Pregnancy and Post-partum Breast Cancer: A Prospective Study

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Abstract. *Background: The concomitant occurrence of breast cancer and pregnancy remains a challenging clinical situation combining ethical and medical problems. There are few prospective data on pregnancy-associated breast cancer (PABC) whose incidence continuously increases. Patients and Methods: Forty patients with PABC were compared with 61 non-pregnant, age-matched patients with infiltrative breast carcinomas (BC) diagnosed and followed since 1982. Results: Although PABC and BC tumor size, grade and type, and lymphovascular and lymph-node invasion were similar, the BC cases showed better overall- (p=0.0001) and disease-free (p=0.015) survival. Moreover, the outcome of pregnant patients was worse than post-partum patients (p=0.017). Importantly, the number of PABC patients receiving hormonotherapy was lower than the BC patients (p<0.0004), due to lower estrogen receptor (ER) (p=0.038) and progesterone receptor (PR) (p=0.008) immunohisto-chemical (IH) levels. Retrospective estrogen-regulated pS2/trefoil factor 1 (pS2/TFF1) immunohistochemitry showed no difference between PABC and BC. All the children delivered were healthy. Conclusion: Pregnancy and the post-partum period increase breast cancer aggressiveness, pregnancy being the most detrimental. PABC hormone-dependence is under-estimated using ER and PR, and pS2/TFF1 might help in its determination. Appropriate treatment does not impair child outcome.*

Pregnancy-associated breast cancer (PABC) occurs during pregnancy or within 1 year after delivery (1). It is a rare disease, but its incidence (1/3,000 to 1/10,000 pregnancies) is increasing as more women choose childbearing at a later age (2). To date, few prospective data on PABC have been reported (reviewed in (3)), and most of the studies have been small (10-15 patients) (4, 5) or corresponded to data compilation from different sources (6, 7).

The prognosis for PABC is unfavorable, although the reasons are still unclear (3). Multiple clinical and biological hypotheses have been proposed (8). Many reports have emphasized the patients' young age (9) or the advanced state of the disease at detection (10). Another hypothesis is a transient immunosuppressive state during pregnancy and childbirth, as well as relative insulin-resistance (11). Prolactin and its receptors have also been implicated as promoters of tumor cell growth and progression (12, 13), whereas human chorionic gonadotropin (hCG) has an antagonistic effect. Russo *et al*. have demonstrated that pregnancy or short-term treatment with hCG protects virgin rats from chemically-induced mammary carcinogenesis (14, 15). Finally, the hormonal status of PABC is unclear. Previous studies have reported that they were hormone-independent or slowly hormone-dependent (4-6). However, high circulating levels of estrogen and progesterone might interfere in estrogen (ER) and progesterone (PR) receptor determination (16).

In the present prospective clinical study, several clinical and biological parameters, tumor hormonal status and patient and child outcome were assessed using a homogeneous series of 40 PABC cases. Sixty-one breast cancer (BC) cases from non-pregnant age-matched patients during the same period served as controls.

Patients and Methods

Patients. Between 1982 and 2004, 40 infiltrative PABC (18 pregnancy, 22 post-partum) were treated at our Breast Center (Centre Hospitalier Régional Universitaire, Strasbourg, France) and 61 age-matched, non-pregnant patients, treated for infiltrative BC during the same period, were selected as controls (Tables I and II). The PABC and BC treatment adhered to the same principles. Informed consent was obtained from all the patients.

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Key Words: Breast cancer, pregnancy, hormonotherapy, patient outcome, child outcome.

Clinical and biological parameters. The studied parameters were age at diagnosis and parity for BC; for PABC, the gestational age, pregnancy outcome, cancer antigen 15.3 (CA 15.3) and carcinoembryonic antigen (CEA) levels in serum (values above 30 IU/ml and 5 ng/ml, respectively, were considered abnormal). Patient follow-up included a yearly clinical examination with mammogram, chest radiography, pelvic and abdominal ultrasound, CA 15.3 and CEA analysis and child health.

