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Clinicopathological Features and Prognosis of Pregnancy Associated Breast Cancer – A Matched Case Control Study

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Abstract Pregnancy Associated Breast Cancer (PABC) manifests during pregnancy or within a year following delivery. We sought to investigate differences in management, outcome, clinical, histopathology and immunohistochemistry (IHC) characteristics of PABC and matched controls in a retrospective case control study. PABC and control patients were selected from breast cancer cases of women ≤45 years, diagnosed in the 2nd Department of Pathology, Semmelweis University, Budapest, Hungary between 1998 and 2012. Histopathology information on tumor type, grade, size, T, N, lympho-vascular invasion (LVI), Nottingham Prognostic Index (NPI), associated in situ lesions and IHC charcteristics: ER, PgR, HER2, Ki67, p53 were recorded, IHC-based subtype was assessed, clinical, management and outcome data were analysed. Thirty-one breast cancer cases were pregnancy related. Clinical management data did not differ in cases and controls. Histopathology of disease at presentation was not significantly different, but NPI assessed the PABC group as having poor, whereas controls as having intermediate prognosis. Associated in situ lesion was more often high grade Extensive Intraductal Carcinoma Component (EIC) in PABC. Triple negative and LuminalB prol tumors predominated in PABC. Disease-free and overall survival was inferior compared to controls. PABC patients with LuminalB prol and Triple negative tumors had inferior outcomes. On multivariate analysis inferior prognosis of PABC was associated with pregnancy. Our study has demonstrated inferior outcome of PABC. Difference in tumor biology is reflected by the predominance of triple negative and LuminalB tumors in PABC. The strength of the study is the analysis of complete pathology and IHC data.

Keywords Breast cancer · Pregnancy associated breast cancer · Pregnancy · Postpartum · Young women

Introduction

Pregnancy associated breast cancer (PABC) has been most commonly defined as breast cancer complicating pregnancy, manifesting during lactation or within 1 year after delivery. The definition is not unanimous however, some authors define the postpartum time frame from 6 months to 2 years after delivery [1, 2].

Pregnancy has dual influence on breast cancer risk, it has long-term protective effect but epidemiologic data also demonstrate a transient increase in breast cancer incidence postpartum. The postpartum time frame for this increased risk ranges from 2 to 15 years, or even longer in case of older first time pregnant women [3–5].

The incidence of PABC is estimated to be 1/10000 to 1/3000 pregnancies in Western countries, which is expected to rise due to the trend of women postponing childbearing to

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later age. It is estimated that 10 % of breast cancer cases affecting women ≤40 years of age are pregnancy related [6].

The situation is complicated at social, ethical, psychological and medical levels, since the diagnosis is often challenging due to physiologic changes occuring in the breast during pregnancy or lactation, and because treatment of the pregnant mother—however essential—can potentially harm the fetus. Clinicians have very limited experience in this complex setting, and this may lead to delayed diagnosis, delayed or undertreatment of maternal breast cancer, controversial termination of pregnancy or induced premature delivery.

The prognosis of PABC is more often reported as being unfavorable [1, 7–10]. Whether this poor prognosis is related to pregnancy or mainly due to the young age of patients—which in itself is known to be a poor prognostic factor [11] - is not clear.

Studies on PABC are rare, with only a few addressing the pathological characteristics of the disease. The majority supply information on the invasive tumor's type, grade and TNM stage, together with only the immunohistochemistry (IHC) assessment of hormonal receptors (with incomplete data provided by most of the studies); data on Her2 status [12–19], Ki67 labeling index [12, 15, 17], p53 [12, 18], IHC-based tumor subtype [15] as well as on the characteristics of eventual associated in situ lesions [19] are scarce. To the best of our knowledge this is the first matched case control study that combines information on the management and outcome of PABC patients together with analysis of all the relevant histopathology and IHC information the pathologist can provide on the multidisciplinary approach to PABC.

The aim of this retrospective matched case control study was to investigate differences in clinicopathological features, immunophenotype, treatment and outcome of PABC and matched control, non-PABC cases.

