EXPRESSION OF CD4, CD8 BIOMARKERS IN INVASIVE CARCINOMA OF BREAST WITH CLINICOPATHOLOGICAL CORRELATION

ABSTRACT

Introduction: Tumor infiltrating lymphocytes (TILs) are widely considered a key sign of the immune interaction between host and tumor, and potentially prognostic biomarkers of good or bad outcome in various cancers, including invasive breast cancer (IBC).**Aim and Objectives:** To correlate the expression of CD4, CD8 T-lymphocytes in invasive carcinoma breast with established markers of prognosis like tumour size, grade, lymph node status and molecular subtypes mainly ER, PR, Her 2Neu, Ki67 status, mainly the triple negative breast cancers(TNBC).**Methodology:** 58 Invasive breast carcinoma proven tissue blocks were subjected to immunohistochemistry and morphometric analysis for positive CD4, CD8 T-lymphocytes were done.**Results:** Triple negative breast cancer subtype shows high TILs than other pathologic subtypes. Tumor interface CD8+ cells very well correlated with the pathological higher nodal stage. Majority CD4, CD8 positive cells were populated more towards the stromal and interface of the tumor microenvironment rather thatintratumoral.**Conclusion:** CD4+ and CD8+ counts may be a valuable independent prognostic tool in predicting the outcome in invasive breast cancer.

Keywords: *Breast cancer, Invasive breast Cancer, CD4 and CD8 Markers, Clinico Pathological Correlation, Tumour infiltrating lymphocytes*.[1]

INTRODUCTION:

Breast cancer (BC) is a neoplasm characterized by molecular and cellular heterogeneity. It is the leading cause of mortality among women globally[1]. Recent advances in diagnosis and treatment of the breast cancer showed the importance of the role of the new markers in prognosis and target therapies will help in patient survival[2].A study reviewing several findings have showed the importance of the microenvironmentfocusing on the function and interaction between immune cells and cancer cells in its

role in cancer progression including Breast cancer[3]. It is generally known that regional lymph nodes are a significant immunological defense for tumor expansion[4].
Tumor infiltrating lymphocytes (TILs) have higher specific immunological reactivity against tumor cells. They play dual role i.e., either suppress or promote growth of the tumor[5]. Tumor infiltrating lymphocytes are part of adaptive immunity that migrate into the tumor and stroma, are considered a manifestation of host antitumor response. Moreover, its role in tumor progression or regression is been studied in various solid tumors[6,7]. Tumor cells can express antigens and become targets for a T cell–mediated adaptive immune response. The ability of CD4+, CD8+ lymphocytes to recognize tumor antigens has been well documented, and tumor regression is observed when tumor-reactive T lymphocytes invade cancers more so by CD8 CTLs[8-10]. The anti- and pro-tumor function of TILs in breast cancer is dependent on the TIL subtype. Definitely cytotoxic T cells (CD8+ T cells) have been described to be associated with better clinical outcome in different phenotypes of Breast Cancer[11-14].The clinical significance of TILs in Breast Cancer is still debatable and the conclusions remain controversial.Generally, assessing the immunogenicity of the neoplasm, and its distribution may determine if an immune response will be prognostically favorable mainly for TNBC and HER2neu overexpressed tumors[15, 16].With successive hormonal and chemotherapeutics, the next modality aims to be immunotherapy in breast cancer. TNBCs are said to have more mutations compared to other subtypes, which could be paramount targets for immunotherapy[8,9,20,].

MATERIALS AND METHODS

58 formalin-fixed and paraffin embedded tissue blocks along with histopathological slides were retrieved from the archives of the Department of pathology, Chettinad Academy of Research and Education. The study protocol was approved by the Ethics committee of this tertiary care hospital. Core biopsies, lumpectomy specimens, neoadjuvant chemotherapy patients were completely exempted from the study. Estrogen receptor, progesterone receptor, Her2 and Ki67 immunopositive staining for each case was doneand evaluated as per American Society of Clinical Oncology/College of American Pathologists Guidelines (ASCO/CAP guidelines**).** Parameters like pTNM staging, lymph node status, morphological and molecular subtypes, Modified Scarff Bloom Richardson grading. All the recorded data was entered on a detailed proforma designed for this study.Immunohistochemistry:- Formalin-fixed, paraffin embedded tissue sections of 3.5 microns thick. Sections were deparaffinised in xylene followed by hydration in graded ethanol. Antigen retrieval was performed by heating process at 100deg Celsius for 20 mins in Tris-EDTA buffer(pH 7.4). Endogenous peroxidize block in the tissue sections was done for 10 minutes. Sections were covered with appropriate primary antibody for 45 minutes at room temperature.After adequate buffers washes.DAB(3,3'-diaminobenzidine tetrahydrochloride) chromogenis added. Sections were counterstained with hematoxylin, dehydrated with ethanol and xylene, and mounted with DPX(Di-n-butylphthalate in xylene).Tonsillar tissue sections served as positive control for markers CD4+,CD8+.

