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ABSTRACT

Background: Breast cancer (BC) is a major public health concern with over 2 million new cases diagnosed and over 600,000 deaths in 2018 in women worldwide. When distant metastases are present at diagnosis, the 5-year survival rate is only 26%. Recent studies have suggested that persistent organic pollutants (POPs) that accumulate in adipose tissue (AT) can influence tumor phenotype and stimulate cellular processes important for metastasis such as invasion. We, therefore, tested the hypothesis that POP exposure is associated with BC metastasis.

Methods: We conducted an exploratory case-control study in which the concentrations of 49 POPs were measured in both AT and serum samples from BC patients, with or without lymph node metastasis, who underwent partial or total mastectomies, lymph node biopsies and sampling of the adipocytic tumor microenvironment. Adjusted, unconditional logistic models were used to study the associations between the POP concentrations and the risk of metastasis and other hallmarks of cancer aggressiveness.

Results: 2.3.7.8-TCDD concentrations in AT are positively associated with the risk of metastasis in 43 patients who have BMIs equal or higher than 25 kg/m2 (odds ratio: 4.48 (1.32–20.71)). Furthermore, the concentrations of 2.3.7.8-TCDD and two coplanar PCBs (77&169) in AT also were positively associated with the risk of lymph node metastasis and the tumor size.

Conclusion: Our study suggests that 2.3.7.8-TCDD and some PCBs contribute to the development of tumor metastasis and other hallmarks of cancer aggressiveness. While these results should be considered with caution, this is the first study to identify such potential risk factors. Larger longitudinal studies are necessary to confirm our results.

Clinical Trial Protocol Record: 2013-A00663-42.

1. Introduction

Breast cancer (BC) is a major health concern with over 2 million new cases diagnosed in 2018 in women worldwide (Bray et al., 2018). If the cancer is located only in the breast, the 5-year survival rate is 99% but this rate decreases if there is spread to lymph nodes (85%). However, if distant metastases are present upon diagnosis the survival rate drops dramatically (26%) (Howlader et al., 1975; Siegel et al., 2017).

The exposure to environmental pollutants has been proposed, recently, as a risk factor for BC (Lauby-Secretan et al., 2013; Leng et al., 2016; Rodgers et al., 2018; Zhang et al., 2015). Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) have been identified as endocrine-disrupting-chemicals (EDC) and/or as carcinogens. Some of these compounds are persistent organic pollutants (POPs) since

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their hydrophobicity favors their bioaccumulation throughout the food chain. Overall, the scientific evidence concerning the associations between EDC and BC risk in humans has been inconclusive (Ochieng et al., 2015).

To date, researchers have been interested mainly in the link between BC initiation and EDC. Only a few recent studies have explored the associations with late stages of cancer, such as metastasis, which is the main cause of BC mortality. We have shown that TCDD (2,3,7,8-Tetrachlorodibenzo-p-dioxin) triggers invasive processes using human tumor cell lines as model systems (Bui et al., 2009; Diry et al., 2006; Pierre et al., 2014; Tomkiewicz et al., 2013). TCDD is best known for its induction of the AhR signalling pathway. AhR is responsive to many POPs (including PCBs) and aromatic hydrocarbons. Two studies have demonstrated an association between smoking and pulmonary metastasis among women with BC (Murin and Inciardi, 2001; Scanlon et al., 1995). Among POPs, TCDD is suspected to act as an endocrine disruptor similarly to organochlorine compounds (OCs). Several studies which focused on the impact of POPs and non-POPs on the initiation of breast cancer did not demonstrate a relationship between xenobiotics exposure and the incidence of BC (Høyer et al., 2000; Muscat et al., 2003; Parada et al., 2016a, 2016b; Roswall et al., 2018). However, p,p′-DDE was found to be associated with a dose-related increased relative risk of both lymph-node involvement and large tumors which suggests a potential relationship with tumor aggressiveness (Dairkee et al., 2008; Demers et al., 2000; Parada et al., 2019). Further, a very recent study found that higher levels of p,p′-DDE and DDT in the blood were associated with worse overall survival (Parada et al., 2019). Also, it has been shown that breast cancer cells exposed to Bisphenol A, another endocrine disruptor (non-POP), exhibit a gene expression profile of tumor aggressiveness which is associated with poor clinical outcomes for breast cancer patients (Dairkee et al., 2008). Finally, several POPs trigger metastatis formation in mice (Castillo-Sanchez et al., 2013; Guo et al., 2015; Liu et al., 2010; Ochieng et al., 2015; Pierozan et al., 2018; Pontillo et al., 2013; Seelbach et al., 2010; Zhang et al., 2016).

Since no observational studies have been conducted to evaluate the link between EDC and BC metastasis, we conducted an exploratory, hospital-based case-control study to analyze the concentrations of 49 POPs in samples of adipose tissue (AT) and serum from breast cancer patients with and without lymph node metastasis. We also analyzed the effect of obesity since this factor is associated with larger tumors, positive lymph node status and overall survival (Picon-Ruiz et al., 2017).

2. Material and methods

2.1. Study design

A monocentric cohort study (Clinical Trial Protocol Record 2013-A00663-42) was conducted in the Department of gynecological-oncological surgery of the Georges-Pompidou European Hospital (Paris, France) from 12/2013 to 11/2017. Participation in the study was proposed to each female newly diagnosed for BC and operated in the department, aged 18 years or more, who possessed a uni- or multifocal lesion with the principal lesion being > 1 cm in size or palpable. All patients (10% of total BC population) gave informed consent to participate in the study, which was approved by the 'Comité de Protection des Personnes' in 2013 [French equivalent of an Institutional Review Board (IRB)]. Patients unable to give informed consent were not included. The reasons for not participating in the study were: 1) Non-palpable lesions. The lesion had to be palpable so that peritumoral adipose tissue could be easily collected; 2) Patients who have an excessive number of solicitations from many studies in the department with a large number of questionnaires; 3) Men were removed from the study for reasons of homogeneity. At enrollment, the participants completed an assisted lifestyle questionnaire and provided a blood sample to assess POP concentrations before any treatment was initiated. Patients underwent partial or total mastectomy. Lymph node biopsy and axillary lymph node removal (depending upon the lymph node biopsy results) were performed according to the American Society of Clinical Oncology (ASCO) guidelines. During the surgery, an adipose tissue sample (1–3 g) was obtained at a distance of 1 cm from the palpable tumor for the measurement of POPs. The cancer tissue removed during the surgery was sent to the pathology laboratory for clinical analyses. Nodal status was determined for each patient. Patients were classified into 2 groups and participants with lymph node involvement were placed in the metastatic group (classification was performed without any information regarding the POP measurement). All the patients' data were anonymized and recorded on a computerized database (Fig. 1).

