The Association of Immune Cell Infiltration with Metastasis Location in De Novo Metastatic Triple Negative Breast Cancer: A Multicenter Cross-Sectional Study in Indonesia

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ABSTRACT

Background: *Triple-negative breast cancer (TNBC) is an aggressive cancer subtype, with limited treatments and a high metastasis risk. The varying location of metastasis in TNBC patients often leads to in prognosis in breast cancer. Therefore, this study aimed to investigate the potential association between immune cells profiles in the tumor microenvironment and metastatic patterns.* ***Methods:*** *We conducted a multicenter cross-sectional study in 2022 to examine formalin-fixed paraffin-embedded (FFPE) and medical record data from 2015 to 2020 in de novo metastatic TNBC patients. The medical records provided crucial information about the sites of metastasis. Immunohistochemistry (IHC) analysis was carried out on primary breast tumor tissues to evaluate the expressions of cluster of differentiation (CD)4 T-cells, CD8 T-cells, CD163, FOXP3 Tregs, and programmed death-ligand 1 (PD-L1), along with immune cells ratios showing antitumor-to-protumor activity (CD4/FOXP3, CD8/FOXP3, CD4/CD163, CD8/CD163). Metastatic locations were grouped into bone-only, visceral, lung, liver, and brain metastasis.* ***Results:*** *A total of 120 metastatic TNBC patients were documented for their metastatic location and IHC report. The clinical and histopathological characteristics showed that the majority of the patients were within the 40-65 years old group, and 34.2% had standard body mass index (BMI). Furthermore, the majority (89.22%) of the patients showed No Special Type (NST), (56.7%) had histopathology*

grade III, high Ki-67 ≥20% (85.8%), and positive PD-L1 expression (30.8%), with visceral metastasis indicating the highest proportion of 75.8%. Patients with a high CD8/FOXP3 and CD4/FOXP3 ratio were significantly prone to have bone-only metastasis compared to visceral metastasis (p= 0.028 and p=0.024, respectively). ***Conclusion:*** *The ratio of antitumor to protumor T-lymphocytes had a significant relevance in the metastatic location patterns in TNBC.*

Keywords*: antitumor, immune cells, metastatic location, protumor, triple-negative breast cancer*

INTRODUCTION

Triple-negative breast cancer (TNBC) is breast carcinomas characterized by the absence of hormone receptors (estrogen and progesterone), and the human epidermal growth factor receptor 2 (HER2). TNBC accounts for 15–20% of all newly diagnosed breast cancer and is the most aggressive, posing a significant challenge for oncologists due to limited curative treatment and a high risk of distant metastasis.[1] Metastatic TNBC patients have the worst prognosis with a median survival of approximately 13.3 months (min 0.8 – max 99 months) and a 12-month mortality rate between 65–75% compared to other types, such as hormonal (HR+) type, which has approximately 37 months.[2–4] The poor prognosis characteristics and aggressive cancer nature can be associated with the tumor microenvironment of metastatic TNBC, which differs from other breast cancer.[5] Visceral metastasis carries a more unfavorable prognosis compared to bone metastasis. Bertho et al. stated that visceral metastasis had a more unfavorable prognosis than bone metastasis in breast cancer.[6]

The components within the microenvironment consist of cancer cells and non-cancerous elements such as stromal and immune cells, leading to the formation of complex interplay.[7] These interactions play a significant role in cancer progression and are essential factors in determining prognosis. Immune cells within the tumor microenvironment primarily comprise lymphocytes known as tumor-infiltrating lymphocytes (TILs) and macrophages, which are called tumor-associated macrophages (TAMs).[8] TILs are the main component of the adaptive immune system present in breast cancer tumor microenvironment, with varying levels across different types. The highest prevalence of lymphocyte-predominant breast cancer (LPBC) is found in TNBC at 30%, while macrophage infiltration reaches 50%. The HR+ type has the lowest LPBC prevalence at 13%.[9] Immunomodulation can be categorized into immunosuppressive and immunoreactive which is primarily mediated by cluster of differentiation (CD) 4 T-cells, and CD8 T-cells, including B-lymphocytes and natural killer (NK) cells. Meanwhile, immunosuppressive activity is majorly mediated by forkhead-box-P3 (FOXP3) regulatory T cells (Treg) and CD163 M2 macrophages, including myeloid-derived suppressor cells (MDSC).[10] The interaction between the immune system and cancer cells plays an important role in tumor development and progression. This role is characterized by a complex interplay of tumor cells and innate as well as adaptive immune system cells, which are influenced by chemical mediators such as cytokines and chemokines.[10]

