THE RELATIONSHIP BETWEEN CTLA-4 (CYTOTOXIC T-LYMPHOCYTE-ASSOCIATED ANTIGEN 4) EXPRESSION AND THE OCCURRENCE OF LYMPH NODE METASTASES IN PATIENTS WITH TRIPLE-NEGATIVE BREAST CANCER

Nisma Yulius[1] , Prihantono[2] , Indra[2]

[1] Specialist Physician Training Program, Department of Surgery, Faculty of Medicine, Hasanuddin University, Indonesia

[2] Consultant in Surgical Oncology, Department of Surgery, Faculty of Medicine, Hasanuddin University, Indonesia

***Corresponding Author:** Nisma Yulius, Department of Surgery, Faculty of Medicine, Hasanuddin University, Jl. Perintis Kemerdekaan Km. 10, Makassar 90245, Indonesia | Email: nismayulius@gmail.com

ABSTRACT

Background: *Breast cancer is the most common cancer among women in Indonesia in terms of new cases (66,271 cases; 30.1%). Triple-negative breast cancer (TNBC) has aggressive biological characteristics, with higher rates of recurrence and metastasis and a poorer prognosis. Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), an immune checkpoint that limits T-cell activation, is expressed not only by immune cells but also by TNBC tumor cells. A study in Egypt reported that CTLA-4 expression is associated with lymph node metastasis; however, because that study population differed, this association needs to be examined in other populations.* ***Objective:*** *To determine the association between CTLA-4 expression and the occurrence of lymph node metastasis (LNM) in patients with TNBC.* ***Methods:*** *An analytical observational study with a retrospective cross-sectional design involving 52 patients with stage I–III TNBC at Hasanuddin University Hospital, Makassar, Indonesia (26 with and 26 without LNM), April–August 2026. CTLA-4 expression was assessed by immunohistochemistry (negative, <1%; positive, ≥1% of tumor cells). Analyses included the chi-square/Fisher's exact test, odds ratio (OR) and logistic regression.* ***Results:*** *Tumor size, lymphovascular space invasion (LVSI) and histopathological grade were significantly associated with LNM ($p < 0.001$). CTLA-4 positivity was found in 25 patients in the metastasis group and 13 patients in the non-metastasis group, whereas CTLA-4 negativity was found in 1 and 13 patients, respectively ($p < 0.001$; OR for negative vs positive expression = 0.040; 95% CI 0.005–0.340). In the multivariate analysis, only tumor size remained significant ($p = 0.007$), whereas LVSI ($p = 0.122$) and CTLA-4 ($p = 0.418$) did not.* ***Conclusion:*** *CTLA-4 expression was significantly associated with LNM in the bivariate analysis, but this association was not significant after adjustment for tumor size and LVSI.*

Keywords: *CTLA-4, Triple-Negative Breast Cancer, Lymph Node Metastasis, Immune Checkpoint, Immunohistochemistry*

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INTRODUCTION

Breast cancer is the most commonly diagnosed cancer in women and remains a major global health issue. According to the GLOBOCAN 2022 estimates of the Global Cancer Observatory, there were 2,296,840 new cases of breast cancer in women worldwide in 2022. In Indonesia, breast cancer ranks first among cancers with the highest number of new cases in women, totaling 66,271 cases, or approximately 30.1% of all cancer cases in women. In addition to its high incidence, breast cancer is a significant issue in oncology because of its heterogeneous biological characteristics and its ability to progress and metastasize.[1,2]

One aggressive subtype is triple-negative breast cancer (TNBC), which is defined by the absence of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) expression. Compared with other subtypes, TNBC tends to have higher rates of recurrence and metastasis and a poorer prognosis. This aggressiveness is related not only to tumor cell proliferation and invasion but also to interactions between tumor cells and the immune system within the tumor immune microenvironment. TNBC is relatively more immunogenic, with more prominent involvement of tumor-infiltrating lymphocytes (TILs), and interactions between tumor cells, T lymphocytes and regulatory T cells (Tregs) can influence tumor development and disease progression.[3–5]