Microscopic assessment of CD4, CD8 Tcells and scoring: In our study, the CD4 and CD8 Tcells were assessed in immunohistochemical(IHC) slides adapted from Ankita et al study, the scoring of immune stained positive CD4,CD8 TILs was done independently by two pathologists on the same microscopic area. CD4+, and CD8+ TILs were counted in same five randomly selected high power fields (40X magnification) and the counts were averaged for tumor, stroma and tumor interface. The tumor infiltrating lymphocyte count was calculated as: + (1-25 cells), ++ (26-50 cells), +++ (≥51 cells) in the tumor, tumor interface and the stroma separately. Positive TILs upto 25 cells were considered as low TIL count and more than 25 cells (*i.e*. ++, +++) were considered as high TIL count in the tumor, tumor interface and stroma separately.

RESULTS

In this study, total 58 tissue blocks of patients who underwent modified radical mastectomy (MRM) were only taken and about 63.8% (37/58) had right MRM, 36.2(21/58) had left MRM. Majority in the study group were females (96.6%). Only 2 male subjects and were diagnosed with invasive ductal carcinoma NST. The mean patient's age was 50.03 years (ranging from 35-74 years) Among the study population, majority 20 (34.5%) of them were belonging to 41 - 50 years.The molecular subtypes showed high triple

negative subtype cases (48.3%) than others. Morphological diagnosis of invasive ductal carcinoma NST [77.5%] were predominant cases, followed by invasive carcinoma with medullary like features[15.50%] followed by invasive papillary carcinoma [3.40%], and mucinous carcinoma[3.40%] were observed. Of all cases, (94.8%) were relapse free survivors, 1 patient had recurrence, and 2 patients did not survive. 53 patients of lymph node positive cases observed. The descriptive statistics of all 58 patients were compiled with tabulation.

Table1. Cross tabulation between Tumour interface CD4 count (High & Low) and other variables:

		Tumor interface CD4		Chi sq p value
		High	**Low**	
pTNMTumour Size	**T1 & T2**	31 (83.8%)	6 (16.2%)	0.295
	T3 & T4	17 (85%)	3 (15%)	
pTNM Nodal Status	**N0 &Nx**	20 (76.9%)	6 (23.1%)	0.115
	N1 & above	28 (90.3%)	3 (9.7%)	
pTNM Metastasis	**M0**	40 (85.1%)	7 (14.9%)	0.315
	Mx	8 (80%)	2 (20%)	
ER	**Positive**	16 (76.2%)	5 (23.8%)	0.17
	Negative	32 (86.5%)	5 (13.5%)	
PR	**Positive**	16 (76.2%)	5 (23.8%)	0.17
	Negative	32 (86.5%)	5 (13.5%)	
Her2Neu	**Positive**	11 (73.3%)	4 (26.7%)	0.159
	Negative	37 (86%)	6 (14%)	
Ki67	**1+ & 2+**	28 (73.7%)	10 (26.3%)	**0.009**
	3+	20 (100%)	0 (0%)	
Pathological Subtype	**TNBC**	24 (85.7%)	4 (14.3%)	0.196
	Luminal A	12 (85.7%)	2 (14.3%)	
	Luminal B	4 (57.1%)	3 (42.9%)	
	Her2 / Neu	8 (88.9%)	1 (11.1%)	

Table2.Cross tabulation between Intratumoral CD8 count (High & Low) and other variables:

