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The predictive and prognostic value of tumor infiltrating lymphocytes in advanced non small cell lung cancer

Mai Ahmed Eissa [1], Ahmed El Saied El Bastawisy[2], Hala Aziz Shokrallah[3], Tarek Nabil EL Bolkainy[4], Rabab Mohamed Gaafar[5]*

[1]*Department of Medical Oncology, Maadi Armed Forces Hospital, Cairo, 11711, Egypt.*

[2]*Department of Medical Oncology National Cancer Institute, Cairo University, Cairo 11796, Egypt.*

[3]*Department of Medical Oncology National Cancer Institute, Cairo University, Cairo 11796, Egypt.*

[4]*Pathology department National Cancer Institute, Cairo University, Cairo 11796, Egypt.*

[5]*Department of Medical Oncology National Cancer Institute, Cairo University, Cairo 11796, Egypt.*

Abstract

Recent studies suggest immune infiltrates in primary tumors and metastases can serve as independent prognostic biomarkers and predict efficacy of platinum-based anticancer drugs in patients with non-small cell lung cancer patients (NSCLC). From January 2016 to Decmber 2018, we conducted a prospective study on 50 chemotherapy naive patients with advanced stage NSCLC. CD3 and CD4 lymphocytes were detected in paraffin embedded tumor tissues. We studied the average density of TILs subgroups using the median values as cut-off. All patients received gemcitabine and platinum agents. Assessment of tumor infiltrating lymphocytes (TILs), correlation with overall response rate and TTP were done. Association of high CD3 TIL was found in males more than females with p. value 0.0032, as well as high CD3 TIL count was associated with smokers patients than non-smokers with p. value 0.005. There was correlation in Low CD3 infiltration with pathological subtypes mainly adenocarcioma with (p. 0.081), while no correlation with CD4 cells. A significant association was seen between the density of CD4 (TILs) and the clinical benefit response to chemotherapy, with p-values of 0.048. However, no correlation was found between the density of CD3 TILs and the response to chemotherapy. There is no correlation between the density of CD3 and CD4 TILs and time to progression. For patients receiving platinum treatments, high CD4 TIL infiltration inside the tumor environment may be regarded as predictive indicators. Understanding the therapeutic significance of the microenvironmental immune milieu may be a critical indictor of prognosis and treatment response and need more studying TILs infiltration inside microenvironment.

Keywords: Non-small cell lung cancer, Tumor infiltrating lymphocytes, chemotherapy.

Full length article **Corresponding Author*, e-mail: maieissa25@yahoo.com

1. Introduction

Worldwide, lung cancer is the primary contributor to mortality -related, resulting in approximately 1.6 million deaths annually [1]. The frequency of advanced disease demonstrated a significant and continuous decrease whereas the rates of localized-stage cancer had a rapid increase yearly. This abrupt increase contributed to improvements in the percentage of diagnoses at the localised stage [2]. The study of the capacity of the immune system to recognise cancer cells and evade immune surveillance has become a topic of concern. Cancer immunotherapy has demonstrated better survival rates and was established as the initial treatment option for various tumours [3]. In a wide range of human neoplasms, there is a correlation with high (TILs) and favorable outcome including colorectal, melanoma and triple- negative breast cancers [4]. Elevated tumor-infiltrating lymphocytes (TILs) in NSCLC patients are linked to a positive prognosis and chemotherapy response, but chromogenic immunohistochemistry (IHC) is controversial in evaluating the prognostic significance of different TIL subtypes [5]. The study of subsets of (TILs) is crucial and there is recent advances in (TILs) immunohistochemistry have been evolved. T lymphocytes are classified into major subtypes based on cell surface markers, encompassing CD8+ cytotoxic T lymphocytes (CTL), CD4+ T helper lymphocytes (Th), CD3, CD45RO+

memory T cells (Tm), and FOXP3+ regulatory cells (Tregs). (TILs) have demonstrated favourable survival outcomes than the TNM staging system [6]. In non-actionable targeted mutations in advanced NSCLC, platinum-based combinations are the initial therapy, adding new approved drugs. However, platinum resistance still poses a challenge [7].

