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HYPOTHESIZED ROLE OF PREGNANCY HORMONES ON HER2+ BREAST TUMOR DEVELOPMENT

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Abstract

Breast cancer incidence rates have declined among older but not younger women; the latter are more likely to be diagnosed with breast cancers carrying a poor prognosis. Epidemiological

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evidence supports an increase in breast cancer incidence following pregnancy with risk elevated as much as 10 years postpartum. We investigated the association between years since last full-term pregnancy at the time of diagnosis (≤10 or >10 years) and breast tumor subtype in a case series of premenopausal Hispanic women (n = 627). Participants were recruited in the United States, Mexico, and Spain. Cases with known estrogen receptor (ER), progesterone receptor (PR), and HER2 status, with one or more full-term pregnancies ≥1 year *prior to* diagnosis were eligible for this analysis. Cases were classified into three tumor subtypes according to hormone receptor (HR+ = ER+ and/or PR+; HR− = ER− and PR−) expression and HER2 status: HR+/HER2−, HER2+ (regardless of HR), and triple negative breast cancer (TNBC). Case-only odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for HER2+ tumors in reference to HR+/HER2− tumors. Participants were pooled in a mixed-effects logistic regression model with years since pregnancy as a fixed effect and study site as a random effect. When compared to HR+/HER2− cases, women with HER2+ tumors were more likely be diagnosed in the postpartum period of ≤10 years (OR=1.68; 95% CI, 1.12–2.52). The effect was present across all source populations and independent of the HR status of the HER2+ tumor. Adjusting for age at diagnosis (≤45 or >45 years) did not materially alter our results (OR=1.78; 95% CI, 1.08–2.93). These findings support the novel hypothesis that factors associated with the postpartum breast, possibly hormonal, are involved in the development of HER2+ tumors.

Keywords

breast cancer; breast tumor subtypes; etiologic heterogeneity; HER2; Hispanic; parity

Introduction

Although incidence rates of invasive breast cancer in the U.S. have decreased slightly over time, this trend has not been observed among younger women, in whom there is evidence for substantial racial/ethnic disparities [1,2]. While the overall proportion of breast cancer occurring in women age <40 years is only 5.6%, it is higher for U.S. Hispanics (10.3%) [1], for whom age at diagnosis has been shown to be as much as ten years earlier than non-Hispanic Whites (NHWs) [3–5]. Furthermore, despite lower incidence rates than in the U.S., the average age at diagnosis in Mexico is 50.1 years [6], approximately six years younger than for U.S. Hispanics [7,3]. In Mexico, it is estimated that 45% of cases are diagnosed in women age <50 years [6], while this proportion is 36% for U.S. Hispanics and 21% for NHWs [6,1].

Clinically, breast cancer represents a heterogeneous disease that is grouped based on its hormone receptor (HR) status (estrogen receptor [ER] or progesterone receptor [PR] positivity) and amplification of the *ERBB2* gene (hereafter referred to as HER2+) [8]. Genomic profiling has identified at least two major lineages (*i.e.*, luminal and basaloid) that split predominantly on their HR status [9,10]. These breast tumor subtypes differ by age at diagnosis, race/ethnicity, reproductive patterns, lifestyle factors, stage at diagnosis, and survival [11–14]. Recent epidemiologic and genetic studies support the concept of underlying etiologic heterogeneity of breast tumor subtypes [15]. For example, breast cancer

risk differs by age at onset and menopausal status [16,17], as well as timing and number of births [18,19], depending on its HR status.

Findings from large, prospective studies provide strong evidence for a “dual” effect of pregnancy, where a transient postpartum increase in breast cancer risk is followed by long-term reduction in risk, relative to nulliparous women [20,18]. Depending on factors such as number of births and age at last birth, the estimated peak in breast cancer risk in the postpartum period ranges from 3–7 years, persisting for 10 or more years [21,20,22]. Further, it has been suggested that two forms of cancer are associated with pregnancy [23–26]. One, referred to as pregnancy associated breast cancer (PABC), is thought to involve those tumors that are diagnosed during or within the first year after a pregnancy; the other consists of tumors diagnosed in the postpartum period (more than one year after birth). The distinction is based on observed differences in mortality risk according to the time of diagnosis relative to pregnancy. Cases diagnosed in the postpartum period experience a higher risk of mortality than those diagnosed during pregnancy, after taking stage and other prognostic markers into account [25]. However, these observations have considered breast cancer as a single entity, and little is known about how, or if, this increase in risk differs by tumor subtype.