One of the mechanisms regulating this immune response is the immune checkpoint, which controls T-cell activation to prevent an excessive immune response. In cancer, tumor cells can exploit this mechanism to evade recognition and elimination by the immune system (immune evasion). CTLA-4 is one of the key immune checkpoint molecules; it is an inhibitory receptor on T cells that limits T-cell activation by regulating interactions between T cells and antigen-presenting cells, and it is closely associated with the function of Tregs. CTLA-4 is also expressed by TNBC tumor cells, showing membranous and cytoplasmic immunohistochemical staining, and CTLA-4-activated-like TNBC tumors display transcriptional features of an immune-escape phenotype.[6–8]

Lymph nodes play a dual role in the relationship between the immune system and breast cancer. Regional lymph nodes are involved in antigen presentation as well as in the activation and regulation of lymphocytes, while also serving as the primary route of breast cancer dissemination via the lymphatic pathway. Lymph node metastasis (LNM) is an indicator of lymphatic spread that plays a role in staging and prognosis. A

study of regional lymph nodes in patients with TNBC showed higher CTLA-4 expression in the lymph nodes of patients with low TIL levels than in those with high TIL levels; in early-stage TNBC, this association may vary according to tumor characteristics, TIL status, the site of examination and the study population.[9,10]

Evidence regarding the association between CTLA-4 and axillary LNM in TNBC has been reported in 100 cases of TNBC in Egypt. By immunohistochemical (IHC) analysis, CTLA-4 was expressed in 45% of cases and was significantly associated with axillary LNM, distant metastasis and advanced TNM stage. However, these findings originate from a population with different clinicopathological characteristics and backgrounds, so they cannot yet be directly generalized to other populations. Thus, the association between CTLA-4 and axillary lymph node status in TNBC still needs to be clarified, especially when CTLA-4 expression is assessed in primary tumor tissue and directly linked to axillary LNM status.[11]

Based on the above, this study aimed to analyze the relationship between CTLA-4 expression, assessed by IHC in tumor tissue, and the occurrence of LNM in patients with TNBC in Makassar, Indonesia.

A. Objectives of the Study

The general objective was to analyze the association between CTLA-4 expression in primary tumor tissue and the occurrence of LNM in patients with TNBC at Hasanuddin University Hospital, Makassar. The specific objectives were (1) to describe the clinicopathological characteristics (tumor size, lymphovascular space invasion [LVSI] and histopathological grade) of patients with and without LNM; (2) to analyze the association between CTLA-4 expression and LNM; and (3) to determine whether this association persists after adjustment for tumor size and LVSI.

B. Research Hypothesis

The research hypothesis (H_1) was that CTLA-4 expression is associated with the occurrence of LNM in patients with TNBC, with positive expression being more frequent among patients with LNM, whereas the null hypothesis (H_0) stated that no such association exists.

C. Significance of the Study

Theoretically, this study extends the evidence on immune checkpoint biology in the lymphatic spread of TNBC to an Indonesian population, in which such data remain limited. Clinically, it provides preliminary evidence on whether CTLA-4 immunohistochemistry, a technique applicable in routine anatomical pathology laboratories, may complement tumor size, LVSI and histopathological grade in the assessment of nodal risk, and it provides a basis for developing CTLA-4 as part of an immune biomarker panel in TNBC.

LITERATURE REVIEW

A. Triple-Negative Breast Cancer and the Tumor Immune Microenvironment

TNBC is defined by the absence of ER, PR and HER2 expression and accounts for a minority of breast cancers, yet it is characterized by high histological grade, an early peak of recurrence and a predilection for visceral and central nervous system metastases. It is biologically heterogeneous, comprising transcriptionally distinct subtypes with different immune and stromal profiles, which partly explains the variable clinical behavior and treatment response. Among the breast cancer subtypes, TNBC shows the most prominent lymphocytic infiltration, and in a pooled analysis of 3,771 patients treated with neoadjuvant therapy, higher TIL levels were associated with higher pathological complete response rates and longer disease-free survival in TNBC.[3–5]

The breast cancer tumor microenvironment (TME) is a dynamic ecosystem of tumor cells, T and B lymphocytes, Tregs, macrophages, dendritic cells, fibroblasts and endothelial cells. Reciprocal interactions among these components exert both pro-tumoral and anti-tumoral effects, shaping invasion, metastasis and therapeutic response; consequently, the prognostic meaning of any single immune marker depends on the cellular context in which it is expressed.[12,13]

