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The relationship of tumour-associated macrophages (CD68, CD163, CD11c) and cancer stem cell (CD44) markers with prognostic parameters in breast carcinomas

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Introduction: In invasive breast cancer (IBC), tumour-associated macrophages and cancer stem cells in the tumour microenvironment are thought to be associated with poor prognosis, tumour growth, and metastasis.
Material and methods: In this study, we evaluated the relationship of CD68, CD163, CD11c, and CD44 expressions in the tumour microenvironment of IBC with prognostic parameters by immunohistochemical method.
Results: A correlation was found between high histological grade, oestrogen receptor/progesterone receptor negativity, and high Ki-67 index in IBC, and the number of CD68-, CD163-, and CD11c-positive macrophages in the tumour microenvironment. In addition, in the tumour microenvironment, a correlation was found between IBC-specific subtypes and CD68, luminal A subtype relative to luminal B and CD163, HER2 score 3 cases, and CD11c-positive macrophage count. It was observed that CD44 expression in the tumour in IBC was not associated with the parameters examined. In addition, a high-level linear correlation was found between CD68- and CD163- and CD11c-positive macrophage counts. Tumour-associated macrophages in the tumour microenvironment in IBC may be a novel prognostic factor and an alternative avenue for targeted therapies.
Conclusions: The fact that CD44 positivity in the tumour is not associated with prognostic parameters will contribute to the literature in this direction.

Key words: macrophages, stem cell, microenvironment, carcinoma, breast.

Introduction

Invasive breast cancer (IBC) is the most frequently diagnosed cancer in women (23%) and the second most common cause of cancer-related death [1]. Its incidence is 24.9% in Turkey, and it is the most common cause of cancer-related death in women [2]. Therefore, many studies have recently been conducted worldwide to demonstrate that the tumour microenvironment and cancer stem cells (CSC) play an important role in tumour initiation and progression in IBC [3–5].

The tumour microenvironment is composed of many cells, such as inflammatory cells, macrophages, and extracellular matrix [3]. In IBC, the tumour microenvironment is rich in macrophages and can reach 50% [6, 7]. Macrophages are divided into subgroups by participating in certain immunological processes according to the environment and growth factors secreted by them. While M1 macrophage is proinflammatory and tumouricidal, M2 macrophages play a role in the release of anti-inflammatory cytokines, tissue repair, wound healing, angiogenesis, and tumour progression [8]. Apart from

these 2 main groups, there are many different types of macrophages [9–11]. Macrophages in the tumour microenvironment and within the tumour are called tumour-associated macrophages (TAM). Tumour-associated macrophages are more common at the margins of tumour invasion and in the perivascular space. Tumour-associated macrophages shape the extracellular matrix in these areas and induce tumour growth, tumour cell migration, angiogenesis, invasion, and metastasis. M2 macrophages are relatively common in the tumour microenvironment [9–12]. Increased M1 macrophages in this area are mostly associated with good prognosis, while increased M2 macrophages are associated with poor prognosis [11]. An increased TAM count in the tumour microenvironment is associated with a poor prognosis in many tumours, including lung carcinomas, renal cell carcinoma, hepatocellular carcinoma, cutaneous squamous cell carcinoma, thyroid papillary carcinoma, and IBC, while colon adenocarcinoma is associated with a good prognosis [7, 11, 13, 14]. Increased TAM count in the tumour microenvironment in IBC has been associated with high histological grade, oestrogen receptor/progesterone receptor (ER/PR) negativity, human epithelial growth factor receptor 2 (HER2) expression, nodal metastasis, and poor prognosis [3, 11, 13, 15, 16]. There are many signs used to identify TAMs, including CD68, which is used for pan-macrophage, CD163 for M2 macrophage, and CD11c for M1 macrophage [3, 17].

Cancer stem cells are the long-lived cell population with high growth potential responsible for pathogenesis in cancer tissue. Cancer stem cells have been identified in many tumours such as IBC, colon adenocarcinoma, and melanoma. These tumours were found to be associated with poor prognostic factors such as advanced pathological stage, clinical stage, lymph node (LN) metastasis, and tumour size [18–25]. CD44 is one of the CSC markers [5, 26]. In many studies on IBC, CD44 expression in tumours has been associated with recurrence, metastasis, and lymphovascular invasion (LVI) [20, 27–30]. However, some studies claim that it is associated with good prognostic factors [31–33]. Therefore, the significance of CD44 expression in IBC is not yet understood.