2. Materials and Methods

This was a prospective, non-randomized study. it included 50 patients with advanced NSCLC presented to National Cancer Institute, Cairo University, in the duration between January 2016 and December 2018. The National Cancer Institute, Cairo University's institutional review board approved the study after obtaining written informed consent from each patient before enrollment in the study.

2.1 Patients and samples

The study included patients who met the specified inclusion criteria, histologically confirmed diagnosis of NSCLC , Stage IV at presentation, age ≥18 years old, Eastern Cooperative Oncology Group (ECOG) Performance status (PS) 0- 2 , adequate bone marrow function, adequate liver function, adequate kidney function and Paraffin embedded tissue specimen should be available. Patients were considered ineligible if they had history of prior malignancy or had history of previous chemotherapy, age≥ 70 years old and Patients who is pregnant or breastfeeding.

2.2 Pathological assessment

Archival paraffin blocks were recruited from the Pathology Department, NCI and checked for adequacy of material. Each paraffin block was identified by examination of hematoxylin and eosin (H&E), stained slides and from each tumor paraffin block. For all cases 5 sections (5 micrometer each) were cut into a sterile, plastic, The quantification of intratumoral lymphocytes was performed by enumerating their numbers inside three high power fields, each observed at a magnification of x400. The enumeration was conducted within regions characterised by the highest degree of lymphocytic infiltration. Foci exhibiting haemorrhage and/or necrosis have been excluded. The expression levels of intratumoral CD4 and CD3+cells were categorised as high or low, depending on whether they above or below the median value.

2.3 Study Endpoints

Objectives

2.3.1 Primary objectives

-Assessment of incidence of tumor infiltrating lymphocytes in advanced non-small
cell lung cancer.
- Assessment of correlation between tumor infiltrating lymphocytes and overall
response rate in advanced non-small cell lung cancer under treatment.

2.3.2 Secondary objectives

Assessment of correlation between tumor infiltrating lymphocytes and:
1-Time to progression
2- Overall survival

The primary endpoint was to assess incidence of TILs in advanced NSCLC and correlation between TILs and overall response rate in advanced NSCLC under treatment. The study evaluated the efficacy of TTP, using the RECIST v1.1 criteria to define radiologic progression. Additional metrics for evaluating effectiveness include overall response rate (ORR), which encompasses (total response and partial response), as well as clinical benefit rate (CBR), which encompasses (complete response, partial response, and stable disease).

2.4 Statistical analysis

The study used SPSS V. 24.0 (SPSS, Inc., Chicago, III., USA) to analyze continuous variables (mean values ± standard deviation (SD) or median and range) by Student's Mann Whiteny test and categorical data (rates and proportion), which were analyzed differences using chi square tests and Fisher's exact tests. The Kaplan and Meier procedure was used to estimate overall survival rates, and the Logrank test was used to compare prognostic factors. For time to progression (TTP) were calculated from date of first visit to date of progression. Statistically significant was considered of P value of≤0.05.

3. Results and Discussion

From January 2016 to December 2018, a total of 50 patients with advanced NSCLC were recruited onto this prospective study with median follow up period of 2 years.

3.1 Patient characteristics

The median age of patients was 56 years old (range 39–70 years) and 76% (n = 38) were men. Thirty-eight (76%) patients were current and ex-smokers, while the remaining 12 % were non-smokers. (PS) of 39 patients was I, whereas 11patients had a PS of II. The median age of patients was 56 years old (range 39–70 years) and 76% (n = 38) were men. Thirty-eight (76%) patients were current and ex-smokers, while the remaining 12 % were non-smokers. (PS) of 39 patients was I, whereas 11patients had a PS of II. Forty-eight patients (96 %) were stage IV disease while (4%) 2 patients had stage IIIC. Most of patients had adenocarcinoma (64%, 32 cases) while 14% of patients harboring squmaous cell carcinoma, and 22% with large cell carcinoma. 30 patients (60%) had a well or moderately differentiated histological grade and 20 (40%) had undifferentiated grade. The patient may have multiple metastases, with positive lymph nodes (N+) in 76% of cases, primarily hilar LNs (58%), mediastinal LNs (38%), and supraclavicular LNs (36%). 36% of patients had pulmonary nodules, 54% had pleural effusion, 12% had brain metastasis, 26% had bone metastasis, 14% had liver metastasis, 10% had adrenal metastasis. The disease characteristics of study population are in Table 1.

