cases (80.4%) and PR-negative cases (72.2%) had high density of TAMs infiltration in tumour stroma. These findings corroborated with earlier research findings that hormone receptor negativity was linked to increased expression of CD68 or CD163.[9,25] An *in vitro* model study demonstrated a novel mechanism of macrophage activation of kinase cascades in the cancer cells is responsible for loss of ERα expression in breast cancer cells.[29]

Our study also demonstrated a significant association between high TAM infiltration with triple-negative breast cancers (TNBC). Most of our TNBC cases (90.9%) is associated with high TAM infiltration within tumour stroma. Our finding is supported by previous study which showed that significant correlation between greater density of CD163-positive TAMs in tumour stroma and TNBC.[9] These findings are particularly important because presence of high TAMs infiltration affects the treatment response in TNBC; as the common chemotherapeutic drugs given to TNBC patients can activate TAMs and induce chemotherapy tolerance and inhibit immune killing of tumour cells by CD8+ T cells.[30]

In the second part of this study, we investigated the association between TAMs and CSF-1 expression in invasive breast carcinoma. CSF-1 stimulates TAMs to polarize from the M1 type to the M2 type; promotes TAM differentiation, proliferation and survival; and attracts monocyte-macrophage lineages to extravasate from peripheral circulation into the tumour tissues.[14] The positive feedback loop between tumour cells and TAMs through CSF-1 and EGF enhances tumour growth and metastasis. In our study, we demonstrated a significant association between the degree of TAM infiltration and CSF-1 expression. We also found that high CSF-1 expression was associated with high TAM infiltration in tumour stroma. This finding supports the crucial role of the surrounding microenvironment in breast cancer progression. CSF-1 and CSF-1R expressions are correlated with poor prognostic parameters in breast cancers. An experimental study demonstrated that breast cancers behaved more aggressively through CSF-1 secretion as more macrophages were recruited into the TME, thus creating a pro-tumorigenic milieu.[31] In our study, we demonstrated that CSF-1 expression was significantly associated with histological grade.

Our findings are in agreement with previous research that revealed significant correlation between high CSF-1 expression levels with higher pathological grade and worse prognosis in breast cancer.[32] We also found that there was a significant association between high CSF-1 expression and positive HER2 status. HER2-positive breast cancer is aggressive and has a poor prognosis if untreated; but due to the effectiveness of HER2-targeted therapies, its prognosis has improved. Studies showed that CSF-1 enhances invasiveness of cancer cells by signalling the macrophages to secrete EGF which causes the alteration of cellular morphology into elongated protrusions, which in turn promote tumour invasion.[33]

In this present study, we observed some discrepant results. An experimental study of hormone-independent breast cancers demonstrated a more aggressive behaviour than hormone-dependent breast cancer through CSF-1 secretion and TAMs recruitment.[28] However, our study failed to find association between CSF-1 expression and negative hormone receptor status. Another study has found significant correlations between CSF-1 expression in breast cancer with lymph node metastasis; however, our finding was nonsignificant.[14] The conflicting data produced may be due to different inclusion and exclusion and different methodologies to study the expression of CSF-1 in breast cancers. There are various methods that can be used to investigate CSF-1 level and expression such as fluorescence in situ hybridization (FISH), polymerase chain reaction (PCR), Western blot, ELISA and immunohistochemistry that can affect and produce variable results.[14,28,34] Immunohistochemistry method is reliable, specific, cost effective and feasible in most diagnostic laboratories. These differing results can also be attributed to the fact that the effect of CSF-1 in breast cancer is influenced by not only the genotype and phenotype of the carcinoma cells but also other cells in the TME. [35] Therefore, more data and further studies are required to better understand the correlation of CSF-1

with clinicopathological parameters and breast cancer prognosis.

CONCLUSION

In conclusion, we demonstrated that different TAM localization within invasive breast carcinoma NST have different degree of association with the clinicopathological parameters. High CD163-positive TAMs infiltration is more prevalent in tumour stroma as compared to tumour nest. High stromal TAMs were significantly associated with poor prognostic parameters. In this study, we also demonstrated that CSF-1 expression has varying degrees of association with TAM infiltration in the tumour nest and tumour stroma. High TAM infiltration in the tumour stroma was strongly associated with high CSF-1 expression. High CSF-1 expression was associated with adverse prognostic factors in breast cancers. Therefore, evaluation of TAMs infiltration and CSF-1 expression in breast cancer while taking into account the histologic localization is important. It has potential to serve as valuable biomarkers for patient prognosis and as targets for personalized cancer treatment strategies.

