BREAST CANCER DIAGNOSED IN THE POST-WEANING PERIOD IS INDICATIVE FOR A POOR OUTCOME

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ABSTRACT

Background: In young women, a breast cancer diagnosis after childbirth increases the risk for metastasis and death. Studies in rodents suggest that post-weaning mammary gland involution contributes to the poor prognosis of postpartum breast cancers. However, this association has not been investigated in humans, mainly due to missing information on the patient's lactation status at diagnosis.

Patients and methods: Clinicopathological data of 1,180 young women with primary invasive breast cancer, diagnosed within two years postpartum (PP-BC), during pregnancy (Pr-BC) or nulliparous (NP-BC), were collected. For PP-BC patients, breastfeeding history was retrieved to differentiate breast cancers identified during lactation (PP-BC$_{DL}$) from those diagnosed post-weaning (PP-BC$_{PW}$). Differences in prognostic parameters, first site of distant metastasis and risks for metastasis and death were determined between patient groups.

Results: Cox proportional hazard models pointed to a 2-fold increased risk of metastasis and death in PP-BC$_{PW}$ patients compared to PP-BC$_{DL}$ (HR 2.1 [P_{DRS}=0.021] and 2.9 [P_{OS}=0.004]), Pr-BC (HR 2.1 [P_{DRS}<0.001] and 2.3 [P_{OS}<0.001]) and NP-BC (HR 2.1 [P_{DRS}<0.001] and 2.0 [P_{OS}<0.001]) patients. Prognosis was poorest for PP-BC$_{PW}$ patients who did not breastfeed or only for ≤3 months prior to diagnosis. This could not fully be attributed to differences in standard prognostic characteristics. In addition, PP-BC$_{PW}$ tumors showed a 3- to 8-fold increased risk to metastasize to the liver, yet this did not correlate with the poor outcome of this patient cohort.

Conclusions: Breast cancer diagnosed shortly after weaning specifically adds to the poor prognosis in women diagnosed with PP-BC. Apart from the importance of an increased awareness, these data show that detailed lactation data need to be registered when breast cancer outcome in young women is investigated.

Key words: postpartum breast cancer, involution, post-weaning, lactation, metastasis, prognosis

1. INTRODUCTION

A postpartum breast cancer (PP-BC) diagnosis is associated with a significant increased risk for metastasis and death compared with breast cancers diagnosed in young premenopausal women beyond the postpartum window.[1-5] Though this effect is most pronounced when the cancer diagnosis is made in the first two years postpartum,[6-8] it may extend up to 10 years.[2,3,9] Most studies show that the poor prognosis of PP-BC is independent of standard prognostic factors, like maternal age, molecular cancer subtype, tumour size, lymph node status or grade.[2,3,6,10,11] The exact mechanisms underlying this high risk of recurrence remain however under investigation.

It has been hypothesized that postpartum mammary gland involution, being a unique biological process in post-weaning breast tissue, may account for the increased metastatic risk and poor survival among PP-BC patients.[12,13] During pregnancy, the mammary gland epithelium undergoes proliferation and differentiation in order to prepare for lactation. After parturition in the absence of lactation, or at weaning, the gland remodels to a state morphologically and functionally similar to pre-pregnancy, a process called postpartum involution.[14] Animal studies revealed that the involution process resembled tissue-remodelling programs that are activate during wound healing, with a characteristic initial inflammatory response followed by an immunosuppressive phase[12,15-17] and that this process could stimulate tumour growth, motility and invasion.[18-21] Although it has been shown in humans that normal postpartum breast tissue is also characterized by an increased immune cell influx,[22,23] a wound-healing-like immune pattern[19,24] and mammary and lymphatic remodelling as seen in rodents,[21,25,26] it has never been explored whether the process of mammary gland involution is effectively associated with a poor prognosis in PP-BC patients. One major limiting factor is the lack of detailed data on lactation status at the time of cancer diagnosis, necessary to define the post-weaning period as a surrogate for mammary gland involution. To this end, we initiated this retrospective study in a large cohort of 1,180 young breast cancer patients with unique breastfeeding information. We compared the outcome of breast cancer patients specifically diagnosed during the post-weaning period with that of patients diagnosed outside this specific window.

2. METHODS

2.1 Patient data collection

Patient data were retrospectively collected from University Hospitals Leuven and 13 centres participating in the International Network on Cancer, Infertility and Pregnancy (INCIP) (eFig. 1 in the data article). Given the prognostic association between age and breast cancer outcome, which may vary for different breast cancer subtypes[27], we only included premenopausal women diagnosed with primary invasive breast cancer, aged 25-40 years. All women were diagnosed between January 1995 and December 2017 (Fig. 1A). Due to an equal distribution of patients across different time frames of diagnosis (1995-1999; 2000-2004; 2005-2009; 2010-2014; 2015-2017), we could eliminate major bias, such as treatment advances, related to period of enrollment (Table 1). The following data were retrieved: (a) therapy-related characteristics: surgery; radiotherapy (RT); chemotherapy (CT); hormonal therapy (HT) and/or anti-HER-2 treatment, (b) patient-related characteristics (if applicable): age at diagnosis; year of diagnosis; date of most recent delivery; gravidity; parity; number of miscarriages; lactation history; date of distant recurrence; site of distant recurrence and clinical outcome, and (c) tumour-related characteristics: clinical stage; tumour size; lymph node (LN) infiltration; pN subtype (N0, N1, N2, N3); grade; histological type and surrogate molecular subtype. ER, PR and HER-2 status were evaluated using immunohistochemistry according to ASCO/CAP guidelines.[28,29] Additional *in situ* hybridization techniques were used to confirm *HER-2* gene amplification according to each participating centre's guidelines. Tumours were classified as Luminal A-like (ER positive, HER-2 negative, grade 1-2), Luminal B-like (ER positive, HER-2 negative, grade 3), Luminal HER-2 (ER positive, HER-2 positive, any grade), HER-2-like (ER negative, HER-2 positive, any grade) or triple-negative breast cancer (TNBC: ER negative, PR negative, HER-2 negative, any grade). Follow-up data were obtained by medical record review. For PP-BC patients specifically, patient files were reviewed thoroughly to assess, for each parity, if and for how long breastfeeding was given. The study was approved by the Ethics Committee Research UZ/KU Leuven (study number: S25470).