A study investigating the influence of immune cells on prognosis has been widely conducted in non-metastatic TNBC. Widodo et al. found a significant association between stromal tumor-infiltrating lymphocytes (sTILs) and a higher grade or a lower stage of tumor in patients.[8] Park et al. also found that TILs provided valuable prognostic insights for early-stage non-metastatic TNBC patients who have not received systemic treatment.[11] However, there is no study regarding the influence of these immune cells on the prognosis of *de novo* metastatic TNBC, specifically in Indonesia. Previous studies also have indicated that differences in metastatic location yield distinct prognoses. Therefore, this study aimed to investigate the potential association between immune cells profiles in the tumor microenvironment and metastatic patterns influencing the prognosis of metastatic TNBC.

METHODS

A multicenter cross-sectional study was conducted at 7 hospitals in Indonesia. The population consisted of all patients diagnosed with *de novo* metastatic TNBC from January 2015 to December 2020. The inclusion criteria were subjects ≥18 years old and *de novo* metastatic TNBC, while exclusion criteria were incomplete medical record data and/or unsuitable fixed paraffin-embedded (FFPE) tissue samples for further examination.

TNBC was defined by immunohistochemistry (IHC) test from available formalin- FFPE tissue blocks with Estrogen Receptor (ER) and Progesterone Receptor (PR) <1% and HER-2 Receptors at 0, 1+, or 2+, and a non-amplified fluorescence in situ hybridization (FISH) test result, according to St. Gallen International Breast Cancer Conference definition and American Society of Clinical Oncology (ASCO) guidelines.[5] Metastatic sites were recorded from medical records and categorized into bone-only and visceral metastasis, as well as bone, lung, liver, and brain. Visceral metastasis was defined as any metastasis event with the lung, liver, or brain site. Bone-only metastasis was characterized by metastasis events affecting only the bone site. Meanwhile, bone, lung, liver, and brain metastasis was defined as an event occurring in the respective organ sites.

The expressions of CD4 T-cells, CD8 T-cells, FOXP3 Tregs, and CD163 were evaluated in immune cells in the invasive tumor area. Staining methods were used to detect CD8 T-cells and CD163, using specific antibodies for CD8 T-cells (Cells Marque, 108R-14) and CD163 (Biocare Medical, ACR353AK). FOXP3 Tregs and CD4 T-cells were evaluated by the double-staining method using antibodies for CD4 T-cells (Biocare Medical, ACI3148) and FOXP3 (Genetex, GTX107737). Subsequently, the MACH 2 Double Stain 2 (Biocare Medical) was used for incubation, where FOXP3 and CD4 T-cells were stained with Vulcan Fast Red (Biocare Medical). CD4 T-cells were defined by the expression of CD4 alone, while FOXP3-positive cells were defined by the co-expression of CD4 and FOXP3. Immune cells were evaluated by direct quantification under the microscope as well as by scanning the slides and subsequently analyzing them using the QuPath open-source software platform (version 0.4.1). The average of the total cell count from five fields with the highest concentration of TILs was quantified under 200x magnification. IHC staining of PD-L1 was performed using a mouse monoclonal primary anti-PD-L1 antibody (clone 22C3; Dako; Agilent Technologies, Inc.). The combined positive score (CPS) was used for evaluating the immunohistochemical expression of PD-L1. The CPS was determined as a ratio of the sum of tumor cells and immune cells in the tumor stroma stained with PD-L1 antibody to the total number of viable tumor cells and multiplied by 100. At least 100 viable tumor cells were observed in each section of the countable array core.

Ethics Approval

We received ethical approval from The Ethics Committee of The Faculty of Medicine, Universitas Indonesia (ethical approval number: KET-1209/UN2.F1/ETIK/PPM.00.02/2021). All principles used were in accordance with the Declaration of Helsinki.