CONFLICT OF INTEREST

There are no potential conflicts of interest for any of the authors.

ACKNOWLEDGEMENT

This research was funded by Sultan Ahmad Shah Medical Centre International Islamic University Malaysia (SASMEC@IIUM) under the SASMEC Research Grant (SRG-21-036-0036). We also would like to extend our sincere gratitude to the staff of the Department of Pathology Hospital Tengku Ampuan Afzan for the support received.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68:394-424.
2. Azizah A, Hashimah B, Nirmal K, et al. Malaysia National cancer registry report (MNCR). National Cancer Institute, Ministry of Health: Putrajaya, Malaysia. 2019.
3. Ellsworth RE, Blackburn HL, Shriver CD, Soon-Shiong P, Ellsworth DL. Molecular heterogeneity in breast cancer: State of the science and implications for patient care. In Seminars in Cell & Developmental Biology 2017 Apr 1 (Vol. 64, pp. 65-72). Academic Press.
4. Soysal SD, Tzankov A, Muenst SE. Role of the tumor microenvironment in breast cancer. Pathobiology 2015; 82:142-52.
5. Hill BS, Sarnella A, D'Avino G, Zannetti A. Recruitment of stromal cells into tumour microenvironment promote the metastatic spread of breast cancer. In Seminars in Cancer Biology 2020 Feb 1 (Vol. 60, pp. 202-213). Academic Press.
6. Lin Y, Xu J, Lan H. Tumor-associated macrophages in tumor metastasis: biological roles and clinical therapeutic applications. Journal of Hematology & Oncology 2019; 12:1-6.
7. Larionova I, Tuguzbaeva G, Ponomaryova A, et al. Tumor-associated macrophages in human breast, colorectal, lung, ovarian and prostate cancers. Frontiers in Oncology 2020; 10:566511.
8. Zhao X, Qu J, Sun Y, et al. Prognostic significance of tumor-associated macrophages in breast cancer: a meta-analysis of the literature. Oncotarget 2017; 8:30576.
9. Mwafy SE, El-Guindy DM. Pathologic assessment of tumor-associated macrophages and their histologic localization in invasive breast carcinoma. Journal of the Egyptian National Cancer Institute 2020; 32:1-1.
10. Ni C, Yang L, Xu Q, et al. CD68-and CD163-positive tumor infiltrating macrophages in non-metastatic breast cancer: a retrospective study and meta-analysis. Journal of Cancer 2019; 10:4463.
11. Yang J, Li X, Liu X, Liu Y. The role of tumor-associated macrophages in breast carcinoma invasion and metastasis. International Journal of Clinical & Experimental Pathology 2015; 8:6656.
12. Yang M, Li Z, Ren M, et al. Stromal infiltration of tumor-associated macrophages conferring poor prognosis of patients with basal-like breast carcinoma. Journal of Cancer 2018; 9:2308.

