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Tumour infiltrating lymphocytes (TILs) in breast cancer during pregnancy

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ABSTRACT

Background: Tumour infiltrating lymphocytes (TILs) is one of the most exciting breast cancer biomarkers, yet no data is available on its prevalence in tumours diagnosed during pregnancy.
Methods: We evaluated the prevalence of TILs (stromal and intratumoural) in pregnant and non-pregnant young breast cancer patients.
Results: 11/116 (9.6%) of the non-pregnant and 2/86 (2.3%) pregnant patients had TILs $\geq$ 50% ($p < 0.001$) with highest prevalence observed in triple negative tumours ($p = 0.01$).
Conclusions: This is the first report on TILs in tumours diagnosed during pregnancy. The low prevalence could reflect the state of low host immunity associated with pregnancy.
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Introduction

Tumour infiltrating lymphocytes (TILs) has recently emerged as one of the most exciting cancer-related biomarkers in the breast cancer field. Tumours with high lymphocytic infiltration were shown to have better prognosis particularly in triple negative breast cancer [1], higher rate of pCR [2] and higher benefit of trastuzumab in the adjuvant setting [3]. These findings have resulted in the call of integrating TILs in standard pathological evaluation and an international consensus for standardizing how TILs should be evaluated in the clinical setting has just been published [4].

Breast cancer diagnosed during pregnancy is rare, comprising less than 1% of all breast cancer cases [5]. Conflicting data exist regarding the prognosis of these patients, yet some series have suggested a tendency towards a worse outcome [6]. Given the altered immune status during pregnancy [7], the prevalence and clinical relevance of TILs are expected to be different in tumours diagnosed during the course of gestation. In this analysis, we report for the first time the prevalence of TILs in breast tumours diagnosed during pregnancy using two datasets and correlate it with known classical clinico-pathological variables, including breast cancer subtypes.

Materials and methods

Study population

We evaluated TILs in two datasets of patients. The first (i.e. Dataset-A) is a previously published dataset comprised of 65 patients diagnosed during pregnancy and 130 matched non pregnant breast cancer controls [8]. Briefly, all patients in this dataset were diagnosed in the period from 1997 to 2010 at the European Institute of Oncology (IEO) in Milan. No differences in classic pathological features were observed between the pregnant and non-pregnant patients, yet patients diagnosed during pregnancy had

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significantly poorer disease-free survival. The second dataset (i.e. Dataset-B) was composed of patients diagnosed with breast cancer during pregnancy at IEO in the period from 2011 to 2014. To avoid any impact of tissue sampling and the potential effect of neo-adjuvant therapy on TILs evaluation, patients who received neo-adjuvant therapy were excluded from both datasets.

Pathological evaluation

All patients were pathologically diagnosed at IEO. Evaluation of oestrogen and progesterone receptors (ER, PgR), Ki-67 and HER2 was performed as per routine practice in the same laboratory using standard testing. Breast cancer subtypes were defined using the St Gallen criteria [9]. TILs were evaluated on haematoxylin and eosin stained slides from primary surgical specimens, as previously defined [1,4], blinded for clinical information. Briefly, the infiltrate had to consist of mononuclear cells while any granulocyte infiltrate in areas of tumour necrosis was excluded. Intratumoral lymphocytes were defined as intraepithelial mononuclear cells within tumour cells nests or in direct contact with tumour cells. Stromal lymphocytes were defined as mononuclear infiltrate within the tumour stroma. Only stroma of invasive carcinoma and not carcinoma in-situ was evaluated. Both intratumor and stromal lymphocytes were scored separately by one pathologist (A. V.). The cases defined as having a high (≥50%) content of lymphocytes were reviewed by a second pathologist (G. P.) for confirmation.

Statistical analysis

In dataset-A, to evaluate the independent association between pregnancy (yes/no) and TILs, we constructed a linear regression model adjusting the association to age, tumour size, nodal status, histological grade and breast cancer subtype. We then evaluated the association between TILs and the different clinico-pathologic variables in patients diagnosed during pregnancy, considering all pregnant patients in Dataset-A and B using Chi-squared test. We finally performed a descriptive analysis of the association between TILs and disease-free survival (DFS) in the pregnant cohort.

Table 1
Patients characteristics.

