Clinical value of peripheral blood M2/M1 like monocyte ratio in the diagnosis of breast cancer and the differentiation between benign and malignant breast tumors

Mustafa M. Fadhil[1], Omar F. Abdul-Rasheed[1]✉ and Manwar Al-Naqqash[2]

[1]Department of Chemistry and Biochemistry, College of Medicine, Al- Nahrain University, Baghdad, Iraq; [2]Department of Surgery, College of Medicine, Baghdad University, Baghdad, Iraq

Background: During tumor progression, circulating monocytes and macrophages are actively recruited into tumors where they alter the tumor microenvironment to accelerate tumor progression. In response to multiple microenvironmental signals from the tumor and stromal cells, macrophages change their functional phenotypes. Based on their function, macrophages are commonly classified into both, classical M1 and alternative M2 macrophages. M2-like tumor-associated macrophages promote breast tumor growth and survival, and may migrate into the peripheral blood. However, the level of circulating M2/M1-like monocyte ratio in the peripheral blood of breast cancer patients has not been yet clarified. **Aim:** To compare peripheral blood M2/M1 monocyte ratio among breast cancer patients, benign breast tumor patients and healthy subjects. Also, to investigate the role of peripheral blood M2/M1 monocyte ratio as a circulating breast cancer tumor marker and to asses the validity of this marker in differentiation between benign and malignant breast tumors. **Methods:** Flow cytometry technique was used to determine the peripheral blood M2/M1 monocyte ratio in three groups of subjects, i.e. 45 patients with breast cancer, 40 patients with benign breast tumor, and 40 healthy subjects as a control group. The results of carbohydrate antigen15-3 (CA15-3) determination were analyzed comparatively. **Results:** The peripheral blood M2/M1 monocyte ratio in patients with breast cancer (0.27±0.1) was significantly higher (*P*<0.001) than that in healthy subjects (0.07±0.05) and than in benign tumor subjects (0.08±0.04). The area under the receiver operating characteristic (ROC) curve of peripheral blood M2/M1 monocyte ratio determination was significantly higher (*P*≤0.001) than that of CA15-3 levels. **Conclusion:** M2/M1-like monocyte ratio is of a high diagnostic value for breast cancer and is a promising differentiating marker between benign and breast cancer tumor groups.

✉e-mail: omar.rasheed@colmed-alnahrain.edu.iq

Abbreviations: TAM, Tumor-associated macrophages; BC, Breast Cancer; EGF, Epidermal growth factor; IHC, Immunohistochemistry; ER, Estrogen receptor; PR, Progesterone receptor; Her-2/neu (Her-2), human epidermal growth factor receptor 2

INTRODUCTION

Breast cancer is the most common cancer diagnosed in women, accounting for more than 1 in 10 new cancer diagnoses each year. It is the second most common cause of death from cancer among women in the world (Bray *et al.*, 2018). Breast cancer has become a major threat to female health in Iraq, where it is the leading cause of death after cardiovascular diseases among women, with a cancer-related mortality rate of 23% (Alwan, 2016). Since 1986, it has been the highest-ranked malignancy among the Iraqi population in general (Alwan, 2016). Breast tumors are broadly classified as a benign or malignant mass. The main fear of many women presenting with breast tumor is the likelihood of cancer, but reassuringly, most breast masses are benign (Degnim *et al.*, 2017). Malignant breast mass encompasses many histological types, but is not limited, such as in situ ductal or lobular carcinoma, infiltrating ductal or lobular carcinoma and inflammatory carcinoma (Degnim *et al.*, 2017). Breast cancer is known as cancer that developed from breast tissues. Breast cancer most commonly develops in cells from the lining of milk ducts and the lobules that supply the ducts with milk (Foster, Jr, 2003). Cancers developing from the ducts are known as ductal carcinomas, while those developing from lobules are known as lobular carcinomas (Calhoun & Anderson, 2017). About 5–10% of cases are due to genes inherited from a person's parents (Kuchenbaecker *et al.*, 2017). Breast cancer is the most prevalent type of cancer among women around the world and has the highest fatality rate (Parkin *et al.*, 2005). The most reliable detection methods are mammography and core needle biopsy. However, these methods are not sensitive or comfortable enough for women to select as routine examinations. For the liquid biopsy, the existing markers, such as carcinoembryonic antigen or carbohydrate antigen 15-3, are not recommended for screening or diagnosis of breast cancer because of their low sensitivity in early detection (Harris *et al.*, 2007). Therefore, a convenient, effective method for early detection is urgently needed. Monocytes are released from the bone marrow into the blood, and after 3 to 4 days in circulation they enter the tissues and serosal cavity where they are considered as macrophages (Sica *et al.*, 2008). Tumor related macrophage (tumor-associated macrophage TAM) plays an important role in the development of tumors (Sica *et al.*, 2008). Studies have shown that TAM usually has 2 states in the tumor tissue which are characterized by a completely different polarization ,

