BMC Research Notes

RESEARCH NOTE

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# The relationship between high ratios of CD4/FOXP3 and CD8/CD163 and the improved survivability of metastatic triple-negative breast cancer patients: a multicenter cohort study

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## Abstract

**Background** Triple-negative breast cancer (TNBC) has been documented as the most aggressive subtype of breast cancer. This study aimed to analyze antitumor and protumor immune activities, and their ratios as significant prognostic biomarkers in metastatic TNBC (mTNBC).

**Methods** A multicenter cohort study was conducted among 103 de novo mTNBC patients. The expression of CD8 and CD163 was evaluated using immunohistochemistry staining, CD4 and FOXP3 using double-staining immunohistochemistry, and PD-L1 using immunohistochemistry and RT-PCR.

**Results** Multivariate analysis revealed that high CD4/FOXP3 (HR 1.857; 95% CI 1.049–3.288; $p=0.034$) and the CD8/CD163 ratio (HR 2.089; 95% CI 1.174–3.717; $p=0.012$) yield significantly improved 1 year overall survival (OS). Kaplan–Meier analysis showed that high levels of CD4 ($p=0.023$), CD8 ($p=0.043$), CD4/FOXP3 ($p=0.016$), CD8/FOXP3 ($p=0.005$), CD8/CD163 ($p=0.005$) ratios were significantly associated with higher rate of 1 year OS. Furthermore, 1 year OS was directly correlated with antitumor CD4 ($R=0.233$; $p=0.018$) and CD8 ($R=0.219$; $p=0.026$) and was indirectly correlated with protumor CD163 and FOXP3 through CD4/FOXP3 ($R=0.282$; $p=0.006$), CD4/CD163 ($R=0.239$; $p=0.015$), CD8/FOXP3 ($R=0.260$; $p=0.008$), and CD8/CD163 ($R=0.258$; $p=0.009$).

**Conclusion** This is the first study to demonstrate that high levels of CD4/FOXP3 and CD8/CD163 significantly improved the 1 year OS in de novo mTNBC patients. Thus, we recommend the application of these markers as prognosis determination and individual treatment decision.

**Keywords** Survival, Metastatic, Triple-negative breast cancer, CD4, CD8, CD163, FOXP3

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## Introduction

Triple-negative breast cancer (TNBC) has been established as the most aggressive subtype of breast cancers and is frequently associated with inflammation in the stroma along with a greater risk of immune cell infiltration compared to other subtypes [1, 2]. The identification of TNBC is based on the cells expression of estrogen receptors (ER) and progesterone receptors (PR) of ≤ 1% and negative expression of human epidermal growth factor receptor 2 (HER2) [1]. The lack of ER, PR, and HER2 protein expression makes TNBC unresponsive to the current endocrine and HER2-targeted therapies and leads to a poor prognosis [1, 3]. TNBC is responsible for 12–17% of all breast cancers and is inevitably recurrent [1, 4]. Its incidence has been documented to be increasing consistently over the past few decades. The median survival for metastatic TNBC (mTNBC) is 13.3 months and the mortality rate at 12 months is 75% [1, 5–7].

Based on current evidence, tumor microenvironment (TME) plays a critical role in TNBC immunomodulation, which can be categorized as immunoreactive (antitumor) or immunosuppressive (protumor). Immunosuppressive TME is majorly comprised of forkhead-box-P3 (FOXP3) regulatory T-cells (Treg), M2 macrophages, and programmed death-ligand 1 (PD-L1) axis. Immunoreactive TME is mainly comprised of CD4 and CD8 T-cells, M1 macrophages, and natural killer (NK) cells [3].

We hypothesized that alterations in protumor and antitumor immune activities might impact disease progression in mTNBC [8]. We aimed to analyze the use of antitumor and protumor immune activities, and their ratios as prognostic markers in de novo mTNBC patients [9–11]. Thus, a comprehensive understanding of protumor and antitumor immune activities can be obtained as a promising strategy for evaluating the prognosis and treatment for mTNBC [9, 12].

## Methods

### Patient samples and methodology

A multicenter cohort study was conducted at two Indonesian National Cancer Centers (Dharmais National Cancer Hospital and Mochtar Riady Comprehensive Cancer Center), Cipto Mangunkusumo National Central General Hospital, Gatot Soebroto Central Army Hospital, Siloam Lippo Village Hospitals, Jakarta Breast Cancer Hospital, and Metropolitan Medical Center Hospital. The study population consisted of all patients diagnosed with mTNBC (stage IV) defined by immunohistochemistry (IHC) from available formalin-fixed paraffin-embedded (FFPE) tissue blocks from January 2015 to December 2020 [13, 14]. The included subjects ≥ 18 years old and their de novo mTNBC status was confirmed by histopathology and IHC with ER and PR < 1% and their HER2 receptors were at either 0, 1+, or 2+with non-amplified fluorescence in situ hybridization (FISH) test result, all of which were in accordance with American Society of Clinical Oncology (ASCO) guidelines [1, 3]. Exclusion criteria were either incomplete medical record data and/or unsuitable FFPE tissue samples for further examination.