3.2 Treatment outcomes

All recruited patients received Gemcitabine and platinum agent, evaluation was done every 3 cycles for maximum 6 cycles then follow up every 3 months. Out of two cases of stage IIIC patients; one patient was candidate for sequential chemo – radiotherapy, the other one received palliative radiotherapy over lung as was presented with Supraclavicular lymph node. Among 50 patients, (50%) twenty-five patients had progressive disease; while 25

patients (50%) had clinical benefit. 10 patients (20%) achieved partial response, while the remaining 15patients (30%) had stable disease. 43 patients (86%) had non-haematological toxicity and haematological toxicity, 38% had presented with Anemia, most of them was grade 1 and only 1 patient hade grade 3. The 2ry endpoint of this study was TTP with median number of completed chemotherapy cycles was 6 with a range (2-6) cycles. The median follow up was 2 years 11.4%.The median TTP in the whole study was 7months CI (4.8-9.2).

3.3 Tumor infiltrating lymphocytes analysis (TILs)

The various lymphocytes in (NSCLC) tissue were characterised by employing particular antibodies to distinguish the subsets of (TILs). The tumour tissue exhibited infiltration of immune cells in a disseminated pattern, appearing as individual cells scattered throughout, as evidenced by histological staining with (H&E). The stained cells displayed in terms of cell density, as well as the presence of CD3 and CD4 cells, with significant differences observed among the samples. In this study, we focused solely on evaluating lymphocyte infiltrates within the tumour nest and the statistical analysis of the average numbers of (TILs). The immunostaining of CD8 revealed the presence of cytomembrane staining in a specific subgroup of (TILs) located surrounding the tumour nests. The study found that the median and mean number of CD4 cells was 22 and 29.6 cells /HPF respectively, and the range was 3– 87 cells/ HPF. The median and mean number of CD3 cells was 50 and 48.5 cells /HPF respectively, and the range was 4– 94 cells/ HPF.

3.4 TIL characteristics and correlation with clinicopathological features

In terms of prognostic impact of TILS in tumor tissue, according to previous reports, the median value is used as the threshold for categorizing TILs sub-groups due to the lack of universally recognized standardized cut-points for clinical outcomes. Hence, to compare low infiltration rates with high ones intra- tumoral tissues and its association with tumor biologic markers. As shown in table (2), twenty-six patients (52%) had low level of CD4+ lymphocytic infiltration, whereas 24 (48%) had high CD4+ infiltration. 24 patients (49%) revealed low CD3+ lymphocytic infiltration rates, whereas (51%) 25 patients had high CD3+ infiltration. Association of high CD3 TIL was found in males (88.5%) more than females with p. value 0.0032, as well as high CD3 TIL count was associated with smokers patients than non-smokers with p. value 0.005. There was correlation in Low CD3 infiltration with pathological subtypes mainly adenocarcioma with (p. 0.081), while no correlation with CD4 cells as in table (2).

3.5 The predictive value of (TIL) subgroups in relation to the platinum chemotherapy efficacy

The study aimed to explore the relationship between tumor density (TILs) and platinum-based chemotherapeutic drugs responses in patients with advanced NSCLC using cut off value by median. The high median point of CD4 among responders to chemotherapy was significantly higher in comparison to non-responders p=**0.048.** The median cut off point of CD3 TILS density did not show a significant difference in response to chemotherapy as shown in table (3).

