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PD-L1 Expression and Tumor-infiltrating Lymphocytes in Breast Cancer: Clinicopathological Analysis in Women Younger than 40 Years Old

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Abstract. *Background/Aim: To evaluate the association between programmed cell death ligand 1 (PD-L1) expression on both tumor cells (TC) and inflammatory cells (IC), tumor infiltrating lymphocytes (TILs), $CD3^+$ and $CD8^+$ lymphocytes and other clinicopathological parameters in primary infiltrative breast cancer (IBC) of young women, a population shown to have a worse prognosis. Materials and Methods: A retrospective study was performed collecting data from patients younger than 40 years old. Forty-five young women with IBC were included. Whole tissue sections were used to evaluate all parameters. Results: Twenty percent (20%) of cases showed PD-L1 expression by tumor cells (PDL1TC) and 44.4% showed PD-L1 expression by immune cells (PDL1IC). Furthermore, 28.88% revealed high stromal TILs. PDL1TC and PDL1IC expression were significantly associated with tumor diameter and expression of estrogen (ER) and progesterone (PR) receptors and Ki67. PDL1TC expression was also associated with grade. High TILs were associated with tumor diameter, ER and Ki67 expression. PDL1TC, PDL1IC expression and TILs were associated with the density of $CD3^+$ and $CD8^+$ lymphocytes. Conclusion: Our results are similar to those of other age groups, as reported in the literature.*

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Breast cancer is the most common malignancy diagnosed among women of all ages and the second malignancy to cause death, after lung cancer (1). Younger women with breast cancer have a higher risk of recurrence and death, compared to older women (2-6). Additionally, this patient group shares considerations that must be taken into account when treated, like fertility preservation, pregnancy, sexual life and external appearance (7-11).

Over the past decade great improvement in diagnosis, prognosis and treatment has been achieved, complementing the already established targeted-therapy against estrogen (ER) and progesterone receptors (PR), as well as human epidermal growth factor receptor 2 (HER2). The molecular subtyping of breast cancer, compared to the morphological features, have opened new horizons to the understanding of the complex pathogenetic pathways leading to this common neoplasm (12). From the available studies it appears that for primary infiltrative breast cancer (IBC) there are not considerable differences regarding the biological profile in various ages, excluding the genetically predisposed cases (13).

The development and progression of IBC is due to a complex system of multiple factors including those of the microenvironment such as stromal and immune cells (14). The latter knowledge in combination with perceiving the cancer – immunity cycle**,** has led numerous studies to focus on the tumor microenvironment of the host in several solid cancers, including IBC (15, 16). Over the decades, it has been proven that the presence of high tumor infiltrating lymphocytes (TILs) in solid tumors, such as lung, ovarian cancer, colorectal cancer, renal cell carcinoma, prostate cancer and head and neck cancers, as well as in breast cancer, is associated with better prognosis (17-25).

Most recently, researchers have been focusing on programmed cell death 1 and programmed cell death ligand 1 (PD1/PD-L1) axis, one of the most common mechanisms of tumor cell escape in cancer (26). The programmed cell PD-1 is a 55-kDa transmembrane protein located on the membrane surface of $CD4^+$ T cells, NK T cells, B lymphocytes and dendritic cells. The most studied ligand of PD-1 is programmed cell death ligand 1 (PD-L1) or B7H1 or CD274 as otherwise known (27). PD-L1 is located on the membrane of various cell types such as hematopoietic cells *e.g.* B and T lymphocytes, dendritic cells, macrophages and mast cells, but also on non-hematopoietic cells such as endothelial, epithelial and muscle cells (28). Its levels in normal tissues are extremely low, whereas it is found overexpressed on neoplastic cells (29). PD-1 is commonly expressed on T regulatory cells found in TILs of several solid cancers. Its interaction with PD-L1 located on the surface of neoplastic cells leads to decreased cytokine expression, suppression of further activation of T lymphocytes gets and elimination of immune response (30, 31).