### Analysis of tumor-infiltrating lymphocytes (TILs)

The infiltration of immune cells was analyzed on H and E stained slides [15, 16]. The average of total cell count from five fields with the highest concentration of TILs was quantified under 200×magnification [17]. All slides were examined by two pathologists who had extensive expertise in mammary pathology (L. and D.S.H.) [15, 18].

### IHC evaluation

The levels of CD4, CD8, FOXP3, and CD163 were evaluated in the immune cells located in the invasive tumor area [15]. CD8 and CD163 were demonstrated by staining methods using antibodies to CD8 (Cell Marque, 108R-14) and CD163 (Biocare Medical, ACR353AK) cells. FOXP3 and CD4 cells were evaluated by the double-staining method using antibodies to CD4 (Biocare Medical, ACI3148) and FOXP3 (Genetex, GTX107737) cells. The MACH 2 Double Stain 2 (Biocare Medical) was utilized for the incubation process. FOXP3 and CD4 cells were stained with Vulcan Fast Red (Biocare Medical) [17].

Immunohistochemical staining of PD-L1 was performed using a mouse monoclonal primary anti-PD-L1 antibody (clone 22C3; Dako; Agilent Technologies, Inc.). Subsequently, the slides were incubated with Novolink Polymer Detection System (Leica Microsystems) as a secondary antibody (Novocastra). The combined positive score (CPS) was used for evaluating immunohistochemical expression of PD-L1 [19].

### PD-L1 mRNA

The mRNA samples were analyzed using the NEXproTM qRT-PCR Master Mix (SYBR) kit in accordance with the manufacturer's instructions. The Bioneer Exicycler™ 96 Real-Time Quantitative Thermal Block was used for the quantitative PCR which was performed in accordance with the manufacturer's instructions [20]. PCR primers were as follows: forward 5′—TATGGTGGTGCCGAC TACAA-3′ and reverse 5′—TGGCTCCCAGAATTA CCAAG-3' [21].

### Statistical analysis

The extracted data was analyzed with Statistical Package for the Social Sciences (SPSS) version 27 for Windows.

ROC curve was used to determine the optimal cut-off for categorizing the low and high levels groups. Survival analysis were performed using Kaplan–Meier and Cox proportional hazard models. Spearman correlation was used to analyze the correlation between each prognostic marker and the 1 year OS.

## Results

Initially, 128 female subjects with de novo mTNBC who fulfilled all inclusion and exclusion criteria were recruited. Among them, 103 subjects were included for IHC and RT-PCR evaluation (Additional file 1: Fig. S1).

The mean age was 51.3 years old, while the mean body mass index (BMI) was 23.2. The most frequent sites of metastasis are the lung (56.3%) and bone (49.5%). Histopathology characteristics showed most subjects had NST type (92.2%), grade III (54.4%), and high Ki-67 (86.4%). The chemotherapy agents were used according to National Comprehensive Cancer Network in oncology (NCCN) guidelines Table 1, [22]. The IHC staining and double-staining are depicted in (Additional file 2: Fig. S2).

Multivariate analysis demonstrated that high CD4/FOXP3 (HR 1.857; 95% CI 1.049–3.288; $p=0.034$) and CD8/CD163 ratios (HR 2.089; 95% CI 1.174–3.717; $p=0.012$) significantly improved 1-year OS. In Kaplan–Meier and univariate Cox regression analyses, high levels of CD4, CD8, CD4/FOXP3, CD8/CD163, and CD8/FOXP3 were significantly associated with higher rates of 1 year OS (Table 2, Fig. 1).