3.6 Association of TIL subtypes with TTP

There was no correlation between Time to progression and the density level of CD4 and CD3 tumor cell infiltration as demonstrated in (table 4). In this prospective study, assessment of the prognostic and predictive significance of TILs in advanced NSCLC in correlation to disease characteristics and chemotherapy were studied. Although this study is prospective, it is important to highlight many limitations that may be present in our study. currently, there is a lack of a standardised cut-off point for assessing levels of TILs. Additionally, the distinct and various locations of subsets of TILs are not consistently accomplished in an established way, there were a different TILs in form of CD8 and FOXP3 cells as well as PDL-1 didn't be assessed. In (NSCLC), the factors considered for prognosis and treatment in standard clinical practise have been staging based on (TNM) system, molecular characterization and histological subtypes (8). These prognostic indicators have demonstrated how valuable they are, but clinical outcome differs although in the same prognostic factors. For which, we need to add more reliable prognostic factors for prediction of treatment outcome.The T-cell-mediated immune response plays a crucial role in the host immune surveillance mechanism for halting tumour progression. In previous literature, the prognosis for a wide range of malignant neoplasms has shown association with the presence of TILs (9).

Higher levels of TILs are indicative of enhanced immune response towards tumor cells, hence signifying the presence of T-cell inflammation in tumour microenvironment and may exhibit increased susceptibility to checkpoint blocking. Consequently, in addition to its prognostic significance, the density of TILs have been investigated as a potential biomarker for predicting the efficacy of immunotherapy (10). In our study, Among 50 patients, after receiving platinum – treatment, the median no, of completed cycles of chemotherapy was 6 cycles with a range (2-6) cycles. The median follow up was 2 years. Twenty-five patients (50%) had progressive disease; while 25 patients (50%) had clinical benefit (20% of cases achieved partial response, while 30% had stable disease). the 2ry endpoint of this study was TTP, The median TTP was 7months CI (4.8-9.2). As consistent with other studies, despite the availability of advanced therapeutic options, the prognosis of advanced NSCLC remains dismal, with a 5-year survival rate of less than 15% (11). With ERA of personalized treatment by targeted agents or immunotherapy, the 5-year survival rates have demonstrated a notable improvement, ranging from 15% to 50%, based on biomarkers (12). In contrary, Gataa ., et al 13 demonstrated that the m PFS was 5.7 months. Most studies conducted on NSCLC and (TILs) were carried out using IHC staining techniques, specifically targeting T cells. Morphological assessment of TILs was performed on hematoxylin and eosin (H&E) slides. However, no standardised approach has yet been established (14). In terms of prognostic and predictive impact of TILS in tumor tissue, the median was employed as cut off value for CD4 and CD3 cells, Hence, to compare high infiltration rates with low ones intra- tumoral and its association with tumor biologic markers as in

agreement with Liu et al., 2012 15 but in contrary to Gataa et al., 2021 13, the criterion for identifying a high TIL count (high-TIL) was set as above 10% using the mean value. (Treg cells) are often viewed as exerting negative regulation on the immune system. However, their role in the prognosis of NSCLC remains uncertain (16). The role of Treg cells in the pathogenesis of lung cancer has been postulated, and their correlation with a poorer prognosis has been established across all histologic subtypes (17). In our study, Low CD3 infiltration was associated with adenocarcinoma with p. value 0.081. But, no correlation was found of CD4 cells with histological subtypes. This is in contrary with Li et al., 2021 18 which showed no correlation with density of CD3 T-cells.

Table 1**:** Baseline characteristics

Charecteristics	value
Age	
≤56	25(50.0)
>56	25(50.0)
Gender	
Male	38(76.0)
Female	12(24.0)
Smoking	
YES	38(76.0)
NO	12(24.0)
PS	
I	39(78.0)
II	11(22.0)
PATHOLOGICAL CHARACTERISTICS	
Pathology	
Adeno	32(64.0)
Squamous	7(14.0)
Large cell	11(22.0)
Grade	
I	2(4.0)
II	28(56.0)
III	20(40.0)
Stage	
3	2(4.0)
4	48(96.0)
T mass	
0	9(18.0)
1	41(82.0)
LNS	
NO	12(24.0)
YES	38(76.0)
TNM CHARACTERESTICS	value%
T.Size	
≤ 6cm	20(48.8)
>6cm	21(51.2)
LNs*	
Hilar	29(58.0)
Mediastinal	19(38.0)
Supraclavicular	10(20.0)
Chest wall mass	1(2.0)
Site of metastases	
Pleura Effusion	27(54.0)
Liver	7(14.0)
Bone	13(26.0)
Brain	6(12.0)
Adrenal	5(10.0)
Pleural _Pericardial nodules	7(14.0)
Lung Nodules	32(36.0)