The aim of the present study was to examine the expression of PD-L1 on both tumor cells (TC) and immune cells (IC), quantify TILs, $CD3^+$ and $CD8^+$ in IBC and investigate the association between these parameters, well as with other clinicopathologic parameters in the population of young women ≤40-years-old.

Materials and Methods

Study group. We retrospectively searched the electronic data-base of the Pathology Department of the University Hospital of Ioannina, Ioannina, Greece. All patients were young women (≤40 years old) with IBC treated surgically at the same Institution between 2011 and 2016. Forty-five cases of breast cancer were found, all of which were of the nonspecific type (NST); several parameters were studied including tumor size, histological grade, estrogen receptor (ER), progesterone receptor (PR), HER2 and Ki67 expression. Due to the limited number of cases of triple-negative cancer (TNC), further analysis of the IBC into molecular subtypes was not performed. Similarly, grade 1 IBC were excluded from the statistical analysis due to their limited number.

Quantification of TILs. Histopathological analysis of the lymphocytic infiltrate was performed according to the guidelines for clinical and research practice (32). Briefly, using the hematoxylin/eosin stained tissue sections, the percentage of stromal mononuclear cells, lymphocytes and plasma cells (polymorphonuclear leukocytes were excluded) were quantified within the tumor border. The evaluation did not include hot spots, TILs outside the tumor border, TILs in tumor zones with crush artifacts, necrosis, regressive hyalinization or areas of previous biopsy site. The quantification was as detailed as possible, dividing TILs in three groups (≤10%, 11-60%, and >60%). Ultimately, they were divided into two groups, low TILs (<60%) and high TILs (>60%). Further analysis was performed, identifying the composition of the TILs, using CD3 and CD8 antibodies. Each antibody was counted in five randomly selected high power fields at 400x magnification, and the counts were averaged (33). Positive CD3 or CD8 TILs up to 25 cells were considered as low (+1-25 cells), whereas medium density was the count of 26 up to 50 cells (++26-50 cells) and high the count of 51 cells or more (+++≥51 cells). As with TILs quantification, CD3 and CD8 were divided in two groups, low (1-25 cells) and high (>26 cells) for statistical purposes.

Table I. *Clinicopathological characteristics of young women with breast cancer.*

Patient characteristics	n (=45)	%
Tumor diameter		
≤2 cm	22	48.89
>2-5 cm	19	42.22
>5 cm	4	8.89
Tumor grade		
1	2	4.45
2	15	33.33
3	28	62.22
In situ coexistence		
Yes	38	84.44
No	7	15.56
No of tumors		
Single	39	86.67
Multiple	6	13.33
Lymphovascular invasion		
Yes	24	53.33
No	21	46.67
Perineural invasion		
Yes	6	13.33
No	39	86.67
Lymph node metastasis		
Yes	21	46.67
No	24	53.33
Estrogen receptors expression		
Positive	32	71.11
Negative	13	28.89
Progesterone receptors expression		
Positive	38	84.44
Negative	7	15.56
MIB1/ki67 expression		
Low (<20)	20	44.44
High (≥20)	25	55.56
HER2 expression		
Positive	12	26.67
Negative	33	73.33
TILs		
Low	32	71.11
High	13	28.89
Stromal CD3 count		
Low (1)	25	55.56
High (2-3)	20	44.44
Stromal CD8 count		
Low (1)	25	55.56
High (2-3)	20	44.44
PDL1TC expression		
Positive	9	20
Negative	36	80
PDL1IC expression		
Positive	20	44.44
Negative	25	55.56