In Fig. 2, our path analysis showed that the recursive patterns of antitumor CD4 (R = 0.233, $p=0.018$) and CD8 cells (R = 0.219, $p=0.026$) were directly correlated with 1 year OS. Remarkably, 1 year OS had indirect correlations with CD163 and FOXP3 through the antitumor/protumor ratios, including CD4/FOXP3 (R = 0.282; $p=0.006$), CD4/CD163 (R = 0.239; $p=0.015$), CD8/FOXP3 (R = 0.260; $p=0.008$), and CD8/CD163 (R = 0.258; $p=0.009$). Furthermore, there were significant positive correlations between CD4 and CD4/CD163 (R = 0.896, $p<0.001$, between CD8 and CD8/CD163 (R = 0.794, $p<0.001$). There were also significant negative correlations between FOXP3 and CD4/FOXP3 (R = − 0.662, $p<0.001$); FOXP3 and CD8/FOXP3 (R = − 0.845, $p<0.001$); and CD163 and CD8/CD163 (R = − 0.293, $p=0.03$).

## Discussion

To the best of our knowledge, this is the first study that evaluated TME of TNBC in metastatic settings. Based on our multivariate analysis, high CD4/FOXP3 and CD8/CD163 ratios were significantly associated with higher 1 year OS. Kaplan–Meier and univariate Cox regression analyses demonstrated that higher rates of 1 year OS were related with higher levels of CD4, CD8, CD4/FOXP3, CD8/CD163, and CD8/FOXP3 (Table 2 and Fig. 1).

**Table 1** Subject characteristics

| Characteristics | N (103) |
|---|---|
| Age, mean ± SD, in years | 51.3 ± 12.6 |
| BMI, mean ± SD, in kg/m$^2$ | 23.2 ± 6.1 |
| Chemotherapy, N (%) | |
| Antimetabolite (5-FU, capecitabine, gemcitabine, methotrexate) | 41 (39.8) |
| Anthracycline (doxorubicin, epirubicin) | 40 (38.8) |
| Alkylating agents (cyclophosphamide) | 34 (33) |
| Taxane (docetaxel, paclitaxel) | 36 (34.9) |
| Platinum (carboplatin, cisplatin) | 23 (22.3) |
| Vinca alkaloid (vinorelbine) | 2 (1.9) |
| Antimicrotubule (eribulin) | 2 (1.9) |
| Histopathology, N (%) | |
| NST | 95 (92.2) |
| Lobular | 3 (2.9) |
| Others (metaplastic, papillary, medullary) | 5 (4.9) |
| Histo grade, N (%) | |
| I | 1 (1) |
| II | 33 (32) |
| III | 56 (54.4) |
| N/A | 13 (12.6) |
| Ki-67, N (%) | |
| < 20% | 10 (9.7) |
| ≥ 20% | 89 (86.4) |
| N/A | 4 (3.9) |
| Site of metastasis, N (%) | |
| Bone | 51 (49.5) |
| Lung | 58 (56.3) |
| Liver | 30 (29.1) |
| Brain | 11 (10.7) |
| Others (adrenal, soft tissue) | 2 (1.9) |

*SD* standard deviation, *BMI* body mass index, *NST* no special type

High level of CD4/FOXP3 ratio was significantly associated with a higher rate of 1 year OS (Table 2 and Fig. 1C). The effect of this antitumor/protumor ratio was more robust than the single effect of CD4 (Table 2). A cohort study by Tavares et al. revealed that non-metastatic TNBC patients with low CD4/FOXP3 ratios had significantly reduced OS compared to patients with high ratios [15]. These findings, including ours, reflected the complex interaction between CD4 and FOXP3 in metastatic and non-metastatic TNBC.

According to the current evidence, CD4 T-cells are vital parts of the tumor immunity. They facilitate antitumor response of CD8 T-cells by supporting the pro-inflammatory cross-presenting dendritic cells (DC)

**Table 2** The ROC curve and cox regression analysis of the prognostic markers

| Variables | ROC Curve | | | | Univariate cox regression | | Multivariate cox regression | |
|---|---|---|---|---|---|---|---|---|
| | p-value | Cut-off | Sensitivity (in %) | Specificity (in %) | HR (95% CI) | p-value | HR (95% CI) | p-value |
| CD4 | 0.067 | 82.2/mm$^3$ | 71.4 | 48.9 | 1.935 (1.083–3.457) | **0.026** | 1.415 (0.638–3.141) | 0.393 |
| CD8 | 0.028 | 179/mm$^3$ | 58.9 | 61.7 | 1.867 (1.095–3.182) | **0.022** | 1.621 (0.870–3.021) | 0.128 |
| CD4/FOXP3 ratio | 0.023 | 8.63 | 60.0 | 61.4 | 2.020 (1.145–3.564) | **0.015** | 1.857 (1.049–3.288) | **0.034** |
| CD8/FOXP3 ratio | 0.038 | 26.2 | 60.0 | 61.4 | 1.821 (1.032–3.231) | **0.039** | 1.467 (0.716–3.006) | 0.295 |
| CD8/CD163 ratio | 0.017 | 0.925 | 64.3 | 63.8 | 2.169 (1.254–3.752) | **0.006** | 2.089 (1.174–3.717) | **0.012** |