TNM characteristics (patient may present by more than one site)

Table 2: Correlation beween TILs and clinicopathological features

characteristics	CD3		P.	CD4		P.
	Low	high		Low	high	
Age						
<56	13(54.2)	12(46.2)	0.571	13(50.0)	12(50.0)	1.000
>56	11(45.8)	14(53.8)		13(50.0)	12(50.0)	
Gender						
Male	15(62.5)	23(88.5)	**0.032**	19(73.1)	19(79.2)	0.614
Female	9(37.5)	3(11.5)		7(26.9)	5(20.8)	
Smoking						
Yes	14(58.3)	24(92.3)	**0.005**	20(76.9)	18(75.0)	0.874
No	10(41.7)	2(7.7)		6(23.1)	6(25.0)	
PS						
1	17(70.8)	22(84.6)	0.240	19(73.1)	20(83.3)	0.382
2	7(29.2)	4(15.4)		7(26.9)	4(16.7)	
Histology						
Adeno	18(75.0)	14(53.8)		16(61.5)	16(66.7)	
Squamous	4(16.7)	3(11.5)		4(15.4)	3(12.5)	0.926
Large cell/undiff.	2(8.3)	9(34.6)	**0.081**	6(23.1)	3(12.5)	
Tumor status						
≤6 cm	8(42.1)	12(54.5)	0.427	12(57.1)	8(40.0)	0.272
>6 cm	11(57.9)	10(45.5)		9(42.9)	12(60)	
Grading						
1-2	16(66.7)	14(53.8)	0.325	16(61.5)	14(58.3)	0.817
3	8(33.3)	12(46.2)		10(38.5)	10(41.7)	
Nodal status						
NO	5(20.8)	7(26.9)	0.614	5(19.2)	7(29.2)	0.411
YES	19(79.2)	19(73.1)		21(80.8)	17(70.8)	
Hilar						
NO	9(37.5)	12(46.2)	0.536	10(38.5)	11(45.8)	0.598
YES	15(62.5)	14(53.8)		16(61.5)	13(54.2)	
Mediastinal			0.944			
NO	15(62.5)	16(61.5)		16(61.5)	15(62.5)	0.944
YES	9(37.5)	10(38.5)		10(38.5)	9(37.5)	

Table 3: Response rates in relation to TIL

	Median Cut off point	No Response (PD) n=10(%)	Response (PR-SD) n=40(%)	p value
TILS_CD3	<50	7(29.2)	17(70.8)	0.119
	≥50	3(11.5)	23(88.5)	
TILS_CD4	≤22	8(30.8)	18(69.2)	0.048
	>22	2(8.3)	22(91.7)	

Table 4: Association of TIL subtypes with TTP

	Median OS (95%CI)	P value	Median TTP (95%CI)	P value
TILS_CD3				
<50	11.0(3.9-18.1)	0.888	7.9(4.4-11.6)	0.770
≥50	13.0(8.2-17.9)		7.0(3.8-10.2)	
TILS_CD4				
≤22	13.0(10.0-16)	0.826	5.0(2.5-7.5)	0.235
>22	14.0(10.5-16.4)		7.0(5.2-8.8)	

