Tumor biology and immunology in patients with breast cancer occurring during pregnancy (BCP) compared to non-pregnant breast cancer patients

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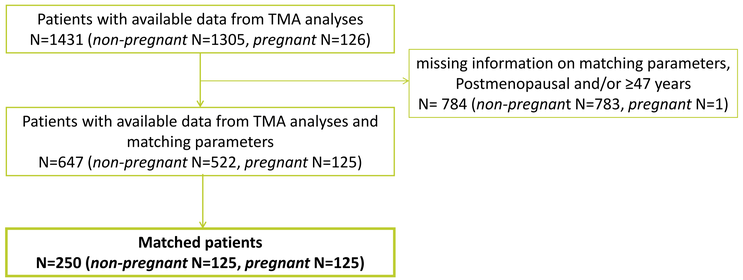
Background

Breast cancer (BC) is one of the most common malignancies during pregnancy. The incidence is likely to increase as more women tend to delay childbearing into later life and the overall lifetime cancer risk increases with age. Pregnancy presents a complex and unique immunological condition. Patients diagnosed with BC during pregnancy usually present more advanced stages than young, non-pregnant patients.[1,2] This study investigated the tumor biology and immunology of a BCP cohort compared to a non-pregnant BC cohort.

Patients and Methods

Tissue microarrays (TMA) of formalin-fixed paraffin embedded core biopsies or surgical specimens from 125 pregnant BC patients treated with (neo-) adjuvant chemotherapy were constructed. The BCP cohort was matched to an appropriate non-pregnant BC cohort with existing TMAs from the GAIN study by variables age, tumour stage, nodal status (N0 patients were not eligible in the GAIN study), grading and subtype. The nearest neighbour matching in a 1:1 ratio was performed in R, version 4.1.0, especially the R package MatchIt, version 4.1.2,[3] by using the Mahalanobis distance without replacement. TMAs were stained via immunohistochemistry (IHC) to assess estrogen and progesterone receptor (ER, PgR), human epidermal growth factor receptor 2 (HER2), Ki-67 (≤20% vs >20%), and immune response relevant markers HLA class I (EMR8-5, heavy chain), HLA-G (4H84), PD-L1 (<1% vs ≥1%), TIGIT (BLR047F), Nectin-4 (EPR15613-68, Abcam) and tumour-infiltrating lymphocytes (TILs, ≤25% vs 26-60% vs >60%). PD-L1 expression was evaluated in tumour and immune cells using the 22C3 antibody (Abcam). H-scores of HLA, HLA-G, TIGIT and Nectin-4 as continuous variables were calculated.[4-7] Comparisons between the pregnant and non-pregnant cohort were performed by using Wilcoxon test (continuous parameters), Fisher's exact test resp. Pearson's χ^2 test (categorical parameters). All statistical tests were considered to be descriptive.