2.2 Patient classification according to timing of diagnosis

Based on breastfeeding history prior to the breast cancer diagnosis, patients were classified as (i) PP-BC$_{PW}$, if diagnosed within two years Post-Weaning, (ii) PP-BC$_{DL}$, if diagnosed During Lactation, (iii) Pr-BC, if diagnosed during PRegnancy or (iv) NP-BC, if never been pregnant (NulliParous patients) (Fig. 1B). Delineating the post-weaning period at 2 years after cessation of lactation, enabled us to collect sufficient patient numbers whilst preserving homogeneity with regard to the postpartum time frame. PP-BC$_{PW}$ cases were further subdivided in (i) PPBC$_{PW/NL}$, if patients Never Lactated, (ii) PP-BC$_{PW/Lshort}$,

when lactating ≤3 months, and (iii) PP-BC$_{PW/LIong}$, when lactating >3 months prior to the cancer diagnosis. No patient breastfed for >24 months.

2.3 Statistical analyses

A priori power calculations indicated that small clinicopathological (10%-15%) and prognostic differences (Hazard Ratio, HR 2.0) between PP-BC$_{PW}$ subgroups and Pr-BC and NP-BC groups, and larger differences (HR ≥3.0) within the PP-BC subgroups could be identified with sufficient power (>70%). Frequencies of prognostic categorical variables were evaluated using Chi-Square testing. Continuous variables were compared via One-Way ANOVA or Kruskal-Wallis analyses. Odds Ratios (OR) with 95% Confidence Interval (CI) were determined using multinomial logistic regression.

The risk of distant recurrence and death of any cause was determined using Kaplan-Meier analyses. Log-rank tests assessed differences between distant recurrence and survival probabilities across groups. Distant recurrence-free survival (DRS) was calculated from the date of diagnosis to first systemic metastasis; overall survival (OS) from the date of diagnosis to death from any cause. Since exclusion of cases with stage IV disease did not influence the outcome of regression modelling (eFig. 5-6 in the data article), cases with stage IV cancer at diagnosis were included in OS analyses to reflect true population outcomes. To determine which prognostic parameters affected DRS and/or OS, univariate Cox regression analyses were performed. Variables that significantly differed between patient groups or that were associated with OS and/or DRS were used in multivariate Cox proportional hazards regression models to assess the association between patient group and prognosis. For each parameter proportional hazards assumptions were examined graphically using residual analyses. The influence of centre of diagnosis on prognostic differences was ruled out using Cox regression analyses (eFig. 4 in the data article).

Binary logistic regression was used to assess the effect of patient group on the frequency of metastasis to different organs. To avoid potential confounding due to multi-site metastasis, we only took into account the primary site of metastatic disease. Patients with multi-site metastatic disease or unknown site of first metastatic recurrence were excluded. The association between site of metastasis and study group was assessed using two-sided Fisher's Exact tests. The association between metastatic site and OS probability was determined using Cox regression analyses. Statistical analyses were performed using R v3.4.4. $P<0.05$ was considered statistically significant.

3. RESULTS

3.1 PP-BCPW had different clinicopathological characteristics than PP-BCDL

Based on the timing of their breast cancer diagnosis relative to their pregnancy history and lactation status at diagnosis, 189 women in our cohort were assigned to the PP-BCPW group, 53 to PP-BCDL, 492 to Pr-BC and 446 to NP-BC (Fig. 1). We then assessed whether host- and tumour-related prognostic parameters differed among these groups (Table 1 and eFig. 2 in the data article). Compared to NP-BC patients, PP-BCPW cases were significantly more often diagnosed with (i) stage IIIC disease, (ii) more LN infiltration and (iii) higher graded disease. When comparing to PP-BCDL and Pr-BC patients, significantly more early stage and luminal-A-like tumours were found in PP-BCPW patients. Strikingly, tumour characteristics of PP-BCDL were resembling those of Pr-BC (eTable 1 in the data article).

When assessing treatment modalities, PP-BCPW patients were found to be significantly more often treated with RT than other patient groups (Table 1). PP-BCPW patients were also more likely to receive adjuvant CT and less HT than NP-BC cases, concurring with observed differences in surrogate molecular subtype and grade between these groups. PP-BCPW cases received less often CT than Pr-BC patients.

3.2 PP-BCPW patients showed a two-fold increased risk for metastasis and death

To investigate whether a post-weaning diagnosis in PP-BC patients was associated with a poor prognosis, we assessed whether the risk of metastasis and death in PP-BCPW patients differed from that in PP-BCDL, Pr-BC and NP-BC patients. PP-BCPW cases demonstrated a higher five-year probability for death and metastases (25% and 34%, respectively) compared to PP-BCDL (16% and 30%), Pr-BC (13% and 18%) and NP-BC (11% and 15%) patients. Unadjusted Kaplan-Meier survival analyses confirmed that PP-BCPW patients had an approximate 2-fold increased risk of metastasis and death (P<0.001) (Fig. 2A and B). Only for PP-BCDL cases the observed differences in OS (P=0.303) and DRS (P=0.381) did not reach statistical significance.

After adjustment for prognostic host- and tumour-related variables that (i) significantly differed between patients groups (Table 1 and eTable 1 in the data article) and/or (ii) were significantly correlated to OS and/or DRS (eTable 2 in the data article), PP-BCPW cases still displayed significantly decreased survival rates compared to PP-BCDL, Pr-BC and NP-BC patients (P-values ranging from 0.021 to <0.001) (Fig. 2C and D). Remarkably, outcome PP-BCDL patients was similar to that of Pr-BC and NP-BC cases and seemed to be mainly driven by poor prognostic characteristics.

3.3 No or short prior breastfeeding correlated with the poorest outcomes in PP-BCPW

Detailed breastfeeding data were originally obtained to differentiate PP-BC$_{PW}$ patients, diagnosed post-weaning, from PP-BC$_{DL}$ patients, diagnosed during lactation. This information also allowed us to assess the influence of prior breastfeeding duration on breast cancer outcome. Among PP-BC$_{PW}$ cases, there were 69 PP-BC$_{PW/NL}$ patients who never lactated, 72 PP-BC$_{PW/Lshort}$ cases who lactated ≤3 months and 48PP-BC$_{PW/Llong}$ case who lactated >3 months prior to their breast cancer diagnosis (Fig. 1). Due to small numbers, PP-BC$_{DL}$ patients, still lactating at diagnosis, were not further subdivided.

