Maternal Reproductive Hormones and Angiogenic Factors in Pregnancy and Subsequent Breast Cancer Risk

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Abstract

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Purpose: Breast cancer risk associated with pregnancy characteristics may be mediated by maternal hormones or angiogenic factors.

Methods: We conducted a prospective breast cancer case-control study among women in the Avon Longitudinal Study of Parents and Children (ALSPAC) and Norwegian Mother and Child Cohort Study (MoBa) related to maternal pregnancy prolactin (n=254 cases and 374 controls), placental growth factor (PlGF, n=252 and 371), soluble fms-like tyrosine kinase-1 (sFlt-1, n=118 and 240) and steroid hormone concentrations (ALSPAC only, n=173 and 171). Odds ratios (OR) and 95% confidence intervals (CI) for a 1 SD change in analytes were estimated using unconditional logistic regression with matching factors (cohort, mother's birth year, serum/plasma, blood collection timing) and gestational age.

Results: Breast cancer ORs (95% CI) were 0.85 (0.51–1.43) for estradiol, 0.86 (0.67–1.09) for testosterone, 0.89 (0.71–1.13) for androstenedione, 0.97 (0.71–1.34) for hCG, 0.93 (0.75, 1.15) for prolactin, 1.00 (0.78–1.27) for PlGF and 1.91 (1.00–3.65 ALSPAC) and 0.94 (0.73–1.21 MoBa) for sFlt-1, and were similar adjusting for potential confounders. Results were similar by blood collection timing, parity, age at first birth or diagnosis, and time between pregnancy and diagnosis.

Conclusion: These data do not provide strong evidence of associations between maternal hormones or angiogenic factors with subsequent maternal breast cancer risk.

Introduction

Parity is an established risk factor for breast cancer, with a reduction in breast cancer risk in the long-term among parous women, but a transient increase in risk directly following the pregnancy lasting up to a decade (http://cancer.gov/cancerinfo/ereworkshop-report, accessed May 9, 2018). Several hypotheses have been raised to explain these observations including, hormonally mediated molecular alterations in breast tissue that reduce its predisposition to carcinogenesis [1], and the possibility of promotion of existing tumors from high concentrations of growth hormones or pro-inflammatory factors associated with breast involution [2], respectively. The full range of hormones involved in breast carcinogenesis is unknown, but there is evidence for oestrogens, androgens and progestins [3], all of which increase during pregnancy. In animal models, the lowest incidence of breast tumors is achieved administering oestrogen and progesterone jointly, or human chorionic gonadotropin (hCG) alone [1]. Prolactin, which also increases in pregnancy, may be involved as well. Several studies using biospecimens from birth cohorts linked with cancer registries have explored these associations for different hormones and timing in pregnancy [4–17]. The strongest evidence so far indicates an association between maternal pregnancy estradiol concentrations and breast cancer risk that appears to depend on characteristics of the mother, pregnancy, and tumor [9, 15–17].

In addition to timing and number of pregnancies, a history of preeclampsia has been consistently linked with an approximate 10–20% reduction in maternal breast cancer risk [18–20]. In preeclampsia, the balance of major angiogenic and antiangiogenic proteins is

altered, with high levels of the antiangiogenic protein soluble fms-like tyrosine kinase-1 (sFLT-1), binding with and neutralizing the proangiogenic effects of free placental growth factor (PlGF) and vascular endothelial growth factor (VEGF), resulting in low levels of their bioactive forms [21]. It has been speculated [22, 23] that pregnancy's profoundly proangiogenic state represents an angiogenic challenge [24] in which women with a less exaggerated response (i.e. a relatively anti-angiogenic profile in pregnancy) would be predicted to react similarly in a state of tumorigenesis, resulting in diminished tumor growth and development.

In this study, we linked antenatal samples collected during pregnancy from mothers in two birth cohorts, the Avon Longitudinal Study of Parents and Children (ALSPAC) and the Norwegian Mother and Child Cohort Study (MoBa) with cancer register data to provide additional data on the association of estradiol concentrations, and of several other maternal reproductive hormones with subsequent development of breast cancer. In addition, in the ALSPAC data, we assessed the associations of angiogenic factors and breast cancer risk.

Materials and Methods

Cohorts

ALSPAC is a prospective birth cohort study which initially recruited 14,541 pregnant women (resulting in 14,062 live births) with expected delivery dates between 1st April 1991 and 31st December 1992 and living in and around the city of Bristol in the south west of England. The study is described in detail elsewhere [25,26] and a searchable data dictionary is available: (http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/, accessed May 9, 2018). MoBa (the Norwegian Mother and Child Cohort Study) is a prospective, population-based birth cohort study conducted by the Norwegian Institute of Public Health. Participants were recruited from all over Norway from 1999–2008. The women consented to participation in 41% of the pregnancies, and the cohort includes 95,200 mothers [27]. The current study is based on version 9 of the quality-assured data files. Blood samples were collected at routine antenatal clinical examinations using a standard protocol occurring between gestational weeks 5 and 41 (median 16 weeks; IQR 10–31 weeks) in ALSPAC and 12 and 33 (median 18 weeks; IQR 17–19 weeks) in MoBa.