2.2. Chemical analysis

The methodologies for the isolation, detection and quantification of the targeted POPs, including dioxins (PCDD/F), polychlorobiphenyls (PCB), polybromodiphenylethers (PBDE), polybromobiphenyls (PBB) and hexabromocyclododecane (HBCD), have been described (Antignac et al., 2006, 2009; Bichon et al., 2015; Cariou et al., 2005; Costera et al., 2006; Ploteau et al., 2016). The complete list of chemicals (IUPAC) can be found in Table S1. Briefly, samples were first submitted to a high pressure and temperature extraction (ASE Dionex, Sunnyvale, CA, USA) (fat) or a liquid/liquid extraction with pentane (serum). The resulting extracts were weighed to measure fat content (gravimetric method for fat, enzymatic determination for serum) and reconstituted in hexane for further sample clean up. PCDD/F, PCB, PBDE and PBB measurements were performed by gas chromatography (Agilent 7890A) coupled to high-resolution mass spectrometry (GC-HRMS) on double sector instruments (JEOL MS 700D and 800D) after electron impact ionization (70 eV; 10% valley) and in the single ion monitoring (SIM) acquisition mode. The three HBCD isomers (alpha, beta and gamma) were quantified using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) on a triple quadrupole instrument (Agilent 6410) using electrospray ionization and selective reaction monitoring as the acquisition technique. All the analyses were conducted in an ISO 17025:2005 accredited laboratory. The lipid content for serum samples was determined with enzymatic kits (Biolabo, Maizy, France) which allowed determination of the concentrations of phospholipids (PHO), triacylglycerides (TAG), total cholesterol (t.CHO) and free cholesterol (f.CHO). Total serum lipids (TSL) were estimated using the following formula (Akins et al., 1989): $TSL = 1.677 * (t.CHO - f.CHO) + (f.CHO + TAG + PHO)$.

2.3. Statistical analysis

Demographic characteristics were summarized using the median and interquartile range, for continuous variables, and the frequency and percentage, for categorical variables. The statistical comparison of demographic characteristics between groups (i.e. metastatic versus non-metastatic) was performed using the Mann-Whitney-Wilcoxon Test and Fisher's exact test, for the numerical and the categorical variables, respectively. The associations calculated with Spearman's correlation coefficients were plotted in heatmaps. The distributions of POPs between groups were summarized by medians and interquartile ranges and compared statistically using Mann-Whitney-Wilcoxon Tests. Those chemicals with detection rates below 75% were excluded from the statistical analysis and for the analytes whose detection rates were between 75 and 100%, the non-detected values were replaced by the limits of detection (Antweiler 2015).

Crude odds ratios (ORs), adjusted ORs (aORs) and corresponding 95% confidence intervals (CI) were calculated for each chemical (transformed to the natural logarithm) using unconditional logistic regression considering the non-metastatic cancer group as referent. Three adjusted models were considered to account for predefined variables known as factors which affect the internal levels of POPs and the risk of

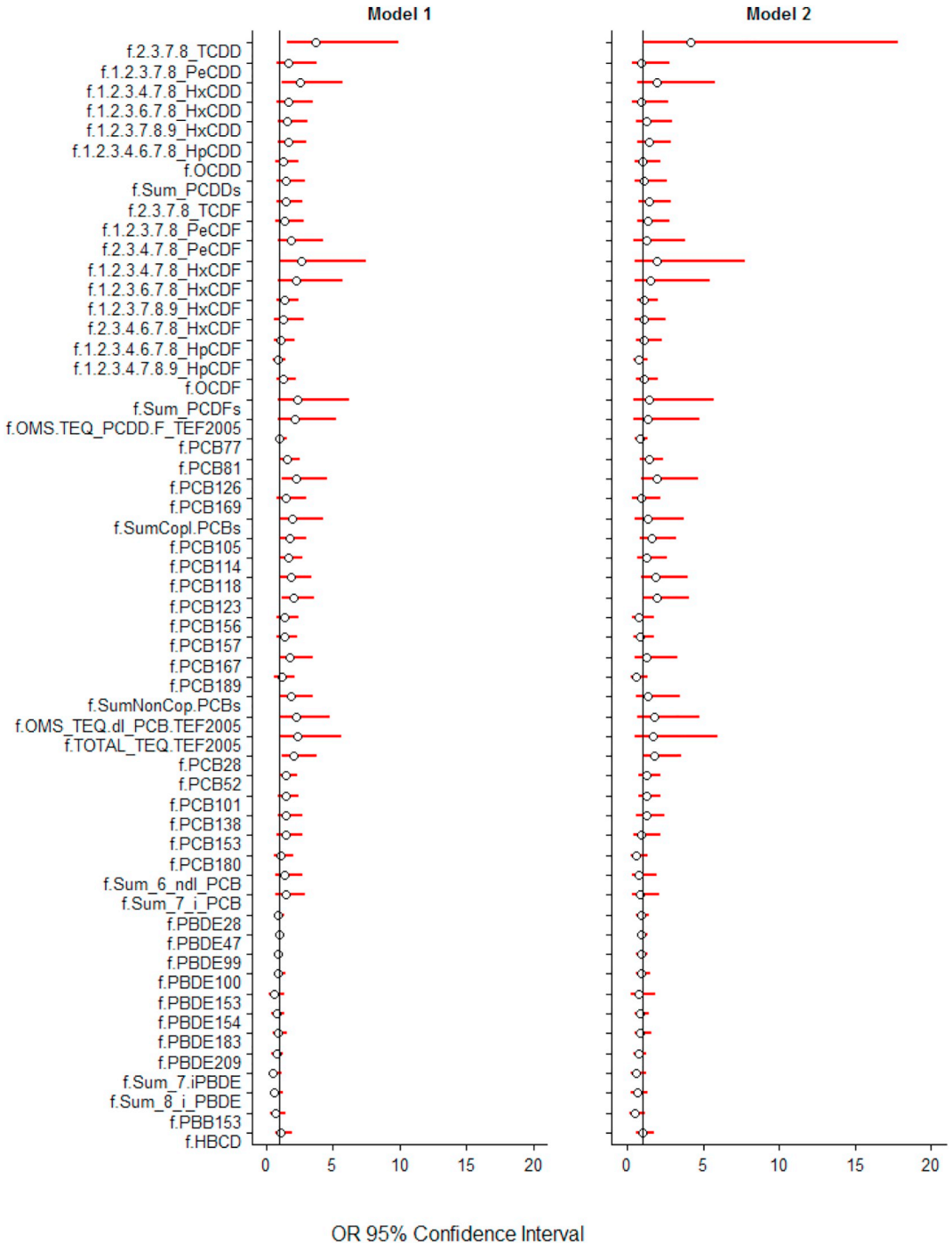

Fig. 1. Associations between the concentrations of persistent organic pollutants in adipose tissue and the presence of large tumor size (≥ 2 cm vs < 2 cm). The forest plots represent the odds ratios and 95% confidence intervals estimated through unconditional logistic regression. Model 1 - Crude model; Model 2 – adjusted for age, body mass index and familial history of breast cancer.

cancer. The confounding variables in the first adjusted model were the age, smoking and menopause. In the second adjusted model, we further included the body mass index (BMI) to evaluate the effect of adiposity as a potential confounder. In the third adjusted model, the familial history of BC also was included in the final model. Due to the small influence of confounding variables in the risk estimates, we only reported the results of the crude and fully adjusted models in the main manuscript.