		Intratumoral CD8		Chi sq p value
		High	**Low**	
pTNMTumour Size	**T1 & T2**	20 (54.1%)	17 (45.9%)	0.77
	T3 & T4	10 (50%)	10 (50%)	
pTNM Nodal Status	**N0 &Nx**	13 (50%)	13 (50%)	0.716
	N1 & above	17 (54.8%)	14 (45.2%)	
pTNM Metastasis	**M0**	24 (51.1%)	23 (48.9%)	0.241
	Mx	6 (60%)	4 (40%)	
ER	**Positive**	6 (28.6%)	15 (71.4%)	**0.008**
	Negative	24 (64.9%)	13 (35.1%)	
PR	**Positive**	6 (28.6%)	15 (71.4%)	**0.008**
	Negative	24 (64.9%)	13 (35.1%)	
Her2Neu	**Positive**	8 (53.3%)	7 (46.7%)	0.885
	Negative	22 (51.2%)	21 (48.8%)	
Ki67	**1+ & 2+**	16 (42.1%)	22 (57.9%)	**0.043**
	3+	14 (70%)	6 (30%)	
Pathological Subtype	**TNBC**	16 (57.1%)	12 (42.9%)	**0.011**
	Luminal A	5 (35.7%)	9 (64.3%)	
	Luminal B	1 (14.3%)	6 (85.7%)	
	Her2 / Neu	8 (88.9%)	1 (11.1%)	

Table3. Cross tabulation between Stromal CD8 count (High & Low) and other variables:

	Stromal CD8	**Chi sq**

		High	Low	p value
pTNMTumour Size	**T1 & T2**	34 (91.9%)	3 (8.1%)	0.393
	T3 & T4	19 (95%)	1 (5%)	
pTNM Nodal Status	**N0 &Nx**	23 (88.5%)	3 (11.5%)	0.204
	N1 & above	30 (96.8%)	1 (3.2%)	
pTNM Metastasis	**M0**	43 (91.5%)	4 (8.5%)	0.452
	Mx	10 (100%)	0 (0%)	
ER	**Positive**	19 (90.5%)	2 (9.5%)	0.356
	Negative	34 (91.9%)	3 (8.1%)	
PR	**Positive**	19 (90.5%)	2 (9.5%)	0.356
	Negative	34 (91.9%)	3 (8.1%)	
Her2Neu	**Positive**	14 (93.3%)	1 (6.7%)	0.404
	Negative	39 (90.7%)	4 (9.3%)	
Ki67	**1+ & 2+**	33 (86.8%)	5 (13.2%)	0.11
	3+	20 (100%)	0 (0%)	
Pathological Subtype	**TNBC**	25 (89.3%)	3 (10.7%)	0.14
	Luminal A	13 (92.9%)	1 (7.1%)	
	Luminal B	6 (85.7%)	1 (14.3%)	
	Her2 / Neu	9 (100%)	0 (0%)	

Table4. Cross tabulation between Tumour Interface CD8 count (High & Low) and other variables:

		Tumor interface CD8		Chi sq p value
		High	**Low**	
pTNMTumour Size	**T1 & T2**	34 (91.9%)	3 (8.1%)	0.266

	T3 & T4	20 (100%)	0 (0%)	
pTNM Nodal Status	**N0 &Nx**	23 (88.5%)	3 (11.5%)	**0.049**
	N1 & above	31 (100%)	0 (0%)	
pTNM Metastasis	**M0**	44 (93.6%)	3 (6.4%)	0.554
	Mx	10 (100%)	0 (0%)	
ER	**Positive**	19 (90.5%)	2 (9.5%)	0.33
	Negative	35 (94.6%)	2 (5.4%)	
PR	**Positive**	19 (90.5%)	2 (9.5%)	0.33
	Negative	35 (94.6%)	2 (5.4%)	
Her2Neu	**Positive**	13 (86.7%)	2 (13.3%)	0.223
	Negative	41 (95.3%)	2 (4.7%)	
Ki67	**1+ & 2+**	34 (89.5%)	4 (10.5%)	0.174
	3+	20 (100%)	0 (0%)	
Pathological Subtype	**TNBC**	26 (92.9%)	2 (7.1%)	0.175
	Luminal A	14 (100%)	0 (0%)	
	Luminal B	5 (71.4%)	2 (28.6%)	
	Her2 / Neu	9 (100%)	0 (0%)	