Case and Control Selection

The ALSPAC mothers were linked to the UK National Health Service's (NHS) central register to obtain NHS ID number. This linkage was used to identify maternal cancer event records held within the English Cancer Registry and Office National Statistics Minimum Cancer Dataset (a legally mandated register of all cancer events (http://www.ncin.org.uk/home, accessed May 9, 2018)). The MoBa cohort was linked using the unique national personal identifying number with the Cancer Registry of Norway (a national registry with compulsory notification) to identify maternal cancer events.

In both birth cohorts, case selection was restricted to mothers of singleton deliveries who had a linked first primary, invasive breast cancer event (coded as ICD v9 174. or ICD v10 C50.) after delivery while enrolled in the cohort, and before the end of follow-up (September

2012 for ALSPAC and December 2008 for MoBa). All cases with an available antenatal sample were considered eligible for this study (ALSPAC n=136 cases had a serum sample, and n=37 had only a plasma sample; MoBa n=81 cases had a plasma sample). Controls were chosen randomly from subsets of women who were: mothers of singleton pregnancies with no record of any cancer in the cancer register up to the time of case selection. In ALSPAC, one control was matched to their case on maternal birth year, trimester of sample draw and sample type (serum or plasma); 2 cases and 4 controls were excluded because of missing gestational age resulting in 2 less controls than cases in the analysis sample. In MoBa, up to three controls (n=203) were matched to each case on maternal birth year; matching on sample type and trimester of blood draw were not necessary as MoBa collected plasma during a short interval: 92% between 16–19 weeks' gestation.

Data Collection

The index pregnancy was defined as the most recent cohort pregnancy (not necessarily the woman's last pregnancy) before the date of cancer diagnosis for cases, and before the matched controls' reference date, (i.e. the date of diagnosis in their matched case). In ALSPAC, follow-up of pregnancies occurring after the 1990–92 enrolment period is incomplete due to subsequent pregnancy questions only being asked periodically and subject to missing responses. In MoBa, information on subsequent pregnancies was only available if the pregnancy occurred while enrolled in the cohort study. Thus, in both cohorts, there is the possibility that we lacked information on pregnancies that occurred between the last study pregnancy and the date of cancer diagnosis.

In MoBa, gestational age was estimated based on ultra sound screening (second trimester). In ALSPAC gestational age was based on the mother's date of last menstrual period. When there was conflicting information regarding last menstrual period, the clinical records were reviewed, and dating was based on the earliest ultrasound scan. ALSPAC serum and heparin plasma samples were isolated from additive-free tubes and frozen at −20C and then transferred to −80C, with one previous freeze-thaw cycle for 62% of samples, two for 24% and three for 14%. In MoBa, EDTA tubes were used for plasma isolation, and then stored at −80C with no freeze/thaw cycles for 70.3%, 1 for 24.3%, 2 for 5.1% and 3 for 0.3%, before aliquoting for the current study. The individual data for freeze/thaw cycles were available for ALSPAC. After taking account of gestational age at sample draw, there was no association between number of freeze-thaw cycles for any of the analytes except prolactin and adjusting for number of freeze-thaw cycles did not affect the estimates for prolactin and breast cancer risk (data not shown).

Information on possible confounders (ages at menarche and first pregnancy, pre-pregnancy body mass index (BMI) defined as height divided by squared weight, oral contraceptive use (ever vs. never), parity up to the point of the study pregnancy and offspring sex) was ascertained in ALSPAC by questionnaires sent to the women at approximately 18 and 32 weeks' gestation, and supplemented by data abstracted from obstetric and birth records. The same information was obtained in MoBa from questionnaires administered by mail (approximately 15, 22 and 30 weeks' gestation), and supplemented by linkage with the National Medical Birth Registry of Norway (MBRN; https://www.fhi.no/en/hn/health-

registries/medical-birth-registry-of-norway/medical-birth-registry-of-norway, accessed May 9, 2018). Complete information on confounders was available for 74% and 89% of the ALSPAC and MoBa participants, respectively, and in both studies, similar proportions of data were missing in cases and controls (76% of cases and 71% of controls in ALSPAC; 90% of cases and 89% of controls in MoBa).