Bold indicates a statistically significant

*HR* hazard ratio, *CI* confidence interval

[22–24]. This ultimately provides activating signals for CD8 T-cells, including the development of cytotoxicity and production of tumoricidal cytokines such as interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) [24–27]. On the other hand, FOXP3 has been considered to reduce or prevent inflammation-mediated tumor progression. It may diminish the activity of the CD4 T-cells through cell-to-cell interaction [28–30]. According to a cohort study conducted by Liu et al. it was also found that Treg exhibits immunosuppressive effects in non-small cell lung cancer (NSCLC) patients. Treg plays a significant role in promoting immunosuppressive mechanisms within malignant diseases, as they effectively impacts the immune response against different types of cancer cells [31]. This evidence validates the previous theory regarding the protumor FOXP3 suppressing the antitumor immune cells rather than targeting the cancer cell directly [32]. Thus, a higher level of CD4/FOXP3 can reflect a significant improvement in the 1 year OS (Table 2, Fig. 1C).

High CD8/CD163 ratio was also significantly associated with a higher rate of the 1 year OS (Table 2, Fig. 1E). This antitumor/protumor ratio was greater in significance than the single effect of CD8 or CD163 (Table 2). A cohort study of non-metastatic TNBC patients by Ren et al. showed that patients with high CD8 and low CD163 had significantly better 1 year OS [33]. These results indicate complex interactions between CD8 and CD163 in metastatic and non-metastatic TNBC microenvironments.

Based on the previous studies, CD8 T-cells are the key players in antitumor adaptive immunity for immunological surveillance and tolerance [2]. CD8 T-cells clear cancer cells directly by releasing perforin and granzymes and inducing apoptosis by activating the FasL pathway [2, 34, 35]. On the other hand, CD163 M2-macrophages are a subset of naive M0-macrophage cells that induce the apoptosis of CD8 via the PD-L1 expression; prevent the CD8 T-cells from migrating to the tumor site; and facilitate the tumor progression, metastatis, and angiogenesis via the secretion of the TGF-β, MMP-2, IL-10, and IL-13 [36, 37]. CD163 triggers an immunosuppressive microenvironment and inhibits the antitumor immune response within the TME of TNBC [38]. Thus, the CD8/CD163 ratio can potentially impact the decision-making process regarding mTNBC therapies, specifically harnessing the full capability of the immune system in combating cancer (Table 2, Fig. 1E) [23, 26].

Surprisingly, PD-L1 IHC and PD-L1 mRNA showed no significant effects on the 1-year OS. PD-L1 is an immune checkpoint inhibitor that could potentially reduce antitumor immune cells by binding to PD-1 [36, 39]. The interaction of PD-1/PD-L1 molecules leads to the apoptosis of CD8 T-cells, increases the conversion of T-reg, and protects the macrophages from the destruction by CD8 T-cells [39–41]. Interestingly, another cohort study by Purwanto et al. revealed that high PD-L1 mRNA level significantly worsened the prognosis of non-metastatic TNBC patients [42]. On the other hand, Tavares et al. showed that PD-L1 level had no significance for the prognosis of non-metastatic TNBC [15]. The differences might be accounted due to the complex immune interactions in a metastatic setting.

In the path analysis (Fig. 2), the recursive pattern of antitumor CD4 and CD8 cells was directly correlated with 1 year OS. Interestingly, 1 year OS had indirect correlations with CD163 and FOXP3 through the antitumor/protumor ratios, including CD4/FOXP3, CD4/CD163, CD8/FOXP3, and CD8/CD163. These findings validated the previous theory regarding the mechanism of action of protumor CD163 and FOXP3 which worked indirectly against tumor cells by suppressing the activity of effector cells [32]. Furthermore, the significant positive correlation between CD4 and CD4/CD163 ratio and between CD8 and CD8/CD163 ratio specifically indicated that an increase in the levels of either CD4 or CD8 was associated with an increase in the 1 year OS. The significant negative correlations