MICROSCOPIC IMAGES

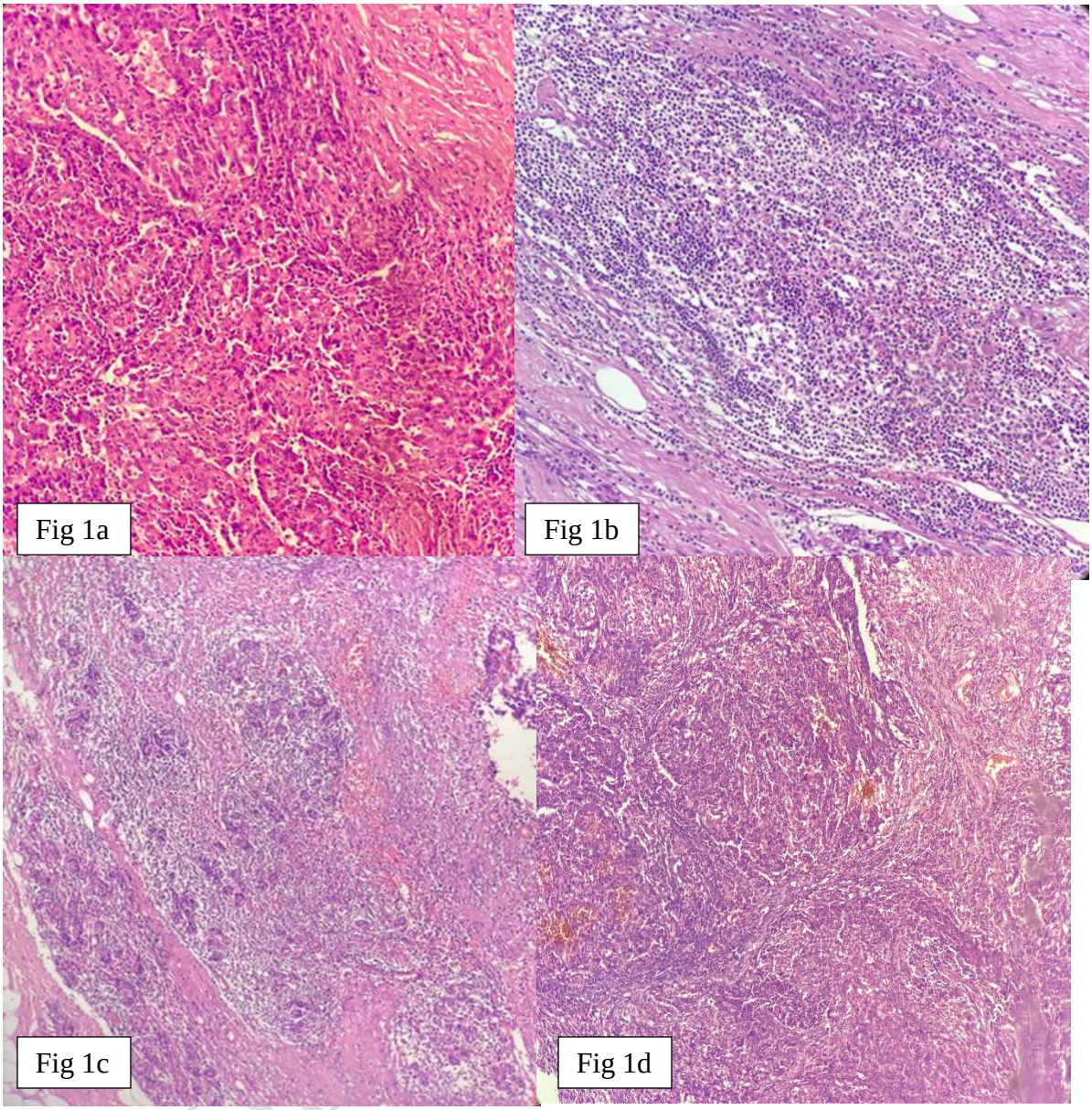

Fig1(a)- Intratumorallymphocytes(x40 magnification, H&E)

Fig 1(b)-Tumor interface lymphocytic infiltrate(x40 magnification, H&E)

Fig 1(c)-Stromal lymphocytic infiltrate seen extending to the adjacent normal breast terminal duct lobular unit(x20),

Fig1 (d) extensive lymphoplasmacytic infiltrates in the stroma and intratumoral(x40)