Quality Assessment Samples

Two types of quality assessment samples were included with the study samples in the assay batches. Residual samples from the Prostate, Lung, Colorectal and Ovarian (PLCO) Screening Trial [29] provided matched pairs of plasma and serum samples from the same individual (n=40) to test inter-sample type correlations. We embedded quality assessment samples in the current study batches to confirm that analyte values were similar when assayed from plasma or serum. Analyte values were reasonably similar when measured in plasma and serum from the same pregnant women (see Supplementary Table 1), consistent with a previous assessment of the concordance of serum and plasma hormone values in the same women which the correlations were close to 1.0 and only minor percent differences in means were observed [30].

ALSPAC and MoBa samples were shipped (in a protective dry-ice environment) directly to NCI's biorepository (PPD, Philadelphia, USA), batched, blinded and sent to the Clinical and Epidemiologic Research Laboratory at Boston Children's Hospital (Boston, USA) for assays. Quality assessment samples were from blood collected in pregnant women in a US birth cohort (Brigham and Women's Hospital (BWH), Boston, USA) within the last 5 years, and stored at −70C. Plasma from a single individual was used to create 60 1ml aliquots; these were designed to assess intra-plate and inter-plate variation.

Assay Preparation

Each assay batch was allocated an equal proportion of cases and controls, ALSPAC and MoBa samples, plasma and serum samples, and quality assessment samples. Because cases and controls were matched on maternal delivery year, this essentially matched for sample storage time. These were randomly assigned to a batch position.

Assay Methods (full assay descriptions are in the Supplemental Material)

Androstenedione was measured by an ELISA assay (Alpco Diagnostics – Salem, NH); the blinded CV% was 3.5%. Testosterone, estradiol and prolactin were measured by an electrochemiluminescence immunoassay on the Roche E Modular system (Roche Diagnostics, Indianapolis, IN); the blinded CV% were 3.5%, 1.8% and 1.5%, respectively. Human chorionic gonadotropin (hCG) was measured by an electrochemiluminescence immunoassay on the Roche E Modular system (Roche Diagnostics, Indianapolis, IN); the blinded CV% was 3.8%. PlGF and sFlt-1 were measured by an ELISA assay (R & D Systems, Minneapolis, MN); the blinded CV% were 3.7% and 3.1%, respectively.

All analytes were measured in ALSPAC cases and controls. In MoBa [28], there was sufficient volume available to measure only the angiogenic factors (PlGF, sFlt-1) and prolactin. sFlt-1 was only measured in plasma. The following numbers of cases and controls

were included in the analyses: 254 cases (173 ALSPAC, 81 MoBa) and 374 controls (171 ALSPAC, 203 MoBa) for prolactin; 252 cases (171 ALSPAC and 81 MoBa) and 371 (168 ALSPAC and 203 MoBa) controls for PlGF; 118 cases (37 ALSPAC and 81 MoBa) and 240 controls (37 ALSPAC and 203 MoBa) for sFlt-1; and 173 cases and 171 controls (all ALSPAC) for the remaining analytes.

Statistical Methods

Data were analysed for the most recent cohort pregnancy before the date of diagnosis in cases and the reference date in their matched controls. Analyte values were transformed to produce approximately normal distributions. Since the analytes generally did not vary linearly with gestational age, fractional polynomials [31] were used to select an appropriate model based on the study data to adjust for gestational age at blood sample draw (the selected models are summarised in Supplementary Table 2). Initially, both conditional and unconditional logistic regression, adjusting for the matching factors (delivery year, and where applicable, cohort and sample type (plasma/serum)), were used to estimate odds ratios (OR) and 95% confidence intervals (CI) for the association between each analyte and maternal breast cancer risk. Since these gave similar results, though with wider confidence intervals for the conditional analyses, unconditional logistic regression was used in all subsequent analyses and for the presented results (results for conditional logistic regression are available from the authors). ORs for breast cancer for tertiles of the analytes based on distributions in the controls were reviewed. However, in the main analysis analytes were converted to z-scores within study and analysed as continuous variables (i.e. in SD units). To check for deviations from linearity, the *lowess* command in Stata was used to examine smoothed plots of the probability of being a case against each analyte. Prolactin, PlGF and sFlt-1, which were measured in both cohorts, were analysed separately in the two cohorts and the results were combined using a fixed-effect meta-analysis. Cochran's Q and the I^2 statistic were used to assess whether there was evidence for heterogeneity between the studies. In a sensitivity analysis, the results from meta-analysis were like those obtained by simply combining the data from the two studies, adjusting for study as a covariate.

In secondary analyses, we stratified by gestational age at sample draw (<18 vs 18+ weeks), parity (1 vs. 2+), age at first birth (<25, 25+ years), time since pregnancy to diagnosis (10, >10 years and 5, >5 years), and age at breast cancer diagnosis (<40 vs. 40+ years).

All analyses were carried out using Stata 14.0 and 15.0.