B. CTLA-4 as an Immune Checkpoint in Breast Cancer

CTLA-4 (CD152) is an inhibitory receptor that is upregulated on activated T cells and constitutively expressed on Tregs. It shares the ligands CD80 and CD86 with the co-stimulatory receptor CD28 but binds them with higher affinity, thereby outcompeting CD28 and raising the threshold for T-cell activation, mainly during the priming phase in lymphoid organs, whereas programmed cell death protein 1 (PD-1) acts predominantly in peripheral tissues during the effector phase.[6,7,14]

In breast cancer, Tregs expressing FoxP3, CD25, CTLA-4 and PD-1 accumulate within tumor tissue and suppress anti-tumor immunity through interleukin-2 deprivation, inhibitory cytokines, cytolytic activity, metabolic disruption and checkpoint signaling, and their enrichment is associated with immune escape, invasion, metastasis and poor prognosis. Persistent inhibitory receptor signaling also contributes to T-cell exhaustion, a pivotal mechanism of immune escape and resistance to immune checkpoint inhibitors.[15,16]

CTLA-4 is not restricted to lymphocytes. CTLA-4-positive breast cancer cells suppress the maturation and antigen-presenting function of dendritic cells and the downstream T helper 1 and cytotoxic T-cell responses, effects that are reversed by CTLA-4 blockade. In TNBC, IHC of 50 tumors demonstrated membranous and cytoplasmic CTLA-4 expression at varying intensities, CTLA-4 stimulation enhanced ERK1/2 signaling, and CTLA-4-activated-like tumors displayed an immune-escape-like state. These observations have supported therapeutic targeting of CTLA-4

in breast cancer with monoclonal antibodies and, more recently, with RNA interference-based approaches.[8,14,17,18]

C. Lymph Node Metastasis in Breast Cancer and Its Determinants

Single-cell lineage tracing has shown that the lymphatic system is the mainstream route for breast cancer dissemination. Tumor-draining lymph nodes (TDLNs) are simultaneously sites of anti-tumor immune priming and the first sites of metastasis, and single-cell studies have revealed extensive immunosuppressive remodeling of the nodal microenvironment during LNM. In TNBC, a Toll-like receptor 4-dependent fibroblast–monocyte axis recruits programmed death-ligand 1 (PD-L1)-high monocytes that suppress T cells within TDLNs; BTG2-deficient mast cells recruited to metastatic nodes induce Treg expansion through interleukin-2; Treg frequencies increase with nodal invasion; and the intratumoral CD8/Treg ratio correlates with LNM in early breast cancer.[9,19–23]

Among clinicopathological determinants, tumor size and lymphovascular invasion are the most consistent predictors of nodal involvement. In 59,400 patients from the Danish Breast Cancer Group, the occurrence of LNM increased with tumor size, and lymphovascular invasion was associated with approximately five-fold higher odds of LNM (OR 5.16); in 2,143 patients with early invasive breast cancer, tumor size >3 cm and lymphovascular invasion were independent risk factors for sentinel node metastasis. Blood-vessel invasion is likewise associated with larger tumors, LNM and higher grade. Histological grade reflects tumor differentiation and proliferative activity and is a robust prognostic factor, and in TNBC, both Nottingham grade and tumor size differ between patients with and without axillary metastasis.[24–28]

D. Previous Studies

Studies addressing CTLA-4 and lymph node status in breast cancer are summarized in Table 1. The evidence is heterogeneous: tumor-cell CTLA-4 assessed by IHC has been associated with nodal and distant metastasis in TNBC, whereas high primary-tumor CTLA4 transcript levels identify node-negative basal-like tumors with an excellent prognosis. These discrepancies probably reflect differences in the compartment assessed (tumor cells, immune cells, lymph nodes or bulk tumor messenger RNA), the method of measurement and the population studied.[8,10,11,17,22,23,29]

Table 1. Summary of Previous Studies on CTLA-4, Tregs and Lymph Node Status in Breast Cancer