With the recognition of what appear to be distinct etiological paths to breast tumorigenesis, the complex role of pregnancy as a risk factor for breast cancer in younger women has gained a renewed interest. *In vitro* and *in vivo* studies provide evidence of critical cross-talk between progesterone/PR and estrogen/ER, possibly ER, and heregulin/HER2 signaling transduction pathways and a contribution of hormones or hormone conditioning of the breast in the activation of HER2 [27–29]. There are no known risk factors for the development of HER2+ tumors, and few epidemiological studies have assessed HER2+ tumors as a separate subgroup. Kwan *et al*. [30], showed that the odds of HER2-overexpressing tumors, defined as $HR^{-}/HER2^{+}$, were significantly greater in younger women, Asian and Hispanic women, and cases with 3 or more children who never breastfed when compared to cases with luminal A (HR+/HER2−) tumors. Analyses for HER2+ tumors independent of HR status were not considered separately.

Data from our previous genomic study of early-stage breast tumors show that HER2+ tumors are genetically more similar to each other than to $HR+/HER2^{-}$ or triple negative breast cancer (TNBC) [31]. We therefore speculate that, at least at the DNA level, HER2+ tumors may represent a subset of breast cancers that arise under distinct etiological influences, regardless of HR status. Here, we hypothesize that hormone exposure during pregnancy or lactation/involution confers a selective pressure for the outgrowth of cells harboring disturbances in HER2 signaling, independent of their hormone receptor status. Given the younger age at diagnosis and higher fertility rates in Hispanics compared with NHWs [32], we explored associations between pregnancy-related factors and HER2+ breast cancer in a case series of Hispanic women. Specifically, we investigated the association between the number of years since the last full-term pregnancy at the time of diagnosis and HER2+ tumors.

Materials and Methods

Study population

We pooled data from two Hispanic case series: the *Ella* Binational Breast Cancer study (*Ella*) and the Breast Oncology Galician Network (BREOGAN) study. *Ella* comprises 1,515 patients diagnosed with invasive breast cancer in the previous 24 months. Participants were recruited between 2007–2010; the present analysis includes data available by July 11, 2011. This multi-center study includes two sites in the U.S. [the Arizona Cancer Center (AZCC) in Tucson, Arizona and the M.D. Anderson Cancer Center (MDACC) in Houston, Texas] and three sites in Mexico [the Universidad de Sonora (UNISON) in Hermosillo, Sonora; the Instituto Tecnológico de Sonora (ITSON) in Ciudad Obregón, Sonora; and the Universidad de Guadalajara (UG) in Guadalajara, Jalisco]. All recruitment sites used a predominately clinic-based recruitment strategy. A detailed description of the organizational structure and methods of the study has been previously described [33]. Women were eligible to participate if they were diagnosed with incident invasive breast cancer, were age 18 years or older, and self-identified as Mexican or Mexican-American. Risk factor characteristics, including a detailed pregnancy history and menopausal status at the time of diagnosis, were collected via an interviewer-administered questionnaire [33]. Age at diagnosis was abstracted from medical records. All participants provided written informed consent. The Institutional Review Board (IRB) from each participating institution approved the study protocol.

The BREOGAN study is a population-based study conducted in the cities of Vigo and Santiago de Compostela, Spain. A total of 979 invasive and *in situ* breast cancer cases diagnosed between 1997–2012 were recruited at the Clinical University Hospital of Vigo and the Clinical University Hospital of Santiago de Compostela. Risk factor information was abstracted from patient medical records using the *Ella* risk factor questionnaire format for consistency between U.S. and Mexico datasets. Age at diagnosis, pregnancy history, and other reproductive factors are routinely obtained as part of the comprehensive patient medical record system in Spain. All participants provided written informed consent. The study was conducted in accordance to the Helsinki Principles of 1975, as revised in 1983. The Galician Ethics and Research Committee (CEIC, Comité Ético de Investigación Clínica de Galicia), responsible for the oversight of both university hospitals, approved the study protocol.