Tumor parameters. Primary tumor characteristics included histological type and size, histological Scarff, Bloom and Richardson grade (17). Lymphovascular and lymph node (LN) invasion were analyzed using Haematoxylin-Eosin (HE) staining.

ER and PR immunohistochemistry (IH) were performed on the formalin-fixed, paraffin-embedded primary tumors using mouse monoclonal antibodies (ER, 1:20 dilution, clone 6F11; PR, 1:100 dilution, clone 16 PR 312; Tebu-Bio, F-Le Perray en Yvelines, France), appropriate second antibodies and the biotin-streptavidin-peroxidase method. All the samples were analyzed by the same pathologist to avoid individual differences. An H-score, including the staining intensity (from 0 to 3+) and the percentage of stained tumor cells, of 20 and above was considered as positive (18).

Retrospective pS2/trefoil factor 1 (pS2/TFF1) IH was performed as described above using conserved paraffin-embedded samples and p28O2 mouse monoclonal antibody (1:50 dilution; IGBMC/Euromedex, F67460 Souffelweyersheim, France).

Statistical analysis. The data are given as mean and standard deviation (SD). The statistical tests used for the comparisons were Fisher's exact test and *t*-test. The overall and disease-free survival curves were established using the Kaplan-Meier method. *P*-values <0.05 were considered to be statistically significant.

Results

Characteristics and treatments for the 40 PABC patients. All the data are summarized in Tables I and II. Twenty-two of the tumors (55%) showed lymphovascular invasion that was previously reported for BC developing in young women (19). Axillary clearance was performed in 39 patients and metastases were found in 18 cases (45%). ER and PR were positive in 19 (47.5%) and 13 (32.5%) cases, respectively.

In 31 patients, surgery was followed by radiation therapy. Thirty women were treated by chemotherapy after delivery (doxorubicin 50 mg/m^2 or epirubicin 100 mg/m^2, fluorouracil 500 mg/m^2 and cyclophosphamide 500 mg/m^2 every 3 weeks, 6 cycles) and 3 received treatment during pregnancy (doxorubicin 50 mg/m^2 or epirubicin 75 mg/m^2 and fluorouracil 500 mg/m^2 every 3 weeks). Taking the ER and PR levels into account, hormonal therapy (tamoxifen 20 mg daily) was administered after delivery in 18 patients (45%).

Characteristics and treatments for the 61 BC controls. All the data are summarized in Tables I and II. Lymphovascular invasion was identified in 31 tumors (51%). An axillary clearance was performed in all 61 cases (100%) and LN metastases were found in 33 cases (54%). ER and PR were positive in 42 (69%) and 37 (61%) cases, respectively.

In 57 cases (93%), surgery was followed by radiotherapy. Fifty-two women (85%) were treated by chemotherapy (doxorubicin 50 mg/m^2 or epirubicin 100 mg/m^2, fluorouracil 500 mg/m^2 and cyclophosphamide 500 mg/m^2 every 3 weeks, 6 cycles). Hormonotherapy (tamoxifen 20 mg daily) was administered in 49 cases (80%).

ER and PR differences between PABC and BC patients. The patient/tumor characteristics for the PABC and BC groups showed only few differences. More of the BC than PABC patients received locoregional radiotherapy (93% *versus* 77.5%, $p=0.0311$). More importantly, ER (47.5% *versus* 69%, $p=0.038$) and PR (32.5% *versus* 61%, $p=0.008$) positivity were lower in the PABC cases. Subsequently, hormonotherapy was administered to 45% of the PABC and 80% of the BC patients ($p=0.0004$). These results prompted us to perform retrospective pS2/TFF1 IH analysis (20) using conserved primary tumors and/or metastases to re-evaluate the PABC hormone dependence, since pS2/TFF1 has been shown to be correlated with hormone dependence in breast tumors from non-pregnant patients (21). pS2/TFF1 was detected in the cytoplasm of cancer cells (Figure 1A and B), as previously reported in breast carcinomas from non-pregnant patients (21). pS2/TFF1 positivity was similar in the PABC (23 cases; 57.5%) and the BC (28 cases; 46%) (Table I). It was observed in 16/19 ER-positive PABC (84%). Among the 21 ER-negative PABC, 7 (33%) were pS2/TFF1-positive (3 during pregnancy and 4 post-partum). Likewise, 25/42 ER-positive BC were pS2/TFF1-positive (60%), and 3/19 (16%) ER-negative BC were pS2/TFF1-positive.