Unadjusted regression analyses indicated that PP-BC$_{PW/NL}$ patients had the poorest prognosis, with an almost 2-fold increased risk for death and metastasis compared to PP-BC$_{PW/Llong}$ (P_{OS}=0.035, P_{DRS}=0.030), Pr-BC ($P_{OS/DRS}$<0.001), NP-PB ($P_{OS/DRS}$<0.001) and PP-BC$_{DL}$ (P_{OS}=0.078, P_{DRS}=0.082) patients, although for PP-BC$_{DL}$ the difference was borderline non-significant (Fig. 3A and B). Similarly, PP-BC$_{PW/Lshort}$ patients also had a significantly poorer prognosis than Pr-BC (P_{OS}=0.022, P_{DRS}=0.006) and NP-BC (P_{OS}=0.001, P_{DRS}<0.001) patients (eTable 3 in the data article). Prognosis did not significantly differ between PP-BC$_{PW/NL}$ and PP-BC$_{PW/Lshort}$ patients (P_{OS}=0.179, P_{DRS}=0.164). Prognosis of PP-BC$_{PW/Llong}$ and PP-BC$_{DL}$ patients was found to be comparable. When adjusting for the same confounding parameters as before, the observed differences in risks for death and metastasis in PP-BC$_{PW/NL}$ compared to all other patient groups, including PP-BC$_{PW/Lshort,}$ increased to up to 5-fold (*P*-values ranging from 0.011 to <0.001) (Fig. 3C and D). Prognosis of PP-BC$_{PW/Lshort}$ patients also remained poorer than that of Pr-BC (P_{OS}=0.036, P_{DRS}=0.038) and NP-BC (P_{OS}=0.044, P_{DRS}=0.035) patients (eTable 3 in the data article). Since there was no significant difference in the length of the period between delivery or cessation of lactation and the time of the cancer diagnosis when comparing the different PP-BC$_{PW}$ subgroups (eFig. 3 in the data article), it could be excluded that PP-BC$_{PW/NL}$ and PP-BC$_{PW/Lshort}$ patients were not in the ability to lactate (extensively) because of their poor prognosis.

3.4 The poor prognosis of PP-BC$_{PW}$ was not associated with a preferential site of first metastasis

As the site of distant breast cancer metastasis is known to influence prognosis,[30,31] we investigated whether PP-BC$_{PW}$ patients had any particular preferential metastatic site that could underlie their poor prognosis. Overall, 47% of PP-BC$_{PW}$, 30% of PP-BC$_{DL}$, 28% of Pr-BC and 26% of NP-BC cases presented with metastatic disease, either at diagnosis or during follow-up. Binary logistic regression models, adjusted for the same confounding variables as before, indicated a significant 3- to 8-fold increased frequency of liver metastases in PP-BC$_{PW}$ patients compared to all other patients (eTable 4 in the data article). To more accurately evaluate the influence of metastatic site on prognosis, analyses were restricted to the first site of metastasis. Fisher's Exact testing showed that PP-BC$_{PW/NL}$, PP-BC$_{PW/Lshort}$ and PP-BC$_{PW/Llong}$ patients had a significant increased risk for primary liver metastasis compared to the PP-BC$_{DL}$, Pr-BC and NP-BC patients (Fig. 4). We then investigated the association between primary

metastatic site and prognosis. Patients with brain metastases were found to have the highest risk for death (*P*=0.0032) (eFig. 7-8 in the data article). Preferential metastasis to liver, as observed in PP-BC$_{PW/NL}$ and PP-BC$_{PW/Lshort}$, did not seem to correlate with the poorer outcomes of these patient subgroups.

4. DISCUSSION

The exact window of risk and prognostic factors associated with the poor outcome typically observed in PP-BC patients are unidentified. We here show for the first time that women with PP-BC, specifically diagnosed during the first two years post-weaning (PP-BC$_{PW}$), have a particular poor prognosis compared to lactating (PP-BC$_{DL}$), pregnant (Pr-BC) and nulliparous (NP-BC) patients, irrespective of standard prognostic characteristics. This poor prognosis was not associated with the unique preference of PP-BC$_{PW}$ cancers to metastasize to the liver. When considering the post-weaning period as a surrogate for the postpartum mammary gland involution window, these data suggest that processes unique to the involuting microenvironment may underlie the poor prognosis of PP-BC$_{PW}$. So far, this hypothesis has only been validated in preclinical models.[18-21,32] In women, the wound-healing like tissue remodelling phase of involution has been found to occur within 18 months after delivery[22] and distinct immune signatures have been found to persist up to 10 years after delivery.[23] Recent examinations in healthy involuting breast tissue discovered mammary gland remodelling mechanisms with tumour promotional potential.[26] In addition, molecular analyses of epithelial and stromal compartments from pregnancy-associated breast cancer patients indicated aberrant expression of several oncogenes, tumour suppressor genes, apoptosis regulators, transcription regulators and genes involved in DNA repair mechanisms, in cell proliferation and the immune response.[33,34] Although evidence in human tissue is sparse, these alterations may be related to prognostic differences in pregnant, postpartum and nulliparous breast cancer patients. It is important to note that these gene expression studies lack detailed data on lactation - thus the key window of weaning-induced involuting has yet to be defined in healthy women.