	Dataset A		Dataset B
	Non-pregnant (n = 116)	Pregnant (n = 48)	Pregnant (n = 38)
Mean age (range)	36 (28–47)	36 (28–47)	36 (23–45)
TILs			
Mean stromal TILs (±SD)	17.8 (19.3)	2.92 (1.01)	12.9 (15.7)
Mean intratumoural TILs (±SD)	1.72 (2.8)	0 (0)	0.76 (0.85)
Mean total TILs (±SD)	19.5 (21.3)	2.98 (0.92)	13.6 (16)
Total TILs ≥ 50%	11 (9.5%)	0	2 (5%)
Tumour size			
≤2 cm	48 (41.4%)	22 (45.8%)	16 (42.1%)
>2 cm	64 (55.2%)	24 (50%)	13 (34.2%)
Missing	4 (3.4%)	2 (4.2%)	9 (23.8%)
Nodal status			
Negative	54 (46.5%)	23 (47.9%)	15 (39.5%)
Positive	62 (53.5%)	25 (52.1%)	18 (47.4%)
Missing	0	0	5 (13.1%)
Histological grade			
I	4 (3.4%)	4 (8.3%)	1 (2.6%)
II	44 (37.9%)	17 (35.4%)	12 (31.6%)
III	64 (55.2%)	25 (52.1%)	17 (44.6%)
Missing	4 (3.4%)	2 (4.2%)	8 (21.2%)
Breast cancer subtypes			
Luminal-A	22 (19%)	10 (20.8%)	10 (26.3%)
Luminal-B	50 (43%)	22 (45.8%)	7 (18.4%)
HER2	22 (19%)	8 (16.7%)	8 (21.2%)
Triple negative	22 (19%)	8 (16.7%)	11 (28.9%)
Missing	0	0	2 (5.2%)

TILs: tumour infiltrating lymphocytes; n: number; SD: standard deviation.

Results

Patient population

Dataset-A included 116 non-pregnant and 48 pregnant patients while dataset B included 38 pregnant patients (Fig. 1). No obvious differences were observed regarding age, nodal status and tumour size, albeit a higher proportion of patients with triple negative disease in dataset-B (28.9%) versus 19% and 16.7% (Dataset-A, non-pregnant & pregnant respectively) (Table 1). Stroma and

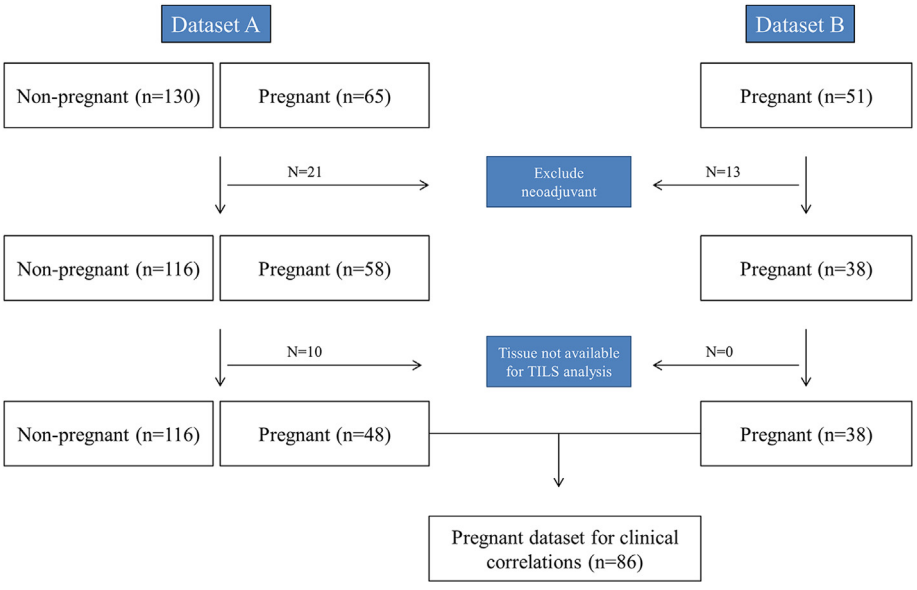

Fig. 1. The CONSRT flow chart showing patients included in this analysis.

intratumoral TILs were evaluated in all patients (n = 202) and their results were highly correlated (r = 0.67; p < 0.001).

Associations between TILs and pregnancy status

In dataset-A, tumours diagnosed during pregnancy had significantly lower TILs, considering stromal TILs (2.9% vs. 17.8%, p < 0.001) or stromal and intraepithelial TILs (2.9% vs. 19.5%, p < 0.001) (Table 1). In a linear regression model, diagnosis during pregnancy was independently associated with lower TILs (p < 0.001). Eleven cases (9.5%) in the non-pregnant groups and none in the pregnant group had TILs ≥ 50%.

We went on to validate this finding in dataset-B, where 38 pregnant patients were evaluated for TILs. The mean TILs was 12%, but only two patients had TILs ≥50%.

Fig. 2A shows the difference between the non-pregnant and pregnant patients considering datasets-A and B.

Association between TILs expression, clinico-pathological features and DFS in the pregnant cohort

For this analysis, we combined all pregnant patients in dataset-A (n = 48) and dataset-B (n = 38) and evaluated the association with known prognostic parameters. No significant correlation was observed with age (p = 0.32), tumour size (p = 0.59), nodal status (0.68), and histological grade. However, there was a positive correlation with Ki67 (p = 0.05) and breast cancer subtypes (p = 0.01), with the highest expression observed within the pregnant patients with triple negative breast cancer (Fig. 2B). In the non-pregnant group, the highest expression was observed in both HER2-positive and triple negative tumours, although the differences were not significant (p = 0.3).

After a mean follow-up of 49.11 months, 22/86 pregnant patients developed a DFS event (25.5%). The mean TILs in patients who developed an event was 2.9% (range: 1–4) compared to 9% (range: 0–67) in those who did not experience an event.