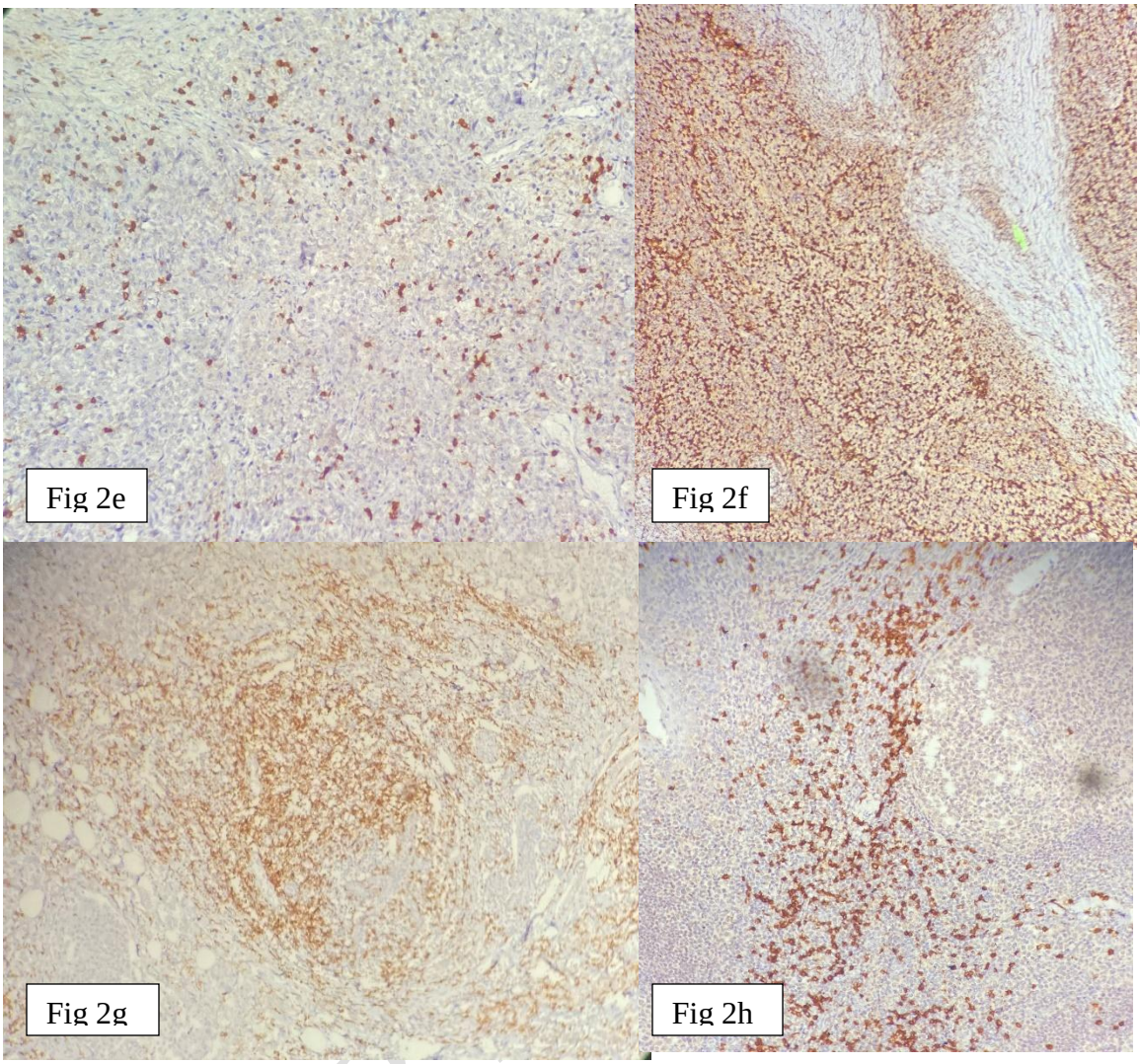

Fig 2(e,f)- Intratumoral CD8, CD4 immunopositive T-lymphocytes(x40,x20)

Fig 2(g)- Tumor interface CD8 positive Tcells(x20)

Fig 2(h)- Stromal CD8 positive Tcells (x40)

DISCUSSION

In the present study, the correlation between Intratumoral, stromal andtumor interface CD4+, CD8+ marker expression with pathological variables and molecular subtypes were as follows:-

Stromal CD4 count when correlated with negative hormonal receptor status(ER,PR) wassignificant(p value =0.05).When compared to the tumor stage, low intratumoral CD4 and high stromal and interface CD4 count was found in TI,T2 stages of tumor and in all positive nodal status as well. Collectively CD4 intratumoral expression was low in relation to all the parameters like tumor size, lymhpnodes and all molecular subtypes. Majority, of TNBC showed low intratumoral CD4 count and high stromal and interface CD4 count. Lowtumorstage(T1,T2) showed high intratumoral CD8 positive expression and also significant correlation was observed with triple negative subtype(ER-,PR-,Her2-) of p value(<0.05) which was statistically significant. Whereas stromal CD8+ didn't show any significant difference compared to any of the parameters, but high stromal and interface CD8 count is seen compared to intratumoral CD8 count. Tumor interface CD8+ cells very well correlated with the pathological higher nodal stage. Again, high CD8 count is seen more in triple negative breast cancers. Also noted that both stromal and interface CD4, CD8 infiltrate were high in Her2 and TNBC cases than the intratumoral sites. CD8 Tcells were high compared to CD4 Tcells around the normal breast TDLU. Moreover, suggests immune response in these two subtypes is different from hormonal receptor subtype.