Outcome in PABC and BC. The follow-up information is summarized in Table II. The follow-up times were similar for the PABC and BC cases. The PABC ten-year survival was only 70% compared with 97% for the BC cases ($p=0.0001$). The overall survival curves are shown Figure 2A; the BC patients had significantly better five-year ($p=0.0347$) and ten-year ($p=0.0001$) overall survival.

Distant metastases occurred in 13 (32%) PABC and 8 (13%) BC cases. The metastases developed more rapidly in PABC than in BC (65 (SD 35) *versus* 90.5 (SD 42.5) months; $p=0.0023$). All the PABC patients who developed metastases died, while 75% of the BC patients with metastases were still alive at the end of the study ($p=0.0205$). Accordingly, the disease-free survival curve was significantly better for the BC patients ($p=0.0150$) (Figure 2B), as were the five-year ($p=0.0478$) and the ten-year ($p=0.0357$) disease-free survival rates.

Pregnancy PABC compared to post-partum PABC. Most of the patient/tumor characteristics were similar in the pregnancy PABC and the post-partum PABC cases (Tables I and II). However, the parity was lower in the pregnancy PABC than in the post-partum cases (1.3 (SD 1.4) *versus* 2.1

Table I. *Characteristics of the 40 PABC and 61 BC tumors*.

Characteristic	Pregnancy	Post-partum	*p*	PABC	BC	*p*
Tumor size (mm)	25.5 (16.2)	27.8 (27.4)	NS	26.1 (23.2)	24 (15.8)	NS
Grade						
Low	2 (11%)	5 (23%)	NS	7 (17.5%)	13 (21%)	NS
Intermediate	2 (11%)	7 (32%)	NS	9 (22.5%)	22 (36%)	NS
High	13 (72%)	9 (41%)	NS	22 (55%)	25 (41%)	NS
ND	1 (5.5%)	1 (4.5%)	NS	2 (5%)	1 (2%)	NS
Tumor type						
Ductal	16 (89%)	17 (77%)	NS	33 (82.5%)	56 (92%)	NS
Lobular	1 (5.5%)	3 (17%)	NS	5 (12.5%)	4 (7%)	NS
Medullar	1 (5.5%)	1 (4.5%)	NS	2 (5%)	0 (0%)	NS
Tubular	0 (0%)	1 (4.5%)	NS	0 (0%)	1 (1%)	NS
Lympho-vascular invasion	7 (39%)	15 (68%)	NS	22 (55%)	31 (51%)	NS
Lymph nodes						
Axillary clearance	17 (94%)	22 (100%)	NS	39 (97.5%)	61 (100%)	NS
Positive lymph node	9 (50%)	9 (41%)	NS	18 (45%)	33 (54%)	NS
1-3	7 (39%)	5 (23%)	NS	12/18 (66%)	25/33 (76%)	NS
>3	2 (11%)	4 (18%)	NS	6/18 (34%)	8/33 (24%)	NS
Hormonal factors						
ER+	8 (44%)	11 (50%)	NS	19 (47.5%)	42 (69%)	0.038
PR+	5 (28%)	8 (36%)	NS	13 (32.5%)	37 (61%)	0.008
pS2/TFFI+	9 (50%)	14 (64%)	NS	23 (57.5%)	28 (46%)	NS

Data are presented as mean values (+/–SD), or frequency (percentage); NS: not significant; ND: not determined; ER: estrogen receptor; PR: progesterone receptor; pS2/TFF1: pS2/trefoil factor 1.

Table II. *Characteristics, treatment and outcome of the 40 PABC and 61 BC patients*.