13. Jeong H, Hwang I, Kang SH, Shin HC, Kwon SY. Tumor-associated macrophages as potential prognostic biomarkers of invasive breast cancer. Journal of Breast Cancer 2019; 22:38-51.
14. Williams CB, Yeh ES, Soloff AC. Tumor-associated macrophages: unwitting accomplices in breast cancer malignancy. NPJ Breast Cancer 2016; 2:15025.
15. Kluger HM, Dolled-Filhart M, Rodov S, et al. Macrophage colony-stimulating factor-1 receptor expression is associated with poor outcome in breast cancer by large cohort tissue microarray analysis. Clinical Cancer Research 2004; 10:173-7.
16. Richardsen E, Uglehus RD, Johnsen SH, Busund LT. Macrophage-colony stimulating factor (CSF1) predicts breast cancer progression and mortality. Anticancer Research 2015; 35:865-74.
17. Riaz N, Burugu S, Cheng AS, et al. Prognostic significance of CSF-1R expression in early invasive breast cancer. Cancers 2021; 13:5769.
18. Anderson NR, Minutolo NG, Gill S, Klichinsky M. Macrophage-based approaches for cancer immunotherapy. Cancer Research 2021; 81:1201-8.
19. Autio KA, Klebanoff CA, Schaer D, et al. Immunomodulatory Activity of a Colony-stimulating Factor-1 Receptor Inhibitor in Patients with Advanced Refractory Breast or Prostate Cancer: A Phase I Study. Clin Cancer Res. 2020; 26:5609-5620. doi:10.1158/1078-0432.CCR-20-0855
20. Yang L, Zhang Y. Tumor-associated macrophages: from basic research to clinical application. Journal of Hematology & Oncology 2017; 10:1-2.
21. Jiang WG, Sanders AJ, Katoh M, et al. Tissue invasion and metastasis: Molecular, biological and clinical perspectives. In Seminars in Cancer Biology 2015 Dec 1 (Vol. 35, pp. S244-S275). Academic Press.
22. Kitamura T, Qian BZ, Soong D, et al. CCL2-induced chemokine cascade promotes breast cancer metastasis by enhancing retention of metastasis-associated macrophages. Journal of Experimental Medicine 2015; 212:1043-59.
23. Ch'ng ES, Tuan Sharif SE, Jaafar H. In human invasive breast ductal carcinoma, tumor stromal macrophages and tumor nest macrophages have distinct relationships with clinicopathological parameters and tumor angiogenesis. Virchows Archiv 2013; 462:257-67.
24. Li C, Xu X, Wei S, et al. Tumor-associated macrophages: potential therapeutic strategies and future prospects in cancer. J Immunother Cancer. 2021; 9:e001341.
25. Gwak JM, Jang MH, Kim DI, Seo AN, Park SY. Prognostic value of tumor-associated macrophages according to histologic locations and hormone receptor status in breast cancer. PloS One 2015; 10:e0125728.
26. Emami Nejad A, Najafgholian S, Rostami A, et al. The role of hypoxia in the tumor microenvironment and development of cancer stem cell: a novel approach to developing treatment. Cancer Cell Int. 2021; 21:62.
27. Sousa S, Brion R, Lintunen M, et al. Human breast cancer cells educate macrophages toward the M2 activation status. Breast Cancer Research 2015; 17:1-4.
28. Tashireva LA, Denisov EV, Gerashchenko TS, et al. Intratumoral heterogeneity of macrophages and fibroblasts in breast cancer is associated with the morphological diversity of tumor cells and contributes to lymph node metastasis. Immunobiology. 2017; 222:631-640.
29. Stossi F, Madak-Erdoğan Z, Katzenellenbogen BS. Macrophage-elicited loss of estrogen receptor-α in breast cancer cells via involvement of MAPK and c-Jun at the ESR1 genomic locus. Oncogene 2012; 31:1825-34.
30. Qiu X, Zhao T, Luo R, Qiu R, Li Z. Tumor-associated macrophages: key players in triple-negative breast cancer. Frontiers in Oncology 2022; 12:772615.
31. Ding J, Guo C, Hu P, et al. CSF1 is involved in breast cancer progression through inducing monocyte differentiation and homing. International Journal of Oncology 2016; 49:2064-74.
32. Achkova D, Maher J. Role of the colony-stimulating factor (CSF)/CSF-1 receptor axis in cancer. Biochemical Society Transactions 2016; 44:333-41.

33. Goswami S, Sahai E, Wyckoff JB, et al. Macrophages promote the invasion of breast carcinoma cells via a colony-stimulating factor-1/epidermal growth factor paracrine loop. Cancer Research 2005; 65:5278-83.
34. Ho J, Peters T, Dickson BC, et al. Detection of CSF1 rearrangements deleting the 3' UTR in tenosynovial giant cell tumors. Genes Chromosomes Cancer. 2020; 59:96-105.
35. Beck AH, Espinosa I, Edris B, et al. The macrophage colony-stimulating factor 1 response signature in breast carcinoma. Clinical Cancer Research 2009; 15:778-87.