Next to identifying a post-weaning diagnosis as a negative prognostic factor, we found that no or short breastfeeding prior to diagnosis was associated with the poorest outcome in PP-BC$_{PW}$ patients. In pre-menopausal women, childbearing is known to have a dual effect on breast cancer risk, being associated with long-term risk reductions, following a transiently increased risk in the early postpartum period that can last up to 10 years.[35] In addition, it is well documented that prolonged breastfeeding decreases the relative risk of breast cancer, especially for TNBC.[36-39]. However, the link between breastfeeding and breast cancer prognosis has been scarcely explored, with conflicting results. A large-scale study in 92,794 Mexican women investigating breast cancer mortality rates and duration of breastfeeding (never, <6 months, 6-11 months, 12-23 months or ≥24 months) found that longer periods of breastfeeding were associated with lower mortality.[40] Yet, the timing of breast cancer diagnosis in relation to the patients' breastfeeding history was not considered. Stensheim *et al.* compared cause-specific survival between 59 pregnant, 138 non-pregnant and 46 lactating breast cancer patients and found a significantly increased risk for death in patients diagnosed during the lactation period, being

defined as the first 6 months following delivery.[10] Whether or not these patients were still breastfeeding or already weaned at time of diagnosis was not investigated. Elshmay postulated that duration of lactation is inversely correlated to the incidence of aggressive breast tumours in young women.[41] Applying this hypothesis, it could be speculated that, in our PP-BC$_{PW/Llong}$ patients who breastfed for longer periods, terminal differentiation of mammary epithelial cells was facilitated. After weaning, these fully differentiated mammary epithelial cells would be removed by programmed cell death occurring during normal postpartum involution.[16,42,43] In contrast, in PP-BC$_{PW}$ patients with no (PP-BC$_{PW/NL}$) or short prior breastfeeding (PP-BC$_{PW/Lshort}$) before weaning, less differentiated cells with concomitant immunosuppressive features would remain present and could escape cell-clearing programs.[26] This in turn could lead to the formation of aggressive tumours with increased invasiveness. Further research into molecular and cellular changes in the involution mammary gland in healthy women and cancer patients is needed to validate this hypothesis.

A major strength of our study is the delineation of the post-weaning window based on unique data on the patients' lactation status at diagnosis. Large patient numbers allowed us to define with high power significant small outcome differences between post-weaning (sub)group(s) and control cohorts. In the smaller patient subgroups, we still had significant power (>70%) to detect larger differences (HR≥3.0). Also, internationally collected data enabled accounting for variations associated with ethnic background. At the same time, local differences in staging and treatment methods might have introduced some heterogeneity among patients diagnosed at different centres. Also due to the retrospective nature of the present study, there might be some bias in the recollection of lactation history. Finally, we lacked complete family history data. For a subset of patients (n=528), *BRCA*-status was known. Primary cox regression analyses did not indicate significant outcome differences between *BRCA*-positive and *BRCA*-negative patients in each patient group.

5. CONCLUSION

We identified the post-weaning period as an independent negative prognostic marker in PP-BC patients. Therefore, the importance of a patient's breastfeeding history with regard to the prognosis of a breast cancer diagnosis during the post-weaning window should not be ignored. Our results may pave the way for future prospective validation studies in larger patients cohorts with known parity and lactation information. Finally, these data call for increased awareness and close surveillance of potential breast cancer symptoms shortly after weaning.

AUTHOR CONTRIBUTIONS SECTION

HL, DL, LL and FA conceived and designed the study. PN, EW, EC, FAP, SL, CM, HDM, KJJ provided clinical data. GF conducted IHC and FISH of Belgian patients and assisted in interpretation of data analyses. HL and MJ performed statistical analyses. HL and LL wrote the manuscript in consultation with FA. All authors provided critical feedback and approved the final version of the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors have declared no conflicts of interest.

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Table 1. Comparison of the frequencies of host- and tumour-related prognostic parameters in PP-BC_{PW} versus the other patient groups