Patients and Methods

First, upon specific approval of the Semmelweis University Institutional Review Board (TUKEB 17/2006, 3/2013) we reviewed the database of the 2nd Department of Pathology, Semmelweis University and recorded all the breast cancer cases of women ≤45 years of age, diagnosed between January 1st, 1998 and November 1st, 2012. Histopathological data of the invasive tumor were obtained: tumor type, Nottingham grade, multifocality, characteristics of eventual associated in situ lesions, invasive tumor size, regional lymph node involvement, TNM stage, Nottingham Prognostic Index (NPI), presence of lympho-vascular invasion (LVI), and the characteristics of IHC analysis were investigated: Estrogen receptor (ER), Progesterone receptor (PgR) and Her2 expression, Ki67 labeling index and p53 expression. Used antibodies, methodology and FISH assessment method are summarized in Supplementary Material 1. ER and PgR statuses were evaluated according to the most recent guidelines [20], Ki67 positivity was measured as the ratio of positive tumor cell nuclei in the tumor and Her2 IHC was evaluated according to current guidelines [21] by a 0–3 scale standard protocol. FISH results were evaluated according to standard protocol [21]: non-amplified if the HER2/CE17 ratio was less than 1.8, equivocal if this ratio was between 1.8 and 2.2 and amplified if the HER2/CE17 ratio was over 2.2. When discordance occurred with Her2 IHC, FISH results were taken into consideration.

A tumor was considered ER and/or PgR positive if 10 % of tumor cells demonstrated intranuclear positivity. In Ki67 IHC assessment, a labeling index of 14 % or more was considered high [22]. Based on IHC characteristics of the invasive tumor, the IHC-based subtype was assessed. ER (and eventually PgR) positive tumors with low Ki67 labeling index were considered Luminal A (LumA), ER (and eventually PgR) positive tumors with high Ki67 labeling index or HER2 expression were considered Luminal B subtype (LumBprol or LumBHer2 respectively). The tumors which were hormone receptor (HR) negative and expressed Her2 were considered as Her2 positive, while tumors that did not express either HRs or Her2 were considered as triple negative (TNBC). [23]

In the second step, we reviewed the patient database of Semmelweis University for recorded breast cancer patients and added all the available loco-regional and systemic treatment information, relevant personal, parity and family history, as well as data on eventual loco-regional and systemic relapses. Finally, we selected all pregnancy associated breast cancer cases manifesting during pregnancy, lactation or within 1 year after delivery.

Control patients were identified through the two databases, each PABC patient was matched by age (±1 year) and year of first breast cancer (BC) diagnosis (±1 year) to a control patient with non-PABC.

Asymmetrical numeric data were analyzed by matched Wilcoxon-test. Categorical data were compared using Chi-square and Fisher's exact test. Overall survival analyses were performed using the Kaplan-Meier method. Overall survival intervals were determined as the time period from initial diagnosis to the time of death or date of last follow up. Disease-free survival was calculated as the time interval from initial breast cancer diagnosis to the date of disease recurrence (loco-regional or systemic) or to time of death from BC, or to the date of last follow-up if the patient was disease free. Comparison between survival functions for different strata was assessed with the log-rank statistic. Multivariate analysis of prognostic factors was performed using Cox's regression model. Differences were considered significant when $p \leq 0.05$. All statistical analyses were performed using Statistica 9.0 software (StatSoft Inc. Tulsa, OK).

Results

Clinical Data Analysis

Thirty-one breast cancer cases were found to be pregnancy related, 10 manifesting during pregnancy and 21 during lactation or postpartum period, within 1 year after delivery. Patient characteristics and management data of all PABC and control patients are summarized in Table 1. Median age at diagnosis was 34 (range: 29–42) years for both the PABC and control group (range: 28–42).

Treatment Modalities During Pregnancy

Among the pregnant patients three were diagnosed with breast cancer during the first trimester, six during the second trimester and one in the third trimester of their pregnancy.

Six patients underwent surgery (four breast conserving surgery/BCS/and two mastectomy, all with axillary lymph node dissection/ALND/) while pregnant, followed by adjuvant therapy after delivery in four cases. One patient received neoadjuvant chemotherapy (2xFAC) followed by surgery (mastectomy with ALND) during pregnancy and adjuvant therapy after delivery. One patient had surgery (BCS with ALND) followed by adjuvant chemotherapy (6xFEC) while pregnant.

Pregnancy Outcome

One patient underwent induced abortion at 23rd week of gestation, five patients had elective cesarean section (between the 29th and 34th week of gestation) and four patients delivered their babies spontaneously (one preterm delivery).