We further explored whether the concentrations of POPs were associated with the number of lymph nodes affected, classified according to the international TNM classification of breast cancer (N1 $\leq$ 3 nodes, N2 = 4–9 nodes, N3 $\geq$ 10 positive nodes). The groups N2 and N3 were combined for the statistical analysis due to the low number of individuals in each group. This classification aims to better anticipate the aggressiveness of the tumor and its spreading potential. Tumor size was evaluated as an independent outcome of cancer aggressiveness by dichotomizing the cases, according to the median of the distribution of tumor sizes, into a group of small tumor size and another with large size (cut off = 2 cm). The concentrations of POPs were compared between the two groups with adjustments to POPs concentrations for BMI, age and familial breast cancer history in unconditional logistic models. The breast cancer expression of estrogen and progesterone receptors was

further considered in the stratification analysis but was limited to the positive phenotypes due to the low number of receptor-negative tumors.

Serum biomarkers were considered as surrogates of peritumoral AT biomarkers and were considered only in the global models to evaluate the coherence of estimates with AT models. For that reason, these proxy circulating markers were not considered in the stratification analysis and were relegated to secondary outcomes in the results section. Since lipid normalized concentrations of POPs in serum may lead to biased estimates when serum lipids may fall in the causal pathway between the exposures and the outcomes, a systematic comparison of different approaches was conducted (Cano-Sancho et al., 2018; O'Brien et al., 2016). Hence, we considered the Model WW, that uses serum concentrations expressed on a wet basis (pg or ng/mL); the Model WWA, that uses serum concentrations expressed on a wet basis (pg or ng/mL) and the TSA included as a covariate in the regression model; Model LW, that uses serum concentrations on a lipid basis (pg or ng/g lipids); Model LWA, that uses serum concentrations on a lipid basis (pg or ng/g lipids) and the TSA also included as a covariate in the regression model. We further tested the associations between metastasis and the ratio of concentrations between both matrices, serum and adipose tissue ($R_{ser:AT}$), as a multi-compartment biomarker (Ploteau et al., 2016). In equilibrated states, the concentrations of both compartments expressed in lipid weight should be equivalent, and the $R_{ser:AT}$ should be close to 1, conversely, altered states would be reflected by estimates > 1.

Mixtures of parent chemicals were generated by sum of concentrations and/or sum of 2005 World Health Organization dioxin-like toxic equivalents (WHO-TEQ). A threshold for the p-value < 0.05 was used to define associations as statistically significant. Considering the hypothesis-driven design of this study, the relatively small number of multiple tests conducted and the high correlation among variables tested, we opted not to correct for the multiple comparisons which minimizes the risk of inflating the type 2 errors (Rothman, 1990). All the statistical analyses were performed using R (v.3.3.1.) software.

3. Results

3.1. Demographic characteristics

A summary of the study population's characteristics is presented in Table 1 and Table S2 (participants with and without metastases). Both datasets reflected the homogeneity of the groups. The median age of participants ranged from 58 to 67 years. A majority of women had undergone menopause (71–85%). The most frequent histological subtype in both groups was infiltrating ductal carcinoma. There was no difference in the expression of ER, PR and HER2. As expected, the number of lymph nodes, the frequency of larger tumors and the Ki-67 proliferation index > 20%, were higher among patients with metastatic cancer. For several participants, the volume of serum or the amount of adipose tissue was insufficient to complete the chemical analyses and vice versa. Therefore, the serum dataset contained the results derived from 40 participants with metastatic cancer and 47 with non-metastatic cancer, different than the 38 participants with metastatic cancer and 53 with non-metastatic cancer recorded in the AT dataset.

3.2. Distribution of persistent organic pollutants

The distributions of POPs in AT and serum are summarized in Table 2 and in Tables S3 and S4. Chemicals with detection rates lower than 75% (i.e. PBB52, PBB101, β-HBCD, γ-HBCD in AT and serum and α-HBCD in addition in serum) were excluded from the statistical analysis. The POPs distribution was not statistically different between both groups.

After normalization by lipid content, the concentrations in AT and serum were highly correlated for most chemicals (Fig. S1A–B). However, most flame retardants in AT exhibited lower correlations with their circulating levels, although there were several exceptions (Fig. S1C). The correlation structures between chemicals appeared to be almost identical between metastatic and non-metastatic cancers in both AT and serum (Figs. S2–3).

Table 1
Population characteristics (Median and interquartile range (IQR) or counts and frequency percentages) from the adipose tissue dataset for individuals with breast cancer with metastasis or without metastasis. The distributions between groups have been compared with Mann–Whitney *U* test for continuous data and Fisher's exact test for categorical data.

	Metastatic N = 38	Non-metastatic N = 53	p-Value
Age (years)	59.8 (52.9–74.3)	64.0 (51.0–73.0)	0.99
BMI (kg/m^2)	24.0 (21.4–27.8)	24.0 (21.0–27.0)	0.63
Parity (number of infants)			0.94
0	8 (21%)	13 (25%)	
1	8 (21%)	13 (25%)	
2	12 (32%)	15 (28%)	
≥3	10 (26%)	12 (22%)	
Breastfeeding	18 (47%)	25 (47%)	1.00
Missing	1	3	
Menopause	27 (71%)	38 (72%)	1.00
Missing	1	0	
Hormonal contraception	9 (24%)	11 (21%)	0.80
Missing	2	1	
Professional category			0.27
White collar	17 (45%)	30 (57%)	
Blue collar	2 (5%)	2 (4%)	
Pink collar	17 (45%)	15 (28%)	
Un-employed/students	1 (3%)	5 (10%)	
Living area			0.17
Rural	5 (13%)	2 (4%)	
Urban	33 (87%)	50 (94%)	
Mixt	0 (0%)	1 (2%)	
First-hand smoking	8 (21%)	9 (17%)	0.60
Missing	1	0	
Second-hand smoking	4 (11%)	10 (19%)	0.38
Missing	1	0	
Personal history of cancer	2 (5%)	5 (9%)	0.69
Family history of breast cancer	8 (21%)	14 (26%)	0.62
Missing	0	1	
Histopathological examinations			
Triple negative	4 (11%)	6 (11%)	1.00
Estrogen receptor positive	33 (87%)	44 (83%)	0.77
Progesterone receptor positive	27 (71%)	36 (68%)	0.82
Tumor size			0.001
0.1–2 cm	12 (32%)	31 (58%)	
3.1–5 cm	20 (53%)	22 (42%)	
> 5 cm	6 (16%)	0 (0%)	
Affected lymph nodes			< 0.0001
≤ 3 nodes	26 (68%)	0 (0%)	
4–9 nodes	9 (24%)	0 (0%)	
≥10 nodes	3 (8%)	0 (0%)	
No applicable	0 (0%)	53 (100%)	
Grade histology			0.68
1	5 (13%)	10 (19%)	
2	21 (55%)	25 (47%)	
3	12 (32%)	18 (34%)	
Histological classification			0.89
Infiltrating ductal carcinoma	31 (82%)	40 (75%)	
Infiltrating lobular carcinoma	6 (16%)	9 (17%)	
Papillary carcinoma	0 (0%)	2 (4%)	
Mucinous (colloid) carcinoma	1 (3%)	1 (2%)	
Apocrin carcinoma	0 (0%)	1 (2%)	
HER2 Positive	2 (5%)	6 (11%)	0.46
Ki-67			0.03
< 20%	12 (32%)	29 (55%)	
> 20%	26 (68%)	24 (45%)	

Table 2
Summary of the (median (interquartile range)) of persistent organic pollutants measured in adipose tissue from individuals with metastatic and non-metastatic breast cancer. The groups were compared with the Mann-Whitney *U* test.