Statistical Analysis

We analyzed the data using Statistical Package for the Social Sciences (SPSS) version 27. Descriptive categorical data of the baseline characteristics were shown in frequency (n) and percentage (%), while averages were presented in the median and its interquartile range (IQR). Subsequently, the Mann-Whitney test or independent T-test was used to compare numerical variables between subgroups. The Mann-Whitney test was used when the data distribution was not normal, while the independent T-test was carried out when the data followed a normal distribution. In cases where the data were not normally distributed, a logX transformation was performed to convert it into a normal distribution. All tests were considered statistically significant at p <0.05, with a 95% confidence interval (CI).

RESULTS

The baseline characteristics of 120 metastatic TNBC patients are presented in **Table 1**. All

patients were female, with a mean age of 51.3 years old and the majority ranged between 40 and 65 years old (71.7%), accounting for a mean age of 51.42 ± 11.94 years. Furthermore, all patients had a normal Asian body mass index (BMI) (34.2%), with a median BMI of 23.2 (IQR 20 – 25.9) kg/m^2. The histopathology characteristics showed that the majority of subjects had the No Special Type (NST) (89.2%), histopathology grade III (56.7%), and high Ki-67 (85.8%), and a median of 60 (IQR 30 – 80). Positive PD-L1 expression occurred in 30.8% of the subjects, with a median value of 0.00 (IQR 0.00 –1.10).

The majority of subjects had visceral metastasis (75.8%), consisting of the lungs as the prevalent site (56.7%). Bone-only metastasis occurred in 24.2% of the subjects, while 10% had brain metastasis, as presented in **Table 1**. IHC examination of immune cells showed that CD4 T-cells, CD8 T-cells, CD163, and FOXP3 had a median of 64.10 (IQR 31.35 – 121.75), 182.30 (IQR 118.15 – 304.25), 193.80 (IQR 148.80 – 281.80), and 6.10 (IQR 1.20 – 16.25), respectively. Examination of antitumor protumor ratio showed that the CD4/FOXP3, CD8/FOXP3, CD4/CD163, and CD8/CD163 ratio had a median of 8.76 (IQR 4.65 – 23.57), 26.73 (IQR 14.92 – 80,70), 0.31 (IQR 0.14 – 0.57), and 0.91 (IQR 0.56 – 1.48), respectively.

The comparison between immune cells and metastasis location was carried out using the Mann-Whitney test or independent T-test.

Table 1. Clinical and Histopathology Characteristics

Variables	n=120
Age (years), median (IQR)	51.42±11.94 years
Age (years), n (%)	
- 18-39	18 (15)
- 40-65	86 (71.7)
- > 65	16 (13.3)
BMI (kg/m^2), median (IQR)	23.2 (20 – 25.9)
BMI (kg/m^2), n (%)	
Underweight	11 (9.2)
Normal	41 (34.2)
- Overweight	31 (25.8)
- Obese I	21 (17.5)
- ·bese II	9 (7.5)
- Unknown	7 (5.8)
CD4 T-cells, median (IQR), cells/m^2	64.10 (31.35 – 121.75)
CD8 T-cells, median (IQR), cells/m^2	182.30 (118.15 – 304.25)
CD163, median (IQR), cells/m^2	193.80 (148.80 – 281.80)
CD4/FOXP3 Ratio, median (IQR), cells/m^2	8.76 (4.65 – 23.57)
CD8/FOXP3 Ratio, median (IQR), cells/m^2	26.73 (14.92 – 80,70)
CD4/CD163 Ratio, median (IQR), cells/m^2	0.31 (0.14 – 0.57)
CD8/CD163 Ratio, median (IQR), cells/m^2	0.91 (0.56 – 1.48)
FOXP3 Tregs, median (IQR), cells/m^2	6.10 (1.20 – 16.25)
Ki-67, median (IQR), %	60 (30 – 80)
Pathology, n (%)	
- NST/Ductal	108 (89.2)
- Lobular	5 (4.2)
- Other (Metaplastic, papillary, medullary)	8 (6.7)
Grade, n (%)	
- I	3 (2.5)
- II	36 (30)
- III	68 (56.7)
- Unknown	13 (10.8)
- < 20%	13 (10.8)
- ≥ 20%	103 (85.8)
- Unknown	4 (3.3)