Treatment Modality Comparison of PABC and Control Cases

There was no significant difference in the modality of surgery (breast conserving surgery versus mastectomy) between cases and controls. Axillary lymph node dissection was the main therapy of choice in both groups. The pregnancy associated group did not show significant difference in elapsed time between diagnosis and surgery compared to controls (p=0.4, data not shown). Neoadjuvant, adjuvant chemo- and radiotherapy frequency or regimen did not differ (p=0.839) between the cases and controls. Adjuvant endocrine therapy was

Table 1 Patients characteristics and management data of PABC and control patients

			PABC N=31	Control N=31	p-value
Age		median (years)	34	34	match
Surgery	Breast	BCS[a]	12 (38.7 %)	14 (45.2 %)	(BCS[a] vs other) 0.448[m]
		mastectomy[b]	18 (58 %)	14 (45.2 %)	
		bilat mastectomy[c]	1 (3.2 %)	1 (3.2 %)	
		subcut mastect[d]	0 (0 %)	2 (6.5 %)	
	Axilla	x[e]	1 (3.2 %)	1 (3.2 %)	1[n]
		SNLB[f]	4 (12.9 %)	3 (9.7 %)	
		ALND[g]	26 (83.9 %)	27 (87.1 %)	
Oncological Treatment	Neoadj[h]	all	15 (48.4 %)	10 (32.3 %)	0.196[m]
		followed by chemotx	11 (35.5 %)	6 (19.4 %)	0.342[m]
	Chemotx[i]	yes	24 (77.4 %)	24 (77.4 %)	0.605[m]
		no	5 (16.1 %)	7 (22.6 %)	
		nd[o]	2 (6.5 %)	0 (0 %)	
	Regimen[j]	antracyclin	6(19.4 %)	7(22.6 %)	0.839[m]
		antracyclin + taxane	9(29 %)	9(29 %)	
		trastuzumab	4(12.9 %)	8(25.8 %)	
		other	5(16.1 %)	6(19.4 %)	
		nd[o]	4(12.9 %)	0(0 %)	
	Radiotx[k]	yes	22 (70.9 %)	22 (70.9 %)	0.668[m]
		no	7 (22.5 %)	9 (29 %)	
		nd[o]	2 (6.5 %)	0 (0 %)	
	Endocr[l]	yes	12 (38.7 %)	24 (77.4 %)	0.004[m]
		no	17 (54.8 %)	7 (22.6 %)	
		nd[o]	2 (6.5 %)	0 (0 %)	

[a] Breast conserving surgery, [b] Modified radical mastectomy, [c] Bilateral mastectomy, [d] Subcutaneous mastectomy, [e] No surgery, [f] Sentinel lymph node biopsy, [g] Axillary lymph node dissection, [h] Neoadjuvant chemotherapy, [i] Adjuvant chemotherapy, [j] Adjuvant chemotherapy regimen [k] Adjuvant radiotherapy, [l] Adjuvant endocrine therapy, [m] Chi-square test, [n] Fisher's exact test, [o] No data

Table 2 Relapse, survival and family history data of PABC and control patients

			PABC N=31	Control N=31	p-value
Relapse		yes	18 (58.1 %)	4 (12.9 %)	0.0003[f]
		no	12 (38.7 %)	25 (80.6 %)	
		nd[a]	1 (3.2 %)	2 (6.5 %)	
	Loco-regional	yes	6 (19.4 %)	1 (3.2 %)	0.102[f]
		no	24 (77.4 %)	28 (90.3 %)	
		nd[a]	1 (3.2 %)	2 (6.5 %)	
	Systemic	yes	14 (45.2 %)	3 (9.7 %)	0.003[f]
		no	16 (51.6 %)	26 (83.9 %)	
		nd[a]	1 (3.2 %)	2 (6.5 %)	
Death	Deceased		13 (41.9 %)	2 (6.5 %)	
	Alive	All	18 (58.1 %)	29 (93.5 %)	0.005[e]
		alive with disease	1 (3.2 %)	2 (6.5 %)	
		no evidence of disease	17 (54.8 %)	27 (87.1 %)	
Death/IHC subtype[b]		LumA	0/0	0/5	See
		LumBprol	6/10	0/8	Fig. 5
		LumBHer2	1/3	0/6	
		Her2+	1/3	2/5	
		TNBC[c]	5/15	0/7	
Family history	All	positive	14 (45.2 %)	15 (48.4 %)	0.558[e]
		negative	7 (22.6 %)	5 (16.1 %)	
		nd[a]	10 (32.3 %)	11 (35.5 %)	
	BC related[d]	positive	8 (25.8 %)	7 (22.6 %)	0.837[e]
		negative	13 (41.9 %)	13 (41.9 %)	
		nd[a]	10 (32.3 %)	11 (35.5 %)	

[a] No data,
[b] Immunohistochemistry-based subtype, [c] Triple negative immunophenotype, [d] Breast cancer related family history, [e] Chi-square test, [f] Fisher's exact test

however administered twice as frequently to controls (p=0.004).