namely M1 and M2. The M1 type macrophages generally appear at the early stage of tumorigenesis, at benign lesions and in the tissues which are surrounding the cancer nest, and are killing the tumor cells, promote inflammatory responses and other functions; M2 macrophages exist during the occurrence and development of tumors, promote tumor growth and functions, such as angiogenesis and tumor metastasis (Sica *et al.*, 2008). Previous studies have confirmed that TAM can allow cancer cells to pass through the capillary wall in the tumor tissue and enter the blood circulation (Roussos *et al.*, 2011; Adams *et al.*, 2014). Wyckoff *et al.* have shown that mammary tumors exhibit a paracrine loop between TAMs and cancer cells (Wyckoff *et al.*, 2004). TAMs express monocyte colony-stimulating factor receptor (M-CSFR, also known as CSF-1R or cFMS), which binds the monocyte colony-stimulating factor (M-CSF, also known as CSF-1) secreted by cancer cells. Conversely, TAMs secrete epidermal growth factor (EGF) and activate the EGF receptor (EGFR) on the cancer cells. This allows co-migration of the two cell types, and thus enhancing motility and subsequent invasion of healthy surrounding tissue and intravasation (Sousa *et al.*, 2015). The immune system is a major player in the cancer cell/tumor microenvironment crosstalk. In solid tumors, 5–40% of the tumor mass consists of tumor-associated macrophages (TAMs). Approximately 80% of the publications in this field report an association between TAMs and poor prognosis (Pollard, 2008). The new nomenclature that groups monocytes into three subsets, based on expression of the surface markers CD14 and CD16, has been approved by the Nomenclature Committee of the International Union of Immunologic Societies (Ziegler-Heitbrock *et al.*, 2010). Based on this nomenclature, the major population of human monocytes (90%) with high CD14 but no CD16 expression ($CD14^{++}CD16^-$ or $CD14^+CD16^-$) are termed classical monocytes, whereas the minor population of human monocytes (10%) is further subdivided into the intermediate subset, with low CD16 and high CD14 ($CD14^{++}CD16^+$ or $CD14^+CD16^+$), and the non-classical subset, with high CD16 but with relatively lower CD14 expression ($CD14^+CD16^{++}$ or CD14 $dimCD16^+$) (Italiani & Boraschi, 2014).

This study was aimed to explore whether the circulating M2/M1-like monocyte ratio might be used as a new circulating tumor marker for Breast Cancer (BC) diagnosis and differentiating between BC and benign breast tumors.

MATERIALS AND METHODS

Patients and ethics. A case-control study was conducted at the Department of Chemistry and Biochemistry at the College of Medicine, Al-Nahrain University, during the period from November 2018 to April 2019. The study involved 125 women who are consulting at the specialized tumor hospital in medical city at Baghdad governorate.

The patients were women having breast tumors. They were divided into two groups. Group 1 included a total of 45 cases of malignant breast tumor patients whose age range was between 18–65 years.

Group 2 included 40 cases of benign breast tumor patients whose age range was between 18–65 years.

Control group included 40 healthy volunteer women with matched age range.

Exclusion criteria of the study included patients with breast cancer after surgical removal, patients after chemotherapy, patients with viral hepatitis, infectious mononucleosis, atherosclerosis, positive human immunodeficiency virus (HIV) patients, as well as patients with any autoimmune disorder.

All patients that were included were interviewed as having breast tumors by using triple assessment, mammography, clinical examination, ultrasonography and some of them by using fine-needle aspiration cytology.

The study was approved by the Institutional Review Board (IRB) and was performed in accordance with the revised Helsinki Declaration of the World Medical Association. Informed consent was acquired from all individuals.

The data were collected using a short structured questionnaire that included information on age, weight, height, family history of breast cancer or other malignancies, and history of other diseases (Table 1).

Flow cytometry detection of M2 and M1-like monocytes. Main Reagents. Fluorescein isothiocyanate (fluorescein isothiocyanate, FITC) labeled CD45 antibody (for cell gating) and phycoerythrin (PE) labeled CD16 antibody were purchased from Biolegend, USA. Allophycocyanin (APC) CD14 antibody was purchased from Biolegend, USA.

Immunophenotyping $CD14^+$, $CD16^+$, and $CD45^+$ expression were investigated using flow cytometry (FCM) technique.