Ethics, consent and permissions

The current analysis makes secondary use of pre-existing data from ALSPAC and MoBa participants who provided written informed consent. Ethical approval in ALSPAC was obtained from the ALSPAC Ethics and Law Committee and NHS Research Ethics Committees. Access and use of the linked ALSPAC cancer registry information was restricted to AB and RC who are ONS Accredited Researchers operating within the ONS 'Safe Research' framework. MoBa has a license from the Norwegian Data Inspectorate (01–4325) and approval from the Regional Committee for Medical Research Ethics (REK), Southern Norway (S-97045, S-95113). The angiogenic factor study received approval from

REK, South-Eastern Norway (S-08541a) and a license from the Norwegian Data Inspectorate (08/01126–4). At the U.S. National Cancer Institute, the project was reviewed by the Office of Human Subjects Research and exempted from institutional review board approval on the basis that the analysis was performed in the U.K. using de-identified data.

Availability of materials and data

The assay data have been returned to the relative cohorts for inclusion in their research repositories. The assay data are available on request from the cohorts, subject to the necessary approvals.

Results

Table 1 shows the median (IQR) value for each analyte among cases and controls included in the analysis. After taking account of gestational age, delivery year and sample type (plasma or serum), levels of PlGF and prolactin were similar (PlGF: ratio of geometric means, ALSPAC vs MoBa = 1.17 95% CI 0.93–1.48; p=0.2 and prolactin: difference in square root = 1.3 95% CI −2.9–5.6; p=0.5) in ALSPAC and MoBa mothers, while sFlt-1 levels were lower in ALSPAC mothers (ratio of geometric means = 0.45 95% CI 0.30–0.68; p<0.001).

In both ALSPAC and MoBa, the mean age at first birth for cases and controls was in the mid-20s, and age at index pregnancy was in the early 30s. Cases were more likely than controls to have ever used oral contraceptives and to have had a younger age at menarche (Table 2) but had a similar mean pre-pregnancy BMI. In ALSPAC, parity was similar in cases and controls but, in MoBa, cases were slightly more likely to be multiparous.

OR and 95% CI adjusted only for matching factors and gestational age, and fully adjusted, are presented in Table 3. There was no strong evidence that any of the analytes were associated with the risk of breast cancer. For prolactin and PlGF, there was little evidence of heterogeneity between the cohorts in the fully adjusted analysis (Q=1.07, p=0.3; I^2 = 6% for prolactin and Q=0.61, p=0.4; I^2 = 0% for PlGF). For sFlt-1, there was some evidence of heterogeneity (Q=2.84, p=0.09; I^2 = 65%); OR=0.96, 95% CI 0.72–1.28 for MoBa and OR=2.44, 95% CI 0.85–6.99 for ALSPAC). However, the numbers with sFlt-1 measured in ALSPAC with complete covariate information were low (n=50) and the confidence intervals consequently wide. For PlGF and prolactin, the results obtained when pooling the data from both cohorts were like those obtained from the meta-analysis (adjusted OR=1.01, 95% CI 0.76–1.34 for PlGF and OR=0.87 95% CI 0.67–1.12 for prolactin). The estimates did not change appreciably with further adjustment for age at menarche, parity, age at first birth, oral contraceptive use, pre-pregnant BMI and offspring sex; therefore, the remainder of the analyses included women regardless of whether they were missing values for these covariates and without adjustment for them.

The associations of the maternal analytes and breast cancer risk (Table 4) showed some variation by gestational timing of blood collection, parity, age at diagnosis and time from the index pregnancy to diagnosis but there was no statistical evidence for heterogeneity by these factors (p>0.05 in all cases; data not shown), although we acknowledge that power to detect

such differences was low. There was the suggestion of an inverse association between estradiol concentrations measured early in the pregnancy (<18 weeks) and breast cancer, and an inverse association with cancer diagnosed at <40 years. Given the small numbers within subgroups for these stratified analyses, caution is required in assuming that these reflect true differences.