Relapse and Survival Comparison of PABC and Control Patients

Table 2 summarizes relapse, survival and family history data of PABC and controls.

Relapse was significantly more common in the PABC group (p=0.003), 14 (45.2 %) patients had systemic relapse as compared to three (9.7 %) control patients (p=0.0003). Systemic relapse was the most common in LumBprol (6 patients out of 10) and triple negative cases (5 patients out of 15); all these patients died of their disease. Disease free and overall survival was significantly worse in PABC cases (p=0.0004 and p=0.0007) (Fig. 1a and c). When survival data of pregnant and postpartum patients were assessed separately, the postpartum patient group showed significantly worse disease free (p=0.001) and overall survival (p=0.00008) as compared with the controls. Disease free survival of pregnant patients was inferior in comparison to control cases (p=0.007), but overall survival was not significantly worse (Fig. 1b and d). The outcome of PABC was inferior, since thirteen PABC patients (41.9 %) died of the disease; 11 of whom had postpartum breast cancer, while 2 patients (6.5 %) died in the control group (p=0.005) (Fig. 2). There was no difference between the PABC and non-PABC group when overall and breast cancer related family history was evaluated.

Pathological Data Analysis

Table 3 summarizes the tumor characteristics of PABC and control patients.

The most common tumor type was high grade invasive ductal carcinoma (IDC) in both groups. There was no significant difference in tumor T or N stage. For assessment of median size of the invasive tumor, when patients were treated with neoadjuvant chemotherapy, tumor size before treatment was considered. Median size of PABC was 24 mm (range 10–100 mm) compared to that of controls (22 mm; range 9–85 mm, p=0.13) (Supplementary Material 2). The Notthingham Prognostic Index (NPI) median value for PABC was 6 (range: 3.24–8.5), and 4.65 (range: 3.24–7.2) for controls (Fig. 3). This finding categorized PABC as a disease of poor prognosis, while the non-PABC group was categorized as having intermediate prognosis (p=0.03). Patients who had complete or partial pathological response after neoadjuvant chemotherapy were excluded from this analysis.