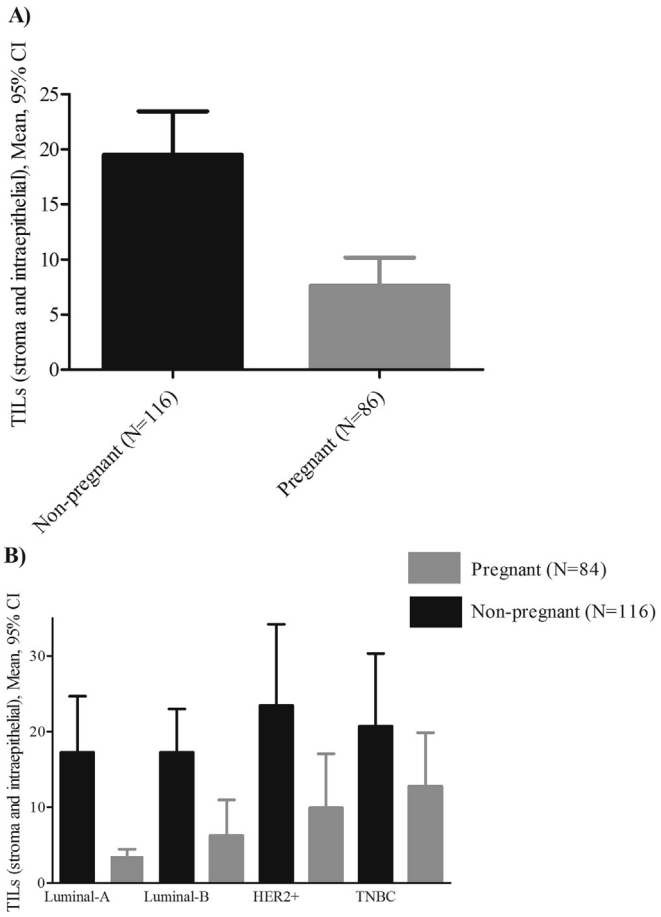

Fig. 2. A) TILs expression in non-pregnant and pregnant breast cancer patients. The y-axis shows TILs expression. Bar plot represent mean TILs expression and the 95% CI. **B)** TILs expression according to breast cancer subtypes in pregnant and non-pregnant breast cancer patients. The y-axis shows TILs expression. Bar plot represent mean TILs expression and the 95% CI.

Discussion

We found that TILs are significantly less represented in tumours diagnosed during pregnancy. Out of 86 pregnant patients, only two had ≥50%TILs (2.3%) compared to 11 out of 116 non-pregnant patients (9.6%). Within the pregnant group, TILs were higher in patients with triple negative disease, which is consistent with earlier studies in the non-pregnant setting [1]. This possibly explains why TILs expression in dataset B was higher than the pregnant cohort in dataset-A, given the higher proportion of triple negative patients in the former (28% vs. 16%).

The interplay between cancer and the immune system is complex, however there is increasing evidence that such interaction is critical for the progression of cancer [10,11]. On the other hand, emerging data suggests that changes occurring during and shortly following pregnancy impacts breast cancer biology [12,13]. During pregnancy, the immune system is suppressed to avoid rejection of the growing foetus and is also evidenced by increased susceptibility for infection in pregnant women [7]. This may explain why a host anti-tumour immune response was less evident in tumours diagnosed during pregnancy.

Of note, the current analysis was solely performed on young patients where no data on TILs has been previously published. Breast cancer arising at a young age is known to have distinct biological features [14,15]. In the non-pregnant cohort, we found relatively higher expression of TILs in HER2 and triple negative tumours like other studies, albeit the differences were not statistically significant which could be due to the low number of patients in our study. Yet, around 10% of patients had extensive lymphocytic infiltration of at least 50% TILs, which is in line with data from the GeparDuo (n = 218) and GeparTrio (n = 840) cohorts [2] where 11% had at least 50% TILs and the median age was 50 years. In the BIG 2-98 (n = 2009) trial, the median age was also 50 years but only 5% of patients had TILs ≥ 50% [1]. This could be due to the lower representation of triple negative disease in the BIG 2-98, which was in the range of 12% compared to around 20% in the current study and both the GeparDuo and GeparTrio [2].

Previous studies have pointed out to the association between TILs and DFS mainly in patients with triple negative breast cancer [1,3]. Owing to the low number of patients in our analysis, formal evaluation according to breast cancer subtype is not possible. However, we observed that patients with relatively high TILs did not develop any DFS event while all patients who developed a DFS event had TILs <5%. Given the favourable prognosis of patients with high TILs, it could be postulated that the poor outcome of pregnancy-associated breast cancer observed in some studies is related in part to the lower levels of TILs and hence lack of anti-tumour immunity in these patients. However, this finding requires further validation in larger cohorts.

In conclusion, we report for the first time the prevalence and clinical relevance of TILs in pregnant and also young breast cancer patients. This adds to the limited available data on the biology of these rare tumours and underscores the potential impact of the pregnancy microenvironment on tumour biology.

Conflict of interest statement

None declared.

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