Assay principle. Proportion of monocytes was defined by the flow cytometry method. The basic principles of flow cytometry are the passage of cells in single file in front of a laser, so they can be detected, counted and sorted. Flow cytometry measures optical and fluorescence characteristics of a single cell. Physical properties, such as size (represented by forward scatter FSC) and inner complexity (represented by side scatter SSC), resolve certain cell populations (Bain & Dacie, 2011). The dyes are bound with different cellular components, such as DNA or RNA. Furthermore, antibodies conjugated to fluorescent dye can bind specific proteins on cell membranes or inside cells. When labeled cells are passed by a light source, the fluorescent molecules are excited to a higher energy state. Upon returning to resting states, the fluorochromes emit light energy at higher wavelengths. The use of multiple fluorochromes, each with similar excitation and different emission wavelengths allows several cell properties to be measured at the same time. The common dyes include phycoerythrin, propidium iodide, and fluorescein. Tandem dyes with internal fluorescence resonance energy transfer can create more colors and longer wavelengths (Bain & Dacie, 2011). Inside a flow cytometer, cells in suspension are drawn into a stream created by a surrounding sheath of isotonic fluid that creates laminar flow, allowing the cells to pass individually through an interrogation point. A laser beam intersects the cells. The emitted light is given off in all directions and collected via optics that direct the light to a series of filters and mirrors that isolate particular wavelength bands. The light signals are detected by photomultiplier tubes and digitized for computer analysis. The resulting information is displayed in a histogram. The entire operation is fully automated: from washing to final reading (Bain & Dacie, 2011).

Determination of M2 monocytes was done by CD16 antibody and of M1 by CD14 antibody, and the gating was done by CD45 antibody. Gating refers to selecting a particular region of data to be analyzed andallows to look at parameters that are specific to only that subset.

Assay procedure. Fresh 100 μl of ethylenediaminetetraaceticacid (EDTA) anti-coagulated blood samples were

Table 1. Anthropometric and clinical characteristics of patients and controls

Variables	Breast cancer patients	Benign breast tumor patients	Controls	P
	N=45	N=40	N=40	
Age (years)[a]	52.0±8.96	52.63±7.5	52.77±7.2	0.923
BMI (kg/m²)[a]	26.02±7.14	25.5±3.2	25.54±5.4	0.746
History of BC[b] No Yes	 26 (57.77%) 19 (42.23%)	 22 (55%) 18 (45%)	 21 (52.5%) 19 (47.5%)	 0.861
Menopausal status[b] Pre-menopausal Post-menopausal	 18 (40 %) 27 (60%)	 17 (42.5%) 23 (47.5%)	 16 (40%) 24 (60%)	 0.621
Histological type Invasive ductal carcinoma Ductal carcinoma *in situ* Others	 29 (64.6%) 8 (17.7%) 8 (17.7%)	 – 	 – 	 –
Histological grade II III	 25 (55.5%) 20 (45.5%)	 – 	 – 	 –
Tumor size (mm) ≤22 >22	 10 (22.22%) 35 (77.87%)	 – 	 – 	 –
Nodal Status pN0 pN+	 23 (51.1%) 22 (49.9%)	 – 	 – 	 –
Estrogen receptor Positive Negative	 17 (37.7%) 28 (62.3%)	 – 	 – 	 –
Progesterone receptor Positive Negative	 20 (44.44%) 25 (55.56%)	 – 	 – 	 –
HER-2/neu receptor Positive Negative	 14 (31.1%) 31 (69.9%)	 – 	 – 	 –

[a]Data are mean ± S.D. ; [b]Chi-square test; BMI, Body mass index

labeled with the following monoclonal anti-human antibodies: FITC anti CD45, 7.5 µl (BioLegend USA), 4.5 µl APC anti CD14 (BioLegend USA) and 6.0 µl PE anti CD16 (BioLegend USA). Samples were incubated for fifteen minutes in the dark, at room temperature. Then, 2 ml of FACS lysing solution were added to lyse erythrocytes. Finally, the antibody-labeled monocytes were detected using a fully-equipped desktop four-color flow cytometer (FCM) (Zhang *et al.*, 2017).

The cell group was gated with CD45. CD16 was used as an M2-like surface marker and CD14 was used as M1 macrophage surface marker.