In our study, we aimed to investigate the relationship between CD68-, CD163-, and CD11c-positive macrophage counts in the tumour microenvironment and CD44 expression in tumours with hormone receptor status, pathological stage, molecular classification, and other prognostic parameters in IBC.

Material and methods

Patients and clinical data selection

The study was initiated with the decision of the Ethics Committee of Afyonkarahisar Health Sciences University, Faculty of Medicine, clinical research no. 2019/324. Between 2010 and 2019, 150 breast carcinomas with appropriate paraffin blocks, the histopathological examination of which was performed in the Pathology Department of our hospital, and which did not receive neoadjuvant chemotherapy and/or radiotherapy, were included. Demographic data of the cases were obtained using the hospital automation system. An appropriate tumour block was selected for each patient.

Histopathologic analysis

Haematoxylin and eosin-stained preparations of the cases were obtained from the archive of the hospital's Medical Pathology Laboratory. The cases were re-evaluated based on the pathology report in terms of demographic information in Table I. Data were updated in case of incompatibility. Only the presence of macrometastases was taken as LN metastasis.

Immunohistochemistry

1–1.5-micron-thick sections were taken from paraffin blocks of tissues fixed in 10% formalin solution to adhesive slides. CD68, CD163, CD11c, and CD44 were applied to the sections taken as primary antibodies. The Bond Polymer Refine Detection (Leica brand, DS9800) kit was used for the primary antibody. All cases were studied on a Leica Bond Max automatic immunohistochemistry (IHC) device. The primary antibodies used in the immunohistochemical study and the applications performed are shown in Table II.

Assessment of immunohistochemistry staining

Estrogen receptor, PR, HER2, and Ki-67 IHC slides in the archive were re-evaluated. Oestrogen receptor and PR IHC staining were evaluated according to American Society of Clinical Oncology (ASCO)/American College of Pathologists (CAP) guidelines. The guidelines recommend classifying all cases with at least 1% receptor-positive cells. HER2 expression was also scored according to ASCO/CAP guidelines with a score of 4 ranging 0–3. Because the HER2 status of the suspected (score 2) cases in our hospital could not be confirmed by fluorescent in situ hybridization, the cases with scores of 2 and 3 were considered positive for HER2. Quantification of Ki-67 was done by counting nuclear stained cells among 1000 tumour cells. The present study was designed to evaluate the role of Ki-67 with a cut-off value of 14% in molecular subgroups and its association with patient prognosis [34]. The molecular classification of IBCs in our study according to the 12th St. Gallen International Breast Cancer Conference is divided into different subtypes. The patients were classified into luminal A, luminal B, HER2 overexpression, and triple-negative breast cancer,

as well as low Ki-67 (≤ 14%) and high Ki-67 (> 14%) expression groups using IHC (Table I).

The publication of Jeong *et al*. was based on the evaluation and scoring of CD68, CD163, and CD11c immunohistochemical stains applied in our study [3]. Cytoplasmic and membranous staining in CD68 and CD163 stains (Figs. 1, 2), and membranous staining in CD11c stain (Fig. 3) were considered positive. Each case was scanned at 40´ magnification. Three sites were selected showing the most intense macrophage infiltration. Macrophages expressing these dyes in the tumour microenvironment were counted in selected areas at 400´ magnification and the average of the 3 areas was calculated. The publication of Ryspayeva *et al*. was used for the evaluation of CD44 immunohistochemical staining [35]. Membrane staining with CD44 was taken into account in immunohistochemical staining. ≥ 10% membranous staining in tumour cells was considered positive, and cytoplasmic and < 10% membranous staining was considered negative (Fig. 4).

Statistical analysis

The obtained data were evaluated with descriptive statistics. The compatibility of the parametric data with the normal distribution was tested with the Kolmogorov-Smirnov test and graphical examinations. Student's T test and the Mann-Whitney *U* test were used to compare the mean of 2 independent groups. One-way ANOVA and the Kruskal-Wallis test was used to compare the mean of 2 or more groups. Percentage distributions between groups were evaluated with the χ^2 test and Fisher's exact test. SPSS v22 software was used in the analysis of the data, and a $p < 0.05$ level was considered significant.

Result

Patients

Our study included 150 breast carcinoma cases, all of which were female. The age range of the patients was 30–83 years, with a mean age of 55.72 ±12.16 years. Detailed clinicopathological data are presented in Table I. To make statistical analyses more meaningful, the diagnosis subgroups other than invasive ductal carcinoma and invasive lobular carcinoma were collected under the name of "other" based on the study of Özmen *et al*. [2].