RafalMatkowski et al (2009) studied 88 breast cancer patients with ductal histology. Along with the CD4 and CD8 expression, the type, density, localization and distribution of tumour infiltrating lymphocytes (TILs) was studied. The patients with high expression of CD4 or CD8 had lymph node involvement and worse cancer specific overall survival in this study[17].

Sahar M A Mahmoud et al (2011)analysed 1334 tissue microarray cores. The study results revealed that the total number of CD8(+) cells was positively correlated with tumour grade, patient's age during the diagnosis, molecular markers expression. They observed that the Stromal CD8+ T cell lymphocytes were independently associated with better prognosis and patient survival[18]. Also in our study, stromal CD8+ counts were high with good prognosis.

Ankita Singh Rathore et al (2014) In this study, immunohistochemistry ofCD3, CD4 and CD8 T-cell markers were used on150 breast cancer tissue sections. On analysing each parameter, it showed that the high (++/+++)intratumoral CD4+ count showed the highest survival compared to CD3+ and CD8+ count the least when compared to respective low (+) counts. In contrast, the high CD8+(P<0.001) stromal count showed the highest survival followed by CD4+ and CD3+ the least. They concluded that each of themare better independent predictors of favourable survival outcome in infiltrating ductal breast carcinoma[19].

Ezzeldin M Ibrahim et al (2014) meta-analysis study showed, the prognostic value of tumour-infiltrating lymphocytes in triple-negative breast cancer(TNBC) by extracting data of 2,987 patients with early stage breast cancer from 8 studies and concluded that the increased TILs were significantly associated with improved survival outcome in early TNBC and further recommended that it can be considered as a strong prognostic factor for TNBC[20].Also stated, Invasive ductal histology is the predominant type to be involved[21].

Hirofumi Matsumoto et al study found that high levels of CD8+ iTILs and CD4+ sTILs were significantly associated with better clinical outcomes in TNBC. The role of CD4+ TILs in antitumour responses is often to stimulate the CD8+ TILs and to attack tumor cells. The CD4+ T-cells benefit the CD8+ CTLs in maintaining the tumour immunity which happens in three phases, viz. by primary induction, effector maintenance, and memory of CD 8+ CTL responses[22].

Kim et al reported that declined number of CD8+ TILs in breast tumours were significantly related with lymph node metastasis (as seen in our study too), high stage and higher proliferative index[23].

Triple negative breast cancers (TNBC) are most likely to have tumors with >50 % lymphocytic infiltrate, has the greatest survival benefit from each 10 % increase in TIL. ER+ve,PR+ve,HER2- tumors tend to have the least immune infiltrate[24]. T cells were the predominant immunophenotype being noted in 81% of

tumors, compared to B cells in predicting the biological behavior. They came to a conclusion that, probably favorsneoplastic progression rather than acting as an antitumor immune response[25].

Kurozumi et al.recently investigated the relationship between TILs and prognosis in 294 cases and reported that high stromal TILs expression was a good prognostic marker in ER-negative cancer subtype[26].

Running text- Clinicopathological comparison of T-cell Biomarkers CD4, CD8 influence on tumorinfitratinglymphocytes(TILs) in invasive breast cancer depicts the immunological interaction of host immunity and tumor, which shows progression or regression of tumor cells in invasive breast carcinoma. The prognostic significance is mainly needed in triple negative breast cancers (TNBCs), where therapy is questionable. Emergence of immunotherapy in various cancers, including breast cancer imply the significance of T-cell population of tumor infiltrating lymphocytes which can promote value to the treatment protocol and predict thelife expectancy of patients with Triple negative invasive breast cancer.As with this study, triple negative breast cancer subtype showed high TILs than other pathologic subtypes.Tumor interface CD8+ cells very well correlated with the pathological higher nodal stage. Majority CD4, CD8 positive cells were populated more towards the stromal and interface of the tumor microenvironment rather being intratumoral.