Immunohistochemistry. IHC methods involve special tissue-staining techniques with labeling antibodies, which requires pathology laboratory infrastructure; quality control is quite important to testing accuracy (Gelband *et al.*, 2015). Immunohistochemistry for ER and PR-formalin (10%) fixed, paraffin-embedded sections of cell blocks were stained for ER and PR using the primary monoclonal antibodies to ER (Clone ID 5; DAKO, Glostrup, Denmark, Cat No. M 7047), and PR (Clone PgR 636; DAKO, Carpinteria – CA, Cat No. M 3569) at a dilution of 1:50, according to the manufacturer's specification. The ASCO/CAP guideline recommendations were used for assessment of the hormone receptors. The tumor cells were considered positive for ER or PR status if more than or equal to 1% of tumor cells demonstrated nuclear staining; less than 1% is negative. Normal breast tissue was used as a positive external control in all cell block preparations (Francis *et al.*, 2019). Immunohis-

tochemistry for HER-2 invloved firstly fixed by formalin, paraffin-embedded sections of cell blocks, which were stained for HER-2 using HER-2/neu antibodies (polyclonal; DAKO, Glostrup, Denmark, Cat No. A 0485) at a dilution of 1:50, according to the manufacturer's specification. Stains were assessed by using the ASCO/CAP 2013 guidelines, HER-2-IHC-3+ was considered when >10% of tumor cells had shown a homogeneous dark circumferential (chicken wire) pattern. Incomplete and/or weak/moderate membrane staining and within more than 10% of tumor cells, or complete membrane staining that is intense and within less than or equal to 10% of the tumor cells, was interpreted as equivocal (HER-2-IHC-2+). Incomplete membrane staining that was faint/barely perceptible and within >10% of the tumor cells was classified as HER-2-IHC-1+, and HER-2-IHC-0 was defined by no staining observed, or membrane staining that is incomplete and is faint/barely perceptible and within ≤10% of the tumor cells. HER-2-IHC-1+ and HER-2-IHC-0 were interpreted as HER-2 negative (Wolff *et al.*, 2013).

Quantification of plasma CA15-3 level. The level of plasma cancer antigen 15-3 was measured by using an ELISA kit (CALBIOTECH, U.S.A) for quantitative measurement of serum CA 15-3.

Statistical analysis. Stasistical analyses were performed using SPSS 17.0. Results were expressed as mean ± standard deviation (S.D.) and all statistical comparisons were carried out by means of independent t-test and analysis of variance (ANOVA) test. All statistical

tests' differences with a *P* level of <0.05 were considered to be significant. In addition, areas under the curve (AUC) of receiver operating characteristics (ROC) curves were calculated, as well as specificity and sensitivity were also estimated (Perkins & Schisterman, 2006).

RESULTS

The Hematological and biochemical characteristics of the three groups (45 breast cancer patients, 40 benign breast disease patients and 40 controls) are summarized in (Table 2).

As shown in Table 2, there is no significant difference among the three groups in the WBC count, monocytes count, neutrophil count, and platelets level. The ratio of M2/M1 monocytes was examined by evaluation of M2-like and M1 percentages.

A generally accepted definition of M2 like macrophages is $CD16^{++}$ and $CD14^+$, and M1 type definition is $CD14^{++}$ and $CD16^-$, while intermediate monocytes are those cells which express $CD14^+$ $CD16^+$ as shown in (Fig. 1).

As expected, the percentages of M2-like macrophages were significantly elevated ($P \leq 0.05$) in BC, and are higher than those in benign and healthy controls (Table 3).

However, there was no significant difference ($P<0.005$) in the percentage of M2 between the benign and healthy subjects.

A preliminary analysis was then performed to identify whether the percentage of M2 and M1-like macrophages were associated with patient's characteristics as shown in Tables 4 and 5.

ROC curve was used to evaluate the diagnostic value of M2 and M2/M1 monocyte ratio and then a comparison with CA15-3 diagnostic value was performed by assessing the sensitivity, specificity, and accuracy of markers.

Each of M2, M2/M1 ratio and CA15-3 had significantly higher values in BC women when compared to BBT. Accordingly, these parameters were evaluated for
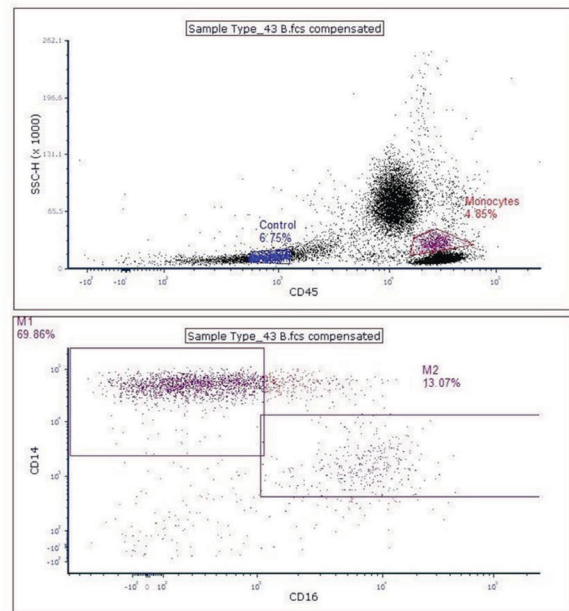
Figure 1. Characterization of circulating M2-like macrophages by flow cytometry

their diagnostic value in the context of discrimination between breast cancer and benign breast tumor group (Fig. 2).