Study group	PP-BC_{PW} (*n* = 189)		PP-BC_{DL} (*n* = 53)		Pr-BC (*n* = 492)		NP-BC (*n* = 446)		PP-BC_{PW} VS		
	n	%	n	%	n	%	n	%	PP-BC_{DL} OR [95% CI] ♦	Pr-BC OR [95% CI] ♦	NP-BC OR [95% CI] ♦
Mean age diagnosis (SD)	33.3	SD 3.6	33.2	SD 3.6	33.7	SD 3.9	34.8	SD 4.3	1.0 [0.9 – 1.1]	1.0 [0.9 – 1.0]	**0.9 [0.9 – 1.0]**
Mean year diagnosis (SD)	2007	SD 6.0	2009	SD 4.6	2009	SD 5.1	2008	SD 5.7	**0.9 [0.9 – 1.0]**	**0.9 [0.9 – 1.0]**	**1.0 [0.9 – 1.0]**
Year diagnosis											
1995-1999	21	11.1%	1	1.9%	20	4.1%	36	8.1%	--	--	--
2000-2004	42	22.2%	6	11.3%	77	15.7%	78	17.5%	2.2 [0.9 – 6.8]	1.5 [0.9 – 2.4]	1.3 [0.9 – 2.1]
2005-2009	49	25.9%	19	35.8%	139	28.3%	115	25.8%	0.6 [0.3 – 1.3]	0.8 [0.6 – 1.2]	1.0 [0.7 – 1.5]
2010-2014	52	27.5%	20	37.8%	158	32.1%	152	34.1%	0.6 [0.3 – 1.3]	0.8 [0.5 – 1.1]	0.7 [0.5 – 1.1]
2015-2017	25	13.2%	7	13.2%	98	19.9%	65	14.6%	1.0 [0.4 – 2.9]	0.7 [0.4 – 1.2]	0.9 [0.5 – 1.5]
Missing											
Stage											
Stage IA	39	21.0%	4	7.5%	70	14.3%	103	23.3%	--	--	--
Stage IB	6	3.2%	1	1.9%	6	1.2%	28	6.3%	0.6 [0.1 – 6.5]	1.8 [0.5 – 6.0]	0.6 [0.2 – 1.5]
Stage IIA	56	30.1%	18	34.0%	169	34.6%	143	32.3%	0.3 [0.1 – 1.0]	**0.6 [0.4 – 1.0]**	1.0 [0.6 – 1.7]
Stage IIB	28	15.1%	12	22.6%	111	22.7%	75	16.9%	**0.2 [0.1 – 0.8]**	**0.5 [0.3 – 0.8]**	1.0 [0.6 – 1.7]
Stage IIIA	26	14.0%	4	7.5%	70	14.3%	48	10.8%	0.7 [0.2 – 2.9]	0.7 [0.4 – 1.2]	1.4 [0.8 – 2.6]
Stage IIIB	11	5.9%	6	11.3%	17	3.5%	18	4.1%	**0.2 [0.1 – 0.8]**	1.2 [0.5 – 2.7]	1.6 [0.7 – 3.7]
Stage IIIC	13	7.0%	4	7.5%	28	5.7%	14	3.2%	0.3 [0.1 – 1.5]	0.8 [0.4 – 1.8]	**2.5 [1.1 – 5.7]**
Stage IV	7	3.8%	4	7.5%	18	3.7%	14	3.2%	**0.2 [0.1 – 0.9]**	0.7 [0.3 – 1.8]	1.3 [0.5 – 3.5]
Missing	3		0		3		3				
LN involvement											
Negative	80	43.5%	21	39.6%	217	44.4%	226	51.1%	--	--	--
Positive	104	56.5%	32	60.4%	273	55.6%	215	48.9%	0.9 [0.5 – 1.7]	1.1 [0.8 – 1.6]	**1.5 [1.1 – 2.1]**
Missing	5		0		3		4				
pN status											
N0	80	43.5%	21	39.6%	217	44.4%	226	51.1%	--	--	--
N1	79	42.9%	25	47.2%	192	39.3%	154	34.8%	0.8 [0.4 – 1.6]	1.1 [0.8 – 1.6]	1.5 [0.9 – 2.1]
N2	15	8.2%	2	3.8%	46	9.4%	35	7.9%	2.0 [0.4 – 9.3]	0.9 [0.5 – 1.7]	1.2 [0.6 – 2.3]
N3	10	5.4%	5	9.4%	34	7.0%	27	6.1%	0.5 [0.2 – 1.7]	0.8 [0.4 – 1.7]	1.1 [0.5 – 2.3]
Missing	5		0		3		4				
Mean tumour size (mm) (SD)	33.9	SD 26.6	32.8	SD 21.4	32.6	SD 24.0	30.1	SD 23.4	1.0 [0.9 – 1.0]	1.0 [0.9 – 1.0]	1.0 [0.9 – 1.0]
Grade											
Grade I	7	3.8%	5	9.4%	15	3.1%	60	13.9%	--	--	--
Grade II	49	26.5%	9	17.0%	111	23.1%	161	37.3%	3.9 [0.9 – 15.0]	0.9 [0.4 – 2.5]	**2.6 [1.1 – 6.1]**
Grade III	129	69.7%	39	73.6%	354	73.8%	211	48.8%	2.4 [0.7 – 7.9]	0.8 [0.3 – 2.0]	**5.2 [2.3 – 11.8]**
Missing	4		0		12		14				
Surrogate Molecular Subtype											
Luminal A-like	36	19.6%	3	5.7%	35	8.1%	127	30.5%	--	--	--
Luminal B-like	47	25.5%	15	28.3%	115	26.6%	106	25.5%	**0.3 [0.1 – 0.9]**	**0.4 [0.2 – 0.7]**	1.6 [0.9 – 2.6]
Luminal HER-2	30	16.3%	10	18.9%	77	17.8%	70	16.8%	**0.3 [0.1 – 0.9]**	**0.4 [0.2 – 0.7]**	1.5 [0.9 – 2.7]
HER-2-like	19	10.3%	10	18.9%	52	12.0%	25	6.0%	**0.2 [0.1 – 0.7]**	**0.4 [0.2 – 0.7]**	1.8 [0.9 – 3.5]
Triple Negative	52	28.3%	15	28.3%	154	35.6%	88	21.2%	0.3 [0.1 – 1.1]	**0.3 [0.2 – 0.6]**	2.1 [0.9 – 3.2]
Missing	5		0		59		30				
Histological Subtype											
IDC	153	81.8%	41	77.4%	404	83.1%	351	79.1%	--	--	--
ILC	23	12.3%	5	9.4%	50	10.3%	67	15.1%	1.2 [0.4 – 3.4]	1.2 [0.7 – 2.1]	0.8 [0.5 – 1.3]
Other (Special)	11	5.9%	7	13.2%	32	6.6%	26	5.9%	0.4 [0.2 – 1.2]	0.9 [0.5 – 1.9]	0.9 [0.5 – 2.0]
Missing	2		0		6		2				
Chemotherapy											
No	29	15.4%	5	9.4%	27	5.5%	92	20.8%	--	--	--
Yes	159	84.6%	48	90.6%	463	94.4%	350	79.2%	0.6 [0.2 – 1.6]	**0.3 [0.2 – 0.6]**	1.4 [0.9 – 2.3]
Adjuvant	103	64.8%	25	52.1%	311	67.2%	252	72.0%	1.0 [0.3 – 3.3]	1.4 [0.8 – 2.6]	**3.8 [1.7 – 8.4]**
Neoadjuvant	39	24.5%	19	39.6%	115	24.8%	87	24.9%	0.5 [0.3 – 1.0]	1.0 [0.7 – 1.6]	1.1 [0.7 – 1.7]
Adj. + Neoadj.	17	10.7%	4	8.3%	37	8.0%	11	3.1%	1.4 [0.5 – 4.0]	**3.2 [1.8 – 5.7]**	0.8 [0.5 – 1.2]
Missing	1		0		2		4				
Surgery											
No	10	5.3%	4	7.5%	19	3.9%	12	2.7%	--	--	--
Yes	178	94.7%	49	92.5%	470	96.1%	430	97.3%	1.5 [0.4 – 4.8]	0.7 [0.3 – 1.6]	0.5 [0.2 – 1.2]
Missing	1		0		3		4				
Radiotherapy											
No	34	18.2%	17	32.1%	170	36.0%	119	26.9%	--	--	--
Yes	153	81.8%	36	67.9%	302	64.0%	323	73.1%	**2.1 [1.1 – 4.2]**	**2.5 [1.7 – 3.8]**	**1.7 [1.1 – 2.5]**
Missing	2		0		20		4				
Hormone therapy											
No	97	51.6%	35	66.0%	275	56.9%	172	39.0%	--	--	--
Yes	91	48.4%	18	34.0%	208	43.1%	269	61.0%	1.8 [0.9 – 3.5]	1.2 [0.9 – 1.7]	**0.6 [0.4 – 0.9]**
Missing	1		0		9		5				
Anti HER-2 therapy											

No	158	83.6%	38	71.7%	379	78.5%	369	83.7%	--	--	--
Yes	31	16.4%	15	28.3%	104	21.5%	72	16.3%	0.5 [0.2 – 1.0]	0.7 [0.5 – 1.1]	1.0 [0.6 – 1.6]
Missing	0		0		9		5				

♦ Multinomial logistic regression model – 2-tailed Wald tests are used to determine Odds Ratio (OR) and 95% Confidence Intervals (95% CI). OR larger (lower) than 1 indicates increased (decreased) occurrence of that parameter in PP-BC$_{PW}$ compared to the reference type (either PP-BC$_{DL}$, Pr-BC or NP-BC). IDC, invasive ductal adenocarcinoma; ILC, invasive lobular adenocarcinoma. Significant values are indicated in bold.