Discussion

In this study, we found little evidence that concentrations of pregnancy hormones and angiogenic factors were associated with subsequent maternal breast cancer risk. The opportunities to directly measure pregnancy hormonal exposure and subsequent breast cancer risk in the mother are rare. The analysis also must consider the dual effect of pregnancy, whereby parous women have a reduction in breast cancer risk overall, but a transient increase directly following the pregnancy lasting up to a couple of decades. These observations suggest that pregnancy exposures may be adverse in the short-term but beneficial in the longer-term and appear to be consistent with the pattern of results in some previous studies for, most markedly, estrogen and hCG concentrations and breast cancer risk. For example, data from the Finnish Maternity Cohort showed an elevated breast cancer risk with higher first trimester estradiol concentrations among primiparous women diagnosed with breast cancer before age 40 (n = 536 [9], n=510 [15]), but a reduction in risk at age 40 or older (n = 682 [15]); results for estrone were weaker but showed a similar pattern [15]. These results, however, were only observed with estrogen receptor (ER) and progesterone receptor (PR) negative (−) breast tumors. More recently, Fortner et al. [16] showed a positive association of estradiol and breast cancer risk but only in the presence of low progesterone and for ER+/PR+ tumors. Cohn et al. [17] also found a positive association for the sum of estradiol and estrone, and an inverse association for estriol with breast cancer risk, associations which were stronger in primiparas, in breast cancer diagnosed within 15 years of the pregnancy and for women who were older at first gravidas. A study of older U.S. women [6], in which most cases occurred 15 years or more after the pregnancy (n = 151), found an overall positive association of third trimester estrone concentrations with risk, but not estradiol. Our results suggested an inverse association between estradiol concentrations measured early in the pregnancy (<18 weeks) and breast cancer, and an inverse association with cancer diagnosed at <40 years, though we acknowledge problems of small numbers of cases in these stratified analyses. Our data did not indicate associations of the androgens testosterone and androstenedione with breast cancer risk. While two previous studies have observed positive associations between maternal pregnancy testosterone concentrations and breast cancer risk, the association has been limited to cases diagnosed at <40 years of age [15], and in another analysis in those women with ER+/PR+ tumors [16]. Also, the lack of data on hormone receptor status of tumors in our study may have led to misclassification in the outcome if associations with hormones are limited to receptor status negative or positive disease.

In the Northern Sweden Maternity Cohort [12], first trimester hCG concentrations were positively associated with breast cancer risk in women who were <40 years at diagnosis (n=11) or diagnosed within 10 years after the pregnancy (n=4). In contrast, higher concentrations were inversely associated with risk among women who were 40+ years at

diagnosis (n = 91) or diagnosed 10 or more years after the pregnancy (n= 99). Our results for hCG are consistent with a recent, larger study in the Finnish Maternity Cohort, however, which found no association between hCG concentration and breast cancer overall, or by hormone receptor subtype, age at first-term birth, age at diagnosis, or time between blood collection and diagnosis [32].

Consistent with our findings, a previous study found little evidence of an associations of serum PlGF and sFlt-1 during pregnancy and breast cancer in the following 10 years (n=145 cases [10], although the women were younger (on average in their early 30s at diagnosis vs. the majority over 50 years in our study), and no postmenopausal breast cancer cases were included. Our data for sFlt-1 and breast cancer risk differed by cohort both overall and within some of the stratified analyses, with elevated risks demonstrated for sFlt-1 measured later in the pregnancy (18+ weeks' gestation) in ALSPAC but not in MoBa, for cancer diagnosed <10 years after the pregnancy, and among women with a later age at first birth (25+ years). These results are not consistent with our prior hypothesis that low sFlt-1 concentrations, as a proxy for reduced angiogenic response, would be inversely associated with breast cancer risk.

We are unaware of other studies on pregnancy prolactin levels and subsequent cancer risk in the mother, and results of studies of the association of prolactin levels in women who are not pregnant and breast cancer risk have been inconsistent. A recent meta-analysis showed a positive association of prolactin with postmenopausal cancer and hormone receptor positive tumors, but not with premenopausal or receptor negative disease [33].

While characteristics of the first full-term pregnancy appear to be the most relevant for the protection afforded against breast cancer in the long-term, our aim in this study was to also provide data on the transient increase in breast cancer risk that occurs in the decade after pregnancy because this information is sparser in the literature. Therefore, we chose the most recent pregnancy prior to the cancer diagnosis to assess the hypothesis that growth factors in pregnancy cause or promote tumorigenesis. The relationship of the hormone concentrations with gestational age was consistent with other studies of these analytes over normal pregnancy [34–37]. The length of follow-up allowed us to assess short- and long-term associations of maternal analytes and breast cancer risk, although it meant that the samples had been stored for several years. While the stability of reproductive hormones is robust [38], there is less information on the effect of long-term storage on angiogenic factors. Previous research publications on angiogenic factors show ranges of values like those observed clinically, suggesting that the angiogenic factors are stable regarding minor sampling variations in handling. As specimen handling was similar for breast cancer cases and controls in each of the cohorts, any resulting measurement error should be random with respect to cancer risk within the cohort. In both cohorts, linkage was made to national cancer registries, but it is possible that some women migrated out of the country and were subsequently diagnosed with breast cancer elsewhere. If migration was random with respect to case status and unrelated to the pregnancy biomarker concentrations under study this would potentially bias the ORs towards the null. Also, the women in the study were relatively young and death from other causes is unlikely to be related to pregnancy hormone concentrations.

The use of a cross-cohort model increased the study size and improved statistical power; however, our sample size was still relatively small, particularly when exploring subsets of the data. More detailed comparison of maternal analytes and their association with cancer risk between trimesters would also be of interest, as many of the currently used pregnancy biomarkers vary with placental aging and stress [24]. In both cohorts, case/control status is informed by linkage to national registers with good quality standards and high levels of population coverage.