Table II. *TILs and PDL1 correlation with clinicopathological parameters.*

	TILs			PDL1TC			PDL1IC		
	High n (%)	Low n (%)	*p*-Value	Positive n (%)	Negative n (%)	*p*-Value	Positive n (%)	Negative n (%)	*p*-Value
Tumor diameter									
≤2 cm	3 (13.64%)	19 (86.36%)	0.016	1 (4.55%)	21 (95.45%)	0.023	7 (31.82%)	15 (68.18%)	0.185
>2-5 cm	10 (52.63%)	9 (47.36%)		7 (36.84%)	12 (63.16%)		10 (52.63)	9 (47.37)	
>5 cm	0	4 (100%)		1 (25%)	3 (75%)		3 (75%)	1 (25%)	
Tumor grade (n=43)*									
2	2 (13.33%)	13 (86.67%)	0.096	0	15 (100%)	0.017	4 (22.22%)	14 (77.78%)	0.199
3	11 (39.28%)	17 (60.71%)		9 (32.14%)	19 (67.86%)		14 (50%)	14 (50%)	
In situ coexistence									
Yes	10 (26.31%)	28 (73.68%)	0.394	7 (18.42%)	31 (81.57%)	0.614	16 (42.11%)	22 (57.89%)	0.682
No	3 (42.86%)	4 (57.14)		2 (28.57%)	5 (71.42%)		4 (57.14%)	3 (42.86%)	
No of tumors									
Single	1 (2.22%)	27 (96.42%)	0.656	8 (20.51%)	31 (79.48%)	1.000	17 (43.59%)	22 (56.41%)	1.000
Multiple	12 (70.58%)	5 (29.41%)		1 (16.66%)	5 (83.33%)		3 (50%)	3 (50%)	
Lymphovascular invasion									
Yes	8 (33.33%)	16 (66.66%)	0.528	7 (29.16%)	17 (70.83%)	0.143	12 (50%)	12 (50%)	0.423
No	5 (23.80%)	16 (76.19%)		2 (9.51%)	19 (90.47%)		8 (38.10%)	13 (61.90%)	
Perineural invasion									
Yes	0	6 (100%)	0.160	2 (33.33%)	4 (66.66%)	0.583	3 (50%)	3 (50%)	1.000
No	13 (33.33%)	26 (66.66%)		7 (17.94%)	32 (82.05%)		17 (43.59%)	22 (56.41%)	
Lymph node metastasis									
Yes	5 (23.80%)	16 (76.19%)	0.528	4 (19.04%)	17 (80.95%)	1.000	10 (47.62%)	11 (52.38%)	0.688
No	8 (33.33%)	16 (66.66%)		5 (20.83%)	19 (79.16%)		10 (41.67%)	14 (58.33%)	
ER expression									
Positive	4 (12.5%)	28 (87.5%)	0.000	2 (6.25%)	30 (93.75%)	0.001	9 (28.13%)	23 (71.87%)	0.001
Negative	9 (69.23%)	4 (30.76%)		7 (53.84%)	6 (46.15%)		11 (84.62%)	2 (15.38%)	
PR expression									
Positive	9 (23.28%)	29 (76.32%)	0.168	4 (10.52%)	34 (89.47%)	0.002	13 (34.21%)	25 (65.79%)	0.001
Negative	4 (57.14%)	3 (42.86%)		5 (71.42%)	2 (28.57%)		7 (100%)	0	
MIB1/ki67 expression									
Low (<20)	2 (10%)	18 (90%)	0.012	1 (5%)	19 (95%)	0.030	6 (30%)	14 (70%)	0.081
High(≥20)	11 (44%)	14 (56%)		8 (32%)	17 (68%)		14 (56%)	11 (44%)	
HER2 expression									
Positive	6 (50%)	6 (50%)	0.076	2 (16.66%)	10 (8.33%)	1.000	7 (58.33%)	5 (41.67%)	0.258
Negative	7 (21.21%)	26 (78.78%)		7 (21.21%)	26 (78.78%)		13 (39.39%)	20 (60.61%)	
Stromal CD3 count									
Low	0	25 (100%)	0.000	2 (8%)	23 (92%)	0.024	7 (28%)	18 (72%)	0.013
High	13 (65%)	7 (35%)		7 (35%)	13 (65%)		13 (65%)	7 (35%)	
Stromal CD8 count									
Low	0	25 (100%)	0.000	2 (8%)	23 (92%)	0.024	7 (28%)	18 (72%)	0.013
High	13 (65%)	7 (35%)		7 (35%)	13 (65%)		13 (65%)	7 (35%)	

*Grade 1 tumors were excluded from the statistical analysis due to their limited number.