FIGURES

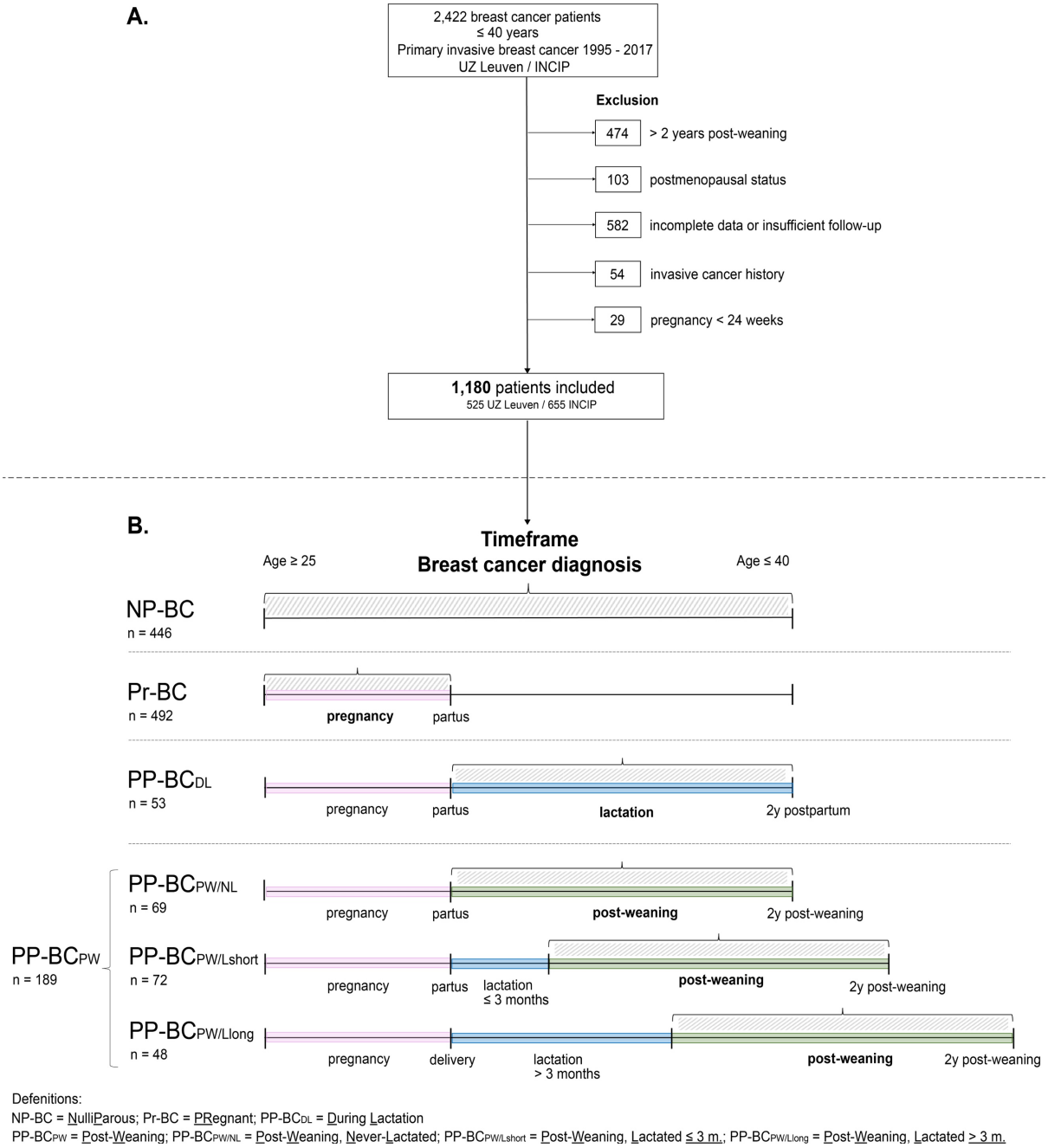

Figure 1. A. Flow chart depicting the number of breast cancer patients included in this study. Exclusion criteria were a diagnosis >2 years post-weaning, postmenopausal status, invasive cancer history, pregnancy lasting <24 weeks and insufficient data on 2 or more parameters or a lack of follow-up (<2 years). Women who became pregnant again within 2 years after delivery were also excluded from these analyses. **B.** Included breast cancer patients were grouped based on pregnancy and lactation status.

NP-BC are NulliParous patients with no history of pregnancy; Pr-BC are women diagnosed during Pregnancy; PP-BC$_{DL}$ are patients diagnosed During-Lactation; PP-BC$_{PW}$ are women diagnosed Post-Weaning. PP-BC$_{PW/NL}$ are women diagnosed Post-Weaning who Never-Lactated; PP-BC$_{PW/Lshort}$ are women diagnosed Post-Weaning who breastfed for ≤3 months; PP-BC$_{PW/Llong}$ are women diagnosed Post-Weaning who breastfed for >3 months prior to diagnosis.