İmmunohistochemical analysis

The number of CD68-, CD163-, and CD11c-positive macrophages in the tumour microenvironment and CD44 expression in the tumour were evaluated in all 150 samples. The relationship between these IHCs and clinicopathological features is shown in Table III. High numbers of TAMs (CD68, CD163, CD11c) in the tu-

Table I. Descriptive features of cases

Age (years)		
Min–max	30–83	
Mean ±SD	55.72 ±12.16	
	n	%
Histological diagnosis		
IDC	130	86.70
ILC	10	6.70
Other	10	6.70
Tumour size [cm]		
≤ 2	64	42.70
2–5	77	51.3
≥ 5	9	6.00
LN metastasis		
Absent	113	75.30
Present	37	24.70
LVI		
Absent	83	55.30
Present	67	44.70
Histological grade		
Grade 1	34	22.70
Grade 2	71	47.30
Grade 3	45	30.00
Pathological stage		
Stage 1	54	36.00
Stage 2A	67	44.70
Stage 2B	24	16.00
Stage 3A	5	3.30
Stage 3B and 3C and 4	0	0.00
ER status		
Absent	26	17.30
Present	124	87.70
PR status		
Absent	38	25.30
Present	112	74.70
HER2 status		
Score 0	137	88.00
Score 1	1	0.70
Score 2	4	2.70
Score 3	13	8.70
Ki-67 (%)		
< 14 (low)	56	37.30
≥ 14 (high)	94	62.70
Molecular subtype		
Luminal A	56	37.30
Luminal B (HER2–)	61	40.60
Luminal B (HER2+)	9	6.00
HER2-overexpressed	8	5.30
Triple-negative	16	10.70

ER – oestrogen receptor, IDC – invasive ductal carcinoma, ILC – invasive lobular carcinoma, LN – lymph node, LVI – lymphovascular invasion, PR – progesterone receptor

Table II. Immunohistochemical administration procedure

Primary antibody	Clone	Dilution	Incubation time (min)	Antigen revealing	Company
CD68	514H12	1 : 100	30	ER2	Novocastra
CD163	10D6	1 : 200	30	ER1	Novocastra
CD11c	5D11	1 : 100	30	ER1	Novocastra
CD44	MRQ-13	1 : 100	30	ER1	Cell marque

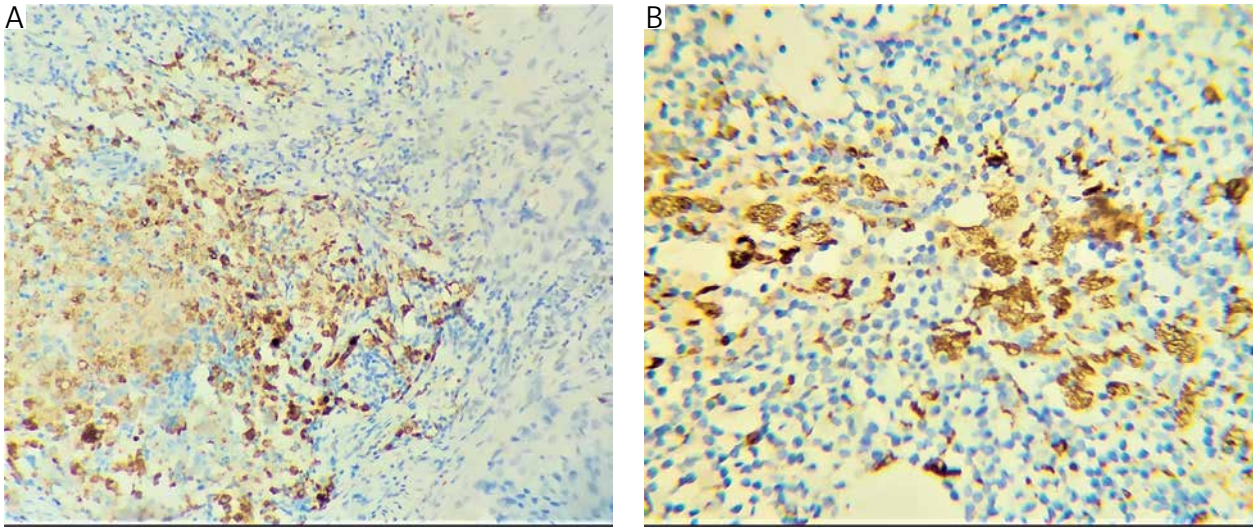

Fig. 1. Cytoplasmic and membranous CD68 positivity in macrophages in the tumour microenvironment: A) 200×; B) 400×