CONCLUSION: In conclusion of this study, it is to highlight that immune markers like CD4,CD8Tcells expression might benefit patients particularly in triple negative breast cancerand Her2 overexpressed subtypes which is more immunogenic than the hormonal subtypes. Understanding the impact of specific subsets of immune cells that infiltrate tumors and its microenvironment is important for making rational decisions in the development of targeted therapies in invasive breast carcinomas. As shown in our study, lymphnode involvement was seen in cases with high CD8 cells in the tumor interface. So, in addition to ER,PR,Her2 and Ki67, CD8 can also be used in patients to assess the prognosis.

ETHICAL CLEARANCE: Ethical Clearance for this study got approved from the Institutional Human Ethical Committee.

REFERENCES:

1. Ghoncheh M, Pournamdar Z, Salehiniya H. Incidence and mortality and epidemiology of breast cancer in the world. Asian Pacific J Cancer Prev [Internet]. 2016 [cited 2020 Sep 19];17(sup3):43–6. Available from: http://dx.doi.org/10.7314/APJCP.2016.17.S3.43
2. Saponaro C, Vagheggini A, Scarpi E, Centonze M, Catacchio I, Popescu O, et al. NHERF1 and tumor microenvironment: a new scene in invasive breast carcinoma. J Exp Clin Cancer Res [Internet]. 2018;37(1):96. Available from: https://doi.org/10.1186/s13046-018-0766-7
3. Shiao SL, Ganesan AP, Rugo HS, Coussens LM. Immune microenvironments in solid tumors: new targets for therapy. Genes Dev. 2011 Dec;25(24):2559–72.
4. GISTEREK I, FRYDECKA I, ŚWIĄTONIOWSKI G, FIDLER S, KORNAFEL J. Tumour-infiltrating CD4 and CD8 T lymphocytes in breast cancer. Reports Pract Oncol Radiother [Internet]. 2008;13(4):206–9. Available from: http://www.sciencedirect.com/science/article/pii/S1507136710600119
5. Badalamenti G, Fanale D, Incorvaia L, Barraco N, Listì A, Maragliano R, et al. Role of tumor-infiltrating lymphocytes in patients with solid tumors: Can a drop dig a stone? Vol. 343, Cellular Immunology. Academic Press Inc.; 2019. p. 103753.
6. Hendry S, Salgado R, Gevaert T, Russell PA, John T, Thapa B, et al. Assessing Tumor-Infiltrating Lymphocytes in Solid Tumors: A Practical Review for Pathologists and Proposal for a Standardized Method from the International Immuno-Oncology Biomarkers Working Group: Part 2: TILs in Melanoma, Gastrointestinal Tract Carcinom. Adv Anat Pathol [Internet]. 2017 Nov;24(6):311–35. Available from: https://pubmed.ncbi.nlm.nih.gov/28777143
7. Gonzalez H, Hagerling C, Werb Z. Roles of the immune system in cancer: from tumor initiation to

metastatic progression. Genes Dev [Internet]. 2018 Oct 1;32(19–20):1267–84. Available from: https://pubmed.ncbi.nlm.nih.gov/30275043

8. Pandya PH, Murray ME, Pollok KE, Renbarger JL. The Immune System in Cancer Pathogenesis: Potential Therapeutic Approaches. J Immunol Res. 2016;2016.

9. Gun SY, Lee SWL, Sieow JL, Wong SC. Targeting immune cells for cancer therapy. Redox Biol [Internet]. 2019/03/20. 2019 Jul;25:101174. Available from: https://pubmed.ncbi.nlm.nih.gov/30917934

10. Pluhar GE, Pennell CA, Olin MR. $CD8^+$ T Cell-Independent Immune-Mediated Mechanisms of Anti-Tumor Activity. Crit Rev Immunol [Internet]. 2015;35(2):153–72. Available from: https://pubmed.ncbi.nlm.nih.gov/26351148

11. Mao Y, Qu Q, Chen X, Huang O, Wu J, Shen K. The Prognostic Value of Tumor-Infiltrating Lymphocytes in Breast Cancer: A Systematic Review and Meta-Analysis. Tagliabue E, editor. PLoS One [Internet]. 2016 Apr 13 [cited 2020 Sep 19];11(4):e0152500. Available from: https://dx.plos.org/10.1371/journal.pone.0152500