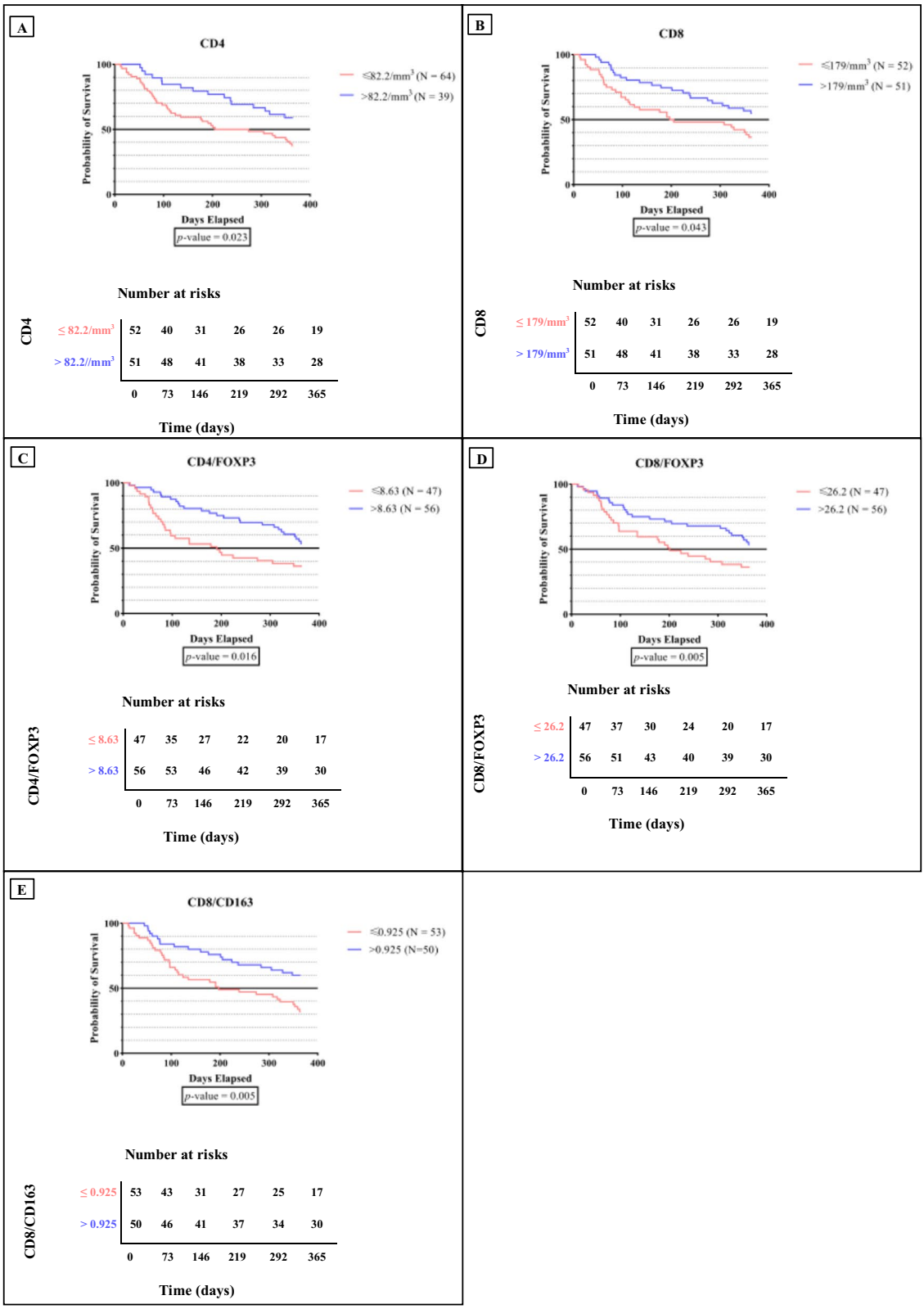


**Fig. 1** Impact of biomarker expression on 1 year OS of mTNBC patients **A** 1 year OS based on CD4 level. **B** 1 year OS based on CD8 level. **C** 1 year OS based on CD4/FOXP3 level. **D** 1 year OS based on CD8/ FOXP3 level. **E** 1 year OS based on CD8/ CD163 level. Survival curves were analyzed using Kaplan-Meier method and compared using log-rank test. *OS* overall survival, *mTNBC* metastatic triple-negative breast cancer.