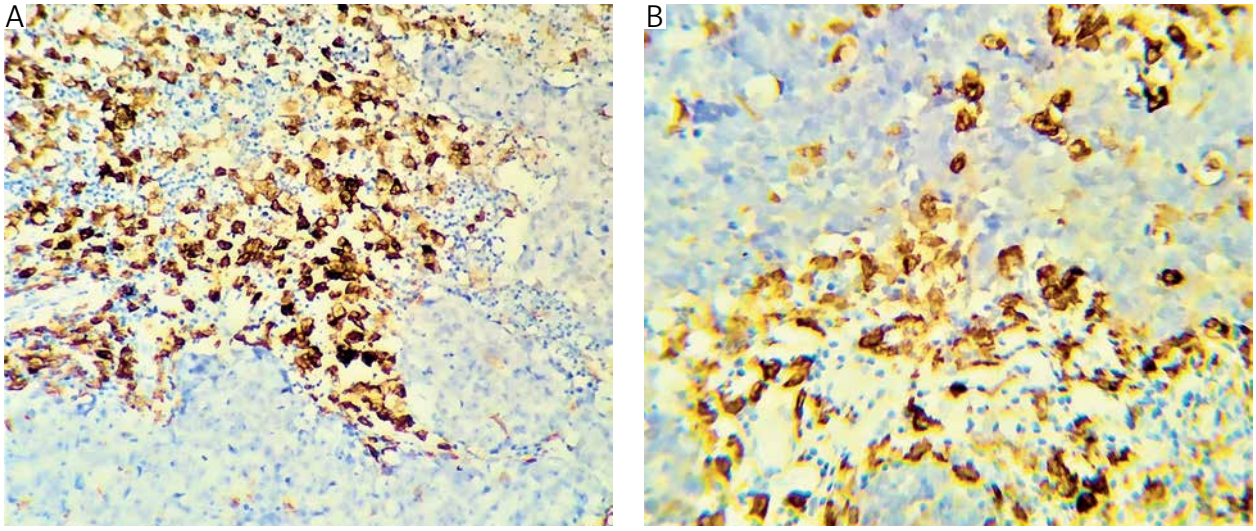

Fig. 2. Cytoplasmic and membranous CD163 positivity in macrophages in the tumour microenvironment: A) 200×; B) 400×

mour microenvironment were associated with high histological grade, ER/PR negativity, and high Ki-67 index. No correlation was observed between the prognostic parameters of CD44 positivity in the tumour.

A significant difference was found between the mean of CD68-positive macrophages in the IBC tumour microenvironment and histological diagnosis, histological grade, ER/PR negativity, and high Ki-67 index. However, no significant difference was observed between tumour diameter, LN metastasis, LVI, pathological stage, HER2 expression, and molecular subtype. When we examine the meaningful relationships in detail, "other" tumours, histological grade 3 tumours, ER/PR negative tumours, and tumours with a high Ki-67 index had higher CD68 positive macrophage averages (Table III).

Asignificant difference was found between the mean of CD163-positive macrophages in the IBC tumour