Author (year)	Design; population	Main findings
Mehrez et al. (2025)[11]	Retrospective cohort, Egypt; 100 primary TNBC	CTLA-4 (IHC) expressed in 45%; associated with LNM (p = 0.009), distant metastasis and advanced TNM stage; independent predictor of DFS and OS
Navarrete-Bernal et al. (2020)[8]	IHC in 50 TNBC (Mexico), TNBC cell lines and in silico analysis	Membranous and cytoplasmic tumor-cell CTLA-4; CTLA-4 activation linked to ERK1/2 signaling and an immune-escape-like state
Quintana et al. (2021)[10]	Regional lymph nodes of 35 localized TNBC (15 high-TIL, 20 low-TIL)	Higher CTLA-4 expression in lymph nodes of low-TIL tumors
Chen et al. (2017)[17]	In vitro co-culture of CTLA-4-positive breast cancer cells with dendritic cells	Tumor-cell CTLA-4 suppressed dendritic cell maturation and T-cell responses; reversed by CTLA-4 blockade
Solís-Castillo et al. (2020)[23]	Flow cytometry; 30 early breast cancers	Tumor Tregs overexpressed CTLA-4; CD8/Treg ratio associated with LNM and stage
Núñez et al. (2020)[22]	Matched tumor-invaded and non-invaded TDLNs and tumors	Treg frequencies increased with nodal invasion; Tregs expressed high levels of co-inhibitory receptors
Røssevold et al. (2025)[29]	45 node-negative basal-like tumors (Oslo1); SCAN-B and METABRIC validation	High tumor CTLA4 messenger RNA identified patients with excellent prognosis

DFS, disease-free survival; IHC, immunohistochemistry; LNM, lymph node metastasis; OS, overall survival; TDLN, tumor-draining lymph node; TIL, tumor-infiltrating lymphocyte; TNBC, triple-negative breast cancer; Treg, regulatory T cell.

E. Research Gap

Several gaps therefore persist. First, direct evidence linking tumor-cell CTLA-4 expression on IHC to axillary LNM in TNBC derives largely from a single Egyptian cohort. Second, many studies have measured CTLA-4 on immune cells, in lymph nodes or as bulk transcript, and few have related membranous CTLA-4 expression in primary tumor cells, graded by a simple tumor-cell scoring system, to nodal status. Third, whether such an association is independent of established determinants such as tumor size and LVSI remains unclear. Fourth, data from Indonesian patients with TNBC are lacking.[8,10,11,29]

F. Novelty

The novelty of this study lies in its translational immunology–clinical approach: it relates CTLA-4 expression in primary TNBC tissue, assessed by IHC using tumor-cell (TC0–TC3) categories applicable in routine anatomical pathology laboratories, to LNM in an Indonesian population, using balanced groups of patients with and without LNM, and it evaluates whether the association persists after adjustment for tumor size and LVSI.

METHODS

A. Research Design

This was an analytical observational study with a retrospective cross-sectional design. Data were extracted from medical records as well as from available histopathology and IHC results, without any intervention involving the study subjects.

B. Study Setting and Period

The study was conducted at Hasanuddin University Hospital, Makassar, South Sulawesi, Indonesia, a teaching hospital affiliated with the Faculty of Medicine, Hasanuddin University, from April to August 2026.

C. Population, Eligibility and Sampling

The target population consisted of all patients with TNBC at the hospital, with an accessible population of 52 histopathologically confirmed patients. Samples were collected by total sampling from patients who met the inclusion criteria: (1) a diagnosis of breast cancer based on histopathological examination; (2) TNBC subtype at clinical stages I–III; (3) availability of tumor tissue for CTLA-4 IHC testing; and (4) availability of data on LNM status. Patients were excluded if they had stage IV disease or distant metastases, a history of immunotherapy or a history of other cancers. A total of 52 patients were analyzed, consisting of 26 patients with LNM and 26 patients without LNM.

D. Variables and Operational Definitions

The independent variable was CTLA-4 expression, defined as the expression of CTLA-4 protein on the tumor cell membrane, assessed by IHC on formalin-fixed, paraffin-embedded tumor tissue. Assessment was based on the percentage of tumor cells showing membranous CTLA-4 staining, evaluated under a light microscope. Expression was categorized as negative when there was no staining or staining in <1% of tumor cells (TC0) and as positive when staining was present in ≥1% of tumor cells, comprising TC1 (≥1% to <5%), TC2 (≥5% to ≤50%) and TC3 (>50%).[8]

The dependent variable was the occurrence of LNM (metastasis or no metastasis) based on medical records. The clinicopathological characteristics analyzed were tumor size (<2 cm, 2–5 cm and >5 cm), LVSI (negative or positive) and histopathological grade (grade 1, 2 and 3). Operational definitions are summarized in Table 2.