PD-L1 immunohistochemistry. PD-L1 immunohistochemistry assessment was performed on whole-tissue sections using the rabbit monoclonal antibody, clone E1L3N (Cell Signaling Technologies, Danvers, MA, USA). Both tumor cells (TC) and immune cells (IC) were evaluated. Human placenta tissue was used as positive control in parallel with the sections. TC staining was defined as partial or complete membranous staining, while IC staining was defined as cytoplasmic or membranous staining in lymphocytes or macrophages, using the number of IC within the stroma of the tumor. Positivity was defined as ≥1% in both TC and IC (34).

Statistical analysis. Statistical analysis was performed using V.22.0 Statistical Package for the Social Sciences (SPSS). The Fisher's exact test was used to examine associations between categorical variables and the Mann Whitney *U*-test to examine differences in groups of quantitative measurements. The significance level was set at <0.05 in all cases.

Results

The mean age of the patients was 34.71 years. The studied parameters included tumor size, histologic grade, estrogen

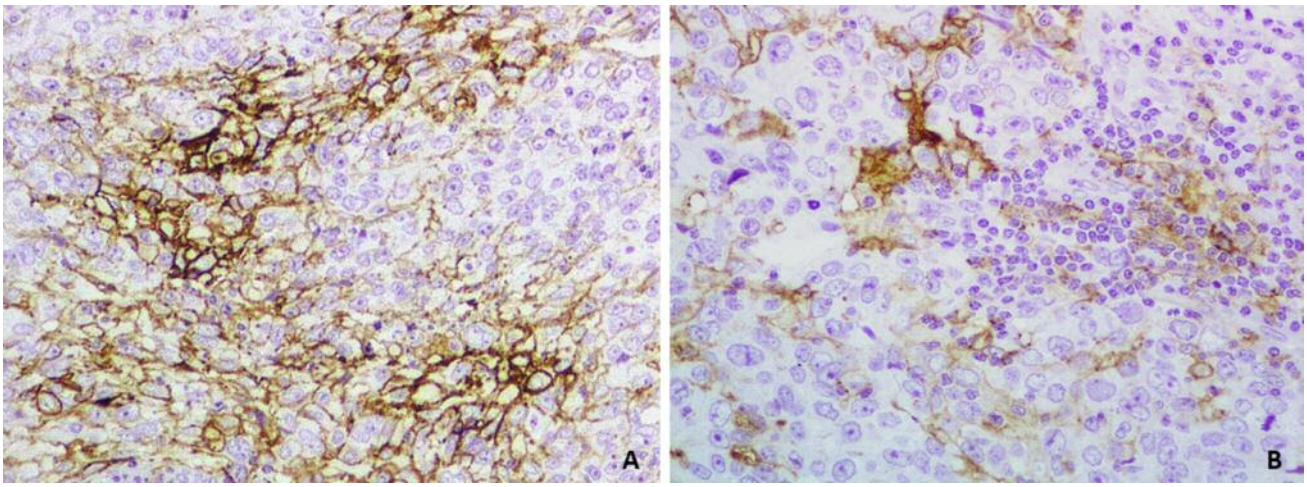

Figure 1. *(A) PD-L1 expression in tumor cells (×20). (B) PD-L1 expression in TILs and tumor cells (×40).*

receptor (ER), progesterone receptor (PR), HER2 and Ki67 expression and are shown in Table I.

Association of PDL1TC expression with clinicopathologic characteristics. Of all forty-five cases of breast cancer collected for this study, 20% were found to have PDL1TC expression. Correlation of PDL1TC with clinicopathologic parameters showed significant association with tumor diameter, breast cancer grade, ER, PR and Ki67 expression (Table II). Specifically, PDL1TC expression revealed a significant association with carcinomas measuring more than 2 cm (36.84%, p=0.023), grade 3 IBC (32.14%, p=0.017%), ER-negative status (53.84%, p=0.001), PR-negative status (71.42%, p=0,002) and high Ki76(32%, p=0.030) expression. PD-L1 expression assessed with continuous variables like the patients' age, tumor diameter as well as Ki67 showed significant association with the latter two (p=0.028, p=0.000) (Table III). No significant association was shown between PD-L1 expression and other clinicopathologic studied parameters.