Chemicals[a]	Units	Metastatic (n = 38)	Non-metastatic (n = 53)	p-Value
2.3.7.8-TCDD	pg/g l.w.	1.4 (1.1–1.8)	1.3 (0.9–2.0)	0.70
1.2.3.7.8-PeCDD	pg/g l.w.	4.6 (3.7–5.9)	4.5 (3.6–6.6)	0.86
1.2.3.4.7.8-HxCDD	pg/g l.w.	2.3 (1.8–2.8)	2.2 (1.4–3.7)	0.72
1.2.3.6.7.8-HxCDD	pg/g l.w.	14.7 (11.9–19.8)	15.4 (9.2–24.4)	0.62
1.2.3.7.8.9-HxCDD	pg/g l.w.	1.6 (1.1–2.3)	1.5 (1.1–2.8)	0.73
1.2.3.4.6.7.8-HpCDD	pg/g l.w.	12.6 (8.1–19.8)	12.9 (6.7–23.8)	0.61
OCDD	pg/g l.w.	91.3 (60.4–123.3)	102.7 (71.8–162.1)	0.18
Sum PCDDs	pg/g l.w.	126.7 (92.4–180.5)	139.8 (96.9–235.1)	0.32
2.3.7.8-TCDF	pg/g l.w.	0.4 (0.3–0.5)	0.4 (0.3–0.6)	0.86
1.2.3.7.8-PeCDF	pg/g l.w.	0.2 (0.1–0.3)	0.2 (0.1–0.3)	0.64
2.3.4.7.8-PeCDF	pg/g l.w.	12.0 (8.7–14.9)	11.2 (8.6–19.3)	0.67
1.2.3.4.7.8-HxCDF	pg/g l.w.	2.0 (1.6–2.6)	2.2 (1.7–3.4)	0.41
1.2.3.6.7.8-HxCDF	pg/g l.w.	2.4 (1.8–2.9)	2.5 (1.9–3.7)	0.46
1.2.3.7.8.9-HxCDF	pg/g l.w.	0.1 (0.1–0.1)	0.1 (0.1–0.1)	0.61
2.3.4.6.7.8- HxCDF	pg/g l.w.	0.8 (0.6–1.0)	0.8 (0.5–1.1)	0.96
1.2.3.4.6.7.8-HpCDF	pg/g l.w.	1.2 (0.8–1.6)	1.3 (1.0–1.8)	0.24
1.2.3.4.7.8.9-HpCDF	pg/g l.w.	0.1 (0.1–0.2)	0.1 (0.1–0.1)	0.83
OCDF	pg/g l.w.	0.3 (0.2–0.6)	0.3 (0.2–0.4)	0.46
Sum PCDFs	pg/g l.w.	19.3 (16.4–26.2)	19.6 (14.9–29.8)	0.62
WHO-TEQ_{2005} PCDD/F	TEF_{2005}/g l.w.	11.8 (10.3–15.1)	11.6 (9.4–17.8)	0.81
PCB 77	pg/g l.w.	4.3 (2.8–7.8)	6.7 (3.4–11.9)	0.16
PCB 81	pg/g l.w.	2.1 (1.1–3.0)	2.4 (1.3–4.7)	0.14
PCB 126	pg/g l.w.	61.0 (42.8–81.5)	62.5 (40.7–103.6)	0.77
PCB 169	pg/g l.w.	79.0 (56.0–117.3)	84.0 (53.4–134.7)	0.94
Sum Copl. PCBs	pg/g l.w.	147.9 (106.9–217.9)	180.8 (103.3–241.4)	0.58
PCB 105	pg/g l.w.	4379.0 (2641.4–6129.3)	5247.4 (2473.8–11,189.3)	0.24
PCB 114	pg/g l.w.	1941.0 (1223.7–3227.5)	2329.2 (857.6–3397.8)	0.75
PCB 118	pg/g l.w.	20,010.1 (13,746.8–28,123.0)	24,288.5 (14,501.8–47,869.5)	0.37
PCB 123	pg/g l.w.	183.2 (132.3–373.8)	268.2 (140.5–573.7)	0.40
PCB 156	pg/g l.w.	21,177.7 (12,023.5–31,398.0)	20,850.0 (11,068.6–37,322.1)	0.88
PCB 157	pg/g l.w.	4496.6 (2325.4–6408.0)	4030.0 (1927.7–7644.8)	0.77
PCB 167	pg/g l.w.	4466.9 (2787.1–7229.4)	4314.9 (2698.6–7531.7)	0.91
PCB 189	pg/g l.w.	2801.2 (1811.9–4222.3)	2871.9 (1639.3–4313.4)	0.81
Sum Non Cop. PCBs	pg/g l.w.	59,011.5 (39,991.2–93,646.3)	62,660.2 (36,089.7–115,909.2)	0.59
WHO-TEQ_{2005} PCB s	TEF_{2005}/g l.w.	10.7 (7.2–15.7)	11.5 (7.8–18.0)	0.57
TOTAL WHO-TEQ_{2005}	TEF_{2005}/g l.w.	22.5 (16.9–30.8)	24.1 (16.1–35.2)	0.71
PCB 28	ng/g l.w.	1.7 (0.9–2.4)	1.9 (1.0–3.0)	0.62
PCB 52	ng/g l.w.	0.3 (0.2–0.6)	0.4 (0.2–0.7)	0.26
PCB 101	ng/g l.w.	0.7 (0.5–1.4)	1.0 (0.5–1.9)	0.17
PCB 138	ng/g l.w.	71.9 (52.9–100.9)	72.3 (40.5–124.7)	0.89
PCB 153	ng/g l.w.	193.0 (131.1–253.3)	166.2 (117.6–283.4)	0.82
PCB 180	ng/g l.w.	168.4 (120.5–270.1)	156.6 (106.1–277.4)	0.63
Sum 6 non dioxin-like PCB	ng/g l.w.	438.6 (291.2–621.3)	391.5 (261.6–710.6)	0.78
PBDE 28	ng/g l.w.	0.021 (0.010–0.036)	0.025 (0.015–0.045)	0.18
PBDE 47	ng/g l.w.	0.238 (0.138–0.491)	0.361 (0.162–0.646)	0.23
PBDE 99	ng/g l.w.	0.067 (0.037–0.112)	0.079 (0.039–0.166)	0.42
PBDE 100	ng/g l.w.	0.123 (0.056–0.207)	0.119 (0.071–0.241)	0.61
PBDE 153	ng/g l.w.	1.440 (1.054–1.899)	1.436 (1.013–1.915)	0.98
PBDE 154	ng/g l.w.	0.025 (0.015–0.034)	0.024 (0.017–0.048)	0.66
PBDE 183	ng/g l.w.	0.122 (0.083–0.201)	0.142 (0.092–0.188)	0.68
PBDE 209	ng/g l.w.	1.761 (1.275–2.199)	1.454 (1.088–1.941)	0.27
Sum penta and hepta BDE	ng/g l.w.	2.296 (1.447–3.039)	2.288 (1.697–3.175)	0.66
Sum penta, hepta and deca BDE	ng/g l.w.	4.381 (3.484–5.537)	3.981 (3.016–5.299)	0.45
PBB 153	ng/g l.w.	0.609 (0.440–0.842)	0.640 (0.488–0.883)	0.47
α-HBCD	ng/g l.w.	1.020 (0.600–1.769)	1.615 (0.965–1.952)	0.18