Table 2. Operational Definitions of Study Variables

Variable	Definition	Scale / categories
CTLA-4 expression	Membranous CTLA-4 staining of tumor cells on IHC of FFPE primary tumor tissue, expressed as the percentage of stained tumor cells	Nominal: negative (TC0, <1%); positive (≥1%: TC1, TC2, TC3)
Lymph node metastasis	Presence of regional lymph node metastasis according to medical records	Nominal: metastasis; no metastasis
Tumor size	Size category of the primary tumor	Ordinal: <2 cm; 2–5 cm; >5 cm
LVSI	Presence of tumor cells within lymphovascular spaces on histopathological examination	Nominal: negative; positive
Histopathological grade	Histological grade recorded in the histopathology report	Ordinal: grade 1; grade 2; grade 3

All variables were obtained from medical records and histopathology/IHC reports. FFPE, formalin-fixed, paraffin-embedded; IHC, immunohistochemistry; LVSI, lymphovascular space invasion; TC, tumor cell.

E. Data Collection and Processing

Data were coded, entered into IBM SPSS Statistics [specify version], tabulated, and presented in tables and narrative form.

F. Statistical Analysis

All variables were categorical and are presented as frequencies and percentages. The associations of clinicopathological characteristics and CTLA-4 expression with LNM were analyzed using the chi-square test, and Fisher's exact test was used when any cell had an expected value <5. The strength of the association between CTLA-4 expression and LNM was expressed as an odds ratio (OR) with a 95% confidence interval (CI), with CTLA-4-positive expression as the reference category. Logistic regression was used to assess the association between CTLA-4 expression and LNM after adjusting for tumor size and LVSI, with results reported as Wald values, ORs and 95% CIs. A p-value <0.05 was considered statistically significant.

G. Ethical Considerations

The research protocol was submitted for ethical approval to [name of the ethics committee; approval number and date], and the confidentiality of patients' identities and medical information was maintained throughout the study.

RESULTS

A. Characteristics of the Study Subjects

This study involved 52 patients with TNBC, who were divided into two groups of equal size: 26 patients (50.0%) with LNM and 26 patients (50.0%) without LNM. Overall, 26 patients (50.0%) had tumors >5 cm, 28 (53.8%) were LVSI-positive, and grade 1 and grade 3 tumors were each found in 18 patients (34.6%) (Table 3).

In the LNM group, no patient had a tumor <2 cm, 1 (3.8%) had a tumor of 2–5 cm and 25 (96.2%) had tumors >5 cm, whereas in the group without LNM, 1 (3.8%), 24 (92.3%) and 1 (3.8%) patients had tumors <2 cm, 2–5 cm and >5 cm, respectively. Positive LVSI was found in 24 patients (92.3%) with LNM and in 4 patients (15.4%) without LNM; conversely, negative LVSI was found in 2 (7.7%) and 22 (84.6%) patients, respectively. In the LNM group, grade 1, 2 and 3 tumors were found in 2 (7.7%), 9 (34.6%) and 15 (57.7%) patients, compared with 16 (61.5%), 7 (26.9%) and 3 (11.5%) patients in the group without LNM.

Bivariate analysis showed that tumor size ($p < 0.001$), LVSI ($p < 0.001$) and histopathological grade ($p < 0.001$) were significantly associated with LNM, with a clinically consistent distribution pattern: the larger the tumor, the more frequent the LVSI positivity and the higher the histopathological grade, the greater the likelihood of LNM.

Table 3. Clinicopathological Characteristics According to Lymph Node Metastasis Status (n = 52)

Variable	Total (n = 52)	LNM (n = 26)	No LNM (n = 26)	p
Tumor size				<0.001†
<2 cm	1 (1.9)	0 (0.0)	1 (3.8)	
2–5 cm	25 (48.1)	1 (3.8)	24 (92.3)	
>5 cm	26 (50.0)	25 (96.2)	1 (3.8)	
LVSI				<0.001

Variable	Total (n = 52)	LNM (n = 26)	No LNM (n = 26)	p
Negative	24 (46.2)	2 (7.7)	22 (84.6)	
Positive	28 (53.8)	24 (92.3)	4 (15.4)	
Histopathological grade				<0.001
Grade 1	18 (34.6)	2 (7.7)	16 (61.5)	
Grade 2	16 (30.8)	9 (34.6)	7 (26.9)	
Grade 3	18 (34.6)	15 (57.7)	3 (11.5)	
Total	**52 (100)**	**26 (100)**	**26 (100)**	

Values are n (%), with column percentages. p values from Pearson's chi-square test unless indicated. †Fisher's exact test. LNM, lymph node metastasis; LVSI, lymphovascular space invasion.