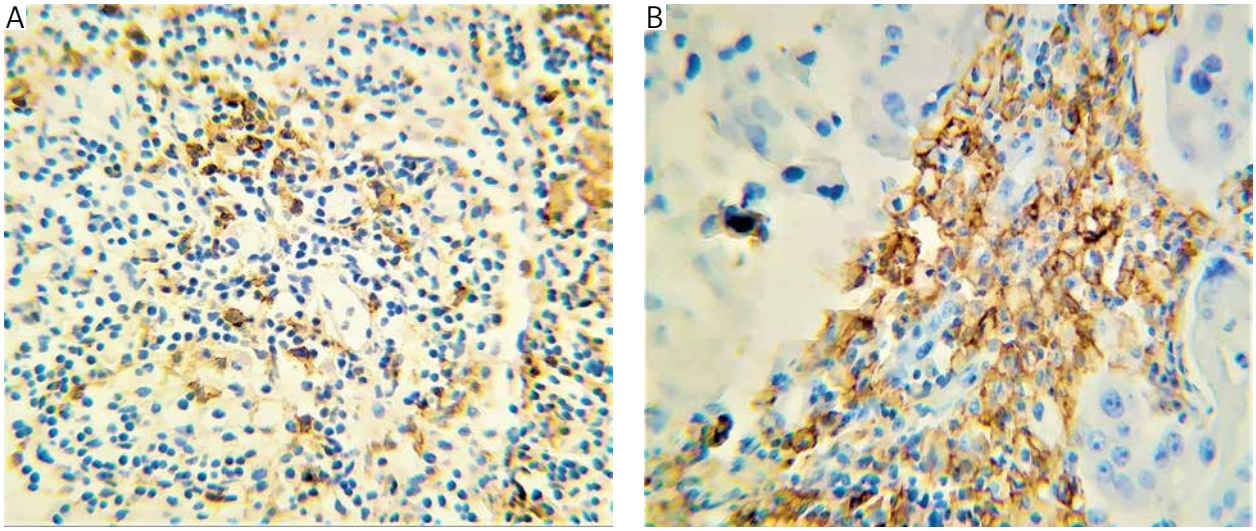

Fig. 3. Membrane CD11c positivity in macrophages in the tumour microenvironment: A) 200×; B) 400×

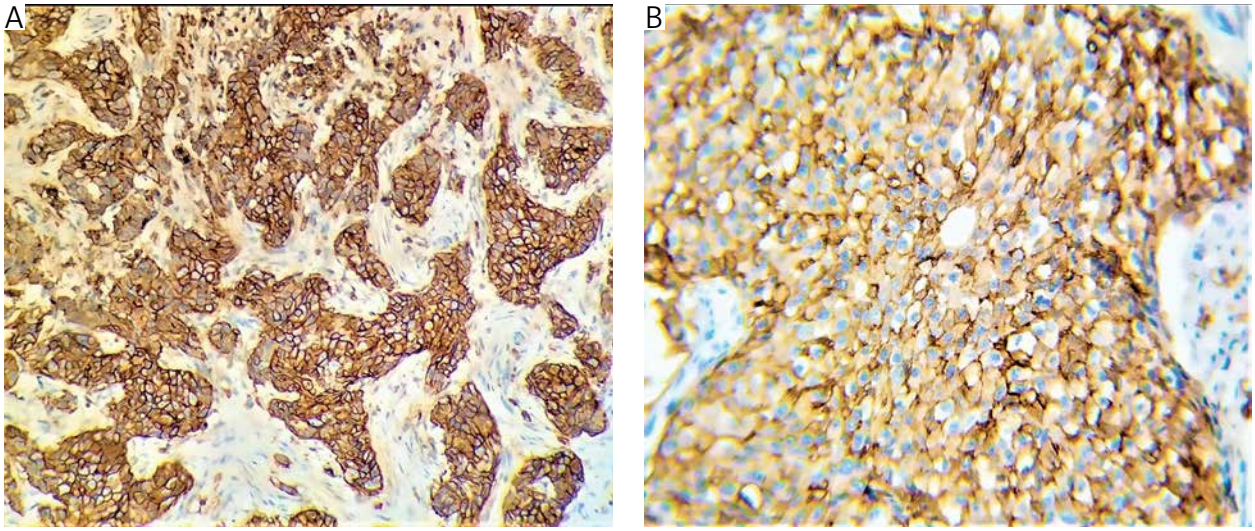

Fig. 4. Positive membranous CD44 in the tumour: A) 200×; B) 400×

microenvironment and histological grade, ER/PR negativity, high Ki-67 index, and molecular subtype. However, no significant difference was observed between histological diagnosis, tumour diameter, LN metastasis, LVI, pathological stage, and HER2 expression. When we examine the meaningful relationships in detail, histological grade 3 tumours, ER/PR negative tumours, tumours with a high Ki-67 index, and luminal A molecular subtype tumours had higher CD163-positive macrophage averages (Table III).

A significant difference was found between the mean of CD11c-positive macrophages in the IBC tumour microenvironment and histological grade, ER/PR negativity, HER2 expression, and high Ki-67 index. However, no significant difference was observed between histological diagnosis, tumour diameter, LN metastasis, LVI, pathological stage, and molecular subtype. When we examine the meaningful relationships in detail, histological grade 3 tumours, ER/PR-negative tumours, score 3 HER2-expressing tumours, and tumours with a high Ki-67 index had higher CD11c positive macrophage averages (Table III).

Considering the immunohistochemical evaluation of breast carcinomas for CD44 in IBC, 55% of the tumours were positive. There was no significant relationship between CD44 positivity in the tumour and the parameters we examined (Table III).