PD-L1, median (IQR), %	0.00 (0.00 –1.10)
PD-L1, n (%)	
- Expressed (1%)	37 (30.8)
- Not Expressed (< 1%)	82 (68.3)
- N/A	1 (0.8)
Metastatic Site, n (%)	
- Bone Involvement	59 (49.2)
- Lung Involvement	68 (56.7)
- Liver Involvement	33 (27.5)
- Brain Involvement	12 (10)
- Others (Adrenal, soft tissue)	2 (1.7)
- Bone-only metastasis	29 (24.2)
- Visceral metastasis	91 (75.8)

When equal variance was assumed, FOXP3 Tregs showed a significant difference in average between cases with bone-only metastasis and visceral metastasis (p=0.047). Although a larger sample of TNBC patients with bone metastasis potentially yielded substantial outcomes, the results obtained in this study were not statistically significant (p=0.095). Subjects with bone-only metastasis had a higher average for the CD8/FOXP3 ratio compared to those with visceral metastasis (26.18 vs 82.50, p= 0.028). The CD4/FOXP3 ratio also had a significant average difference between the bone-only metastasis group and the visceral group (8.63 vs 16.50, p=0.024), as presented in **Table 2.**

Table 2. Difference of Immune Infiltration Cells in Bone-only and Visceral Metastasis Group

Immune Cells	Metastatic Group	N	Median [IQR]	p
CD8 T-cells	Visceral metastasis	91	187.20 [126.20 - 304.80]	0.975[a]
	Bone-only metastasis	29	157.20 [109.10 - 301.30]	
CD4 T-cells	Visceral metastasis	91	63.40 [32.00 - 121.00]	0.773[a]
	Bone-only metastasis	29	81.60 [24.50 - 148.00]	
FOXP3 Tregs	Visceral metastasis	91	7.00 [1.60 - 17.20]	0.095[a]
	Bone-only metastasis	29	3.20 [0.30 - 15.80]	0.047[c]
CD163	Visceral metastasis	91	194.80 [145.20 - 280.60]	0.957[b]
	Bone-only metastasis	29	188.00 [153.60 - 300.20]	
CD8/FOXP3 Ratio	Visceral metastasis	84	26.18 [14.79 - 49.93]	**0.028[a]**
	Bone-only metastasis	25	82.50 [15.93 - 262.50]	
CD4/FOXP3 Ratio	Visceral metastasis	84	8.63 [3.81 -22.47]	**0.024[a]**
	Bone-only metastasis	25	16.50 [5.83 - 44.75]	
CD8/CD163 Ratio	Visceral metastasis	91	0.92 [0.59 - 1.45]	0.946[a]
	Bone-only metastasis	29	0.93 [0.47 - 1.81]	
CD4/CD163 Ratio	Visceral metastasis	91	0.29 [0.15 - 0.58]	0.789[a]
	Bone-only metastasis	29	0.36 [0.11 - 0.58]	

[a] Analyzed using t-test after logX transformation.
[b] Analyzed using Mann-Whitney U test.
[c] If equal variances are assumed

DISCUSSION

The emergence of metastases in visceral organs due to breast carcinoma is strongly associated with an unfavorable prognosis, characterized by significantly diminished overall survival and reduced metastasis-free survival rates.[12] Recently, two studies have reported that bone-only metastatic patients exhibit improved outcomes in terms of overall survival (OS) and progression-free survival (PFS) compared to other subgroups, such as metastatic breast cancer patients with visceral disease.[13,14] Specific metastatic sites or the involved organs could predict the prognosis of breast cancer patients. Studies have revealed that breast cancer with bone-only metastasis had a better prognosis than the visceral one.[15] The median OS between bone-only and visceral metastasis is 38 and 21 months, respectively.[16,17] Among the visceral organ sites, brain metastasis had the poorest prognosis, followed by liver and lung. These results could improve clinical judgment involving the more unfavorable prognosis of visceral organ metastasis compared to bone-only metastasis. To the best of our knowledge, no current studies have examined the influence of immune cells on the prognosis of the metastatic TNBC population.