Association of PDL1IC expression with clinicopathological characteristics. Inflammatory expression of PD-L1 was observed in 44.44% (20/45) of IBC. PD-L1 positivity in TILs showed significant association with ER, PR expression, large tumor diameter and Ki67 expression. More detailed, PDL1IC expression is most likely to be found in ER- and PR-negative cases (84.62%, p=0.001, 100%, p=0.001, irrespectively) (Table II). Furthermore, PDL1IC expression is most commonly found in large tumor diameters and high Ki67 (p=0.019, p=0.04), as was shown estimating the latter two parameters as continuous variables (Table III). No other significant association was shown between PDL1IC expression and other clinicopathological parameters studied.

Association of stromal TILs with clinicopathological characteristics. Of all forty-five cases collected for this study, thirteen (28.88%) revealed high stromal TILs. The presence of high stromal TILs was significantly increased in IBC measuring more than 2 cm (52.63%, p=0.016) and in ER-negative cases (69.23%, p=0,000) and cases with high Ki67 (44%, p=0.012). Furthermore, associating stromal TILs as a continuous variable with the patients' age, tumor diameter and Ki67, revealed that high stromal TILs are more likely to be seen in cases of IBC with a high Ki67 (p=0.000) (Table III). No significant association was found between stromal TILs and other clinicopathologic parameters.

Association of PDL1TC and PDL1IC expression, stromal TILs, CD3 and CD8 lymphocytes. Both PDL1TC and PDL1IC expression in IBC cases revealed a significant association with high stromal TILs (77.78%, p=0.001, 79.92%, p=0.005, respectively) (Table IV). All PDL1TC expression (35%, p=0.024), PDL1IC (65%, p=0.013), and high TILs (100%, p=0.000) showed a significant association with the presence of CD3 lymphocytes. Similarly, PDL1TC expression is significantly associated with the presence of high CD8 infiltrate (35%, p=0.024), as is PDL1IC expression (65%, p=0.013) and high stromal TILs (100%, p=0.000) (Table II).

Discussion

Breast cancer in premenopausal women is being increasingly detected (8, 9). This subgroup of women draws particular attention, because compared to older women, young age itself is a negative prognostic factor, associated with a higher risk of relapse and death (2-6). Moreover, additional concerning

Table III. *Relationship of PD-L1 expression and TILs with continuous variables.*

	Mean	Standard deviation	*p*-Value
PDL1/Age			
Negative	35.22	4.599	0.076
Positive	32.67	3.742	
Total	34.71	4.521	
PDL1/Tumor max diameter			
Negative	2.53	2.126	0.028
Positive	3.34	1.516	
Total	2.69	2.030	
PDL1/Ki67			
Negative	33.95	21.473	0.000
Positive	79.00	11.402	
Total	42.30	26.653	
TILs/Age			
Low	34.81	4.935	0.126
High	34.46	3.455	
Total	34.71	4.521	
TILs/Tumor max diameter			
Low	2.70	2.363	0.527
High	2.67	0.825	
Total	2.69	2.030	
TILs/Ki67			
Low	24.28	19.06	0.000
High	56.77	27.58	
Total	33.67	26.18	
PDL1IC/Age			
Negative	35.12	4.92	0.069
Positive	34.20	4.03	
Total	34.71	4.52	
PDL1IC/Tumor max diameter			
Negative	2.17	1.08	0.019
Positive	3.34	2.69	
Total	2.69	2.03	
PDL1IC/Ki67			
Low	22.76	17.09	0.04
High	47.30	29.42	
Total	33.67	26.18	

issues regarding the quality of life of this population are being encountered such as fertility preservation, pregnancy, sexual life and external appearance (8, 11). It is therefore important, to enrich our knowledge in the tumor microenvironment, a hallmark of cancer in this particular group (14). Although various studies exist examining PD-L1 expression and TILs in breast cancer, this is one of the few studies to focus on this specific age subgroup.