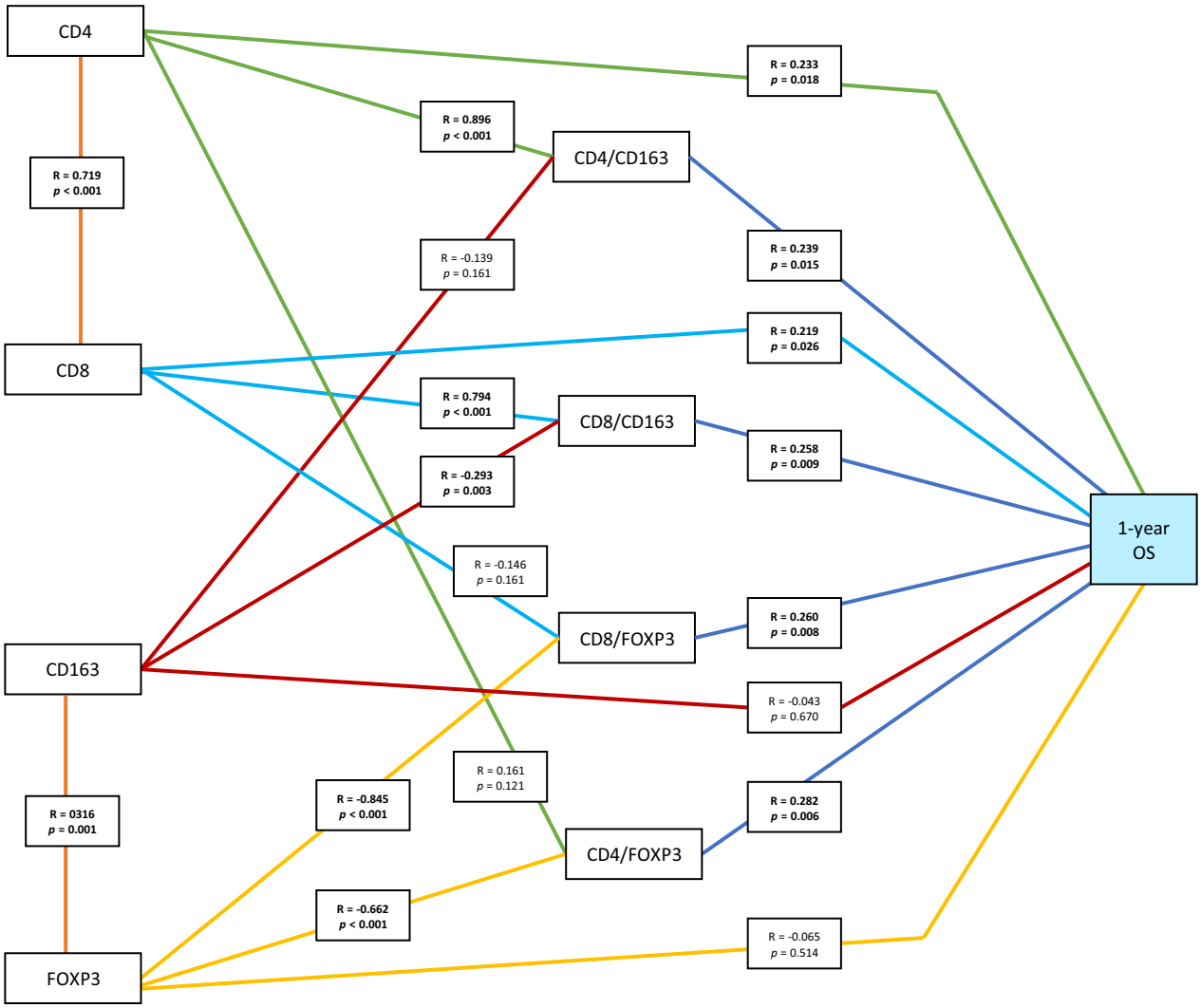


**Fig. 2** The correlation path analysis between each antitumor and protumor marker towards 1 year OS. *OS* overall survival. Analyzed using Spearman's correlation test. Bold indicates a statistically significant correlation. Green lines indicate the path of CD4. Light blue lines indicate the path of CD8. Red lines indicate the path of CD163. Yellow lines indicate the path of FOXP3. Orange lines indicate the correlation either between each antitumor or protumor. Dark blue lines indicate the correlation between antitumor/protumor ratio and 1 year OS

between FOXP3 and CD4/FOXP3; FOXP3 and CD8/FOXP3; and CD163 and CD8/CD163 also indicated that a decline in the levels of either CD163 or FOXP3 was associated with an increase in 1 year OS. Thus, we concluded that antitumor activity had a stronger impact than protumor activity on the 1 year OS.

In accordance with these findings, we propose a mechanism to explain how the antitumor and protumor immune systems work. Antitumor immune system works directly against the cancer cells, whereas protumor immune system works indirectly by inhibiting the antitumor immune system (Fig. 3).