[a] Note: PBB 52, PBB 101, β-HBCD and γ-HBCD presented < 75% of detected samples and excluded from the statistical analysis.

3.3. Associations between POPs and risk of breast cancer metastasis

The summary of adjusted ORs (95% CI) for the major chemical groups is presented in Table 3 whereas the detailed graphical summary of ORs and 95% CIs for the different models and chemicals is plotted in Fig. S4. Overall, the results from the different models using AT biomarkers did not show any significant association between POPs and the risk of metastasis. The ORs and respective 95% CI were uniformly distributed around the null. The Seveso dioxin 2.3.7.8-TCDD exhibited substantially higher adjusted ORs (1.72 (0.56–6.03)) than the rest of PCDDs or their sum (0.67 (0.28–1.54)) (model 4, Fig. S4, Table 3). Moreover, when these analyses were restricted to a sub-group with a high BMI ($\geq 25\,kg/m^2$), the ORs were larger and the risk of BC metastasis was significantly larger for 2.3.7.8-TCDD (aOR 4.48 (1.32–20.71)). Other chemicals such as PBDE 153 (aOR 2.27 (1.06–6.00)), 1.2.3.4.7.8 HxCDD or PCB114 also displayed higher ORs (> 2) with p values below 0.1 (Table 4). The results for the subgroup with a normal BMI ($< 25\,kg/m^2$) displayed null associations for all chemicals with ORs shifting below one for a large list of chemicals including 2.3.7.8-TCDD, the WHO-TEQ Sum of PCDD/F or the non-coplanar PCBs, which supports the concept that BMI modifies effects.

The stratification analyses taking into account only the sub-groups of individuals with breast cancer and the expression of estrogen and progesterone receptors are summarized in the Table S5. The results from both sub-groups showed very similar association patterns, with a global modification of the ORs towards the null or below. Remarkably,

Table 3
Summary estimates for the associations between the sums of concentrations of PCDDs, PCDFs, PCBs, PBDEs and PBB153 in adipose tissue and the risk of breast cancer metastasis represented with the odds ratios and 95% confidence intervals estimated from unconditional logistic regression (N = 91, 38 cases with metastatic cancer and 53 with non-metastatic cancer). The model was adjusted for age, smoking, body mass index, menopause and familial history of breast cancer. Abbreviations: WHO-TEQ, World Health Organization Dioxin-like Toxic Equivalents. NA: not applicable because the low detection rates. Ndl, non-dioxin like.

Chemical groups	Adipose tissue adjusted OR (95% CI)	p value	Serum adjusted OR (95% CI)	p value
Sum PCDDs	0.67 (0.28–1.54)	0.36	0.81 (0.41–1.59)	0.55
Sum PCDFs	0.70 (0.20–2.45)	0.57	0.93 (0.26–3.31)	0.91
Sum PCDD/Fs WHO-TEQ	0.90 (0.28–3.02)	0.86	0.96 (0.34–2.70)	0.93
Sum Coplanar PCBs	0.85 (0.33–2.19)	0.74	1.15 (0.46–2.96)	0.77
Sum Non-Coplanar PCBs	0.93 (0.38–2.25)	0.86	1.11 (0.57–2.20)	0.75
Sum PCBs WHO-TEQ	0.83 (0.33–2.08)	0.70	1.15 (0.52–2.59)	0.73
Sum Ndl-PCB	1.18 (0.48–2.99)	0.72	1.23 (0.58–2.67)	0.60
Sum PBDEs	1.32 (0.61–3.02)	0.49	1.73 (0.65–4.78)	0.27
PBB 153	0.80 (0.33–1.92)	0.62	1.23 (0.44–3.70)	0.70
α-HBCD	1.02 (0.57–1.80)	0.93	NA	NA

the negative associations with PCB 101, PBDE28, PBDE 47 and PBDE99 became statistically significant for both groups ($p < 0.01$). The number of participants with a negative expression of the estrogen receptor (n = 14) and triple negative breast cancer (n = 10) were too small to allow the computation of regression models, but the metastatic group exhibited, in general, larger concentrations of all POPs with statistically significant differences (Mann-Whitney U test, summary in Table S6) for 1.2.3.4.6.7.8-HpCDF ($p < 0.01$) and PCBs 101, 138, 153, 167 and the sum Ndl-PCBs and the sum of 8 PBDEs ($p < 0.05$).

With respect to serum biomarkers, the ORs and 95% CIs also were distributed around the null for most chemicals with minor effects for confounding variables (Fig. S5). Also, the systematic comparisons of serum models did not reveal a major effect of TSL on the risk estimates for any of the models for most chemicals (See details in Fig. S6). These results also suggested a lack of consistency among the risk models using AT biomarkers with the serum biomarkers for some chemicals where the associations shifted in the opposite direction (e.g. 2.3.7.8-TCDD or OCDF). To further explore the potential implications of the chemical dynamics among both compartments on the risk estimates, we included the ratio among compartments $R_{Ser:AT}$ as an independent variable in the risk models. The results from the logistic regression using $R_{Ser:AT}$ are summarized in the Fig. S7. In general, the ORs for the ratios were close to the null, exhibiting similar trends to those results reported for AT and serum biomarkers; that is, the $R_{Ser:AT}$ of Seveso dioxin, 2.3.7.8-TCDD consistently associated with the risk of metastasis (1.72 (1.02–3.09)) (Table 5).

4. Discussion

Given the role of the cellular microenvironment on BC metastasis, we have explored, for the first time, the associations between the internal dose of a large panel of POPs and the risk of BC metastasis. Our study shows the widespread presence of low amounts of POPs in human tissues despite the fact that most of the chemicals were banned many years ago. The concentrations of these chemicals are similar to those previously reported in France which supports a background exposure in our study individuals comparable with that of the general population (Ploteau et al., 2017).