B. CTLA-4 Immunohistochemical Expression

Representative CTLA-4 staining patterns for each tumor-cell category are shown in Figure 1. Overall, CTLA-4 expression was positive in 38 of 52 patients (73.1%) and negative in 14 patients (26.9%).

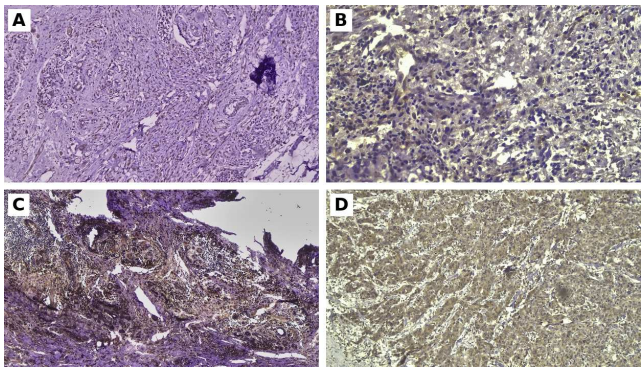


Figure 1: Representative immunohistochemical staining for CTLA-4 in triple-negative breast cancer tissue according to the tumor-cell (TC) categories: (A) TC0, negative (<1% of tumor cells); (B) TC1 (≥1% to <5%); (C) TC2 (≥5% to ≤50%); (D) TC3 (>50%).

C. Association between CTLA-4 Expression and Lymph Node Metastasis

Among patients with LNM, CTLA-4-positive expression predominated (25 of 26 patients), and only 1 patient showed negative expression, whereas among patients without LNM, CTLA-4-negative and CTLA-4-positive expression were each found in 13 patients. Accordingly, LNM was present in 25 of 38 CTLA-4-positive patients (65.8%) but in only 1 of 14 CTLA-4-negative patients (7.1%); conversely, the absence of metastasis was more frequent in the CTLA-4-negative group (92.9%) than in the CTLA-4-positive group (34.2%) (Table 4).

Pearson's chi-square test showed a statistically significant association between CTLA-4 expression and LNM ($p < 0.001$). With CTLA-4-positive expression as the reference category, CTLA-4-negative expression had an OR of 0.040 (95% CI 0.005–0.340), indicating lower odds of LNM in patients with CTLA-4-negative tumors.

Table 4. Association of CTLA-4 Expression with Lymph Node Metastasis

CTLA-4 expression	LNM n (%)	No LNM n (%)	OR (95% CI)	p
Negative	1 (7.1)	13 (92.9)	0.040 (0.005–0.340)	<0.001
Positive	25 (65.8)	13 (34.2)	Reference	
Total	**26 (50.0)**	**26 (50.0)**		

Row percentages. Pearson's chi-square test. CI, confidence interval; LNM, lymph node metastasis; OR, odds ratio.

D. Multivariate Analysis

In the logistic regression model, tumor size overall had a Wald value of 9.816 (df = 2; $p = 0.007$), indicating a statistically significant association with LNM after controlling for LVSI and CTLA-4 expression (Table 5). The estimate for the Tumor (1) category (OR 615,259,339.087; $p = 1.000$) had an extremely large standard error (40,192.969) and no estimable upper confidence limit; this estimate is therefore unstable and was not used for substantive interpretation. The Tumor (2) category had an OR of 0.008 (95% CI 0.000–0.166; $p = 0.002$), indicating a statistically significant association with LNM compared with the reference category of the model.

For LVSI, the OR was 11.239 (95% CI 0.522–242.123; $p = 0.122$), and for CTLA-4 expression (1) the OR was 6.331 (95% CI 0.073–552.222; $p = 0.418$). Because both p-values exceeded 0.05 and both confidence intervals included 1, neither LVSI nor CTLA-4 expression showed a statistically significant association with LNM after adjustment for the other variables. Overall, tumor size was the only variable independently associated with LNM in this model.