Tumor marker classification

In both studies, trained physicians abstracted tumor marker data from medical records. In the *Ella* study, ER and PR were classified as positive or negative according to the most recent guidelines [34]. In the abstraction, priority was given to a numeric value for the percent of cells staining. Any positive staining (≥1% of cells) resulted in ER/PR classified as positive. In 13.1% of cases, no specific number for percent of cells staining was available. We therefore used the interpretation value (“negative”, “positive”, or “low positive”) with any positive staining interpreted as positive. For the BREOGAN study, the cut-point for ER/PR was set at <10% as negative and ≥ 10% as positive. Had *Ella* used the higher cut-point, an additional 2% of cases would have been classified as ER/PR negative. For HER2 status, priority was given to determination by fluorescent *in situ* hybridization over

immunohistochemistry (IHC). IHC values of 0 and 1+ were considered negative, 2+ equivocal, and 3+ positive [35].

Statistical methods

Analyses were restricted to premenopausal women with a known diagnosis of invasive breast cancer (n=898). We limited our focus to early-onset, premenopausal cases to minimize heterogeneity introduced by menopause on breast cancer risk and to assess the effect of pregnancy during reproductive years. Time (years) since last full-term pregnancy was calculated using age at diagnosis and age at last full-term pregnancy, where a full-term pregnancy was defined as one lasting >5 months, regardless of outcome. Cases with equivocal HER2 status not confirmed by FISH (n=31), missing tumor marker information (n=122), or zero full-term pregnancies (n=106) were excluded. We further excluded participants with missing information on age at first and/or last pregnancy (n=2) and those with a full-term pregnancy <1 year prior to diagnosis (n=10). After applying inclusion and exclusion criteria, 627 cases were available for analysis (414 from *Ella* and 213 from BREOGAN).

We dichotomized time since last full-term pregnancy as ≤10 or >10 years, based on evidence supporting a window of increased risk and poor survival in the postpartum period [36,37]. Surrogates of tumor subtypes were approximated according to joint ER, PR, and HER2 status. Breast tumors were classified as HR+ if they were ER+ and/or PR+ and HR- negative if ER− and PR−. Tumors were grouped into the following subtypes: HR+/HER2−, HER2+ (independent of HR), and TNBC (HR− and HER2−). We classified HER2+ tumors independent of their HR status under the *a priori* hypothesis that *ERBB2* amplification arises as a consequence of an unknown, but distinct, etiological event. Our analysis is based on the working assumption that the aforementioned tumor subtypes represent three different forms of breast cancer.

All statistical analyses were performed using Stata 12.1 (StataCorp, College Station, TX). Descriptive statistics (mean ± SD and proportions) for reproductive and clinical characteristics were calculated separately by country of recruitment (USA, Mexico, or Spain). Potential associations between each risk factor and HER2+ status were tested using *t*-tests (continuous variables) or Fisher's exact tests (categorical variables) in each country separately. The crude association between time since last full-term pregnancy (≤ 10 vs. > 10 y) and HER2+ status was tested using logistic regression, with either HR+/HER2− tumors or TNBC as the reference group.

Participants from all 3 countries were pooled in a mixed-effects logistic regression model, with time since last full-term pregnancy and age at diagnosis (≤ 45 or > 45 y) included as fixed effects and study site as a random effect. This approach is advantageous as it allows us to combine smaller study groups into a larger dataset and model distributions of exposure variables across sites. Importantly, the pooled approach is defensible in this context because BREOGAN used the same risk factor instrument to abstract pregnancy history as *Ella*. In addition, to examine the robustness of our findings and to test for heterogeneity of effects across study sites, we also conducted a 2-stage random effects meta-analysis where site-specific effects were calculated in the first stage, and site-specific estimates were aggregated

using a random effects model in the second stage [38]. In order to assess heterogeneity of effect by study site, we calculated the I^2 and χ^2 (Q) statistics using the metan command in Stata [39].

Reported associations between HER2+ tumors and reproductive factors include age at menarche [40,41], parity [30,42], age at first birth [12,30,43], lifetime duration of breastfeeding [12], and ever using oral contraceptives [30]. We considered all of these factors plus additional characteristics listed in Table 1 as potential confounders in our analysis. We did not find any variables associated with both HER2+ tumors and time since last full-term pregnancy. Previous studies have reported an association between HER2+ tumors and age at diagnosis [12,30]. Although age at diagnosis was not associated with HER2+ in our data, we report age-adjusted pooled estimates as a sensitivity analysis. Additionally, in order to assess whether categorization of HER2+ tumors independent of HR status affected our estimates, we compared HER2+/HR+ and HER2+/HR− cases separately to the referent category. We considered a two-sided p-value of < 0.05 to be statistically significant.