The biological mechanisms that underlie the associations of pregnancy with breast cancer risk are unknown, although there are several hypotheses, mainly untested in humans [1], including molecular changes to breast tissue initiated by pregnancy. The findings from the previous studies cited earlier, if true, could suggest that hormonal profiles in pregnancy that are associated with protection in later life, e.g., higher estradiol, may also be associated with an increased risk in the period following the pregnancy, especially for hormone receptor negative tumors which are more common in that period. Additionally, exposure which occurs in the first pregnancy may be the most relevant, consistent with the observation that breast cancer risk is lower in parous compared with nulliparous women, and not as strongly associated with the number of subsequent pregnancies women experience. Nevertheless, the results of these studies, including ours, are inconsistent. Additional investigations are warranted with information on receptor status, and that are large enough to evaluate interactions of hormone profiles by other characteristics of the pregnancy. In our study, which included all breast cancer cases at the time of sample identification, power was limited. A larger consortium of birth cohorts or samples could overcome limitations on power and increase the diversity of pregnancy exposures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1.

Median (interquartile range) of raw values for maternal analytes in ALSPAC and MoBa

Analyte	ALSPAC			MoBa	
Median (IQR) blood draw week[a]	16 (10–31) weeks			18 (17–19) weeks	
	Cases	Controls		Cases	Controls
N	173	171		81	203
PlGF[b,c] (pmol/L)	7.3 (4.7 – 12.3)	7.7 (3.5 – 11.2)		6.6 (4.5 – 8.3)	6.3 (4.7 – 8.9)
sFlt-1[b,d] (pmol/L)	6.6 (1.9 – 28.0)	8.1 (2.1 – 30.1)		8.6 (6.6 – 11.9)	8.3 (6.1 – 11.9)
Prolactin[b] (mIU/L)	706 (269 – 2226)	757 (267 – 2760)		1633 (1128 – 2233)	1614 (1108 – 2474)
Estradiol (pmol/L)	20530 (6230 – 66666)	23128 (5622 – 58683)			
Androstenedione (nmol/L)	5.2 (4.3 – 7.6)	5.6 (4.2 – 8.2)			
Testosterone (nmol/L)	2.1 (1.5 – 2.7)	2.2 (1.5 – 3.1)			
hCG (IU/L)	31790 (14965 – 76325)	32420 (15000 – 68870)			

Abbreviations: IQR=interquartile range; sFlt-1=soluble FMS-like tyrosine kinase-1; PLGF=placental growth factor; hCG=human chorionic gonadotropin.

[a]The distribution in ALSPAC was bimodal with blood draws mainly at 9–12 weeks and at 31–34 weeks.

[b]Only sFlt-1, PlGF and prolactin were measured in the MoBa samples.

[c]Measured in only 171 cases and 168 controls in ALSPAC; all MoBa cases and controls.

[d]sFlt-1 was measured in plasma samples only (n=37 cases, 37 controls in ALSPAC; all MoBa cases and controls).

Table 2:

Maternal characteristics (N (%) or mean (standard deviation (SD))) of breast cancer cases and controls in ALSPAC and MoBa

N[a]		ALSPAC		MoBa	
		Controls N 171	Cases N 173	Controls N 203	Cases N 81
Age at menarche (years (y))	<12	25 (17.9%)	26 (18.3%)	15 (7.9%)	10 (13.7%)
	12–13	64 (45.7%)	80 (56.3%)	106 (56.1%)	39 (53.4%)
	14+	51 (36.4%)	36 (25.4%)	68 (36.0%)	24 (32.9%)
Parity[b]	1	60 (36.8%)	63 (38.4%)	54 (26.7%)	18 (22.2%)
	2	64 (39.3%)	62 (37.8%)	85 (42.1%)	33 (40.7%)
	3+	39 (23.9%)	39 (23.8%)	63 (31.2%)	30 (37.0%)
Ever used oral contraceptives	No	7 (4.5%)	5 (3.2%)	43 (21.2%)	11 (13.6%)
	Yes	149 (95.5%)	152 (96.8%)	160 (78.8%)	70 (86.4%)
Age at first birth (y)[a]	Mean (SD)	26 (5.5)	26 (5.4)	27 (5.5)	27 (4.8)
Age at birth (index pregnancy; y)[a]	Mean (SD)	31 (4.5)	31 (4.4)	34 (4.4)	33 (4.3)
Pre-pregnant BMI (kg/m²)[a]	Mean (SD)	23 (4.3)	23 (3.1)	25 (4.8)	23 (3.9)
Age at diagnosis (y)	Mean (SD)	n/a	44 (6.4)	n/a	36 (5.1)
Time to diagnosis from index pregnancy (y)	Mean (SD)	n/a	13 (5.2)	n/a	3 (1.8)

[a] Numbers vary due to missing information. Data available on: age at first birth for 164 cases, 166 controls in ALSPAC, 75 cases and 188 controls in MoBa; age at index birth for 172 cases and 171 controls in ALSPAC, and all cases and controls in MoBa; pre-pregnant body mass index (BMI) for 152 cases and 144 controls in ALSPAC, 74 cases and 189 controls in MoBa.