Cancer-immune cell interactions are critical in tumor progression and metastasis, yet their specific roles remain unclear. Immune surveillance and tolerance help maintain overall bodily balance but are disrupted in tumors, allowing unchecked growth, invasion, and metastasis.[18] Tumor microenvironments attract various immune cells, notably CD4 T-cells, CD8 T-cells, and FOXP3 Tregs. CD8 T-cells and FOXP3 Tregs play key roles in immune surveillance and tolerance, countering uncontrolled tumor growth.[18,19] This study, unique in the Indonesian population, compares immune cell infiltration in *de novo* metastatic TNBC with bone or visceral metastasis, advancing our understanding of these processes. This study reveals significant discrepancies in the CD8/FOXP3 ratio, favoring bone-only metastases over visceral ones. CD8 T-cells drive cancer cell apoptosis, while FOXP3 Tregs dampen CD8 T-cells activity, promoting tumor growth. Effective anti-tumor immunity hinges on CD4 T-cells and CD8 T-cells, with CD4 T-cells supporting CD8 T-cells proliferation and priming.[19,20] Early breast tumors are characterized by CD4 T-cells and CD8 T-cells predominance, potentially enhancing immunosurveillance. In contrast, advanced cancer exhibits an influx of CD4 T-cells, possibly fostering tumor progression. CD8 T-cells are pivotal for improved clinical outcomes, activated by CD4 T-cells derived IL-2, and producing tumor-suppressing cytokines like interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α).[20]

This study presented the clinical and histopathological characteristics of metastatic TNBC cases, with a mean age of subjects of 51.42 years, predominantly consisting of the 40 to 65 years old group. This report was different from several multinational studies indicating that TNBC was mostly diagnosed in the age group of < 40 years.[21,22] A previous TNBC study from Indonesia showed a similar mean age of the subjects (51.09 years).[8] These variations could determine racial differences, dissimilar health screening programs, and early diagnosis systems across various countries.[23] In this study, the majority of the subjects had normal and median BMI similar to a previous report in the Indonesian population, indicating low domination of hormonal stimulation from high adipose tissue in TNBC. Histopathological characteristics also showed a high proportion of NST/ductal carcinoma, elevated Ki-67, and high-grade tumors. Other studies also reported a high proportion of ductal type in TNBC, Ki-67 expression, and tumor-grade histopathology among TNBC subjects.[24] High Ki-67 levels and high-grade tumors indicated its aggressive nature, suggesting that tumor invasion and metastasis were progressing. The prevalence of the PD-L1 expression was similar to several other investigations of TNBC which ranged from 20%-59.5%.[25] This study showed a high proportion of visceral metastasis compared to the other sites, with the lung becoming the most common metastatic site and the brain being the least. Several studies of metastatic patterns in TNBC reported similar results, which consistently reported the lung, liver, bone, and brain as the most common metastatic sites.[16,17] These results presented the

distinctive characteristics of metastatic patterns in TNBC.[12,14]

This study indicates a significant difference in the average for the CD8/FOXP3 ratio between bone-only metastasis and visceral metastasis.[26] Patients with bone-only metastasis have a higher average for the CD8/FOXP3 ratio compared to patients with metastasis to visceral organs. Additionally, the CD4/FOXP3 ratio also shows a significant difference in average between the bone-only metastasis group and the metastasis to visceral organs group. CD8 T-cells induces the apoptosis of cancer cells by producing proteins including perforin and granzyme.[15,26] On the other hand, FOXP3 Treg cells are immunosuppressive cells that can induce the cytotoxic dysfunction of CD8 T-cells and NK cells through the production and expression of various factors, including β-galactosidase-binding protein (βGBP), PD-L1, and B7-H4, which increase tumor growth, invasion, and migration from the primary site.[27]

A previous study discovered that in most grade III breast cancer cases, the ratio of FOXP3 Tregs to CD8 T-cells consistently exceeds the observed in grade I/II breast cancer, indicating the well-established role of FOXP3 Tregs in facilitating the progression of breast cancer.[20] According to a recent investigation, the quantity of Tregs infiltrating tumors is associated with the initiation and advancement of breast cancer.[28] The average ratio of FOXP3 T regs gradually increases from 0.005 in normal breast tissues to 0.019 in ductal carcinoma in situ and 0.030 in invasive ductal cancer, mirroring their malignant progression.[29]