Patient characteristic	Pregnancy	Post-partum	*p*	PABC Total	BC	*p*
Number of patients	18	22	-	40	61	-
Age at presentation (years)	33.8 (5.4)	33.3 (3.9)	NS	33.5 (4.6)	35.4 (3.8)	NS
Parity	1.3 (1.4)	2.1 (1.0)	0.041	1.8 (1.3)	1.7 (1)	NS
Time at diagnosis (gestational weeks)	22 (10)	-	-	-	-	-
CA 15-3 (IU/ml)	31.9 (9.3)	18.5 (12.5)	0.0006	24.1 (27.7)	20.3 (17.2)	NS
CEA (ng/ml)	1.9 (3.4)	3.1 (6.3)	NS	2.6 (5.3)	1.3 (1)	NS
Treatment						
Treatment delay (weeks)	7.1 (6.9)	4 (1.1)	0.04	5.6 (4.8)	4.8 (3.6)	NS
Breast conservative surgery	9 (50%)	7 (32%)	NS	16 (40%)	27 (44%)	NS
Mastectomy	9 (50%)	15 (68%)	NS	24 (60%)	34 (56%)	NS
Axillary clearance	17 (94%)	22 (100%)	NS	39 (97.5%)	61 (100%)	NS
Chemotherapy	16 (89%)	17 (77%)	NS	33 (82.5%)	52 (85%)	NS
Radiotherapy	15 (83%)	16 (73%)	NS	31 (77.5%)	57 (93%)	0.0311
Hormonotherapy	8 (44%)	10 (45%)	NS	18 (45%)	49 (80%)	0.0004
Patient outcome						
Mean follow-up period (months)	76.8 (60.5)	79.9 (47)	NS	78.5 (52.8)	85.7 (24.2)	NS
Contralateral breast cancer	0 (0%)	1 (5%)	NS	1 (2.5%)	2 (3%)	NS
New primary breast cancer	0 (0%)	0 (0%)	NS	0 (0%)	5 (8%)	NS
Distant metastases	5 (28%)	8 (36%)	NS	13 (32%)	8 (13%)	0.0247
Alive with metastases	0/5 (0%)	0/8 (0%)	NS	0/13 (0%)	6/8 (75%)	0.0005
5-Year overall survival	15 (83%)	20 (91%)	NS	35 (87.5%)	60 (98%)	0.0347
10-Year overall survival	13 (72%)	15 (68%)	NS	28 (70%)	59 (97%)	0.0001
Mean time to death	49.4 (26)	75.2 (37)	0.017	65.3 (35)	62 (44)	NS
Overall survival rate	13 (72%)	14 (63%)	NS	27 (67.5%)	59 (97%)	0.0001
Children outcome						
Abortions	4	2	NS	6	-	-
Healthy children	14	20	NS	34	-	-

Data are presented as mean values (± SD), or frequency (percentage); NS: not significant; CA: cancer antigen; CEA: carcinoembryonic antigen.