[b] Parity refers to the index pregnancy, i.e. the pregnancy from which the blood was sampled. Thus, if parity = 1, the index pregnancy is the woman's first pregnancy resulting in a delivery.

Table 3.

Odds ratios (OR) and 95% confidence intervals (CI) for breast cancer according to a one standard deviation change in maternal analytes

Analyte	N cases / N controls[a]	Model 1 OR (95% CI)[a]	N case / N controls[b]	Model 2 OR (95% CI)[b]
ln PLGF[c]	252/371	1.00 (0.78, 1.27)	205/303	0.92 (0.68, 1.25)
ln sFlt-1[c]	118/240	ALSPAC MoBa 1.91 (1.00, 3.65) 0.94 (0.73, 1.21)	99/205	ALSPAC MoBa 2.44 (0.85, 6.99) 0.96 (0.72, 1.28)
ln Testosterone[d]	173/171	0.86 (0.67, 1.09)	133/125	0.88 (0.66, 1.18)
ln Estradiol[d]	173/171	0.85 (0.51, 1.43)	133/125	0.98 (0.55, 1.77)
ln Androstenedione[d]	173/171	0.89 (0.71, 1.13)	133/125	0.87 (0.65, 1.17)
sqrt hCG[d]	173/171	0.97 (0.71, 1.34)	133/125	0.82 (0.56, 1.19)
sqrt Prolactin[c]	254/374	0.93 (0.75, 1.15)	206/306	0.85 (0.65, 1.11)

Abbreviations: PLGF=placental growth factor; sFlt-1=soluble FMS-like tyrosine kinase-1; hCG=human chorionic gonadotropin.

[a] Model 1 is adjusted for matching variables (cohort, sample type, gestational age and delivery year).

[b] Model 2 is adjusted for pre-pregnant BMI, offspring sex, age at first birth, parity (as of index pregnancy), ever use of oral contraceptives, and age at menarche. Complete covariate information was available for 206 cases (133 ALSPAC, 73 MoBa) and 306 kcontrols (125 ALSPAC, 181 MoBa).

[c] For these analytes results are from ALSPAC and MoBa cohorts; for sFlt-1 results are given separately for each cohort due to cohort differences.

[d] For these analytes results are from the ALSPAC study only.

Table 4.

Odds ratios (OR) and 95% confidence intervals (CI) for breast cancer for a one standard deviation change in maternal analytes by selected factors

	N cases / N controls	Model 1 OR (95%CI)[a]	N cases / N controls	Model 2 OR (95%CI)[a]
	<18 weeks' gestation		18+ weeks' gestation	
ln PlGF[b]	119/148	1.30 (0.80, 2.10)	133/223	0.87 (0.65, 1.17)
ln sFlt-1[b]	A 5/5 M 22/60	A[c] M 1.41 (0.86, 2.32)	A 32/32; M 59/143	A 2.70 (1.15, 6.36) M 0.80 (0.60, 1.07)
ln Testosterone[d]	98/91	0.87 (0.64, 1.18)	75/80	0.88 (0.59, 1.32)
ln Estradiol[d]	98/91	0.47 (0.22, 1.02)	75/80	1.45 (0.65, 3.27)
ln Androstenedione[d]	98/91	0.90 (0.66, 1.21)	75/80	0.93 (0.64, 1.34)
sqrt HCG[d]	98/91	1.05 (0.69, 1.61)	75/80	0.96 (0.53, 1.74)
sqrt prolactin[b]	120/151	1.03 (0.68, 1.58)	134/223	0.94 (0.72, 1.23)
	No previous pregnancies		1+ previous pregnancy	
ln PlGF[b]	81/113	0.92 (0.53, 1.57)	162/249	1.02 (0.78, 1.34)
ln sFlt-1[b]	A 18/11 M 18/54	A[c] M 0.93 (0.55, 1.57)	A 17/23; M 63/148	A 1.62 (0.59, 4.48) M 0.97 (0.72, 1.29)
ln Testosterone[d]	63/60	1.09 (0.71, 1.67)	101/103	0.81 (0.59, 1.12)
ln Estradiol[d]	63/60	1.29 (0.45, 3.72)	101/103	0.76 (0.40, 1.43)
ln Androstenedione[d]	63/60	1.00 (0.65, 1.55)	101/103	0.87 (0.65, 1.16)
sqrt HCG[d]	63/60	0.85 (0.51, 1.42)	101/103	1.08 (0.71, 1.63)
sqrt prolactin[b]	81/114	1.38 (0.87, 2.19)	164/251	0.85 (0.64, 1.12)
	Age at first birth <25 years		Age at first birth 25+ years	
ln PlGF[b]	87/123	1.23 (0.81, 1.88)	150/228	0.98 (0.71, 1.36)
ln sFlt-1[b]	A 13/12 M 25/61	A[c] M 0.98 (0.61, 1.59)	A 21/24; M 50/127	A 2.62 (1.02, 6.75) M 0.93 (0.68, 1.27)
ln Testosterone[d]	63/62	1.04 (0.66, 1.63)	101/104	0.87 (0.64, 1.17)
ln Estradiol[d]	63/62	0.55 (0.20, 1.50)	101/104	1.09 (0.59, 2.04)
ln Androstenedione[d]	63/62	0.95 (0.62, 1.45)	101/104	0.92 (0.69, 1.24)