Results

Descriptive characteristics of the study populations by country

Table 1 presents reproductive and clinical characteristics by country of recruitment. The mean interval of time between last full-term pregnancy and breast cancer diagnosis for the U.S., Mexico, and Spain was 13.1, 13.8, and 15.5 years, respectively. Approximately one-third of cases (range 28.2% to 39.4%) were diagnosed ≤10 years after their last full-term pregnancy. U.S. cases were younger at diagnosis and had the youngest age at first full-term pregnancy compared with cases recruited in Mexico or Spain. On average, Mexican participants had a greater number of full-term pregnancies (3.0), followed by U.S. and Spanish women. The majority of women in all countries reported breastfeeding their children, with prevalence ranging from 89% in Mexico to 67.4% in Spain. Not considering number of births, Spanish women had a shorter mean interval between the first and last pregnancy than those in the U.S. or Mexico. The majority of cases in all three countries were classified as HR+/HER2−: 70.0% in Spain, 61.0% in the U.S., and 55.2% in Mexico; TNBCs were the least common subtype: 17.1%, 22.1%, and 11.3% in U.S., Mexican, and Spanish women, respectively. Relatively little variation was observed for HER2+ tumors, which made up 22.8%, 21.9%, and 18.8% of cases in Mexico, the U.S. and Spain, respectively.

Reproductive and clinical characteristics and HER2+ tumors

The characteristics of HR+/ HER2− and HER2+ cases by country are presented in Table 2. No statistically significant differences were observed in reproductive characteristics between HR+/HER2− and HER2+ cases, with the exception of time since last full-term pregnancy (≤10 vs. >10 y) in Spain. When we evaluated the interval between last full-term pregnancy and breast cancer diagnosis, we observed no difference in the prevalence of HER2+ tumors for women whose diagnosis occurred within 5 years following a pregnancy (24.1%) and those occurring in 5–10 years (26.9%). This was our justification for selecting 10 years after

pregnancy as the interval to define postpartum cases. Furthermore, we excluded cases within one year of pregnancy or pregnant at diagnosis to differentiate post-partum breast cancers from PABC. Participants with HER2+ tumors were more likely to be diagnosed ≤10 years since their last full-term pregnancy than HR+/HER2− tumors. The proportion of HER2+ tumors diagnosed ≤10 years was 42.5% in Spain, 36.4% in Mexico, and 45.8% in the U.S., while the respective proportions for HR+/HER2− tumors were 22.8%, 26.3%, and 39.6%. Although not reaching statistical significance at the 5% level, HER2+ cases in Mexico were more likely to be younger at first and last pregnancies than HR+/ HER2− cases.

Time since last full-term pregnancy and HER2+ tumors

Table 3 presents country-specific and pooled odds ratios (ORs) for the association between time since last full-term pregnancy (≤ 10 vs. > 10 years) and tumor subtype (HER2+ vs. HR +/HER2−). Country-specific ORs varied in magnitude but not in direction of effect. ORs (95% confidence intervals) for each study site ranged from 1.04 (0.40–2.67) for AZCC to 4.50 (0.49–41.25) for UNISON (Figure 1). The pooled point estimate calculated using a random effects model of HER2+ versus HR+/HER2− tumors was OR=1.68 (1.12–2.52). The 2-stage meta-analytic point estimate of the OR was also 1.68 with heterogeneity statistics I^2 = 0.0% and χ^2 (Q) =3.13 (p=0.68). Adjusting for age at diagnosis (≤45 or >45 y) only marginally affected the estimate (OR=1.78; 95% CI, 1.08–2.93). Results remained significant in analyses treating age at diagnosis as a continuous variable (OR=2.15; 95% CI, 1.26–3.67).

Further, when we considered the HR status of HER2+ tumors in the association between time since last full-term pregnancy and HER2 status, we found no evidence of a difference by HR status. The OR (95% CI) for HR+/HER2+ was 1.71 (1.05–2.82) and that for HR−/ HER2+ was 1.54 (0.84–2.80). Lastly, when we compared the odds of having HER2+ tumors compared to TNBCs for time since last full-term pregnancy, a positive, albeit non-significant association was observed. Country-specific positive associations between time since last full-term pregnancy and HER2+ compared to TNBCs were observed for the U.S. (OR=1.93; 95% CI, 0.86–4.34) and Spain (OR=1.31; 95% CI, 0.47–3.68) but not Mexico (OR=0.57; 95% CI 0.21–1.54). The pooled OR (95% CI) was 1.21 (0.71–2.05), and the age-adjusted pooled OR (95% CI) was 1.84 (0.97–3.50).