To date, only a few studies have explored the associations between a large panel of POPs and BC metastasis. For example, positive associations have been found between the internal concentrations of OCP and tumor size (Demers et al., 2000; Høyer et al., 2001). Women with higher concentrations of PCBs (particularly PCB118) were found to have a higher risk of BC recurrence, (Muscat et al., 2003). PCB 118 was one of the PCBs that we found to be associated with tumor size. Our results suggest that 2.3.7.8-TCDD may be a risk factor for BC aggressiveness and a predictor of poor prognosis, especially among overweight women. Obesity is recognized as a factor for poor prognosis in BC due to aggressive features of the tumor and resistance to treatment (Barba et al., 2016; Jiralerspong and Goodwin, 2016). In previous work, we showed that AT releases POPs chronically and slowly (Kim et al., 2011). This internal release may be responsible for the chronic toxicity associated with POPs exposure (Joffin et al., 2018). Moreover, we have shown that POPs promote an epithelial-to-mesenchymal transition which is suspected to promote the formation of metastatic cells (Bui et al., 2009; Pierre et al., 2014). Therefore, in this study, we evaluated the potential involvement of adiposity through stratification of a sub-group of the study population with high BMI. The subgroup analysis revealed a different pattern of associations which enlarged the ORs (95% CI) for 2.3.7.8-TCDD, the most toxic of dioxins.

TCDD (and several PCBs, such as PCB 77 and 169) activate the Aryl hydrocarbon Receptor (AhR) signalling pathway. The AhR regulates the expression of xenobiotic metabolism enzymes (such as cytochromes P450) upon exposure to different types of hydrocarbons including dioxins or various PCBs. Recently, other gene targets have been identified which include transcriptional regulators of the EMT such as Slug and Snail (Pierre et al., 2014). As a consequence, the induction of Slug or Snail by TCDD through the activation of the AhR signalling pathway down-regulates the epithelial marker, E-Cadherin. This facilitates the migration of tumor cells (Diry et al., 2006). The AhR also regulates non-genomic pathways (Src and Focal Adhesion Kinase) which are important for the stability of focal adhesion sites (Tomkiewicz et al., 2013). Thus, the activation of the AhR signalling pathway by xenobiotics has been suspected to play a potential role in tumor progression. This suspicion is supported by the identification of TCDD as a potential biomarker of metastasis. In addition to TCDD, we also have found that two coplanar PCBs (77&169) in AT are positively associated with the risk of lymph node metastasis and tumor size. These two PCBs are two DLCs. The toxic equivalent factor (TEF) of PCB 169 is 0.01, one of the highest among all coplanar PCBs. The identification of these two activators of the AhR signalling pathway as potential biomarkers of tumor size and risk of lymph node metastasis also is consistent with the previous suspicion.

Several hypotheses can be formulated to explain the contribution of the adipocytic microenvironment to tumor aggressiveness which include the secretion of pro-migratory cytokines and chemokines or pro-inflammatory molecules the expression of which could be potentiated by the presence of AhR ligands (Diedrich et al., 2015). Emerging evidence also has shown that POPs may contribute to a dysfunctional adipocyte phenotype. This phenotype is characterized by an increased production of pro-inflammatory cytokines, oxidative stress and lipolytic rates which favor the local over-release of stored chemicals and pro-tumoral molecules by the peritumoral adipocytes (Artacho-Cordón et al., 2016; Howell and Mangum, 2011; Pestana et al., 2017; Ruzzin et al., 2010). These findings suggest that some chemicals may synergistically contribute to the metastatic risk through peritumoral adipocyte dysfunction in addition to the direct effects on breast cancer cells. Such a hypothesis would help to elucidate the gaps between

Table 4
Associations between concentrations of persistent organic pollutants in adipose tissue and metastatic breast cancer for the sub-population group with BMI ≥ 25 kg/m^2 (n = 44) and BMI < 25 kg/m^2 (n = 47). The models were adjusted for age, smoking, body mass index, menopause and familial history of breast cancer.

Chemical	BMI ≥ 25 kg/m^2			BMI < 25 kg/m^2		
	OR	95% CI	p-Value	OR	95% CI	p-Value
2.3.7.8_TCDD	4.48	(1.32–20.71)	0.03	0.90	(0.38–2.5)	0.83
1.2.3.7.8_PeCDD	2.07	(0.78–6.45)	0.17	0.66	(0.28–1.57)	0.33
1.2.3.4.7.8_HxCDD	2.92	(0.94–11.31)	0.09	1.07	(0.45–2.58)	0.88
1.2.3.6.7.8_HxCDD	1.70	(0.67–5.13)	0.29	0.73	(0.31–1.72)	0.45
1.2.3.7.8.9_HxCDD	0.97	(0.40–2.29)	0.95	0.87	(0.4–1.86)	0.71
1.2.3.4.6.7.8_HpCDD	0.89	(0.39–2.00)	0.77	0.71	(0.3–1.62)	0.42
OCDD	0.75	(0.33–1.56)	0.45	0.70	(0.33–1.42)	0.33
Sum_PCDDs	0.79	(0.35–1.73)	0.56	0.74	(0.34–1.53)	0.42
2.3.7.8_TCDF	0.83	(0.40–1.64)	0.59	0.89	(0.46–1.66)	0.71
1.2.3.7.8_PeCDF	0.92	(0.45–1.83)	0.80	0.94	(0.5–1.75)	0.85
2.3.4.7.8_PeCDF	2.09	(0.72–6.92)	0.19	0.60	(0.26–1.36)	0.22
1.2.3.4.7.8_HxCDF	1.01	(0.37–2.81)	0.99	0.72	(0.34–1.52)	0.39
1.2.3.6.7.8_HxCDF	1.36	(0.50–3.91)	0.55	0.65	(0.3–1.37)	0.26
1.2.3.7.8.9_HxCDF	1.33	(0.61–2.91)	0.45	1.15	(0.6–2.31)	0.68
2.3.4.6.7.8_HxCDF	1.09	(0.50–2.47)	0.83	1.15	(0.6–2.22)	0.68
1.2.3.4.6.7.8_HpCDF	0.90	(0.39–1.86)	0.79	0.96	(0.49–1.89)	0.90
1.2.3.4.7.8.9_HpCDF	0.83	(0.35–1.81)	0.65	1.29	(0.68–2.66)	0.45
OCDF	1.66	(0.80–3.92)	0.19	1.28	(0.65–2.76)	0.49
Sum PCDFs	1.48	(0.52–4.46)	0.46	0.71	(0.32–1.57)	0.39
WHO-TEQ Sum PCDD/F	2.22	(0.75–7.69)	0.17	0.70	(0.29–1.7)	0.40
PCB77	0.85	(0.36–1.88)	0.69	0.76	(0.37–1.49)	0.43
PCB81	0.87	(0.40–1.77)	0.70	0.62	(0.28–1.25)	0.20
PCB126	1.17	(0.51–2.74)	0.70	0.87	(0.39–1.9)	0.72
PCB169	2.84	(0.85–12.62)	0.12	0.82	(0.36–1.95)	0.64
Sum Coplanar PCBs	1.44	(0.53–4.24)	0.48	0.80	(0.35–1.83)	0.59
PCB105	1.06	(0.45–2.48)	0.90	0.70	(0.32–1.45)	0.35
PCB114	2.70	(0.94–9.31)	0.08	0.67	(0.29–1.53)	0.33
PCB118	1.39	(0.55–3.64)	0.49	0.67	(0.3–1.45)	0.31
PCB123	1.33	(0.55–3.29)	0.52	0.67	(0.3–1.46)	0.32
PCB156	2.95	(0.89–12.76)	0.10	0.84	(0.37–2)	0.68
PCB157	2.09	(0.80–6.46)	0.16	0.93	(0.4–2.45)	0.88
PCB167	3.61	(0.98–17.00)	0.07	0.70	(0.3–1.62)	0.40
PCB189	1.86	(0.69–5.96)	0.25	1.11	(0.48–2.74)	0.80
Sum Non-Coplanar PCBs	1.94	(0.67–6.33)	0.23	0.71	(0.31–1.64)	0.42
WHO-TEQ PCB	1.33	(0.51–3.59)	0.55	0.79	(0.35–1.78)	0.56
TOTAL WHO-TEQ	1.80	(0.61–5.95)	0.30	0.74	(0.31–1.8)	0.49
PCB28	1.13	(0.56–2.31)	0.72	0.86	(0.41–1.73)	0.68
PCB52	0.69	(0.29–1.41)	0.34	0.66	(0.31–1.33)	0.26
PCB101	0.67	(0.28–1.43)	0.33	0.74	(0.36–1.46)	0.38
PCB138	1.86	(0.73–5.52)	0.22	0.89	(0.44–1.76)	0.72
PCB153	2.01	(0.74–6.41)	0.20	0.96	(0.46–2.05)	0.92
PCB180	1.65	(0.66–4.73)	0.31	1.22	(0.55–2.94)	0.64
Sum 6 non dioxin-like PCB	1.92	(0.70–6.06)	0.22	0.99	(0.47–2.24)	0.99
Sum 7 non dioxin-like PCB	1.98	(0.71–6.34)	0.21	0.97	(0.46–2.19)	0.95
PBDE28	0.68	(0.33–1.33)	0.26	0.70	(0.35–1.29)	0.27
PBDE47	0.64	(0.29–1.31)	0.24	0.88	(0.46–1.64)	0.67
PBDE99	0.71	(0.33–1.47)	0.37	0.87	(0.44–1.66)	0.67
PBDE100	0.90	(0.43–1.89)	0.77	0.89	(0.46–1.68)	0.71
PBDE153	2.27	(1.06–6.00)	0.06	0.63	(0.27–1.26)	0.22
PBDE154	0.68	(0.31–1.39)	0.29	1.02	(0.54–1.94)	0.96
PBDE183	0.78	(0.38–1.54)	0.48	0.96	(0.49–1.88)	0.90
PBDE209	1.17	(0.57–2.43)	0.67	1.07	(0.57–2.08)	0.82
Sum 7 PBDE	1.19	(0.59–2.48)	0.63	0.73	(0.35–1.39)	0.35
Sum 8 PBDE	1.14	(0.56–2.37)	0.72	1.14	(0.6–2.23)	0.68
PBB153	1.40	(0.64–3.50)	0.42	0.58	(0.28–1.13)	0.12
α-HBCD	1.55	(0.60–4.67)	0.39	0.88	(0.37–1.99)	0.76