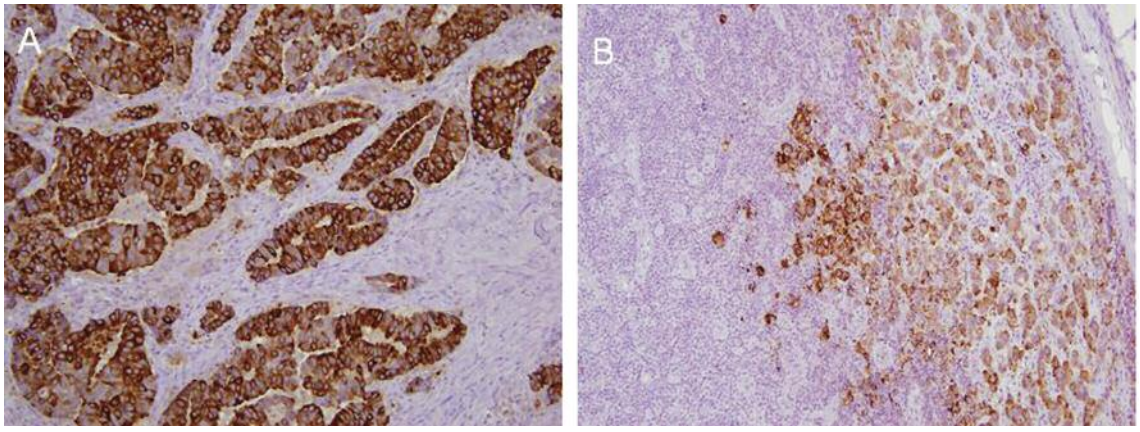

Figure 1. *Immunohistochemistry for pS2/TFF1. Strong cytoplasmic staining in PABC: A. an infiltrative ductal carcinoma; B. an axillary node containing ductal cancer cells. Magnification: ×200.*

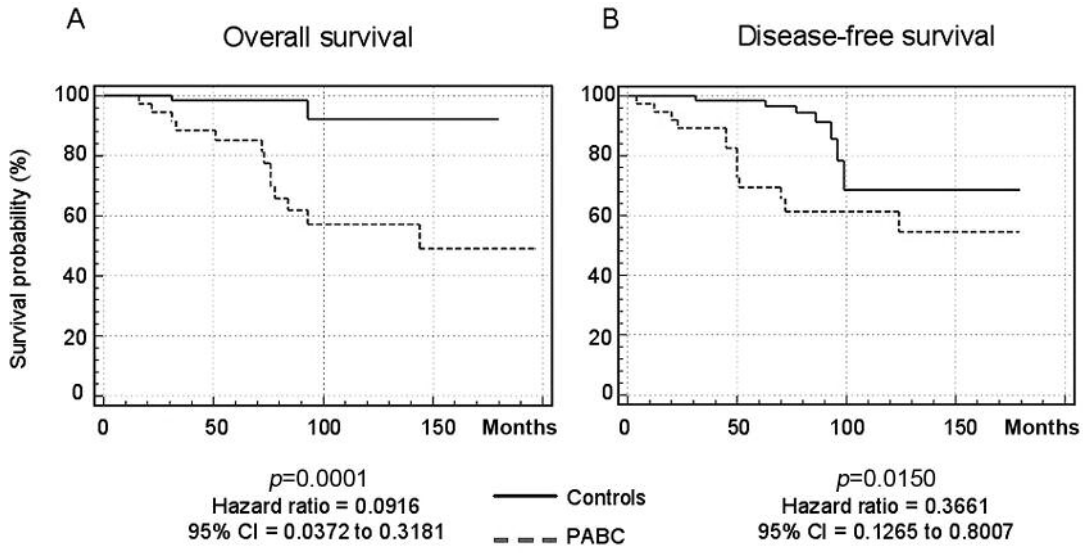

Figure 2. *Survival curves for the 40 PABC and 61 BC. A. Overall survival; B. Disease-free survival. Curves were established using the Kaplan-Meier method.*

(SD 1.0) children, p=0.041). As previously reported (22), higher CA 15.3 levels were found during pregnancy (31.9 IU/ml) compared with the post-partum period (18.5 IU/ml) (p=0.0006). The CEA levels were higher in the post-partum group compared with the pregnant group (3.1 ng/ml *versus* 1.3 ng/ml), but not statistically significantly so. There was also an increased delay in the treatment of pregnancy PABC patients (7.1 *versus* 4 weeks, p=0.04). Finally, the mean time to death was lower in the pregnancy cases than in the post-partum cases (49.4 *versus* 75.2 months, p=0.017).

PABC treatment and child outcome. There was one spontaneous abortion and one extra-uterine pregnancy. Four patients opted for therapeutic abortion and the rest for pregnancy continuation. The genders of the 38 offspring were 24 girls (63%) and 14 boys (37%). Thirty-four children were alive at the end of the study and all but one, treated for hydrocephaly diagnosed before breast cancer treatment, were healthy.

Discussion

The present study benefited from a highly homogeneous PABC series, with the controls similarly treated over the same time period and all the histopathology analysed in the same laboratory by the same pathologist.