Discussion

To our knowledge, this is the first case-only report examining the distribution of tumor subtypes by time since last full-term pregnancy in a large sample of premenopausal women with high parity. Our results indicate that HER2+ tumors have higher odds of being diagnosed in the 10-year period following a full-term pregnancy than HER2− tumors, an increase in odds that appears to be independent of HR status. The results were consistent across all three countries, increasing the validity of these findings.

Several groups have investigated the association between reproductive factors and tumor subtypes [12,44,41,43,42,45,46,40,30,14], but results for HER2+ tumors have been inconclusive. Few studies have reported on the association between a recent pregnancy and HER2+ tumors [12,47,48]. Two case-control studies [47,48] reported no association

between time since pregnancy and HER2+ tumors. In a third, a case-only analysis [12] using HR+/HER2− tumors as the reference, HR−/HER2+ tumors were more prevalent in cases diagnosed ≤5 years (OR= 5.05; 95% CI, 1.43–17.86) and >5 years (OR=2.14; 95% CI, 0.86, 5.34) since last pregnancy than in nulliparous women. However, no association was observed with HR+/HER2+ tumors. Only one of the previous studies [47] excluded pregnant cases and no exclusions were made based on time since pregnancy in any other study. All three studies categorized time since last pregnancy into shorter intervals of 2 [48,47] or 5 years [12], and all three were consistent in finding increased odds of HR- or TNBC tumors during this time period. Also consistent across studies, the association with recent pregnancy and TNBC or HR- tumors disappeared after the first couple of years postpartum.

There are numerous reasons for inconsistency between these and our findings, including choice of reference group, sample size and case-control or case-only study design. A limitation of our study is the lack of a nulliparous referent group. The small proportion of nulliparous cases in our study precluded their inclusion in our analyses. Other considerations include the differences in HER2 classification. In most epidemiological studies conducted to date, HER2+ tumors have been separated by their HR status, with HR+/HER2+ classified as luminal B [11,49,30,12,14,40,45,42]. This hierarchical-based classification of breast tumors is not entirely consistent with gene expression studies [10], and the appropriateness of such grouping for etiological studies has been debated [50]. Our results indicate that the positive association between time since last full-term pregnancy and HER2+ tumors (relative to HR +/HER2− tumors) is independent of HR status. Confirmation of our findings would suggest that HER2+ tumors may derive from a distinct set of etiological factors than either HR+/ HER2− or TNBC that would be more consistent with the genomic character of the disease at the genome level [31].

While our study provides evidence for higher odds of HER2+ tumors in the postpartum period relative to HR+/HER2− tumors, future larger studies would be valuable to assess finer postpartum intervals, as well as the effect of other reproductive characteristics (*e.g.*, age at first birth, number of births, interval between births, and breast feeding) as potential modifiers of the associated risk. Replication of our findings of differential risk by time since last pregnancy in the context of parity may provide a partial explanation for the reported disparities observed among certain populations that have a higher rate of parity (*i.e.*, African American and Hispanic women) when compared to those that exhibit lower parity rates (NHWs).

While novel, our findings need confirmation in studies with different control populations (*i.e.* non-diseased, nulliparous), particularly because ours can address heterogeneity in the association by tumor subtype only [15]. It is possible our results are due to selection bias. The exclusion of cases with incomplete tumor markers and difference in tumor marker classification might be a source of confounding that is difficult to address. By country, the proportion of otherwise-eligible participants missing tumor markers was 2% (n=7) in the U.S., 23% (n=50) in Mexico, and 14% (n=34) in Spain. Although the direction of effect is consistent across all studies, it is important to note that there is variability in the site-specific ORs (Figure 1: AZCC OR=1.04 versus UNISON OR=4.5). An advantage of our meta-

analytic approach is that it provides an estimate derived as a weighted average of results across study sites. The pooled OR of 1.68 reflects the greater weight given to larger sites such as Arizona relative to smaller sites such as UNISON. Furthermore, the consistency of our findings across the three countries is striking, especially as it is highly likely that a number of sources of residual confounding are present in our data.