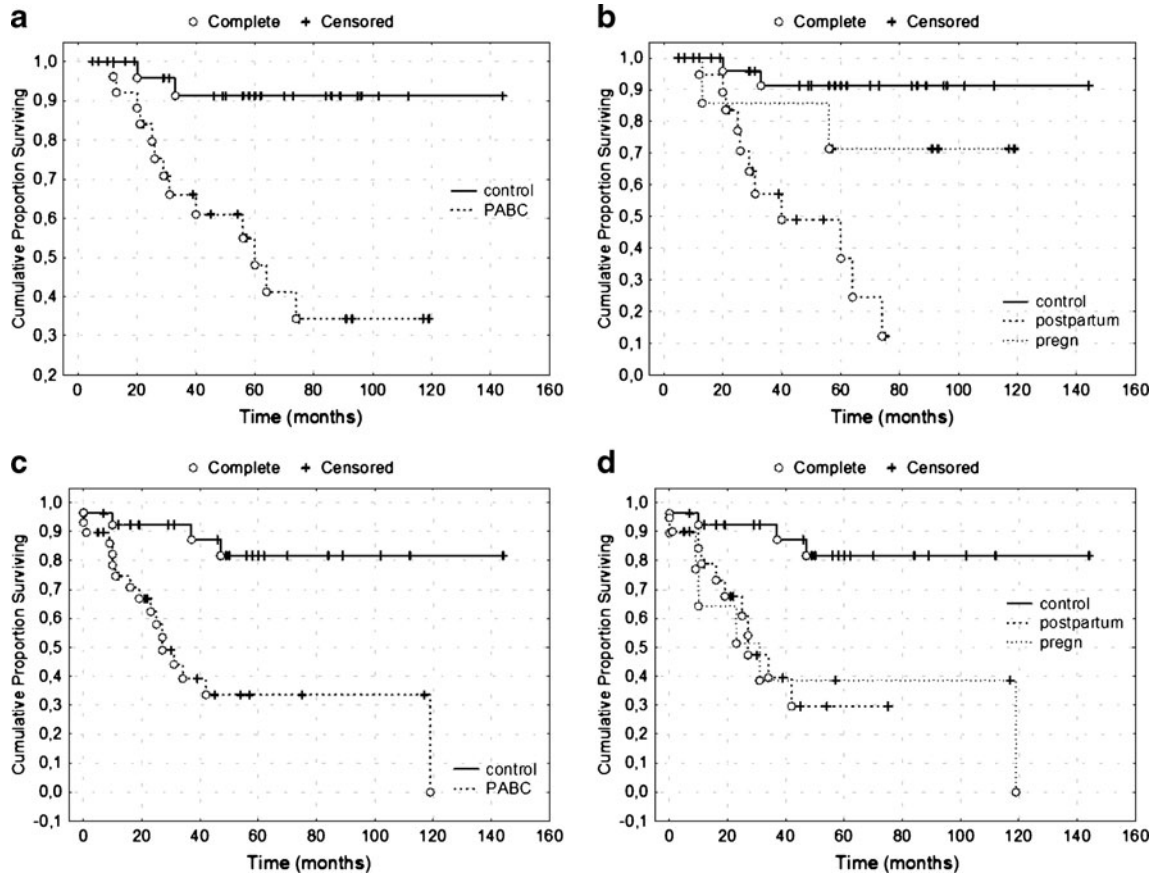

Fig. 1 **a** Overall survival of PABC and control patients ($p= 0.0007$). **b** Overall survival of pregnant, postpartum and control patients ($p= 0.002$) (control vs. postpartum $p= 0.00008$, control vs. pregnant p=0.203, postpartum vs. pregnant $p= 0.049$). **c** Disease free survival of PABC and control patients ($p= 0.0004$). **d** Disease free survival of pregnant, postpartum and control patients ($p= 0.007$) (control vs. postpartum $p= 0.001$, control vs. pregnant $p= 0.007$, postpartum vs. pregnant p=0.979)

Lympho-vascular invasion was detected in 61.3 % of cases and 48.4 % of controls (p=0.228).

PABC and control cases were ER negative in 58 % and 38.8 %, respectively. The difference in PgR expression was significant: 87.1 % of PABC cases, whereas 61.3 % of controls did not express PgR (p=0.04). No significant difference was noticed in Her2 status and by p53 immunostaining. All PABC cases were highly proliferating by Ki67 labeling index (p=0.01). When analyzing IHC-based subtypes, triple negative tumors predominated in PABC (48.4 %), followed by LumBprol tumors (32.3 %). There were no LumA tumors in this group.

Associated in situ lesions showed significant differences in patient cases and controls (p=0.017). In 45.2 % of PABC cases, the invasive tumor was associated with high grade

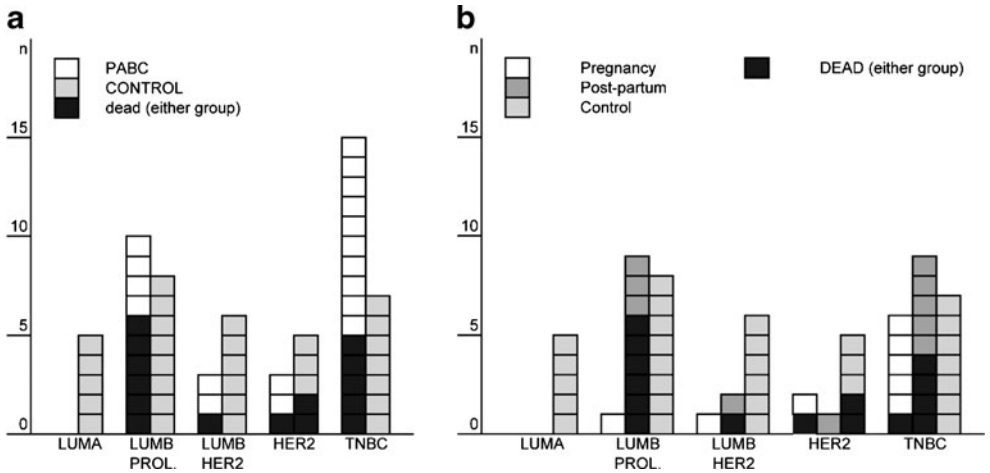

Fig. 2 **a** IHC-based subtype and death of PABC and control patients (n: number of patients). **b** IHC-based subtype and death of pregnant, postpartum and control patients (n: number of patients)