Correlation analysis

In the evaluations, a high level of linear correlation was found between the mean of CD68-positive macrophages and the mean of CD163- ($p < 0.001$, $r = 0.878$) and CD11c- ($p < 0.001$, $r = 0.602$) positive macrophages, and between the mean of CD163-positive macrophages and the mean of CD11c-positive mac-

Table III. Mean values of macrophages (CD68+, CD11c+, or CD163+) in the tumour and CD44 expression in the tumour in the relevant clinicopathological features of the patients

Clinicopathologic features	N	CD68		CD163		CD11c		CD44		
	150	Mean ±SD	p-value	Mean ±SD	p-value	Mean ±SD	p-value	Positive	Negative	p-value
Histological diagnosis										
IDC	130	30.19 ±14.64	0.010	23.31 ±13.09	0.070	12.00 ±8.13	0.079	78	52	0.699
ILC	10	20.50 ±9.54		15.00 ±7.78		9.30 ±6.14		5	5	
Other	10	41.50 ±24.71		28.10 ±15.24		17.60 ±14.80		5	5	
Tumour size [cm]										
≤ 2	64	30.83 ±15.39	0.712	23.38 ±13.15	0.826	11.56 ±8.59	0.669	34	30	0.310
2–5	77	29.75 ±15.97		22.57 ±13.44		12.55 ±8.85		47	30	
≥ 5	9	34.11 ±14.66		25.22 ±8.94		13.67 ±8.35		7	2	
LN metastasis										
Absent	113	31.00 ±15.98	0.471	23.48 ±13.02	0.511	12.53 ±9.22	0.407	68	45	0.320
Present	37	28.86 ±14.40		21.84 ±13.55		11.16 ±6.75		20	17	
LVI										
Absent	83	31.07 ±16.67	0.106	23.52 ±13.22	0.881	12.01 ±9.17	0.334	54	29	0.077
Present	67	29.73 ±14.22		22.53 ±13.09		12.42 ±0.08		34	33	
Histological grade										
Grade 1	34	24.03 ±14.34	< 0.001	17.44 ±11.76	< 0.001	11.15 ±8.40	< 0.001	20	14	0.821
Grade 2	71	28.90 ±13.86		21.42 ±10.97		9.55 ±6.17		40	31	
Grade 3	45	37.82 ±16.48		29.93 ±14.52		17.16 ±10.20		28	17	
Pathological stage										
Stage 1	54	20.41 ±15.30	0.820	22.87 ±13.02	0.770	11.86 ±8.59	0.781	30	24	0.290*
Stage 2A	67	30.75 ±16.54		23.66 ±13.62		12.28 ±9.42		39	28	
Stage 2B	24	31.21 ±15.04		23.23 ±13.16		13.00 ±7.42		14	10	
Stage 3A	5	24.00 ±8.45		17.20 ±7.85		10.40 ±2.96		5	0	
ER status										
Absent	26	41.73 ±17.72	< 0.001	32.62 ±14.08	< 0.001	18.19 ±10.75	< 0.001	16	10	0.744
Present	124	28.11 ±14.07		21.07 ±11.80		10.94 ±7.65		72	52	
PR status										
Absent	38	36.08 ±18.69	0.032	28.71 ±15.20	0.006	15.26 ±10.77	0.037	24	14	0.515
Present	112	28.57 ±13.97		21.16 ±11.82		11.15 ±7.62		64	48	

Table III. Cont.

Clinicopathologic features	N	CD68		CD163		CD11c		CD44		
	150	Mean ±SD	p-value	Mean ±SD	p-value	Mean ±SD	p-value	Positive	Negative	p-value
HER2 status										
Score 0 and 1	133	30.06 ±15.55	0.806	23.11 ±10.08	0.934	11.39 ±8.43	0.001	79	54	0.612
Score 2 and 3	17	31.35 ±16.32		22.82 ±13.84		18.47 ±8.19		9	8	
Ki-67 (%)										
< 14	56	24.43 ±12.76	< 0.001	18.46 ±9.71	< 0.001	9.07 ±5.97	< 0.001	35	21	0.462
≥ 14	94	34.07 ±16.05		25.82 ±14.13		14.05 ±9.49		53	41	
Molecular subtype										
Luminal A	44	37.11 ±17.42	0.054	29.07 ±14.35	0.040	14.11 ±10.51	0.109	29	15	0.170
Luminal B	55	29.22 ±13.88		22.18 ±14.06		11.62 ±7.46		36	19	
HER2-overexpressed	8	29.13 ±13.72		19.38 ±10.01		12.75 ±8.29		4	4	
Triple-negative	16	28.75 ±13.19		24.25 ±12.15		8.13 ±4.84		6	10	

ER – oestrogen receptor, IDC – invasive ductal carcinoma, ILC – invasive lobular carcinoma, LN – lymph node, LVI – lymphovascular invasion, PR – progesterone receptor
**The test result is unreliable because there are cells with a CD44 cell value of 0 (not expressing).*

rophages ($p < 0.001$, $r = 0.502$). There was no statistically significant correlation between CD44 immunohistochemical positive staining and other immunohistochemical staining (Table IV).