There are several strengths of this study. First, this is the first study to provide the evidence that de novo mTNBC patients who had high levels of CD4/FOXP3 or CD8/CD163 ratios had significantly improved 1-year OS. Second, this is the first study that used the double-stain IHC technique to assess CD4 and FOXP3 in mTNBC to differentiate T-helper cells from the Treg cells. IHC staining has the advantage of being highly accessible and has a greater possibility of clinical applications [43]. Third, we successfully recruited 103 de novo mTNBC patients from different hospitals in Indonesia. In fact, the identification of mTNBC in breast cancer patients is challenging due to its rarity. Consequently, the collection of the samples necessitates an extensive screening [1, 3, 4]. We believe these substantial samples accurately represent the mTNBC population.

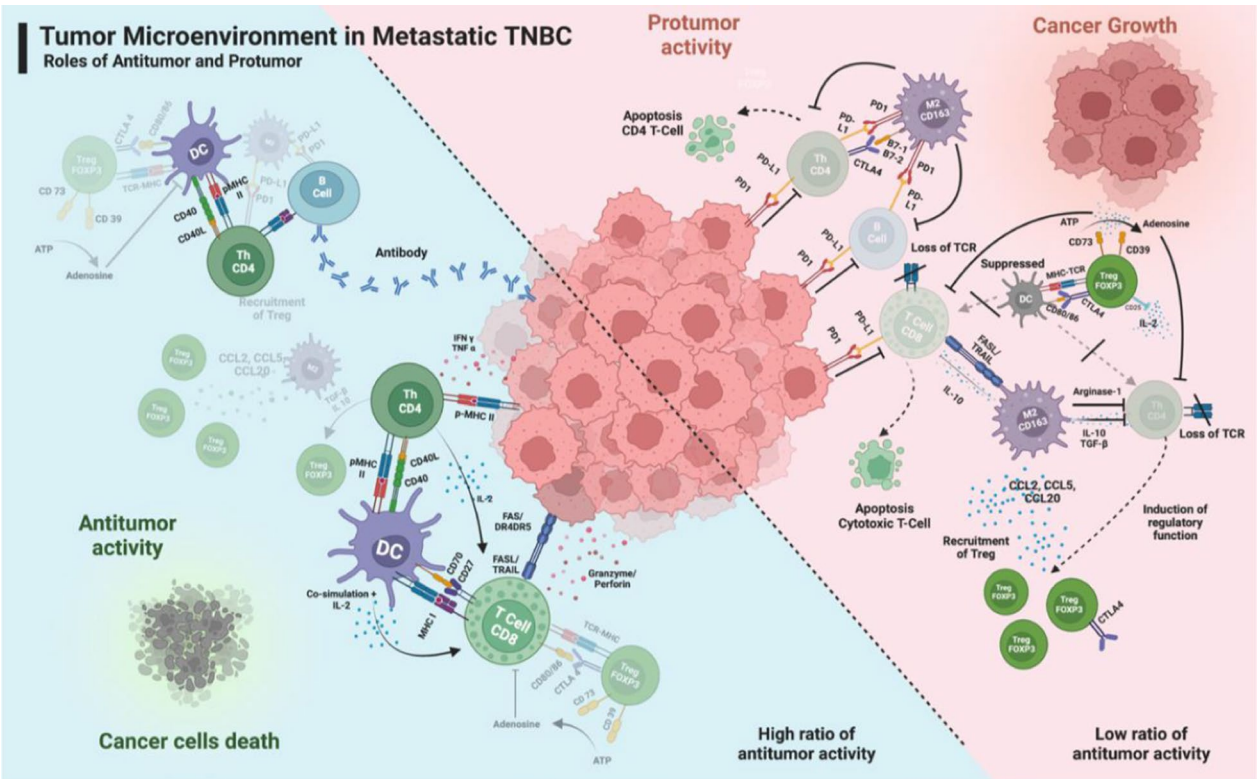


**Fig. 3** The proposed mechanisms of tumor microenvironment in metastatic TNBC. *DC*,dendritic cells, *MHC* major histocompatibility complex, *TGF-β* transforming growth factor-beta, *Treg* T-regulatory cells, *M2* macrophage type 2, *Th* T-helper cell, *TCR* T-cell receptor TRAIL, tumor necrosis factor-related apoptosis-inducing ligand. The antitumor immune system works directly against cancer cells, whereas the protumor immune system works indirectly by suppressing the antitumor immune system. The activities of CD4 and CD8 immune cells have a direct impact on tumor cells. The activation of DC is initiated by CD4, which subsequently triggers the activation of CD8. CD4 cells induce apoptosis in cancer cells by secreting IFNγ, TNFα, and via p-MHC II, whereas CD8 cells induce apoptosis by producing granzyme, perforin, and activating the FasL/TRAIL pathway. Protumor immune cells of CD163 and FOXP3 exert their effects indirectly by inhibiting the antitumor immune cells mediated by the PD-L1/PD-1, CTLA/B7, FasL/TRAIL pathway, and IL-10 secretion. The CD163 secretes CCL2, CCL5, and CCL20 which attract the FOXP3 cells in the TME. It also secretes IL-10 and TGF-β which suppress the TCR expression. Adenosine induces the apoptosis of CD8 and suppresses the TCR expression. Created with biorender.com. Figure courtesy of Jeffry Beta Tenggara. Permission to reuse the figure in any form must be obtained directly from Jeffry Beta Tenggara.