According to Liu et al., the correlation between Tregs and CD8 T-cells in 1270 cases of invasive breast carcinoma showed a significant impact on patient survival, histopathological characteristics, and molecular subtypes.[15] The results indicated that an increased presence of Tregs and T-cells within the tumor was related to adverse features, such as high histologic grade and negative ER and PR status. A high level of Treg infiltration was also connected to reduced OS and PFS. Meanwhile, a high ratio of T-cells to Tregs in the tissue surrounding the tumor was significantly related to improved OS and PFS. Miyan et al. assessed the expression of CD8, FOXP3, and CD3 in 177 patients with primary, invasive, unilateral early-stage breast cancer including all molecular subtypes.[30] The results indicated that T-cells infiltration was associated with hormone receptor-negative tumors, a high rate of proliferation, an elevated histological grade, and larger tumor sizes. Basal-like tumors showed the highest number of FOXP3 T-regs, which was associated with an unfavorable ratio compared to cytotoxic CD8 T-cells.[30]

This study also had a substantial number of samples despite the rarity of *de novo* metastatic TNBC cases. This study's limitation is that it only subjects to the *de novo* metastatic TNBC subjects. As such, it may not apply to progressive metastatic TNBC cases. The implication result of this study could assist clinicians in improving treatment judgment for metastatic TNBC cases.

CONCLUSION

In this study, the ratio of antitumor to protumor T-lymphocytes had a significant relevance with the metastatic location patterns in TNBC. This study shed a light on the immunopathological aspect of the metastatic TNBC, revealing the unique features in the Indonesian population.

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CONFLICT OF INTEREST

All authors have no conflict of interest.

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REFERENCES

1. Sutandyo N, Kurniawati SA, Siregar NN, Sari NK. Three years survival of elderly cancer patients in Indonesia: Do we need a different approach? Acta Med Indones-Indones J Intern Med. 2020;52.
2. Sánchez C, Domínguez F, Galindo H, et al. Survival rates of advanced estrogen-receptor positive breast cancer. Analysis of 211 cases. Rev Med Chil. 2020;148(9):1233-8.