The results of this study indicate that high stromal TILs are only found in a minority of cases, which is consistent with other reports studying TILs in IBC in women of all ages (26, 35). PD-L1 on the contrary, has a very wide spectrum of expression on TC and IC in IBC cases, as is reported in the literature, with a positivity range approximately from 20% to almost 60% and 2% to 90%, respectively (36-41). Of all forty-five cases of IBC included in our study, we observed nine (20%) cases of PDL1TC expression and twenty cases of PDL1IC expression (44.44%), both of which apply in the range. A possible explanation for having only a minority of cases expressing PDL1TC and high stromal TILs, is the fact that high stromal TILs and PD-L1 expression are most commonly encountered in TNC, which are less frequent (37). Thus, a possible explanation for having only a minimum positivity of PDL1TC in this study is the limited number of TNC in our cohort. Furthermore, we have shown that PDL1TC positive cases are more seldom found than PDL1IC positive cases, which is consistent with other studies (42).

Associations between PDL1TC and clinicopathological parameters revealed a positive correlation between this expression and grade 3 IBC, ER and PR negativity, as well as large tumor diameter and higher Ki67 expression. No statistically significant associations were found between PDL1TC and other clinicopathologic parameters. These findings are consistent with most studies found in the literature (43-46). The association between PDL1TC and HER2 status remains controversial, though (41, 47). In our study we did not find any correlation between PDL1TC expression and HER2 positive IBC which is consistent with a meta-analysis performed from Huang *et al*. (41) in which 47 studies were included, conducted between the years 2006-2018. A possible explanation for this heterogeneity between PDL1TC expression and HER2 status in the various studies could be the lack of standardized methodology of measurement of PD-L1 (41).

There are not many studies focusing on the expression of PD-L1 on TILs, but the results regarding the relationship between the latter with various clinicopathologic parameters are consistent (39, 48, 49). Similarly, the results of our study indicated that patients with PDL1IC IBC are most likely to have ER or PR negative carcinomas, large tumor diameter and high Ki67, all of which are established indicators of poor prognosis.

High stromal TILs, PLD1TC and PDL1IC expression were found to be associated with each other, which validates previous reports (35, 37, 39, 50). Furthermore, we indicated that high stromal TILs are more commonly answered in ER negative cases and high Ki67 expression, which shows no deviation from existing reports (51, 52). We already know that high stromal TILs are more commonly answered in HER2+ IBC, but this conclusion could not have been conducted in our study, possibly due to the limited number of HER2+ cases (52).

Further analysis of stromal TILs revealed a statistically significant association between high stromal TILs, PDL1TC and PDL1IC with the presence of high $CD3^+$ and $CD8^+$T

Table IV. *PDL1TC and PDL1IC expression and TILs correlation.*

	PDL1TC			PDL1IC		
	Positive (n=9)	Negative (n=36)	*p*-Value	Positive (n=20)	Negative (n=25)	*p*-Value
TILS						
High (n=13)	7 (77.78%)	6 (16.67%)	0.001	10 (79.92%)	3 (23.07%)	0.005
Low (n=32)	2 (22.22%)	30 (83.33%)		10 (31.25%)	22 (68.75%)	

lymphocytes. TILs are composed by several B and T types of lymphocytes, each of them with a different prognostic value (53).There are various types of T lymphocytes and it is worth mentioning that most $CD8^+$ T lymphocytes are also $CD3^+$ positive (54, 55). Denkert *et al.* (26) indicate that TILs, regardless of their composition, are linked to an improved prognosis, but there are also many studies focusing on the prognostic value of the various types of cells composing TILs. For instance, Seo *et al.* (56) showed that the increased number of $CD8^+$ T cells is linked to a better clinical outcome and $CD3^+$ lymphocytes are associated with a better prognosis in IBC while Teschendorff *et al.* (57) indicated that Th2 cells, a $CD4^+$ subpopulation, are linked to mediating the antitumor response, thus having a negative prognostic value. Mori *et al.* (58) conjectured that the subtypes of TILs should be considered in future studies, because their variations lead to a heterogeneity of conclusions conducted regarding the RFS and OS. Nevertheless, despite having an heterogeneity of opinions between various studies, it is safe to say that our results indicate that young women with high stromal TILs and/or PDL1TC and/or PDL1IC expression are more likely to have a high $CD8^+$ lymphocyte and a high $CD3^+$ infiltrate. Both findings are linked to a better clinical outcome as proven from the studies mentioned above.