exposure to POPs, adiposity and breast cancer (Blücher and Stadler, 2017).

Furthermore, the OR (95% CI) was also enlarged in the sub-group of patients with > 3 metastatic lymph nodes which suggests an association between the metastatic stage and TCDD concentration. Finally, larger tumor size was associated with the presence of higher concentrations of TCDD and some PCBs for which statistically significant associations were observed after confounding adjustment. The statistically significant negative associations found for the ER + and PR + subgroups suggest receptor status can modify effects. They also highlight the need for studies with larger numbers of individuals with breast cancers with negative expression which are already acknowledged to be the most aggressive forms with the worst prognosis.

Although AT is accepted as the gold standard matrix for the evaluation of the internal dose of POPs, little is known about the dynamics of lipophilic pollutants in the breast microenvironment. In the current study, we made a substantial analytical effort to decipher the complex relationships between the concentrations of chemicals in two major compartments, AT and serum. Although the results showed a strong correlation of chemicals between both compartments, the application of such biomarkers in the regression models resulted in divergent risk estimates. Overall, these results suggest that the use of serum

Table 5
Summary of the levels (median (interquartile range)) of persistent organic pollutants measured in adipose tissue from the population dichotomized on the basis of the median of tumor size distribution. The groups were compared with the Mann-Whitney test.