3. Kazmi S, Chatterjee D, Raju D, Hauser R, Kaufman PA. Overall survival analysis in patients with metastatic breast cancer and liver or lung metastases treated with eribulin, gemcitabine, or capecitabine. Breast Cancer Res Treat. 2020;184(2):559–65.
4. Kassam F, Enright K, Dent R, et al. Survival outcomes for patients with metastatic triple-negative breast cancer: Implications for clinical practice and trial design. Clin Breast Cancer. 2009;9(1):29–33.
5. Han HS, Vikas P, Costa RLB, Jahan N, Taye A, Stringer-Reasor EM. Early-stage triple-negative breast cancer journey: Beginning, end, and everything in between. American Society of Clinical Oncology Educational Book [Internet]. 2023;(43):1–12. Available from: https://ascopubs.org/doi/10.1200/EDBK_390464
6. Bertho M, Fraisse J, Patsouris A, et al. Real-life prognosis of 5041 bone-only metastatic breast cancer patients in the multicenter national observational ESME program. Ther Adv Med Oncol. 2021;13.
7. Jiang X, Wang J, Deng X, et al. Role of the tumor microenvironment in PD-L1/PD-1-mediated tumor immune escape. Mol Cancer. 2019;18(1):1–10.
8. Widodo I, Ghozali A, Purwanto I, Ferronika P. Stromal tumor infiltrating lymphocytes (sTILs) were associated with a higher grade and a lower stage of Indonesian triple negative breast cancers. Asian Pacific Journal of Cancer Prevention. 2022;23(8):2749–54.
9. Dieci MV, Miglietta F, Guarneri V. Immune infiltrates in breast cancer: Recent updates and clinical implications. Cells. 2021;10(2):1–27.
10. Wang Z, Wang H, Sun X, et al. A risk stratification model for predicting overall survival and surgical benefit in triple-negative breast cancer patients with de novo distant metastasis. Front Oncol. 2020;10.
11. Park JH, Jonas SF, Bataillon G, et al. Prognostic value of tumor-infiltrating lymphocytes in patients with early-stage triple-negative breast cancers (TNBC) who did not receive adjuvant chemotherapy. Annals of Oncology. 2019;30(12):1941–9.
12. Savci-Heijink CD, Halfwerk H, Koster J, Horlings HM, van de Vijver MJ. A specific gene expression signature for visceral organ metastasis in breast cancer. BMC Cancer. 2019;19(1):333.
13. Wedam SB, Beaver JA, Amiri-Kordestani L, et al. US food and drug administration pooled analysis to assess the impact of bone-only metastatic breast cancer on clinical trial outcomes and radiographic assessments. Journal of Clinical Oncology. 2018;36(12):1225–31.
14. Parkes A, Clifton K, Al-Awadhi A, et al. Characterization of bone only metastasis patients with respect to tumor subtypes. NPJ Breast Cancer. 2018;4(1):2.
15. Liu F, Lang R, Zhao J, et al. CD8+ cytotoxic T cell and FOXP3+ regulatory T cell infiltration in relation to breast cancer survival and molecular subtypes. Breast Cancer Res Treat. 2011;130(2):645–55.
16. Yao Y, Chu Y, Xu B, Hu Q, Song Q. Risk factors for distant metastasis of patients with primary triple-negative breast cancer. Biosci Rep. 2019;39(6).
17. Mori R, Futamura M, Asano Y, Nakakami A, Yoshida K. Similar prognosis of patients with bone-only metastatic breast cancer and visceral metastasis. Archives of Breast Cancer. 2021;8(1):51–6.
18. Tavares MC, Sampaio CD, Lima GE, et al. A high CD8 to FOXP3 ratio in the tumor stroma and expression of PTEN in tumor cells are associated with improved survival in non-metastatic triple-negative breast carcinoma. BMC Cancer. 2021;21(1).
19. Tay RE, Richardson EK, Toh HC. Revisiting the role of CD4+ T cells in cancer immunotherapy—new insights into old paradigms. Cancer Gene Ther. 2021;28(1–2):5–17.
20. Droeser R, Zlobec I, Kilic E, et al. Differential pattern and prognostic significance of CD4 +, FOXP3 +and IL-17 +tumor infiltrating lymphocytes in ductal and lobular breast cancers. BMC Cancer. 2012;12.
21. Purwanto I, Heriyanto DS, Ghozali A, et al. Overexpression of programmed death-ligand 1 receptor mRNA as an independent negative prognostic factor for triple negative breast cancer. World J Oncol. 2020;11(5):216–22.
22. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209–49.
23. Scott LC, Mobley LR, Kuo TM, Il'yasova D. Update on triple-negative breast cancer disparities for the United States: A population-based study from the United States Cancer Statistics database, 2010 through 2014. Cancer. 2019;125(19):3412–7.
24. Srimuninnimit V, Pornpraserthsuk P, Chaiwerawattana A, et al. Real-life clinical pattern, management, and survival in Thai patients with early-stage or metastatic triple-negative breast cancer. PLoS One. 2018;13(12).
25. Kim H, Kwon HJ, Han YB, et al. Increased CD3+ T cells with a low FOXP3+/CD8+ T cell ratio can predict anti-PD-1 therapeutic response in non-small cell lung cancer patients. Modern Pathology. 2019;32(3):367–75.
26. Jørgensen N, Hviid TVF, Nielsen LB, et al. Tumour-infiltrating CD4-, CD8- and FOXP3-positive immune cells as predictive markers of mortality in BRCA1- and BRCA2-associated breast cancer. Br J Cancer. 2021;125(10):1388–98.
27. Peng L, Li L, Guo YW, Yin XJ, Wang S, Liu CP. CD8 + cytotoxic and FoxP3 + regulatory T lymphocytes serve as prognostic factors in breast cancer [Internet]. Am J Transl Res. 2019;11. Available from: www.ajtr.org
28. Leone BA, Vallejo CT, Romero AO, et al. Prognostic impact of metastatic pattern in stage IV breast cancer at initial diagnosis. Breast Cancer Res Treat. 2017;161(3):537–48.
29. García-Martínez E, Gil GL, Benito AC, et al. Tumor-infiltrating immune cell profiles and their change

after neoadjuvant chemotherapy predict response and prognosis of breast cancer. Breast Cancer Research. 2014;16(1).
30. Miyan M, Schmidt-Mende J, Kiessling R, Poschke I, Boniface J. Differential tumor infiltration by T-cells characterizes intrinsic molecular subtypes in breast cancer. J Transl Med. 2016;14(1).