For M2, the AUC was 0.968, 95% CI=0.932–1.0, *P*<0.001. The sensitivity and specificity of the test at cut off value of M2=10.45% was 0.933 and 0.867, respectively. For M2/M1, the AUC was 0.967, 95% CI=0.93–1.0, *P*<0.001. The sensitivity and specificity at cut off value of the M2/M1 ratio=0.145 was 86.7% and 93.1%, for CA15-3, the AUC was 0.639, 95% CI=0.497–0.782, *P*<0.064. The sensitivity and specificity of the test at the cut off value of CA15-3=39.9 U/mL was 0.633 and 0.667, respectively.

Table 2. Hematological and biochemical characteristics of subjects.

Variables	Breast cancer patients (n=45)	Benign breast tumor (n=40)	Controls (n=40)	*p*-value
WBC x) 10³/mm³)	8.12±4.03	7.86±3.06	6.7±2.05	0.128
Monocyte x) 10³/mm³)	0.96±0.66	0.63±0.36	0.67±0.28	0.015
Neutrophils x) 10³/mm³)	5.3±3.1	5.1±2.45	3.7±1.7	0.073
Platelets x) 10³/mm³)	277.4±78.15	267.93±66.15	260.77±62.46	0.648
CA15-3 (pg/mL)	49.07±22.89	37.39±14.32	24.34±8.76	<0.001

Data are listed as mean ± S.D.

Table 3. M2, M1, and M2/M1 monocyte ratio status among breast cancer, benign breast tumor and healthy subjects

Variables	Breast cancer patients (n=45)	Benign breast tumor (n=40)	Controls (n=40)	*p*-value
M1(%)	73.74±6.4[a]	84.72±4.85[b]	86.52±5.82[b]	<0.001
M2(%)	19.32±6.14[a]	6.65±3.3[b]	5.65±3.5[b]	<0.001
Intermediate M(%)	6.97±2.95[a]	8.72±3.08[b]	7.86±3.76[ab]	0.123
M2/M1 ratio	0.27±0.1[a]	0.08±0.04[b]	0.07±0.05[b]	<0.001

Table 4. M1, M2-like monocyte levels in different breast cancer characteristics

Characteristics	M1 (%)	p-value	M2 (%)	p-value
Histological type				
Invasive ductal carcinoma	73.97±7.23		19.03±6.55	
Ductal carcinoma in situ	71.45±2.37	0.742	20.3±6.31	0.913
Others	74.72±4.0		19.95±4.64	
Nodal Status				
pN0	73.57±6.4		20.05±7.1	
pN+	73.9±6.63	0.892	18.6±5.14	0.526
Histological grade				
II	74.45±6.31		19.22±6.2	
III	72.67±6.67	0.465	19.47±6.3	0.917
Tumor size (mm)				
≤22	72.64±7.45		20.42±7.1	
>22	73.96±6.32	0.68	19.1±5.83	0.668

Table 5. M1 and M2-like macrophages in different hormonal receptor status of breast cancer patients

Hormonal Receptor Status	HER-2	No	M1%	M2%
Both negative	Negative	13	72.09±6.64	20.87±6.1
Both positive	Positive	6	71.73±4.64	18.67±6.54
Both positive	Negative	5	74.86±5.53	19.9±6.45
Both negative	Positive	6	78.43±9.92	14.8±8.97
Either of which negative	Negative	10	73.28±6.75	19.38±6.73
Either of which positive	Positive	5	76.63±4.32	18.6±3.56
P-value			0.686	0.825
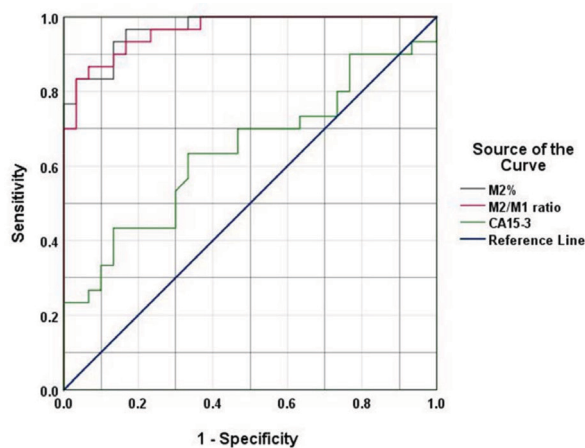
Figure 2. M2 monocytes, M2/M1 monocyte ratio, CA15-3 ROC curve between breast cancer and benign breast tumors