Table 5. Multivariate Logistic Regression of Factors Associated with Lymph Node Metastasis

Variable	B	S.E.	Wald	df	p	OR	95% CI
Tumor size	–	–	9.816	2	0.007	–	–
Tumor (1)	20.238	40,192.969	0.000	1	1.000	6.15×10^{8}‡	0.000–NE
Tumor (2)	−4.797	1.531	9.816	1	0.002	0.008	0.000–0.166
LVSI (1)	2.419	1.566	2.386	1	0.122	11.239	0.522–242.123
CTLA-4 expression (1)	1.846	2.280	0.655	1	0.418	6.331	0.073–552.222
Constant	0.965	1.363	0.502	1	0.479	2.626	–

Binary logistic regression including tumor size, LVSI and CTLA-4 expression; (1) and (2) denote the indicator-coded categories compared with the reference category of each variable in the model. ‡Exact value 615,259,339.087 (unstable estimate). B, regression coefficient; CI, confidence interval; df, degrees of freedom; LVSI, lymphovascular space invasion; NE, not estimable; OR, odds ratio [Exp(B)]; S.E., standard error.

DISCUSSION

This retrospective cross-sectional study showed that CTLA-4 expression was significantly associated with LNM in patients with TNBC in the bivariate analysis. Of the 52 patients, CTLA-4 expression was positive in 25 of 26 patients with LNM, compared with 13 of 26 patients without metastasis ($p < 0.001$; OR 0.040; 95% CI 0.005–0.340 for negative vs positive expression). Tumor size, LVSI and histopathological grade were also significantly associated with LNM (all $p < 0.001$). In the multivariate analysis, only tumor size remained significant ($p = 0.007$), whereas CTLA-4 ($p = 0.418$) and LVSI ($p = 0.122$) did not.

These findings are consistent with those of Mehrez et al., who reported in 100 TNBC cases in Egypt that CTLA-4 was significantly associated with LNM, distant metastasis and advanced TNM stage, and the present study extends that evidence to a population in Makassar, Indonesia. They also accord with evidence that CTLA-4 is expressed by TNBC tumor cells and that CTLA-4-activated-like tumors display an immune-escape-like state. Quintana et al. offer a complementary perspective: in early-stage TNBC, CTLA-4 expression in regional lymph nodes was higher in tumors with low lymphocytic infiltration, whereas Røssevold et al. found that high primary-tumor CTLA4 transcript expression identified node-negative basal-like tumors with an excellent prognosis. The context of this association may therefore vary according to tumor characteristics, the compartment and site of examination, the method of measurement and the population.[8,10,11,29]

Biologically, CTLA-4 is an immune checkpoint molecule that competes with CD28 for binding to CD80/CD86 on antigen-presenting cells and, owing to its higher affinity, inhibits T-cell activation, proliferation and effector function. This mechanism creates an immunosuppressive TME, and persistent inhibitory signaling is associated with T-cell exhaustion. Tumor-draining lymph nodes are the primary site of initial T-cell activation; thus, increased CTLA-4 activity and the accumulation of Tregs may inhibit the anti-tumor response even before nodal metastasis is detected. Tregs with high CTLA-4 expression maintain an immunosuppressive microenvironment and facilitate immune escape, and CTLA-4-positive breast cancer cells suppress dendritic cell maturation, which is thought to support cancer cell survival during lymphatic spread. This interpretation is supported by studies linking Treg infiltration and the CD8/Treg ratio to LNM in breast cancer, and by spatial transcriptomic and multi-omic studies showing that early nodal colonization occurs within an immunosuppressive, tumor-promoting microenvironment.[14–17,19,22,23,30,31]

LNM was found primarily in tumors >5 cm (25 of 26 patients with metastasis), whereas the non-metastatic group was dominated by tumors measuring 2–5 cm (24 of 26 patients). Tumor size reflects tumor burden, which increases the likelihood of lymphatic invasion and is among the most consistent predictors of nodal involvement, including in TNBC; nevertheless, nodal spread is also shaped by tumor biology, such as proliferative activity, rather than by size alone. Tumor size remained significant in the multivariate model (Wald = 9.816; df = 2; p = 0.007); however, the estimate for the Tumor (1) category was unstable (S.E. = 40,192.969), so its OR was not used as the basis for conclusions.[24,25,28,32]