Discussion

There is a complex and dynamic relationship between cancer cells and the immune system. Macrophages in the tumour microenvironment play a very important role in this relationship [13]. In many types of cancer, including IBC, TAMs have been proven to play a key role in regulating various steps of tumour initiation, progression, and metastasis, resulting in poor prognosis and lower survival rates in cancer patients [5, 11, 16, 36]. It has been suggested that TAMs can be used as targets in the treatment of many cancers, and many studies have been conducted on this subject [3, 5, 8, 11, 13, 14, 16, 36, 37]. While many markers such as CD163, CD204, and CD206 were used for M2 macrophages, markers such as CD11c, CD80, and CD86 were used for M1 macrophages. CD68 is the most commonly used marker to show all macrophages regardless of phenotype [3, 13, 17].

Invasive breast cancer is also rich in stem cells [27]. CD44, CD24, ALDH1, CD133, and BMI-1 are markers used for CSC. CD44 plays important roles in processes such as loss of adhesion, penetration, invasion of the extracellular matrix, and LVI required for metastasis of a neoplastic cell. Many studies have suggested that high CD44 expression in tumours is associated with poor prognosis [27, 29, 38].

The main characteristics of the included studies based on CD68-positive macrophages (TAM) are summarized in Table V. Contrary to our study, studies in the literature indicated that the mean of CD68-positive macrophages in the tumour microenvironment was associated with a basal-like molecular subtype. Findings other than the molecular subtype in these studies support the findings in our study.

The main characteristics of the included studies based on CD163-positive macrophages (M2 macrophages) are outlined in Table VI. The findings in these studies support the findings in our study.

The main characteristics of the included studies based on CD11c-positive macrophages (M1 macrophages) are outlined in Table VII. The findings in these studies directly or indirectly support the findings in our study.

54% of tumours were CD44 positive in the study by Han *et al*. [28], and 55% of tumours were CD44 positive in the study of Ma *et al*. [32]. CD44 expression rates in breast carcinomas in these studies are similar to those in our study. The main characteristics of the included studies based on CD44-positive tumour (CSC) are outlined in Table VIII. Considering

Table IV. Correlation analysis of immunohistochemical staining properties

Protein		CD68	CD163	CD11c	CD44
CD68	*r*	1.000			
	p	–			
CD163	*r*	0.878	1.000		
	p	< 0.001	–		
CD11c	*r*	0.602	0.502	1.000	
	p	< 0.001	< 0.001	–	
CD44	*r*	0.074	0.082	0.091	1.000
	p	0.366	0.317	0.269	–

the findings in these studies, many publications confirm and contradict our study's findings. Considering the other studies in the literature on CD44 expression in tumours in breast carcinomas, the findings are highly variable and contradictory.

When we look at the correlations between the immunohistochemical stainings in our study, because CD68 is a pan-macrophage marker, the mean of CD68-positive macrophages was expected and confirmed to be higher than the mean of CD163- and CD11c-positive macrophages. Because TAMs generally show an M2-like phenotype, M2 phenotype macrophages are expected to be more dominant in the tumour microenvironment; therefore, it was not surpris-

Table V. Main characteristics of included studies based on CD68-positive macrophages (tumour-associated macrophages)

CD68	Our study	Jeong *et al.* [3]	Zhao *et al.* [39]	Ni *et al.* [14]	Campbell *et al.* [37]	Medrek *et al.* [4]
Diagnosis	+ (ILK)					
Tumour size	–	–	+	–	–	+
LN metastasis	–	–	–	+	–	–
LVI	–	–	+			
Histological grade	+	+	+	+	+	
Pathological stage	–			–	–	
ER/PR negativity	+	+	+	+	+	–
HER2 status	–	–	–	–		–
High Ki-67 index	+	+		+		+
Molecular subtype	–	+	+ (basal like type)		+ (basal like type)	

ER/PR – oestrogen receptor/progesterone receptor, LN – lymph node, LVI – lymphovascular invasion no significant association, + significant association

Table VI. Main characteristics of included studies based on CD163-positive macrophages (M2 macrophages)