	Units	Small size < 2 cm N = 43	Large size ≥ 2 cm N = 48	p-Value
2.3.7.8-TCDD	pg/g l.w.	1.2 (0.9–1.5)	1.6 (1.1–2.3)	0.003
1.2.3.7.8-PeCDD	pg/g l.w.	4.4 (3.4–5.7)	5.0 (3.7–6.7)	0.25
1.2.3.4.7.8-HxCDD	pg/g l.w.	1.9 (1.3–2.9)	2.6 (1.9–4.0)	0.017
1.2.3.6.7.8-HxCDD	pg/g l.w.	14.3 (9.1–19.7)	15.8 (11.9–25.6)	0.24
1.2.3.7.8.9-HxCDD	pg/g l.w.	1.4 (1.0–2.0)	1.7 (1.1–2.7)	0.15
1.2.3.4.6.7.8-HpCDD	pg/g l.w.	11.1 (6.2–16.9)	14.5 (8.6–24.0)	0.098
OCDD	pg/g l.w.	89.4 (62.6–132.8)	98.6 (72.1–159.3)	0.37
Sum PCDDs	pg/g l.w.	126.2 (92.8–186.8)	142.7 (102.5–217.4)	0.21
2.3.7.8-TCDF	pg/g l.w.	0.3 (0.2–0.5)	0.4 (0.3–0.6)	0.17
1.2.3.7.8-PeCDF	pg/g l.w.	0.2 (0.1–0.2)	0.2 (0.1–0.3)	0.41
2.3.4.7.8-PeCDF	pg/g l.w.	10.9 (7.9–15.7)	12.2 (9.3–19.3)	0.15
1.2.3.4.7.8-HxCDF	pg/g l.w.	2.0 (1.5–2.6)	2.3 (1.8–3.4)	0.063
1.2.3.6.7.8-HxCDF	pg/g l.w.	2.1 (1.7–3.0)	2.6 (1.9–3.9)	0.096
1.2.3.7.8.9-HxCDF	pg/g l.w.	0.1 (0.1–0.1)	0.1 (0.1–0.1)	0.26
2.3.4.6.7.8- HxCDF	pg/g l.w.	0.7 (0.5–0.9)	0.8 (0.6–1.1)	0.28
1.2.3.4.6.7.8-HpCDF	pg/g l.w.	1.3 (0.8–1.6)	1.2 (0.9–1.6)	0.94
1.2.3.4.7.8.9-HpCDF	pg/g l.w.	0.1 (0.1–0.2)	0.1 (0.1–0.1)	0.52
OCDF	pg/g l.w.	0.2 (0.2–0.4)	0.3 (0.2–0.6)	0.16
Sum PCDFs	pg/g l.w.	18.1 (14.3–29.2)	21.7 (16.8–29.8)	0.11
Sum WHO-TEQ PCDD/F	TEF_{2005}/g l.w.	11.3 (8.6–15.8)	12.6 (10.3–18.4)	0.15
PCB 77	pg/g l.w.	5.9 (3.2–11.9)	5.3 (3.3–10.9)	1.00
PCB 81	pg/g l.w.	1.9 (0.9–3.8)	2.5 (1.5–3.9)	0.11
PCB 126	pg/g l.w.	52.0 (34.3–80.9)	68.2 (49.9–112.5)	0.020
PCB 169	pg/g l.w.	79.9 (47.3–125.0)	85.9 (59.4–140.1)	0.22
Sum Coplanar PCBs	pg/g l.w.	137.1 (95.6–202.9)	180.6 (115.6–279.9)	0.064
PCB 105	pg/g l.w.	3397.2 (2315.0–6149.0)	5020.7 (3321.5–10,981.6)	0.044
PCB 114	pg/g l.w.	1633.8 (811.1–2883.6)	2565.9 (1468.1–4056.5)	0.051
PCB 118	pg/g l.w.	18,165.3 (11,329.7–29,421.4)	25,612.6 (15,978.2–47,962.2)	0.027
PCB 123	pg/g l.w.	179.6 (94.5–288.0)	301.3 (150.7–576.7)	0.009
PCB 156	pg/g l.w.	19,468.7 (9259.6–30,824.5)	23,091.2 (13,586.1–37,411.0)	0.27
PCB 157	pg/g l.w.	3602.2 (1868.1–6655.3)	4584.9 (2438.8–7011.3)	0.34
PCB 167	pg/g l.w.	3814.3 (2330.8–5996.2)	5220.7 (3174.9–7808.8)	0.056
PCB 189	pg/g l.w.	2726.2 (1616.2–4279.3)	3020.1 (1723.9–4290.8)	0.62
Sum Non Coplanar PCBs	pg/g l.w.	56,735.1 (33,999.6–85,933.3)	72,402.4 (46,204.6–115,654.6)	0.081
WHO-TEQ dl-PCB	TEF_{2005}/g l.w.	10.2 (6.2–13.6)	12.4 (8.1–20.6)	0.039
TOTAL WHO-TEQ	TEF_{2005}/g l.w.	21.7 (15.8–30.0)	25.7 (18.7–38.1)	0.048
PCB 28	ng/g l.w.	1.1 (0.8–2.3)	2.2 (1.2–3.5)	0.007
PCB 52	ng/g l.w.	0.3 (0.2–0.6)	0.4 (0.3–0.7)	0.056
PCB 101	ng/g l.w.	0.7 (0.5–1.5)	1.0 (0.6–2.0)	0.13
PCB 138	ng/g l.w.	67.1 (39.9–101.5)	81.0 (56.1–125.5)	0.15
PCB 153	ng/g l.w.	163.6 (119.8–280.4)	201.9 (126.9–289.1)	0.27
PCB 180	ng/g l.w.	160.2 (104.0–277.1)	164.9 (114.0–246.8)	0.74
Sum 6 ndl-PCB	ng/g l.w.	388.5 (275.9–683.3)	474.2 (280.4–717.4)	0.32
PBDE 28	ng/g l.w.	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.86
PBDE 47	ng/g l.w.	0.3 (0.1–0.7)	0.4 (0.2–0.5)	0.72
PBDE 99	ng/g l.w.	0.1 (0.0–0.2)	0.1 (0.0–0.1)	0.53
PBDE 100	ng/g l.w.	0.1 (0.1–0.3)	0.1 (0.1–0.2)	0.57
PBDE 153	ng/g l.w.	1.6 (1.0–2.1)	1.4 (1.0–1.8)	0.28
PBDE 154	ng/g l.w.	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.22
PBDE 183	ng/g l.w.	0.2 (0.1–0.2)	0.1 (0.1–0.2)	0.44
PBDE 209	ng/g l.w.	1.5 (1.3–2.2)	1.7 (0.9–2.1)	0.42
Sum 7 i PBDE	ng/g l.w.	2.6 (1.7–3.6)	2.2 (1.5–2.8)	0.12
Sum 8 i PBDE	ng/g l.w.	4.5 (3.7–5.7)	3.6 (3.0–5.3)	0.098
PBB 153	ng/g l.w.	0.6 (0.5–0.9)	0.6 (0.4–0.9)	0.44
α-HBCD	ng/g l.w.	1.1 (0.6–2.0)	1.3 (0.9–2.0)	0.34

biomarkers may result in inconsistent findings if used as surrogate biomarkers in peritumoral AT. These results are consistent not only with those of our previous studies in the field of endometriosis where serum biomarkers exhibited substantial disagreement with the estimates using AT biomarkers (Cano-Sancho et al., 2018) but also with growing evidence about the complexity of use and interpretation of circulating biomarkers (O'Brien et al., 2016). Future studies need take into account the use longitudinal markers of exposure that would approximate lifetime trajectories either by optimally using multiple sample time-points or by reconstruction of exposure trajectories through PBPK modeling (Verner et al., 2011).

The results from this study should be considered with caution due to some methodological limitations. For instance, this exploratory study has a limited sample size and was conducted within a clinical setting to deliver definitive inferential conclusions at a population level. For that reason, large population-based studies with greater statistical power and a lower risk of selection bias are necessary to confirm the findings. Furthermore, we purposely opted not to adjust for the multiple tests performed in this exploratory analysis because the hypothesis-based approach, of the high correlation between variables and to avoid the consequential inflation of type 2 errors (Rothman, 1990). For that reason, the results should be interpreted with caution in the light of potential type 1 errors which result in some associations due to chance. Another limitation is the measurement error derived from using proxy biomarkers instead of target tissue before the onset of the metastasis. The design of the present study may not rule out some potential reverse

causality as the main cause of the associations. In addition, in this first analysis we have evaluated the associations for each chemical individually and only using sums of concentrations or bioactivities to evaluate the effect of mixtures. Hence, we emphasize the need for further research to evaluate the simultaneous effects of these chemicals through more efficient statistical models. However, to date, there are few statistical approaches that are available to efficiently identify the combined associations of highly correlated chemicals reaching an optimal balance between bias and variance (Agier et al., 2016).

5. Conclusion

This exploratory study has investigated, for the first time, the association between lymph node metastasis, the first step of BC dissemination, and the internal exposure to POPs. Although the study is preliminary and consists of a limited sample size that precludes drawing definitive conclusions, the results suggest that the internal exposure to 2.3.7.8-TCDD and some PCBs could be associated with the risk of aggressive BC, in particular in obese patients and in patients with receptor negative cancers, conditions which merit further research. Thus, larger population-based longitudinal studies are necessary to confirm these associations. In addition, fundamental research must develop to improve our understanding at the mechanistic level of the effect of POPs on metastatic cellular processes and potential cross-talk with peritumoral adipose tissue.

Declaration of Competing Interest

The authors declare they have no actual or potential competing financial interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.105028.

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