Positive LVSI was found in 24 of 26 patients with metastasis, whereas negative LVSI was found in 22 of 26 patients without metastasis. LVSI reflects the ability of cancer cells to enter the lymphovascular space and travel to regional lymph nodes. In cervical cancer, LVSI is a recognized predictor of lymph node involvement, and in breast cancer, lymphovascular invasion is associated with approximately five-fold higher odds of LNM, while blood-vessel invasion is associated with nodal metastasis and other adverse features. In this study, four patients with positive LVSI (15.4%) did not have metastases, and LVSI was not significant in the multivariate analysis (p = 0.122), indicating that LVSI is not the sole determinant. Metastasis is also influenced by lymphangiogenesis, the immune response and the microenvironment; therefore, nodal risk assessment should integrate LVSI with other clinicopathological parameters.[24–26,33–35]

Grade 3 tumors predominated in the metastatic group (15 of 26 patients), whereas grade 1 tumors predominated in the non-metastatic group (16 of 26 patients). Higher grades are associated with more proliferative and invasive tumor cells, but their predictive value may change after other factors such as tumor size and lymphovascular invasion are taken into account.[24,27,28]

The multivariate results indicate that CTLA-4 cannot be identified as an independent determinant of LNM (B = 1.846; p = 0.418; OR = 6.331; 95% CI 0.073–552.222); therefore, the bivariate association cannot yet be considered independent. This is consistent with the understanding that the breast cancer TME is a complex system with both pro-tumoral and anti-tumoral effects, and that the prognostic value of CTLA-4 varies according to subtype, the site of expression and the composition of the immune microenvironment, as illustrated by the contrasting associations reported for tumor-cell protein and bulk tumor transcript expression. CTLA-4 is more appropriately viewed as an immunological marker associated with LNM that may reflect suppression of the T-cell response.[11–14,29]

These findings have clinical implications. First, CTLA-4 expression in primary tumor tissue has the potential to serve as an additional marker for assessing the risk of LNM in TNBC, but it needs to be evaluated in conjunction with tumor size, LVSI and grade. Second, future studies should assess CTLA-4 together with immune cell profiles, particularly Tregs and CD8+ T lymphocytes. Third, these data may serve as a basis for developing CTLA-4 as part of an immune biomarker panel.

The strengths of this study include its translational immunology–clinical approach; the use of IHC applicable in routine anatomical pathology laboratories with the TC0–TC3 categories referenced in the literature; the provision of preliminary data from Indonesia, where such evidence remains limited; and the use of total sampling with a balanced number of subjects (26 vs 26).

LIMITATIONS AND FUTURE WORK

This study has several limitations. First, the semi-quantitative assessment of CTLA-4 is subject to inter-observer variability. Second, the retrospective cross-sectional design demonstrates associations only, without long-term outcomes such as disease-free survival (DFS) and overall survival (OS). Third, the sample size was small and derived from a single center, and the logistic regression estimates were unstable, with very wide confidence intervals. Fourth, CTLA-4 assessment was

limited to primary tumor cells, without evaluation of Tregs and CD8+ T lymphocytes.

Future studies are recommended to use larger sample sizes and a multicenter design, to apply standardized or quantitative scoring with assessment of inter-observer agreement, to include concurrent assessment of immune cell profiles, and to follow patients prospectively for DFS and OS.

CONCLUSION AND RECOMMENDATIONS

Conclusion

This study shows that CTLA-4 expression was positive in the majority of TNBC tumors. In the bivariate analysis, CTLA-4 expression was significantly associated with LNM, with positive expression more commonly found in patients with metastasis and negative expression more commonly found in patients without metastasis. However, after adjustment for tumor size and LVSI in the multivariate analysis, CTLA-4 expression did not show an independent significant association with LNM. Thus, the association between CTLA-4 and LNM in this study was not independent after clinicopathological characteristics were accounted for in the analysis model.

Recommendations

1. CTLA-4 immunohistochemistry of primary TNBC tissue should be interpreted together with tumor size, LVSI and histopathological grade, rather than as a stand-alone predictor of LNM.

2. Larger, multicenter and preferably prospective studies should evaluate CTLA-4 together with Treg and CD8+ T-lymphocyte profiles and long-term outcomes (DFS and OS).

3. These data may be used as a basis for developing CTLA-4 as part of an immune biomarker panel for nodal risk stratification in TNBC.

DECLARATIONS

Ethics approval: [Name of the ethics committee; approval number; date].

Author contributions: NY: conceptualization, data collection, analysis and drafting; P and I: supervision, clinical interpretation and critical revision. All authors approved the final manuscript. [to be confirmed by the authors]

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