Schulze et al.,2020 19 reported a correlation of high infiltration of CD4+ cells in (ADC) and large cell carcinoma (LCC) with a significant improvement in (OS) . However, this was not in (SCC). In contrary, Zeng et al., 2016 20, showed that there was no association was found in TILs count with pathological types and grading. In our study, Association of high CD3 TIL was found in males (88.5%) more than females with p. value 0.0032. in contrary to data by Liu et al., 2012 15, no correlation of TILs subtypes of clinic-pathological features. Our study is aimed to explore the correlation between the abundance of (TILs) and the effectiveness of platinum-based chemotherapy in treating advanced (NSCLC) patients using cut off value by median. And we demonstrated that there is a correlation between high CD4 TIL density and response to chemotherapy of p. Value 0.048 but, no correlation between CD3 TILS and response to chemotherapy were found. The therapeutic efficacy of cancer depends on the strength of the immune response against tumours. (TILs), including CD3, CD4, CD8, and FOXP3 T cells, have been demonstrated to reflect the immune response and correlate with patient survival across various types of tumours. Immune-mediated chemotherapy response and better survival rates in malignancies like breast cancer and melanoma is strongly correlated with lymphocyte infiltration (21). The survival of cancer patients is predicted to be influenced by the density, type, and locations of immune cells within tumor specimens (22). Chen et al., 2020 23, demonstrated that TILs may both promote or suppress tumor progression. For which, there are difference in survival benefit of TILs as a single entity.

4. Conclusion

TILs play a pivotal part in immunoediting, especially in the elimination phase. Diagnostic biomarkers are widely used in clinical practice, but research is expanding to identify new biomarkers for clinical trials, leading to an increase in predictive biomarker and therapeutic options. Our study has provided evidence supporting the employing of (TILs) including CD4 cells as an indicator for predicting response in patients with (NSCLC). Additionally, more analysis is warranted to explore the correlations between (TILs) and other clinical and pathological features, such as the expression of PD-L1, FOXP3 and CD8 cells .

Disclosure

No conflict of interest

Reference

[1] K.C. Thandra, A. Barsouk, K. Saginala, J.S. Aluru & A. Barsouk. (2021). Epidemiology of lung cancer. Contemporary Oncology/Współczesna Onkologia. 25(1), 45-52.

[2] R.L. Siegel, K.D. Miller, H.E. Fuchs & A. Jemal. (2021). Cancer statistics. CA: Cancer Journal for Clinicians. 71(1), 7-33.

[3] A.D. Waldman, J.M. Fritz & M.J. Lenardo. (2020). A guide to cancer immunotherapy: from T cell basic science to clinical practice. Nature Reviews Immunology. 20(11), 651-668.

[4] Li, F. Li, C. Cai, X. Xie, Z. Zhou, L. Cheng, B. Zhong, R. Xiong, S. Li, J. Chen & Zhuxing. (2021). The association between CD8+ tumor-infiltrating lymphocytes and the clinical outcome of cancer immunotherapy: A systematic review and meta-analysis. E Clinical Medicine. 41.

[5] G. Corredor, X. Wang, Y. Zhou, C. Lu, P. Fu, K. Syrigos & A. Madabhushi. (2019). Spatial architecture and arrangement of tumor-infiltrating lymphocytes for predicting likelihood of recurrence in early-stage non–small cell lung cancer. Clinical cancer research. 25(5), 1526-1534.

[6] R.V. Uzhachenko & A. Shanker. (2019). CD8+ T lymphocyte and NK cell network: circuitry in the cytotoxic domain of immunity. Frontiers in immunology. 10, 1906.

[7] S. Dasari & P.B. Tchounwou. (2014). Cisplatin in cancer therapy: molecular mechanisms of action. European journal of pharmacology. 740, 364-378.

[8] F.C. Detterbeck, P.E. Postmus & L.T. Tanoue. (2013). The stage classification of lung cancer: Diagnosis and management of lung cancer: American College of Chest Physicians evidence-based clinical practice guidelines. Chest. 143(5), e191S-e210S.

[9] H.E. Lee, S.W. Chae, Y.J. Lee, M.A. Kim, H.S. Lee, B.L. Lee & W.H. Kim. (2008). Prognostic implications of type and density of tumour-infiltrating lymphocytes in gastric cancer. British journal of cancer. 99(10), 1704-1711.