Figure 3. M2, M2/M1 monocyte ratio, CA15-3 ROC curve between breast cancer subjects and healthy controls

The same parameters were used for discrimination between BC and healthy controls. For M2, the AUC was 0.974, 95% CI=0.943–1.00, P<0.001. The sensitivity and specificity of the test at cut off values of M2=10.1% was 0.967 and 0.90, respectively. For M2/M1, the AUC was 0.963, 95%CI=0.924-1.0, P<0.001. The sensitivity and specificity of the test at M1/M2 ratio=0.14 was 0.876 and 0.90, respectively, as shown in Fig. 3.

DISCUSSION

Macrophages continuously infiltrate into the microenvironment of solid tumors and are induced to form TAMs by cancer cells and other factors (Heusinkveld & van der Burg, 2011). Several studies have reported that the polarize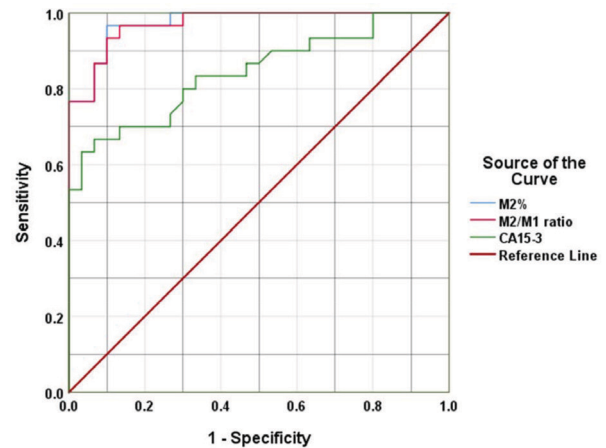d states and levels of TAMs in pretreatment biopsies are altered in BC (Tang, 2013). TAMs resemble M2-like macrophages, and M2-like TAMs facilitate tumor progression, angiogenesis, metastasis, matrix remodeling, and treatment resistance (Sica *et al.*, 2008; Obeid *et al.*, 2013). TAMs derived from the tumor tissue disseminate into the peripheral blood circulation in large numbers, in conjunction with circulating tumor cell (CTCs) *via* transendothelial migration (Adams *et al.*, 2014). Recently, circulating M2-like monocytes have been reported as a useful marker for diagnosis of many cancers, such as non-small-cell lung cancer and colorectal cancer (Zhang *et al.*, 2017). However, until now, few studies have reported the use of circulating M2-like monocytes as a diagnostic marker for breast cancer. Furthermore, data on this cell type are limited, and there have been contradictory results regarding the value of circulating tumor-associated monocytes as potential biomarkers in cancer

patients (Jóźwik *et al.*, 2015).Thus, this study focuses on evaluating the diagnostic value of circulating M2/M1-like monocytes in breast cancer.

Our data indicated that the ratio of circulating M2/M1-like monocytes was increased in the peripheral blood of BC patients and this ratio was promising in differentiating malignant tumor from benign breast tumor patients. Zhang and coworkers (Zhang *et al.*, 2017) stated that the number of M2-like macrophages was increased in histological BC sections.

Accordingly, this research investigated whether M2-like monocytes could be detected in the circulating blood of BC patients. We found that the levels of circulating M2-like monocytes and M2/M1 monocyte ratio were significantly elevated in patients with breast cancer when compared with healthy controls and benign patients. This result confirmed previous findings that M2-like TAMs may leak into circulation from tumor sites, and the subsequent increase in circulating M2/M1-like monocyte ratio may serve as a tumor marker (Jóźwik *et al.*, 2015).

Most of the currently used tumor markers are not suitable as diagnostic tools for the early detection of cancers. CA15-3 are a common marker of BC; however, it lacks sensitivity for early diagnosis (Mirabelli & Incoronato, 2013). Additionally, the American Society of Clinical Oncology (ASCO) guidelines do not recommend the use of CA15-3 for BC screening or directing treatment (Harris *et al.*, 2007).