CD163	Our study	Jeong *et al.* [3]	Zhao *et al.* [39]	Medrek *et al.* [4]	Ni *et al.* [14]	Esbona *et al.* [40]	Miyasato *et al.* [41]
Diagnosis	–	–				+ (IDK)	–
Tumour size	–	+	+		–	+	–
LN metastasis	–	–	–	–	+		–
LVI	–	–	+				
Histological grade	+	+	+	+	+	+	+
Pathological stage	–				–	–	
ER/PR negativity	+	+	+	+	+	+	+
HER2 status	–	–	–	–	–	–	
High Ki-67 index	+	+		+	+	+	+
Molecular subtype	+ (luminal A type)	+	+ (basal like type)	+ (luminal A and basal like type)			–

ER/PR – oestrogen receptor/progesterone receptor, LN – lymph node, LVI – lymphovascular invasion – no significant association, + significant association

ing that the mean of CD163-positive macrophages was higher than the mean of CD11c-positive macrophages [3, 8, 12, 15, 48]. This high-level correlation is present in the study of Jeong *et al*. and supports our study [3].

There was no statistically significant correlation between CD44 immunohistochemical positive staining and other immunohistochemical stainings (TAM markers: CD68, CD163, CD11c). In the study of Tiainen *et al*., contrary to our study, it was observed that there was a correlation between CD163 used for TAM marker and CD44 [8]. In the study of Guoliang Zhang *et al*. on breast carcinomas, contrary to our study, it was stated that there is a correlation between CD204 and CD206 (which are used as TAM markers) and CD44 [49]. In the study of Witschen *et al*. on breast carcinomas in rats, no relationship was found between CD206 used for TAM and CD44 [38]. There are a limited number of studies on this subject, and its importance is not understood.

In our study with all these data, it was found that high TAM averages (both M1 and M2 macrophages) in the tumour microenvironment in IBC were associated with poor prognostic parameters such as high histological grade, ER/PR negativity, and high Ki-67

Table VII. Main characteristics of included studies based on CD11c-positive macrophages (M1 macrophages)

CD11c	Our study	Jeong *et al*. [3]	Lee *et al*. [42]	Laforest *et al*. [43]	Fang *et al*. [44]*	Zhang *et al*. [45]**	Shu *et al*. [46]***
Diagnosis	–	–					
Tumour size	–	+		–		–	–
LN metastasis	–	–	–	–		–	–
LVI	–		–	–			–
Histological grade	+	+	+	+		–	–
Pathological stage	–		–	–		–	–
ER/PR negativity	+	+		–			
HER2 status	+ (score 3)	–					
High Ki-67 index	+	+					
Molecular subtype	–	+			–		

ER/PR – oestrogen receptor/progesterone receptor, LN – lymph node, LVI – lymphovascular invasion
– no significant association, + significant association
**CD80 was used to display M1 phenotype macrophages.*
***Gastric carcinomas have been studied.*
****Hepatocellular carcinoma has been studied.*

Table VIII. Main characteristics of included studies based on CD44-positive tumour (cancer stem cells)

CD44	Our study	Ricardo *et al*. [22]	Ryspayeva *et al*. [35]	Sanmartin *et al*. [31]	Zhong *et al*. [24]	Collina *et al*. [47] *
Diagnosis	–					–
Tumour size	–	+	–	–	–	–
LN metastasis	–	–	–	–	+	–
LVI	–			+		
Histological grade	–	+		+		–
Pathological stage	–		+	–	–	
ER/PR negativity	–	+			–	
HER2 status		+			–	
High Ki-67 index	–	+				–
Molecular subtype	–	–	–	+	–	–

ER/PR – oestrogen receptor/progesterone receptor, LN – lymph node, LVI – lymphovascular invasion no significant association, + significant association
**It was studied in triple-negative breast carcinomas.*

index. In addition, M2 macrophages were found to be associated with molecular subtypes and M1 macrophages with HER2 status. These findings support studies suggesting that TAMs promote tumour formation and progression in breast cancers, reduce response to treatment, and consequently adversely affect prognosis and disease-free survival [3, 18, 22, 25, 35].

Conclusions

The degree of TAM infiltration may come to the fore as a new prognostic marker in the coming days, and treatment modalities targeting TAMs may be developed. With these treatment methods, tumour progression can be prevented and the response to treatment can be increased, so the prognosis can become more favourable and the disease-free survival time can be extended. More studies are needed to prove all these predictions.

In addition, we think that the lack of a significant relationship in staining with CD44 for the detection of CSC in breast carcinomas may contribute to the contradictory findings in the literature.

The authors declare no conflict of interest.

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