12. Ali HR, Provenzano E, Dawson SJ, Blows FM, Liu B, Shah M, et al. Association between CD8+ T-cell infiltration and breast cancer survival in 12 439 patients. Ann Oncol [Internet]. 2014 Jun 9 [cited 2020 Sep 19];25(8):1536–43. Available from: https://nottingham-repository.worktribe.com/output/1102892/association-between-cd8-t-cell-infiltration-and-breast-cancer-survival-in-12439-patients

13. Miyashita M, Sasano H, Tamaki K, Hirakawa H, Takahashi Y, Nakagawa S, et al. Prognostic significance of tumor-infiltrating CD8+ and FOXP3+ lymphocytes in residual tumors and alterations in these parameters after neoadjuvant chemotherapy in triple-negative breast cancer: a retrospective multicenter study. Breast Cancer Res [Internet]. 2015;17(1):124. Available from: https://doi.org/10.1186/s13058-015-0632-x

14. Liu S, Lachapelle J, Leung S, Gao D, Foulkes WD, Nielsen TO. CD8+ lymphocyte infiltration is an independent favorable prognostic indicator in basal-like breast cancer. Breast Cancer Res [Internet]. 2012;14(2):R48. Available from: https://doi.org/10.1186/bcr3148

15. Nagarajan D, McArdle SEB. Immune Landscape of Breast Cancers. Biomedicines [Internet]. 2018 Feb 11;6(1):20. Available from: https://pubmed.ncbi.nlm.nih.gov/29439457

16. Planes-Laine G, Rochigneux P, Bertucci F, Chrétien A-S, Viens P, Sabatier R, et al. PD-1/PD-L1 Targeting in Breast Cancer: The First Clinical Evidences Are Emerging. A Literature Review. Cancers (Basel) [Internet]. 2019 Jul 22;11(7):1033. Available from: https://pubmed.ncbi.nlm.nih.gov/31336685

17. Matkowski R, Gisterek I, Halon A, Lacko A, Szewczyk K, Staszek U, et al. The prognostic role of tumor-infiltrating CD4 and CD8 T lymphocytes in breast cancer. Anticancer Res. 2009 Jul;29(7):2445–51.

18. Yang X, Ren H, Sun Y, Shao Y, Zhang L, Li H, et al. Prognostic significance of CD4/CD8 ratio in patients with breast cancer. Int J Clin Exp Pathol. 2017;10(4):4787–93.

19. Rathore AS, Kumar S, Konwar R, Makker A, Negi MPS, Goel MM. CD3+, CD4+ & CD8+ tumour infiltrating lymphocytes (TILs) are predictors of favourable survival outcome in infiltrating ductal carcinoma of breast. Indian J Med Res. 2014 Sep;140(3):361–9.

20. Ibrahim EM, Al-Foheidi ME, Al-Mansour MM, Kazkaz GA. The prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancer: a meta-analysis. Breast Cancer Res Treat. 2014 Dec;148(3):467–76.

21. Breast cancer incidence (invasive) statistics | Cancer Research UK [Internet]. [cited 2020 Sep 22]. Available from: https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/breast-cancer/incidence-invasive#ref1

22. Matsumoto H, Thike AA, Li H, Yeong J, Koo SL, Dent RA, et al. Increased CD4 and CD8-positive T

cell infiltrate signifies good prognosis in a subset of triple-negative breast cancer. Breast Cancer Res Treat. 2016;156(2):237–47.

23. Kim ST, Jeong H, Woo OH, Seo JH, Kim A, Lee ES, et al. Tumor-infiltrating lymphocytes, tumor characteristics, and recurrence in patients with early breast cancer. Am J Clin Oncol. 2013 Jun;36(3):224–31.

24. Stanton, S.E., Disis, M.L. Clinical significance of tumor-infiltrating lymphocytes in breast cancer. j. immunotherapy cancer(2016) 4, 59

25 Helal TE, Ibrahim EA, Alloub AI. Immunohistochemical analysis of tumor-infiltrating lymphocytes in breast carcinoma: Relation to prognostic variables. [Indian J Pathol Microbiol (2013);56:89-93].

26. Kurozumi S, Matsumoto H, Kurosumi M, Inoue K, Fujii T, Horiguchi J, et al. Prognostic significance of tumour-infiltrating lymphocytes for oestrogen receptor-negative breast cancer without lymph node metastasis. Oncol Lett. 2019;17:2647–56.