These findings extend previous observations [11,30] that the relative proportion of specific breast tumor subtypes in a population may arise from differences in reproductive factors to include consideration of time since last pregnancy. In addition, our results support the possibility that hormonal influences related to pregnancy may contribute to the development of HER2+ tumors. These results are consistent with recent mechanistic studies demonstrating cross-talk between HER2 and certain pregnancy-associated hormones. While a hypothesis, we believe these findings are significant and warrant additional study since HER2+ tumors have no recognized risk factor(s).

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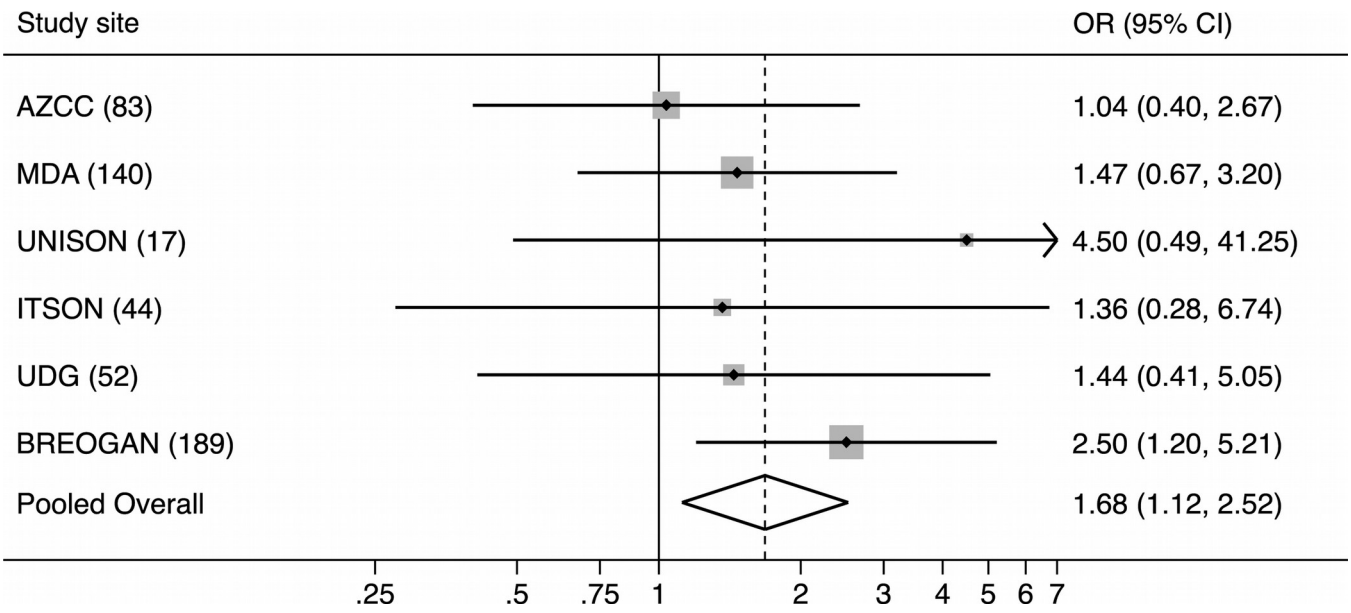

Fig. 1. Forest plot of the association between time since last full-term pregnancy (≤10 vs. 10 years) and HER2+ tumors versus HR+/HER2− tumors

Study sites: AZCC (Arizona Cancer Center, USA), MDA (M.D. Anderson Cancer Center, USA), UNISON (Universidad de Sonora, Mexico), ITSON (Instituto Technológico de Sonora, Mexico), UDG (Universidad de Guadalajara, Mexico), and BREOGAN (Breast Oncology Galician Network, Spain).