[10] J.N. Bodor, Y. Boumber & H. Borghaei. (2020). Biomarkers for immune checkpoint inhibition in non–small cell lung cancer (NSCLC). Cancer. 126(2), 260-270.

[11] R.A. de Mello, D.S. Marques, R. Medeiros & A.M. Araújo. (2011). Epidermal growth factor receptor and K-Ras in non-small cell lung cancer-molecular pathways involved and targeted therapies. World Journal of Clinical Oncology. 2(11), 367.

[12] Howlader, N. Forjaz, G. Mooradian, M.J Meza, R. Kong, C.Y. Cronin, K. A. Mariotto, A. B Lowy, D. R. Feuer & J. Eric. (2020). The effect of advances in lung-cancer treatment on population mortality. New England Journal of Medicine. 383(7), 640-649.

[13] Gataa, I. Mezquita, L. Rossoni, C. Auclin, E. Kossai, M. Aboubakar, F.L. Moluec, S. Masse, J. Masson, M. Radosevic-Robin & Nina. (2021). Tumour-infiltrating lymphocyte density is associated with favourable outcome in patients with advanced non–small cell lung cancer treated with immunotherapy. European Journal of Cancer. 145, 221-229.

[14] Salgado, R. Denkert, C. Demaria, S. Sirtaine, N. Klauschen, F. Pruneri, G. Wienert, S.V. den Eynden, G. Baehner, F.L. Pénault-Llorca, & Frederique. (2015). The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Annals of oncology. 26(2), 259-271.

[15] H. Liu, T. Zhang & J. Ye. (2012). Tumor-infiltrating lymphocytes predict response to chemotherapy in patients with advance non-small

cell lung cancer. Cancer Immunology Immunotherapy.;61(10):1849-56.
doi: 10.1007/s00262-012-1231-7.

[16] Ruffini, E. Asioli, S. Filosso, P. L. Lyberis, P. Bruna, M. C. Macrì, L.Daniele, L. Oliaro & Alberto. (2009). Clinical significance of tumor-infiltrating lymphocytes in lung neoplasms. The Annals of thoracic surgery. 87(2), 365-372.

[17] R.J.. Deleeuw, S.E. Kost, J.A. Kakal & B.H. Nelson. (2012). The prognostic value of FoxP3+ tumor-infiltrating lymphocytes in cancer: a critical review of the literature. Clinical cancer research. 18(11), 3022-3029.

[18] Li, R. Lin, Y. Wang, Y. Wang, S. Yang, Y. Mu, X. Chen, Y. Gao & Zhancheng. (2021). Characterization of the tumor immune microenvironment in lung squamous cell carcinoma using imaging mass cytometry. Frontiers in oncology. 11, 620989.

[19] Schulze, A. B. Evers, G. Görlich, D. Mohr, M. Marra, A. Hillejan, L. Rehmkamper, J. Schmidt, L. H. Heitkötter & Birthe. (2020). Tumor infiltrating T cells influence prognosis in stage I–III non-small cell lung cancer. Journal of thoracic disease. 12(5), 1824.

[20] Zeng, D. Q. Yu, Y.F. Ou, Q.Y. Li, X.Y. Zhong, R. Zhi Xie, C.M. Hu & Q. Gen. (2016). Prognostic and predictive value of tumor-infiltrating lymphocytes for clinical therapeutic research in patients with non-small cell lung cancer. Oncotarget. 7(12), 13765.

[21] Iwamoto, T. Bianchini, G. Booser, D. Qi, Y. Coutant, C. Ya-Hui Shiang, C. Santarpia, L. Matsuoka, J. Hortobagyi, G. N. Symmans & W.Fraser. (2011). Gene pathways associated with prognosis and chemotherapy sensitivity in molecular subtypes of breast cancer. Journal of the National Cancer Institute. 103(3), 264-272.

[22] Chen, B. Li, H. Liu, C. Xiang, X. Wang, S. Wu, A. Shen, Y. Li & Gaofeng. (2020). Prognostic value of the common tumour-infiltrating lymphocyte subtypes for patients with non-small cell lung cancer: A meta-analysis. PLoS One. 15(11), e0242173.