In this study, plasma CA15-3 levels were significantly higher in BC patients than in healthy controls and benign patients. In accordance with findings from previous studies, our results showed that CA15-3 had a relatively low sensitivity in detecting BC. When patients with BC were compared with the healthy population, the ratio of circulating M2/M1-like monocytes had a higher AUC and sensitivity. Furthermore, when comparing BC patients with benign patients, we found that the sensitivity of circulating M2/M1-like monocyte ratio was higher than CA15-3. Accordingly, in terms of diagnostic value in BC, circulating M2/M1-like monocytes were superior to CA15-3. Furthermore, there were no associations between M2/ M1-like monocyte ratio, and cancer characteristics or hormonal receptor statuses in our study. Therefore, it is reasonable to state that there are no possible interactions between circulating M2-like monocytes and BC progression.

In conclusion, our data showed that circulating M2/M1-like monocyte ratio may represent a novel blood-based biomarker for breast cancer and may have a potential utility as a diagnostic tool. The high sensitivity of circulating M2/M1-like monocyte ratio for detecting BC patients indicates that this ratio may be helpful to the currently used biomarkers in breast cancer diagnosis and differentiating malignancy from benign cases. It should be noted, however, that our analyses only focused on monocytes and the sample size was relatively small. Therefore, further studies with a larger population and the collection of matching histological specimens are necessary to confirm our results.

Conflict of interest

The authors declare that there is no conflict of interest with respects to the publication of this article.

Acknowledgements

REFERENCES

Adams DL, Martin SS, Alpaugh RK, Charpentier M, Tsai S, Bergan RC, Ogden IM, Catalona W, Chumsri S, Tang C-M, Cristofanilli M (2014) Circulating giant macrophages as a potential biomarker of solid tumors. *Proc Natl Acad Sci* 111: 3514–3519. https://doi.org/10.1073/pnas.1320198111

Alwan NAS (2016) Breast cancer among iraqi women: preliminary findings from a regional comparative breast cancer research project. *J Global Oncol* 2: 255–258. https://doi.org/10.1200/jgo.2015.003087

Bain BJ, Dacie JV (2011) *Dacie and Lewis practical haematology.* Churchill Livingstone

Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer J Clinicians* 68: 394–424. https://doi.org/10.3322/caac.21492

Calhoun KE, Anderson BO (2017) Lobular carcinoma in situ of the breast. In: *The Breast: Comprehensive Management of Benign and Malignant Diseases.* 553–561.e2. https://doi.org/10.1016/B978-0-323-35955-9.00038-6

Degnim AC, Hoskin TL, Arshad M, Frost MH, Winham SJ, Brahmbhatt RA, Pena A, Carter JM, Stallings-Mann ML, Murphy LM, Miller EE, Denison LA, Vachon CM, Knutson KL, Radisky DC, Visscher DW (2017) Alterations in the immune cell composition in premalignant breast tissue that precede breast cancer development. *Clin Cancer Res* 23: 3945–3952. https://doi.org/10.1158/1078-0432.CCR-16-2026

Foster, Jr RS (2003) Breast cancer detection and treatment. *Arch Surgery* 138: 397. https://doi.org/10.1001/archsurg.138.4.397

Francis I, Altemaimi R, Al-Ayadhy B, Alath P, Jaragh M, Mothafar F, Kapila K (2019) Hormone receptors and human epidermal growth factor (HER2) expression in fine-needle aspirates from metastatic breast carcinoma – Role in patient management. *J Cytol* 36: 94. https://doi.org/10.4103/JOC.JOC_117_18

Gelband H, Jha P, Sankaranarayanan R, Horton S (2015) Disease Control Priorities, Cancer. In: *Cancer: Disease Control Priorities,* edn 3, vol 3. The International Bank for Reconstruction and Development/The World Bank. https://doi.org/10.1596/978-1-4648-0349-9

Harris L, Fritsche H, Mennel R, Norton L, Ravdin P, Taube S, Somerfield MR, Hayes DF, Bast RC (2007) American society of clinical oncology 2007 update of recommendations for the use of tumor markers in breast cancer. *J Clin Oncol* 25: 5287–5312. https://doi.org/10.1200/JCO.2007.14.2364

Heusinkveld M, van der Burg SH (2011) Identification and manipulation of tumor associated macrophages in human cancers. *J Trans Med* 9: 216. https://doi.org/10.1186/1479-5876-9-216

Italiani P, Boraschi D (2014) From monocytes to M1/M2 macrophages: Phenotypical *vs.* functional differentiation. *Front Immunol* 5: 514. https://doi.org/10.3389/fimmu.2014.00514

Jóźwik M, Okungbowa OE, Lipska A, Jóźwik M, Smoktunowicz M, Semczuk A, Jóźwik M, Radziwon P (2015) Surface antigen expression on peripheral blood monocytes in women with gynecologic malignancies. *BMC Cancer* 15: 129. https://doi.org/10.1186/s12885-015-1136-x