Table 3 Tumor characteristics of PABC and control cases

		PABC *N*=31	Control *N*=31	*p*-value
Type	IDC[a]	26 (83.9 %)	30 (96.8 %)	0.195[p]
	Other	5 (16.1 %)	1 (3.2 %)	
Associated in situ carcinoma	none	11(35.5 %)	14(45.2 %)	0.017[o]
	EIC non-high[b]	3(9.7 %)	3(9.7 %)	
	EIC high[c]	14(45.2 %)	3(9.7 %)	
	DCIS non-high[d]	1(3.2 %)	4(12.9 %)	
	DCIS high[e]	2(6.5 %)	7(22.6 %)	
Multifocal disease	No	24 (77.4 %)	28 (90.3 %)	0.306[p]
	Yes	7 (22.6 %)	3 (9.7 %)	
Grade	1	0 (0 %)	2 (6.5 %)	0.199[o]
	2	5 (16.1 %)	8 (25.8 %)	
	3	26 (83.9 %)	21 (67.7 %)	
T	T1	6 (19.4 %)	8 (25.8 %)	0.522[o]
	T2	9 (29 %)	12 (38.7 %)	
	T3	1 (3.2 %)	1 (3.2 %)	
	yT0	2 (6.5 %)	3 (9.7 %)	
	yT1	5 (16.3 %)	4 (12.9 %)	
	yT2	4 (12.9 %)	3 (9.7 %)	
	yT3	4 (12.9 %)	0 (0 %)	
N	Nx	1(3.2 %)	1(3.2 %)	0.646[o]
	N0	6(19.4 %)	9(29 %)	
	N1	4(12.9 %)	7(22.6 %)	
	N2	4(12.9 %)	2(6.5 %)	
	N3	2(6.5 %)	2(6.5 %)	
	yN0	4(12.9 %)	5(16.1 %)	
	yN1	6(19.4 %)	4(12.9 %)	
	yN2	2(6.5 %)	1(3.2 %)	
	yN3	2(6.5 %)	0(0 %)	
ER	ER+	13 (41.9 %)	19 (61.3 %)	0.127[o]
	ER-	18 (58.1 %)	12 (38.7 %)	
PgR	PgR+	4 (12.9 %)	12 (38.7 %)	0.04[p]
	PgR-	27 (87.1 %)	19 (61.3 %)	
ER + PgR+		4	12	0.149[p]
ER + PgR-		9	7	
Her2	Her2+	6 (19.4 %)	11(35.5 %)	0.155[o]
	Her2-	25(80.6 %)	20(64.5 %)	
Ki67	high	31 (100 %)	24 (77.4 %)	0.01[p]
	low	0 (0 %)	6 (19.4 %)	
	nd[r]	0 (0 %)	1 (3.2 %)	
P53	positive	15 (48.4 %)	17 (54.8 %)	0.774[o]
	negative	9 (29.3 %)	12 (38.8 %)	
	nd[r]	7 (22.6 %)	2 (6.5 %)	
NPI[f]	median (range)	6(3.24–8.5)	4.65(3.24–7.2)	0.03[q]
	excellent (2–2.4)[g]	0 (0 %)	0(0 %)	0.172[o]
	good(>2.4–3.4)[h]	2(6.5 %)	5(16.1 %)	
	intermed(>3.4–5.4)[i]	7(22.6 %)	12(38.8 %)	
	poor(>5.4)[j]	14(45.1 %)	9(29 %)	
	na[k]	8(25.8 %)	5(16.1 %)	
LVI[l]	no	10 (32.3 %)	15(48.4 %)	
	yes	19 (61.3 %)	15 (48.4 %)	0.228[o]
	nd[r]	2 (6.5 %)	1 (3.2 %)	

Table 3 (continued)

		PABC *N*=31	Control *N*=31	*p*-value
IHC-Subtype[m]	LumA	0 (0 %)	5 (16.1 %)	0.015[o]
	LumBprol	10 (32.3 %)	8 (25.8 %)	
	LumBHer2	3 (9.7 %)	6 (19.4 %)	
	Her2	3 (9.7 %)	5 (16.1 %)	
	TNBC[n]	15(48.4 %)	7 (22.6 %)	