Table 1

Reproductive and clinical characteristics of premenopausal women, by country

Characteristic	USA (*n* = 269)	Mexico (*n* = 145)	Spain (*n* = 213)
Time since last full-term pregnancy (y), *n* (%)			
> 10	163 (60.6)	96 (66.2)	153 (71.8)
≤ 10	106 (39.4)	49 (33.8)	60 (28.2)
Mean ± SD	13.1 ± 7.8	13.8 ± 7.3	15.5 ± 8.1
Total full-term pregnancies, *n* (%)			
1–2	119 (44.2)	56 (38.6)	177 (83.1)
3+	150 (55.8)	89 (61.4)	36 (16.9)
Mean ± SD	2.8 ± 1.3	3.0 ± 1.4	1.9 ± 0.7
Age at first full-term pregnancy (y), *n* (%)			
≤ 23	169 (62.8)	83 (57.2)	97 (45.5)
> 23	100 (37.2)	62 (42.8)	116 (54.5)
Mean ± SD	22.5 ± 5.4	23.0 ± 5.3	25.1 ± 5.4
Age at last full-term pregnancy (y), *n* (%)			
≤ 30	149 (55.4)	68 (46.9)	122 (57.3)
> 30	120 (44.6)	77 (53.1)	91 (42.7)
Mean ± SD	29.7 ± 5.7	30.5 ± 5.2	29.6 ± 5.9
Interval between first and last pregnancy (y)			
Mean ± SD (if parity > 1)	8.5 ± 5.5	8.5 ± 5.3	6.4 ± 4.7
Lifetime breastfeeding[a] (mo), *n* (%)			
Never	79 (29.4)	16 (11.0)	69 (32.6)
≤ 12	115 (42.8)	60 (41.4)	113 (53.3)
> 12	75 (27.9)	69 (47.6)	30 (14.2)
Mean ± SD	11.5 ± 16.2	21.5 ± 29.2	6.4 ± 9.8
Age at menarche (y)			
Mean ± SD	12.6 ± 1.7	12.8 ± 1.5	12.8 ± 1.6
Hormone contraceptive use, *n* (%)			
Never	84 (31.2)	45 (31.0)	82 (38.5)
Ever	185 (68.8)	100 (69.0)	131 (61.5)
Age at diagnosis (y), *n* (%)			
≤ 45	163 (60.6)	69 (47.6)	101 (47.4)
> 45	106 (39.4)	76 (52.4)	112 (52.6)
Mean ± SD	42.8 ± 6.4	44.3 ± 7.0	44.9 ± 5.1
Family history of breast cancer[b], *n* (%)			
No	225 (84.6)	127 (88.8)	182 (85.9)
Yes	41 (15.4)	16 (11.2)	30 (14.2)
Tumor subtype, *n* (%)			
HR+/HER2−	164 (61.0)	80 (55.2)	149 (70.0)
HER2+	59 (21.9)	33 (22.8)	40 (18.8)

Characteristic	USA (*n* = 269)	Mexico (*n* = 145)	Spain (*n* = 213)
TNBC	46 (17.1)	32 (22.1)	24 (11.3)
Tumor markers, *n* (%)			
ER+	185 (68.8)	82 (56.6)	175 (82.2)
PR+	173 (64.3)	85 (58.6)	155 (72.8)
HER2+	59 (21.9)	33 (22.8)	40 (18.8)

[a] Missing data for lifetime breastfeeding (Spain, *n* = 1)

[b] Family history of breast cancer in a first-degree relative; missing data (USA, *n* = 3; Mexico, *n* = 2; Spain, *n* = 1)

Table 2

Bivariate associations between reproductive/clinical characteristics and HER2+ status (excluding TNBC cases), by country