Kuchenbaecker KB, Hopper JL, Barnes DR, Phillips K-A, Mooij TM, Roos-Blom M-J, Jervis S, van Leeuwen FE, Milne RL, Andrieu N, Goldgar DE, Terry MB, Rookus MA, Easton DF, Antoniou AC, Consortium and the B and BC (2017) Risks of breast, ovarian, and contralateral breast cancer for BRCA1 and BRCA2 mutation carriers. *JAMA* 317: 2402–2416. https://doi.org/10.1001/jama.2017.7112

Mirabelli P, Incoronato M (2013) Usefulness of traditional serum biomarkers for management of breast cancer patients. *BioMed Res Internat* 2013. https://doi.org/10.1155/2013/685641

Obeid E, Nanda R, Fu YX, Olopade OI (2013) The role of tumor-associated macrophages in breast cancer progression (review). *Internat J Oncol* 43: 5–12. https://doi.org/10.3892/ijo.2013.1938

Parkin DM, Bray F, Ferlay J, Pisani P (2005) Global Cancer Statistics, 2002. *CA: A Cancer Journal for Clinicians* 55: 74–108. https://doi.org/10.3322/canjclin.55.2.74

Perkins NJ, Schisterman EF (2006) The inconsistency of "optimal" cutpoints obtained using two criteria based on the receiver operating characteristic curve. *Am J Epidemiol* 163: 670–675. https://doi.org/10.1093/aje/kwj063

Pollard JW (2008) Macrophages define the invasive microenvironment in breast cancer. *J Leukocyte Biol* 84: 623–630. https://doi.org/10.1189/jlb.1107762

Roussos ET, Balsamo M, Alford SK, Wyckoff JB, Gligorijevic B, Wang Y, Pozzuto M, Stobezki R, Goswami S, Segall JE, Lauffenburger DA, Bresnick AR, Gertler FB, Condeelis JS (2011) Mena invasive (MenaINV) promotes multicellular streaming motility and transendothelial migration in a mouse model of breast cancer. *J Cell Sci* 124: 2120–2131. https://doi.org/10.1242/jcs.086231

Sica A, Larghi P, Mancino A, Rubino L, Porta C, Totaro MG, Rimoldi M, Biswas SK, Allavena P, Mantovani A (2008) Macrophage polari-

zation in tumour progression. *Seminars in Cancer Biology* **18**: 349–355. https://doi.org/10.1016/J.SEMCANCER.2008.03.004

Sousa S, Brion R, Lintunen M, Kronqvist P, Sandholm J, Mönkkönen J, Kellokumpu-Lehtinen PL, Lauttia S, Tynninen O, Joensuu H, Heymann D, Määttä JA (2015) Human breast cancer cells educate macrophages toward the M2 activation status. *Breast Cancer Res* **17**: 101. https://doi.org/10.1186/s13058-015-0621-0

Tang X (2013) Tumor-associated macrophages as potential diagnostic and prognostic biomarkers in breast cancer. *Cancer Lett* **332**: 3–10. https://doi.org/10.1016/j.canlet.2013.01.024

Wolff AC, Hammond MEH, Hicks DG, Dowsett M, McShane LM, Allison KH, Allred DC, Bartlett JMS, Bilous M, Fitzgibbons P, Hanna W, Jenkins RB, Mangu PB, Paik S, Perez EA, Press MF, Spears PA, Vance GH, Viale G, Hayes DF (2013) Recommendations for human epidermal growth factor receptor 2 testing in breast. *J Clin Oncol* **31**: 3997–4013. https://doi.org/10.1200/JCO.2013.50.9984

Wyckoff J, Wang W, Lin EY, Wang Y, Pixley F, Stanley ER, Graf T, Pollard JW, Segall J, Condeelis J (2004) A paracrine loop between tumor cells and macrophages is required for tumor cell migration in mammary tumors. *Cancer Res* **64**: 7022–7029. https://doi.org/10.1158/0008-5472.CAN-04-1449

Zhang B, Cao M, He Y, Liu Y, Zhang G, Yang C, Du Y, Xu J, Hu J, Gao F (2017) Combination of plasma HA and circulating M2-like monocytes may serve as a diagnostic marker for breast cancer. *J Cancer* **8**: 3522–3530. https://doi.org/10.7150/jca.20227

Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, Hart DN, Leenen PJM, Liu YJ, MacPherson G, Randolph GJ, Scherberich J, Schmitz J, Shortman K, Sozzani S, Strobl H, Zembala M, Austyn JM, Lutz MB (2010) Nomenclature of monocytes and dendritic cells in blood. *Blood* **116**: e74 LP-e80. https://doi.org/010.1182/blood-2010-02-258558.