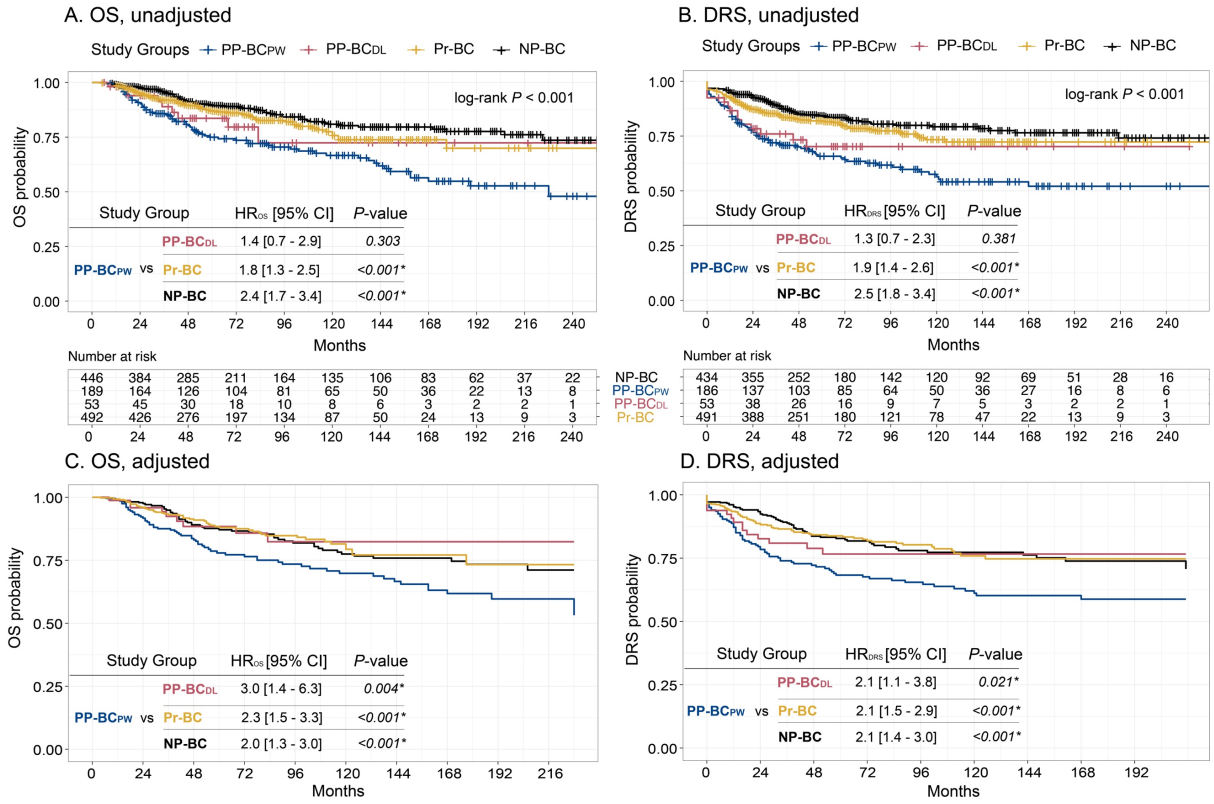

Figure 2. Unadjusted probability of OS and DRS in PP-BC$_{PW}$ (n=189), PP-BC$_{DL}$ (n=53), Pr-BC (n=492) and NP-BC (n=446) patients demonstrated an increased risk of death (**A**) and metastasis (**B**) in PP-BC$_{PW}$. Univariate analyses in all patients (n=1,180) and patients with complete data only (n=1,045) showed similar results, indicating no bias of removal of patients with missing data in the multivariate model. Adjusted probability in PP-BC$_{PW}$ (n=179), PP-BC$_{DL}$ (n=53), Pr-BC (n=409) and NP-BC (n=404) patients also indicates an increased risk of death (**C**) and metastasis (**D**) in PP-BC$_{PW}$. Multivariate Proportional Hazards models were adjusted for age at diagnosis, year of diagnosis, stage (accounting for tumour size and LN infiltration), grade, surrogate molecular subtype, surgery, and (neo-)adjuvant CT, RT, HT and anti-HER-2 therapy. Grade, HT and surgery were stratified to comply with the proportional hazard's assumption. Hazard Ratio (HR) and 95% Confidence Interval (CI) for the OS and DRS proportional hazards models were determined using Cox regression analyses and Kaplan-Meier curves. HR of more (less) than 1 indicates higher (lower) risk of death or metastasis. Significant values are indicated with an asterisk (*).