Many studies have been conducted in order to evaluate the prognostic value of PDL1TC, PDL1IC and TILs in IBC, and even after many meta-analyses some results remain conflicting (41, 59, 60). Kim *et al.* (60), conducted an meta-analysis which included 7,877 cases and was led to the conclusion that PDL1TC expression is associated with poorer DFS. No association was found between PDL1 expression and OS, to the contrast with the meta-analysis conducted by Huang *et al.* which found that PDL1TC expression is linked to a poorer DFS but also OS. In the present study PDL1TC expression was proven to be linked to several unfavorable prognostic factors, which could indicate a poor prognosis. Although the PD-L1 expression on TILs has not yet been thoroughly examined in the literature, it seems from the existing studies that it is of great prognostic value (61). From these studies PDL1IC expression has shown to have opposite prognostic value compared to PDL1TC expression, since most cases are related to higher DFS, RFS as well as OS (41, 62). Zhao *et al.* (61) studied the prognostic value of PDL1IC expression on several solid tumors, and indicated that it is a positive prognostic factor, especially in IBC. Briefly, a possible explanation for this result, is the fact that PDL1TC expression is mostly driven intercellularly through the tumor-intrinsic mechanism, whereas the PDL1IC expression is driven *via* adaptive mechanisms, thus related to high stromal TILs which are an anti-tumor answer as a result of an activated PD1/PDL1 pathway (63). The diversity of prognosis between these two factors indicates the importance of the evaluation of PD-L1 expression on both TC and IC in carcinomas. High stromal TILs *per se* are linked to an improved patient survival and numerous studies focusing on treating such patients with neoadjuvant systemic therapy have shown that the presence of high stromal TILs is in favor of complete pathological response, thus excellent prognosis. Similarly results were found in patients with high $CD3^+$ infiltrate (37, 39).

Undeniable, the discovery of TILs as well as the PD1/PD-L1 axis has opened new horizons in understanding the response of the host immune system and the way it associates with tumor progression. Thus, established targeted therapy against ER and PR, as well as human epidermal growth factor receptor 2 (HER2), are already getting enriched with the introduction of immunotherapy, showing very promising results (64). Our opinion is that as in other age groups, stromal TILs and PDL1TC and PDL1IC could be used as prognostic factors and immunotherapy will complement the existent targeted therapies.

Our study has certain limitations especially because of its retrospective nature and its small number of cases studied, as well as the absence of prognostic information. However, it is conducted in a particular patient group where scarce data actually exist. Breast cancer in younger women was rarely identified in the past but now shows an increased number of diagnoses and unfavorable prognosis (8, 9). This indicates that although major progress has been achieved in prognosis, diagnosis and treatment in elderly women, this does not yet apply in young women. Several studies have been conducted focusing on whether young breast cancer could have special characteristic or not (13, 65). This is the first study to compare clinicopathologic parameters with PD-L1 expression and stromal TILs focusing on this specific age group.

In conclusion, the results of our study show that the PD-L1, CD3 and CD8 expression status in younger women are

similar to other age groups as reported in the literature. Thus, the latter parameters should be evaluated and reported in order to be used in cases of young women with IBC and complement existing targeted therapies with immunotherapy, aiming for a better outcome.

Conflicts of Interest

All Authors declare that they have no conflicts of interest.

Authors' Contributions

Zoi Evangelou and Anna Batistatou have designed the study. Zoi Evangelou, Alexandra Papoudou-Bai, Georgia Karpathiou, Helen Kourea, Sevasti Kamina and Anna Goussia have contributed to the data. Haralambos Harissis has contributed to the acquisition and examination of the original material. Dimitrios Peschos has contributed to the analysis of data. All Authors have read and approved the manuscript.

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