[a] Invasisve ductal carcinoma, [b] Extensive intraductal carcinoma component non-high nuclear grade, [c] Extensive intraductal carcinoma component high nuclear grade, [d] Ductal carcinoma in situ non-high nuclear grade, [e] Ductal carcinoma in situ high nuclear grade, [f] Nottingham Prognostic Index, [g] Excellent prognosis by NPI, [h] Good prognosis by NPI, [i] Intermediate prognosis by NPI, [j] Poor prognosis by NPI, [k] NPI not assessable- partial or complete pathological response after neoadjuvant chemotherapy, [l] Lympho-vascular invasion, [m] Immunohistochemistry-based subtype, [n] Triple negative immunophenotype,[o] Chi-square test, [p] Fisher's exact test, [q] Wilcoxon test, [r] No data

extensive intraductal carcinoma component as compared with 9.7 % in controls. Forty-five percent of controls did not have in situ carcinoma associated with the invasive tumor. The predominant in situ lesion of controls was focal, high grade ductal carcinoma in situ (22.6 %).

Upon multivariate Cox proportional model analysis pregnancy related status was associated with both relapse and survival, while age was associated with relapse (Supplementary Material 3).

Discussion

As far as outcome of PABC is concerned data are conflicting: some authors showed no difference in outcome of PABC and non-PABC cases [14, 16, 24], while others consider PABC as having unfavorable prognosis [1, 7–9]. In a recent meta-analysis of 30 studies, Azim et al. found that PABC patients had poor overall survival especially if breast cancer was diagnosed within 1 year postpartum [25]. Our study also demonstrates inferior outcome of PABC patients, systemic relapse and death being significantly more common in this group. Disease free survival of both pregnant and postpartum patients was worse than that of controls, and overall survival of the postpartum group was inferior compared to that of controls.

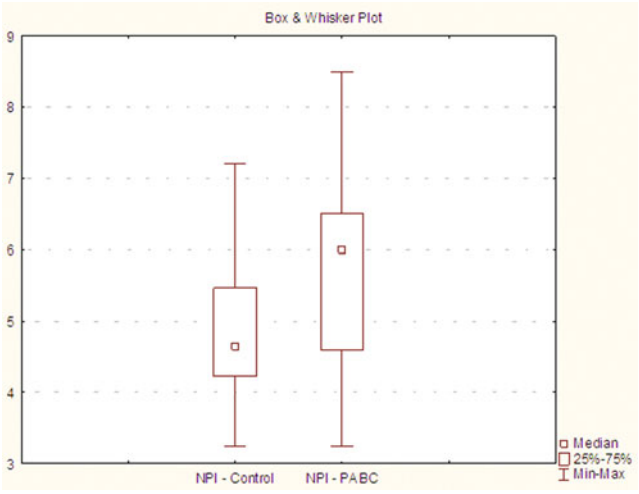

Fig. 3 Nottingham Prognostic Index of PABC and control cases (p=0.03, Wilcoxon)

Previous studies have addressed the hypothesis that the unfavorable outcome of PABC is related to delayed diagnosis resulting in advanced disease at presentation [2, 26]. We found no significant delay in surgery and initiation of oncological treatment of PABC versus control patients, although we could not assess the time elapsing between manifestation of first symptoms and time of initial diagnosis. Disease at presentation (size, T, N) was not found to be significantly different between the two groups; treatment modalities (surgery, neo-adjuvant or adjuvant treatment frequency or regimen) did not differ except for the frequency of endocrine therapy, which was more common in the control group.

NPI is a continuous variable that combines several prognostic factors of BC (grade, tumor size, number of metastatic lymph nodes) [27, 28]. Although there was no significant difference between PABC and non-PABC when tumor size, T, N and grade at initial presentation were assessed separately, the combination of these factors by NPI resulted in significant difference: based on median NPI value the PABC group was categorized as having poor prognosis, while the non-PABC group as having intermediate prognosis. This finding suggests differences in tumor aggressiveness, which can not be captured by individual assessment of classical histopathological prognostic factors.

Progesterone receptor expression showed significant difference between PABC cases and controls, with PABC being mainly PgR negative. Although there were more ER + PgR- LuminalB tumors in PABC patients, the difference was not statistically significant, thus the detected difference in PgR expression is driven mainly by the over-representation of triple negative tumors in PABC, since Her2 expression did not show major differences.