Despite numerous patient characteristics and treatment similarities, the progression of the PABC cases was dramatically worse than that of the BC cases. It has been

shown that pregnancy and the post-partum period induce intense modifications in cell proliferation and survival as well as tissue angiogenesis and remodeling (8), all processes known to favor tumor progression (23). The occurrence of invasive cancer cells in such a favorable microenvironment might therefore be responsible for increased tumor aggressiveness. Moreover, the tumors occurring during pregnancy were more aggressive than those occurring during the post-partum period, and the patients died around two years earlier (p=0.017). This indicated that pregnancy and post-partum are distinct PABC sub-groups, and that the cellular and/or molecular modifications specific to either pregnancy or the post-partum period impact tumor progression differently. It has been proposed that when pregnancy occurs in women whose breast tissue already contains *in situ* tumor or cancer cells, increased proteolysis enhances the invasive and metastatic potential of the breast carcinomas, leading to a poorer clinical outcome (24).

The worse PABC outcome might also result from less aggressive treatments, particularly when pregnancy is continued. In the present study, the treatment delay and surgical treatments were similar in the PABC and the BC cases. Breast conservation was performed in 16 PABC without recurrence, confirming that it is an alternative to radical surgery if radiotherapy can be scheduled after delivery (25). Radiotherapy administration was less frequent in the PABC than in the BC cases (p=0.0311). However, since this treatment concerns locoregional areas, it might be expected that this difference would not be the major factor responsible for the poor patient outcome. The adjuvant chemotherapy based on stage, age and pathological findings was not greatly different between the PABC and BC cases, but was used during pregnancy only when delay in treatment initiation was likely to impact maternal survival (26). It was never given during the first pregnancy trimester to avoid teratogenicity and is well tolerated by the fetus at later stages (27).

The most important treatment discrepancy in our series concerned the hormonotherapy. First, hormonotherapy was usually delayed until the resumption of menstruation since tamoxifen can lead to teratogenesis (Goldenhar syndrome or ambiguous genitalia) and thrombosis (28). Second, the number of PABC patients receiving hormonotherapy was dramatically lower than the BC patients (p<0.0004). Indeed, consistent with previous studies (5-7, 29), significantly less ER (p=0.038) and PR (p=0.008) were detected in the PABC than in the BC patients. However, using retrospective pS2/TFF1 IH analysis the low ER and PR levels observed in PABC were demonstrated to be artefactual (4) and, at diagnosis, PABC hormone-dependence had been underestimated using ER and PR IH. pS2/TFF1 IH was developed in the late nineteen eighties (17), and routinely used in the early nineties. It has been reported that about 50% of primary breast tumors and metastases overexpress pS2/TFF1 (20) and pS2/TFF1 positivity was a predictive factor of hormonotherapy response (30). In contrast, pS2/TFF1 negativity in ER-positive breast carcinomas was associated with reduced hormonotherapy response (31). To date, only one series including 12 PABC cases has reported pS2/TFF1 positivity in 67% of the tumors (32). In our series, 57.5% of the PABC cases were pS2/TFF1-positive, a percentage similar to that observed in BC and clearly higher than those observed for ER (47.5%) and PR (32.5%) positivity. Thus, pS2/TFF1 determination might be particularly useful during pregnancy and the post-partum period to better determine the hormone-dependence of breast tumors and optimize hormonotherapy prescription. Finally, the pregnant PABC patients had a higher delay in hormonotherapy administration than did the post-partum PABC patients (p=0.04). Since they also had the worst outcome, this strongly argues for the beneficial effects of hormonotherapy on PABC outcome.

Finally, in our study, 34 children were born and remained healthy. There were 24 girls (63%), whereas 49% would have been expected (32). To date, no data are available concerning the relationship between offspring gender and risk of breast cancer during pregnancy. However, Hsieh *et al*. (33) have reported that pregnancies with male fetuses have a protective effect for young women. Thus, although not statistically significant, the higher number of female fetuses in the present study suggests a similar protective effect of male fetuses on PABC.

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