The implications of this study can influence not only the TME as a suggestive prognostic marker but also potentially assist healthcare professionals in making personalized and precise treatment decisions.

## Conclusions

This is the first study to demonstrate that high levels of CD4/FOXP3 and CD8/CD163 were significantly associated with the 1 year OS in de novo mTNBC patients. The 1 year OS was directly correlated with CD4 and CD8 and was indirectly correlated with CD163 and FOXP3. Thus, we strongly suggest the introduction of these prognostic markers into clinical practice as their application might be beneficial to maximize treatment in mTNBC.

## Limitations

The potential weakness of the study was in its application which was limited on de novo mTNBC patients. Further research are required for non-de novo mTNBC patients. On the other hand, this multicenter retrospective study has limitations due to differences in local policies across hospitals regarding the determination of metastasis. These different policies are influenced by the capabilities and limitations of each hospital's facilities. Therefore, it became unreachable to conduct a comprehensive mapping of metastasis data across all samples [44]. Despite this limitation, we can conclusively state that high levels of CD4/FOXP3 and CD8/CD163 significantly improved the 1 year OS in de novo mTNBC patients. Thus, this should be considered to improve the study design in further studies.

**Abbreviations**

| | |
|---|---|
| ASCO | American society of clinical oncology |
| BCR | B-cell receptor |
| CPS | Combined positive score |
| DC | Dendritic cells |
| ER | Estrogen receptors |
| FasL | Fas ligand |
| Il | Interleukin |
| Th | T-helper cell |
| TNBC | Triple-negative breast cancer |
| FFPE | Formalin-fixed paraffin-embedded |
| FISH | Fluorescence in situ hybridization |
| FOXP3 | Forkhead-box-P3 |
| HER2 | Human epidermal growth factor receptor 2 |
| IHC | Immunohistochemistry |
| MHC | Major histocompatibility complex |
| mRNA | Messenger ribonucleic acid |
| mTNBC | Metastatic triple-negative breast cancer |
| NK | Natural killer |
| OS | Overall survival |
| *PD-1* | Programmed death-1 |
| *PD-L1* | Programmed death-ligand 1 |
| PR | Progesterone receptor |
| ROC curve | Receiver operating characteristic curve |
| *RT-PCR* | Real-time polymerase chain reaction |
| TCR | T-cell receptor |
| TGF-β | Transforming growth factor-beta |
| *TILs* | Tumor-infiltrating lymphocytes |
| TRAIL | Tumor necrosis factor-related apoptosis-inducing ligand |
| Treg | Regulatory T-cells |
| TME | Tumor microenvironment |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13104-024-06704-z.

**Additional file 1: Fig. S1.** Flowchart of subjects included in the present study.

**Additional file 2: Fig. S2.** IHC staining.

**Acknowledgements**
The authors gratefully acknowledged all staff of the medical record, the Department of Radiology, the Department of Hematology and Medical Oncology, the Department of Anatomical Pathology (Deny Suprihatin), and the Clinical Epidemiology Division, Department of Internal Medicine (Utami Susilowati).

**Author contributions**
JBT, AR, and AWS designed the study and performed the analysis. JBT, AR, AWS, JP, LR, SSP, DSH, NS, IRN, FBR contributed to the data collection. JBT, RS, RB, and SJ wrote the manuscript. All authors read and approved the final manuscript.

**Funding**
The authors declared that there was no funding involved in this study.

**Availability of data and materials**
The dataset analysed during the current study is available in the Figshare repository, https://doi.org/https://doi.org/10.6084/m9.figshare.23694270. The unprocessed data are available from the corresponding author on reasonable request.

## Declarations

**Ethics approval and consent to participate**
Ethical approval for this study was granted by 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 in this study were in accordance with the Declaration of Helsinki. Due to the retrospective and non-interventional nature of the study, a waiver for the application of a free informed consent was allowed by The Ethics Committee of The Faculty of Medicine, Universitas Indonesia.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declared that they had no competing interests.

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Received: 19 July 2023 Accepted: 24 January 2024
Published online: 02 February 2024

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