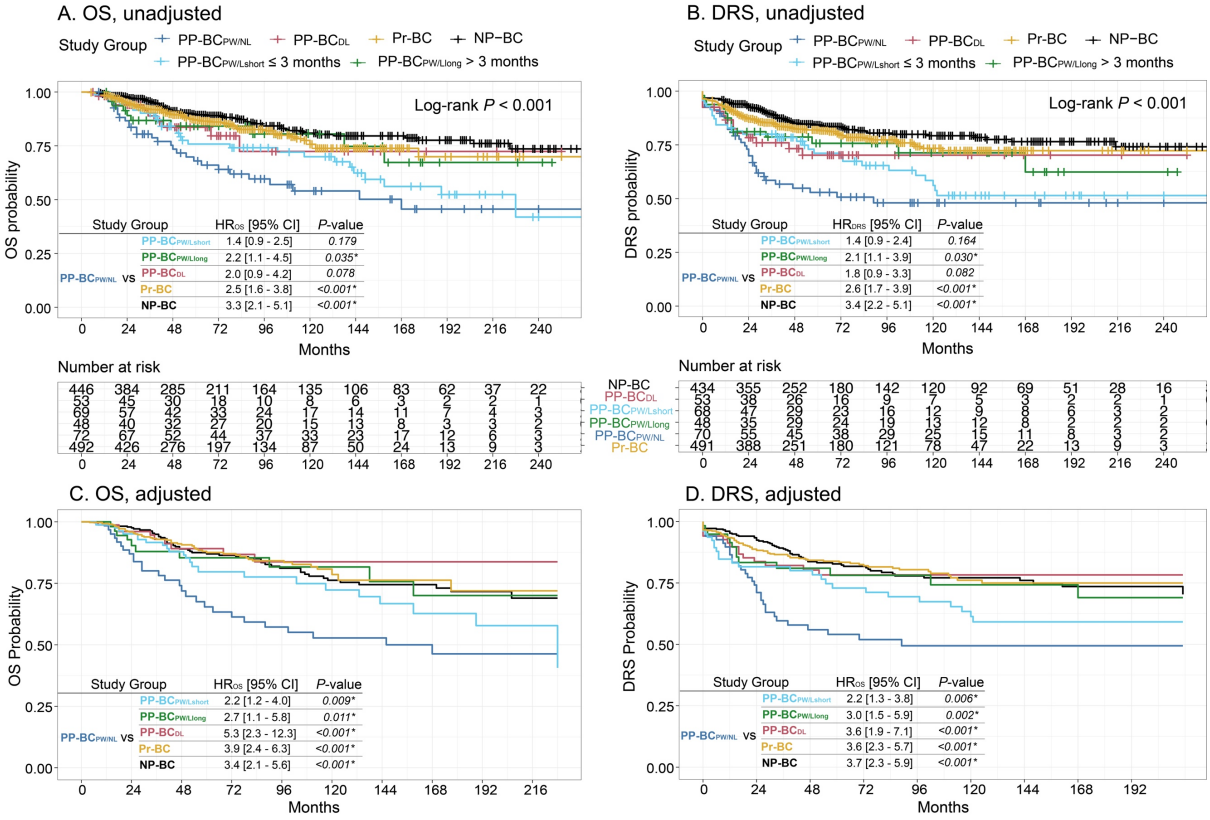

Figure 3. Unadjusted probability of OS and DRS in PP-BCPW/NL (n=72), PP-BCPW/Lshort (n=69), PP-BCPW/Llong (n=48), PP-BCDL (n=53), Pr-BC (n=492) and NP-BC patients (n=446) demonstrated an increased risk of death (**A**) and metastasis (**B**) in PP-BCPW/NL and PP-BCPW/Lshort patients. Adjusted OS and DRS probability in PP-BCPW/NL (n=67), PP-BCPW/Lshort (n=64), PP-BCPW/Llong (n=48), PP-BCDL (n=53), Pr-BC (n=409) and NP-BC (n=404) patients indicated an increased risk of death (**C**) and metastasis (**D**) in both PP-BCPW/NL and PP-BCPW/Lshort patients. Multivariate Proportional Hazards models were adjusted for age at diagnosis, year of diagnosis, stage (accounting for tumour size and LN infiltration), grade, surrogate molecular subtype, surgery, and (neo-)adjuvant CT, RT, HT and anti-HER-2 therapy. Grade, HT and surgery were stratified to comply with the proportional hazard's assumption. Hazard Ratio (HR) and 95% Confidence Interval (CI) for the OS and DRS proportional hazards models were determined using Cox regression analyses and Kaplan-Meier curves. HR of more (less) than 1 indicates higher (lower) risk of death or metastasis. Significant values are indicated with an asterisk (*).

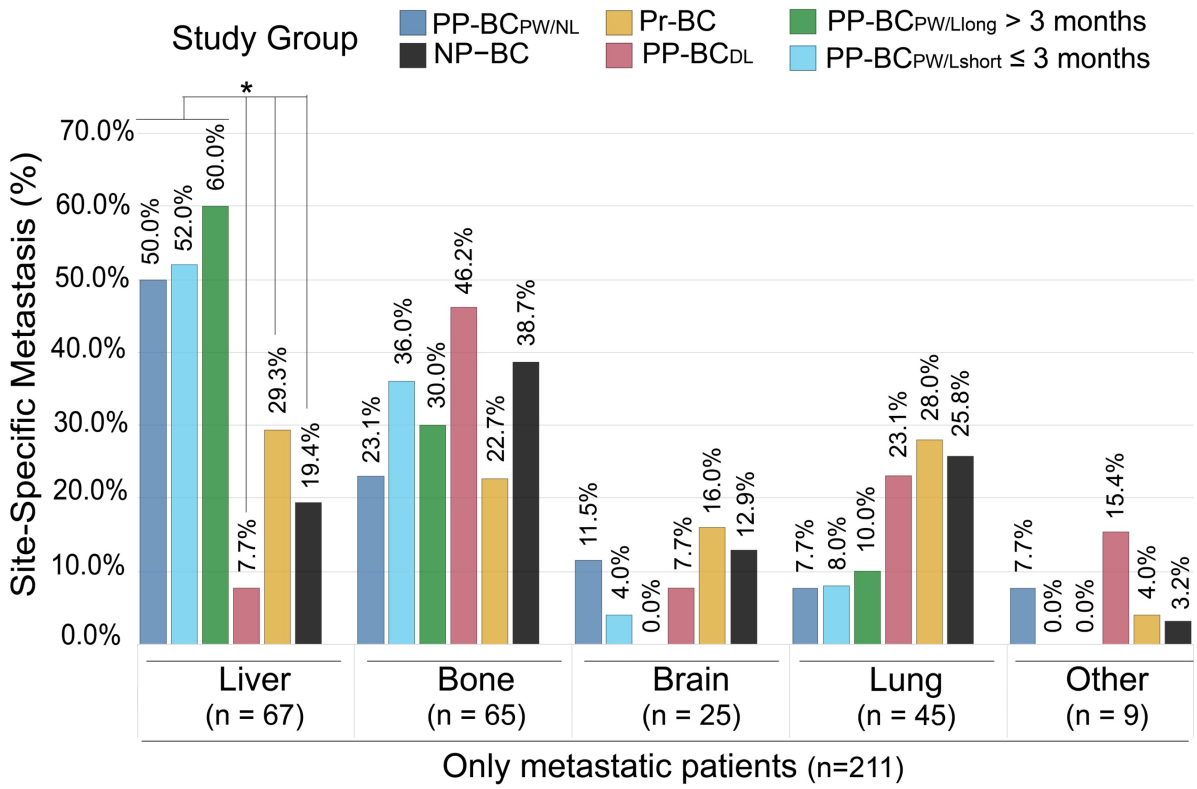

Figure 4. Subset analysis of site-specific metastasis (liver, bone, brain, lung and other sites) in women with metastatic disease (at diagnosis or during follow-up) that presented with only one site of primary distant metastasis (n=211: PP-BCPW/NL (n=26), PP-BCPW/Lshort (n=25), PP-BCPW/Llong (n=10), PP-BCDL (n=13), Pr-BC (n=75) and NP-BC (n=62)). Other sites of metastasis mainly include skin and ovaries. PP-BCPW/NL patients had a significant increased risk in liver metastatic disease compared to PP-BCDL (*P*=0.013), Pr-BC (*P*=0.049) and NP-BC (*P*=0.008). PP-BCPW/Lshort patients also had a significant increased risk in liver metastatic disease compared to PP-BCDL (*P*=0.012), Pr-BC (*P*=0.048) and NP-BC (*P*=0.004). Finally, also PP-BCPW/Llong patients presented with significant increased liver metastases compared to PP-BCDL (*P*=0.019), Pr-BC (*P*=0.049) and NP-BC (*P*=0.013). No significant differences in frequencies of bone, brain, lung or other metastases were observed between our different study groups. All p-values were determined by means of two-sided Fisher's Exact testing. Significant values are indicated with an asterisk (*).