Characteristic	USA		Mexico		Spain	
	HR+/HER2− (*n* = 164)	**HER2+ (*n* = 59)**	**HR+/HER2− (*n* = 80)**	**HER2+ (*n* = 33)**	**HR+/HER2− (*n* = 149)**	**HER2+ (*n* = 40)**
Time since last full-term pregnancy (y), *n* (%)						
> 10	99 (60.4)	32 (54.2)	59 (73.8)	21 (63.6)	115 (77.2)	23 (57.5)[a]
≤ 10	65 (39.6)	27 (45.8)	21 (26.3)	12 (36.4)	34 (22.8)	17 (42.5)[a]
Mean ± SD	13.0 ± 7.8	12.9 ± 8.1	14.8 ± 6.6	14.9 ± 8.0	16.0 ± 7.6	13.8 ± 8.4
Total full-term pregnancies, *n* (%)						
1–2	72 (43.9)	30 (50.9)	33 (41.3)	13 (39.4)	123 (82.6)	34 (85.0)
3+	92 (56.1)	29 (49.2)	47 (58.8)	20 (60.6)	26 (17.5)	6 (15.0)
Mean ± SD	2.7 ± 1.2	2.8 ± 1.7	3.0 ± 1.4	3.2 ± 1.7	1.9 ± 0.7	1.9 ± 0.7
Age at first full-term pregnancy (y), *n* (%)						
≤ 23	99 (60.4)	33 (55.9)	43 (53.8)	19 (57.6)	71 (47.7)	17 (42.5)
> 23	65 (39.6)	26 (44.1)	37 (46.3)	14 (42.4)	78 (52.4)	23 (57.5)
Mean ± SD	22.9 ± 5.5	23.3 ± 5.7	23.5 ± 5.7	22.6 ± 5.1	24.6 ± 4.9	25.6 ± 6.0
Age at last full-term pregnancy (y), *n* (%)						
≤ 30	92 (56.1)	28 (47.5)	34 (42.5)	20 (60.6)	89 (59.7)	19 (47.5)
> 30	72 (43.9)	31 (52.5)	46 (57.5)	13 (39.4)	60 (40.3)	21 (52.5)
Mean ± SD	29.7 ± 5.6	30.1 ± 5.8	30.8 ± 5.3	30.2 ± 5.2	29.2 ± 5.7	30.5 ± 6.3
Interval between first and last pregnancy (y)						
Mean ± SD (if parity > 1)	8.0 ± 5.1	8.3 ± 6.2	8.4 ± 5.6	8.3 ± 4.6	6.4 ± 4.4	7.9 ± 6.1
Lifetime breastfeeding[b] (mo), *n* (%)						
Never	47 (28.7)	19 (32.2)	10 (12.5)	5 (15.2)	45 (30.2)	12 (30.8)
≤ 12	71 (43.3)	26 (44.1)	35 (43.8)	12 (36.4)	83 (55.7)	20 (51.3)
> 12	46 (28.1)	14 (23.7)	35 (43.8)	16 (48.5)	21 (14.1)	7 (18.0)
Mean ± SD	11.5 ± 16.9	10.7 ± 15.7	22.3 ± 32.1	24.5 ± 33.1	6.9 ± 10.4	6.8 ± 9.0
Age at menarche (y)						
Mean ± SD	12.6 ± 1.6	12.3 ± 1.8	12.8 ± 1.5	12.9 ± 1.3	12.8 ± 1.6	12.7 ± 1.5
Hormone contraceptive use, *n* (%)						

Characteristic	USA		Mexico		Spain	
	HR+/HER2− (*n* = 164)	**HER2+ (*n* = 59)**	**HR+/HER2− (*n* = 80)**	**HER2+ (*n* = 33)**	**HR+/HER2− (*n* = 149)**	**HER2+ (*n* = 40)**
Never	54 (32.9)	17 (28.8)	27 (33.8)	6 (18.2)	61 (40.9)	12 (30.0)
Ever	110 (67.1)	42 (71.2)	53 (66.3)	27 (81.8)	88 (59.1)	28 (70.0)
Age at diagnosis (y)						
≤ 45	98 (59.8)	37 (62.7)	30 (37.5)	13 (39.4)	66 (44.3)	22 (55.0)
> 45	66 (40.2)	22 (37.3)	50 (62.5)	20 (60.6)	83 (55.7)	18 (45.0)
Mean ± SD	42.6 ± 6.8	43.0 ± 6.0	45.6 ± 6.6	45.1 ± 7.4	45.2 ± 5.1	44.3 ± 4.9
Family history of breast cancer[c], *n* (%)						
No	143 (88.3)	51 (86.4)	71 (88.8)	31 (93.9)	124 (83.8)	38 (95.0)
Yes	19 (11.7)	8 (13.6)	9 (11.3)	2 (6.06)	24 (16.2)	2 (5.00)

[a] Significantly different from HR+ (Fisher's exact test, $p = 0.016$)

[b] Missing data for lifetime breastfeeding (Spain, $n = 1$)

[c] Family history of breast cancer in a first-degree relative; missing data (USA, $n = 3$; Mexico, $n = 2$; Spain, $n = 1$)

Table 3

Association between time since last full-term pregnancy and HER2+ status, by country

Country	Subtype	*n*	OR (95% CI)
USA			
	HR+/HER2−	164	1.00
	HER2+	59	1.29 (0.71–2.34)
Mexico			
	HR+/HER2−	80	1.00
	HER2+	33	1.61 (0.67–3.82)
Spain			
	HR+/HER2−	149	1.00
	HER2+	40	2.50 (1.20–5.21)
Pooled[a]			
	HR+/HER2−	393	1.00
	HER2+	132	1.68 (1.12–2.52)
Pooled[b]			
	HR+/HER2−	393	1.00
	HER2+	132	1.78 (1.08–2.93)

[a] Study site included as a random effect in logistic regression model

[b] Adjusted for age at diagnosis (≤ 45 or > 45 y); study site included as a random effect in logistic regression model