Figure 1: Flow diagram for patient selection



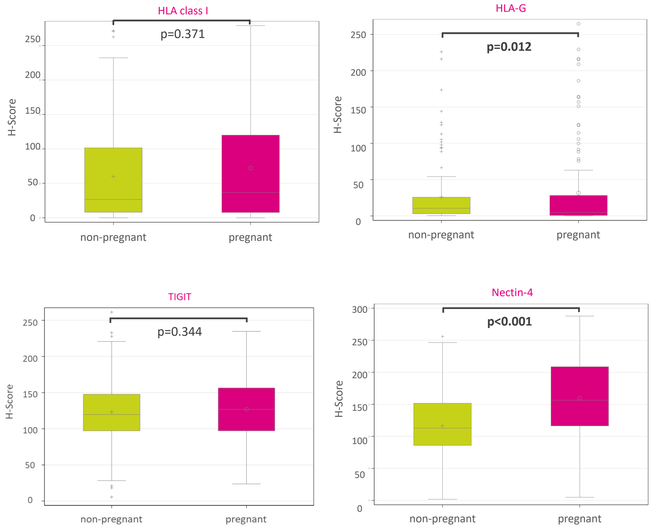
Results

Table 1: Baseline characteristics in BC patients

Parameter	Category	Non-pregnant N=125 N(%)	Pregnant N=125 N(%)	Overall N=250 N(%)	P-value
Age at diagnosis, years	18-29	5 (4.0)	14 (11.2)	19 (7.6)	<.001
	30-34	32 (25.6)	56 (44.8)	88 (35.2)	
	35-39	51 (40.8)	44 (35.2)	95 (38.0)	
	≥40	37 (29.6)	11 (8.8)	48 (19.2)	
HR status combined**	ER and PgR negative	50 (40.0)	52 (41.6)	102 (40.8)	0.898
	ER and/or PgR positive	75 (60.0)	73 (58.4)	148 (59.2)	
HER2 status**	negative	98 (81.0)	95 (78.5)	193 (79.8)	0.749
	positive	23 (19.0)	26 (21.5)	49 (20.2)	
	missing	4	4	8	
Biological subtype**	TNBC	44 (35.2)	45 (36.0)	89 (35.6)	0.989
	HER2+/HR-	6 (4.8)	7 (5.6)	13 (5.2)	
	HER2+/HR+	19 (15.2)	19 (15.2)	38 (15.2)	
	HER2-/HR+	56 (44.8)	54 (43.2)	110 (44.0)	
Histological tumor type**	ductal or ductal-lobular invasive	101 (80.8)	111 (90.2)	212 (85.5)	0.058
	lobular invasive	9 (7.2)	7 (5.7)	16 (6.5)	
	other	15 (12.0)	5 (4.1)	20 (8.1)	
	missing	0	2	2	
Tumor grading**	G1	1 (0.8)	1 (0.8)	2 (0.8)	0.991
	G2	40 (32.0)	39 (31.2)	79 (31.6)	
	G3	84 (67.2)	85 (68.0)	169 (67.6)	
T stage*	T1	38 (30.4)	38 (30.4)	76 (30.4)	0.947
	T2	65 (52.0)	63 (50.4)	128 (51.2)	
	T3	17 (13.6)	17 (13.6)	34 (13.6)	
	T4	5 (4.0)	7 (5.6)	12 (4.8)	
N stage*	N0	0 (0.0)	58 (46.4)	58 (23.2)	<.001
	N1	89 (71.2)	47 (37.6)	136 (54.4)	
	N2	16 (12.8)	14 (11.2)	30 (12.0)	
	N3	20 (16.0)	6 (4.8)	26 (10.4)	
Ki67**, at diagnosis	≤20%	70 (61.9)	56 (46.7)	126 (54.1)	0.025
	>20%	43 (38.1)	64 (53.3)	107 (45.9)	
	missing	12	5	17	
Pregnancy trimester	1st trimester	0 (n.a.)	23 (18.5)	23 (18.5)	n.a.
	2nd trimester	0 (n.a.)	43 (34.7)	43 (34.7)	
	3rd trimester	0 (n.a.)	58 (46.8)	58 (46.8)	
	missing	125	1***	126	

*pT resp. pN, if not available cT and cN; **assessed from stained TMAs (via IHC) by the central pathology, Marburg; *** in 1 patient BC was histologically diagnosed 6 days post partum; Data are N (valid %)

Figure 2: Expression of selected immunomarkers as continuous variables



- Pregnant BC patients were younger than non-pregnant (median 34 [26-47] vs 37 [27-47] years).
- Pregnant BC patients had a higher Ki-67 expression (>20%: 53.3% vs 38.1%, p=0.025).
- The H-score of HLA-G was significantly lower (median: 4.9 vs 10.6, p=0.012), in the BCP cohort compared to the non-pregnant BC cohort.
- H-scores of HLA class I showed no significant differences in both cohorts (median: 36.8 vs 26.7, p=0.371).
- There is weak correlation between the H-scores of HLA class I and HLA-G in the non-pregnant cohort and a moderate correlation for both markers in the pregnant cohort (Spearman`s ρ = 0.27 resp. 0.45).
- The H-score of Nectin-4 (median: 156 vs 113, p<0.001) was significantly higher in the BCP cohort compared to the non-pregnant BC cohort.
- No significant differences were found for TILs, PD-L1 or H-score of TIGIT.

Table 2: Expression of immunomarkers PD-L1 and TILs as categorical variables

Parameter	Category	Non-pregnant N=125 N(%)	Pregnant N=125 N(%)	Overall N=250 N(%)	p-value
PD-L1 IC	negative	103 (85.1)	91 (76.5)	194 (80.8)	0.102
	positive	18 (14.9)	28 (23.5)	46 (19.2)	
	missing	4	6	10	
PD-L1 TC	negative	111 (91.7)	108 (90.8)	219 (91.3)	0.823
	positive	10 (8.3)	11 (9.2)	21 (8.8)	
	missing	4	6	10	
TILs	0-25%	112 (92.6)	53 (86.9)	165 (90.7)	0.46
	26-60%	8 (6.6)	7 (11.5)	15 (8.2)	
	>60%	1 (0.8)	1 (1.6)	2 (1.1)	
	missing	4	64	68	

Conclusions

- **TILs, TIGIT and PD-L1 seem to be pregnancy independent factors of BC in young women.**
- **The effect of pregnancy on the expression patterns of HLA and HLA-G is inconclusive and needs further investigation.**
- **The significantly higher Ki-67 expression in the BCP cohort suggests an increased proliferation in BC cells during pregnancy.**
- **The higher Nectin-4 expression in the BCP cohort could be a sign of an altered anti-tumour response.**
- **Together, the results suggest differences in tumor proliferation and tumor/host immunogenicity in BCP cohort vs non-pregnant BC cohort.**

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