	N cases / N controls	Model 1 OR (95%CI)[a]	N cases / N controls	Model 2 OR (95%CI)[a]
sqrt HCG[d]	63/62	1.44 (0.87, 2.38)	101/104	0.71 (0.46, 1.11)
sqrt prolactin[b]	88/123	0.82 (0.54, 1.24)	151/231	1.00 (0.76, 1.31)
		10 years to diagnosis		**>10 years to diagnosis**
ln PIGF[b]	132/206	1.04 (0.79, 1.38)	118/118	0.91 (0.50, 1.68)
ln sFlt-1[b]	A 16/16 M 81/157	A[c] M 0.90 (0.69, 1.17)	A 21/21; M0/0	A 1.18 (0.51, 2.70) M[c]
ln Testosterone[d]	52/52	0.94 (0.57, 1.54)	120/120	0.84 (0.63, 1.11)
ln Estradiol[d]	52/52	0.47 (0.18, 1.22)	120/120	1.18 (0.61, 2.30)
ln Androstenedione[d]	52/52	0.93 (0.60, 1.44)	120/120	0.89 (0.68, 1.18)
sqrt HCG[d]	52/52	0.86 (0.49, 1.53)	120/120	1.09 (0.74, 1.62)
sqrt prolactin[b]	132/207	0.94 (0.73, 1.21)	120/120	0.75 (0.47, 1.20)
		5 years to diagnosis		**>5 years to diagnosis**
ln PIGF[b]	82/141	1.18 (0.85, 1.64)	164/181	0.70 (0.45, 1.10)
ln sFlt-1[b]	A 1/1 M 68/126	A[c] M 0.95 (0.72, 1.26)	A 34/36 M 13/31	A 1.96 (1.01, 3.79) M 0.60 (0.27, 1.04)
ln Testosterone[d]	14/16	*	153/152	0.92 (0.71, 1.18)
ln Estradiol[d]	14/16	*	153/152	1.01 (0.59, 1.74)
ln Androstenedione[d]	14/16	*	153/152	0.94 (0.74, 1.20)
sqrt HCG[d]	14/16	*	153/152	1.04 (0.74, 1.45)
sqrt prolactin[b]	82/142	0.98 (0.74, 1.30)	166/183	0.76 (0.53, 1.09)
		Age at diagnosis <40 years		**Age at diagnosis 40+ years**
ln PIGF[b]	99/150	1.18 (0.86, 1.63)	15299/176	0.78 (0.50, 1.22)
ln sFlt-1[b]	A9/9M 61/113	A[c] M 0.90 (0.65, 1.25)	A 28/28; M 20/44	A 1.57 (0.77, 3.19) M 0.88 (0.56, 1.40)
ln Testosterone[d]	38/38	0.85 (0.50, 1.43)	134/134	0.86 (0.65, 1.14)
ln Estradiol[d]	38/38	0.24 (0.07, 0.83)	134/134	1.18 (0.66, 2.14)
ln Androstenedione[d]	38/38	0.85 (0.49, 1.47)	134/134	0.91 (0.70, 1.18)

	N cases / N controls	Model 1 OR (95%CI)[a]	N cases / N controls	Model 2 OR (95%CI)[a]
sqrt HCG[d]	38/38	1.07 (0.58, 1.98)	134/134	0.98 (0.67. 1.43)
sqrt prolactin[b]	99/151	0.96 (0.71, 1.30)	154/178	0.80 (0.57, 1.12)

Abbreviations: PLGF=placental growth factor; sFlt-1=soluble FMS-like tyrosine kinase-1; hCG=human chorionic gonadotropin.

[a]Model 1 is adjusted for the matching variables (cohort, sample type, gestational age and delivery year).

[b]For these analytes, results are from ALSPAC (A) and MoBa (M) cohorts; for sFlt-1 results are given separately for each cohort due to differences in risk estimates.

[c]Insufficient numbers to obtain a meaningful estimate

[d]For these analytes results are from the ALSPAC study only