Ki67 labeling index was above 15 % in all PABC cases, reflected also by the difference in IHC-based tumor subtypes, namely that there were no LumA tumors in this group. From all PABC cases 48.4 % showed triple negative and 32.3 % LumBprol phenotype. These two subtypes represented around 80 % of all PABC cases, while only 48 % of the control cases. Our finding on the over-representation of TNBC in PABC is in concordance with that of other authors [18]. Survival assessed by IHC-based subtypes showed the worst survival of patients with LumBprol and triple negative phenotype, especially in postpartum cases. These findings underline the importance of Ki67 testing in ER + Her2- tumors, in order to detect tumors of LumBprol subtype with an eventual worse prognosis.

Associated ductal in situ lesions are known to have an impact on local control of breast cancer. There is a higher risk of local recurrence if ductal carcinoma in situ demonstrates high nuclear grade, comedo necrosis, is extensive, involves surgical margins or the patient is young [29–31]. These factors are not entirely unrelated though, since young patients (≤45 years) tend to have high nuclear grade in situ lesions with comedo necrosis and the extent of DCIS is significantly greater compared to older women [29, 31].

In our study, PABC as compared with non-PABC was more often associated with an extensive intraductal carcinoma component showing high nuclear grade and comedo necrosis, but there was no difference in local relapse. Our finding on the one hand can underline the unfavorable biology of PABC, since increasing molecular evidence suggest that high grade in situ ductal lesions harbour complex and heterogeneous genomic alterations that are highly similar to that of high grade IDC and these aberrations are different from that of low grade DCIS and IDC [32, 33]. On the other hand, this finding can also incite thoughts about the significantly more extensive involvement of ducts by high grade in situ lesions in PABC, which might be a further consequence of the altered milieu related to pregnancy and the postpartum condition.

Possible explanations for the detected outcome differences between PABC and non-PABC besides different tumor biology may involve the relative immunosuppressed state associated with pregnancy, the altered hormonal milieu (the placenta and the fetus as new endocrine "organs", higher estrogen, progesterone and growth hormone levels during pregnancy, increased prolactin level during lactation). Changes in tumor microenvironment during pregnancy (increased responsiveness of the breast to growth factor stimulation) or during postpartum remodeling—when extracellular matrix modifications associated with post-lactational involution create a milieu that resembles wound-healing [34] - are all known factors enhancing tumor growth and metastasis. O'Brien et al. have demonstrated the role of post-lactational involution-associated, alternatively activated M2-type macrophages that share characteristics with Tumor-associated macrophages (TAMs). TAMs, by secreting growth factors, enhance tumor growth. Immature macrophages might also suppress cytotoxic T-cell function, thus creating an immunosuppressed microenvironment [35, 36]. Fornetti et al. have proposed a model showing how post-lactational epithelial cells transition to become phagocytic and engulf the apoptotic (formerly milk-producing) cells during involution while secreting cytokines and growth factors, thus resulting in a tumor-supportive microenvironment [34].

The weakness of our study is its retrospective nature and the lack of information on the time elapsed between first symptoms and first diagnosis of breast cancer. Its strength is the combination of management data and survival analysis with detailed histopathology and IHC data analysis, including not only HR status, but Her2, Ki67, p53, IHC-based subtype, NPI and associated in situ lesion analysis as well. To the best of our knowledge these factors have not been investigated so far together in a matched case control study on PABC.

In conclusion, our study has demonstrated inferior outcome of PABC, especially when detected postpartum. Unfavorable tumor biology was reflected by the predominance of triple negative and LumBprol tumors and PABC was commonly associated with high grade EIC. To our best knowledge, this latter finding has not been previously addressed. Individual analysis of classical prognostic factors could not detect inferior prognosis of PABC, but combination of these factors by NPI has predicted the aggressive nature and poor prognosis of pregnancy associated breast cancer. Based on the literary data, it seems that besides tumor biology, the tumor microenvironment altered by pregnancy and the postpartum state, most probably also plays important role in the inferior outcome of the disease. This issue is still underinvestigated though, new treatments (e.g. successful targeting of tumor microenvironment) however might contribute to improving the prognosis of PABC.

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Conflict of interest The authors declare that they have no conflicts of interest.

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