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ATMOSPHERIC FINE PARTICULATE MATTER AND BREAST CANCER MORTALITY: A POPULATION-BASED COHORT STUDY

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ABSTRACT

Objectives

Atmospheric fine particulate matter ($PM_{2.5}$) has multiple adverse effects on human health. Global atmospheric levels of $PM_{2.5}$ increased by 0.55 $\mu g/m^3$/year (2.1%/year) from 1998 through 2012. There is evidence of a causal relationship between atmospheric $PM_{2.5}$ and breast cancer (BC) incidence but few studies have investigated BC mortality and atmospheric $PM_{2.5}$. We investigated BC mortality in relation to atmospheric $PM_{2.5}$ levels among patients living in Varese Province, northern Italy.

Methods

We selected female BC cases, archived in the local population-based cancer registry, diagnosed at age 50-69 years, between 2003 and 2009. The geographic coordinates of each woman's place of residence were identified and individual $PM_{2.5}$ exposures were assessed from satellite data. Grade, stage, age at diagnosis, period of diagnosis, and participation in BC screening were potential confounders. Kaplan-Meir and Nelson-Aalen methods were used to test for mortality differences in relation to $PM_{2.5}$ quartiles. Multivariable Cox proportional hazards modeling estimated hazard ratios (HR) and 95% confidence intervals (CI) of BC death in relation to $PM_{2.5}$ exposure.

Results

Of 2021 BC cases, 325 died during follow-up to 31/12/2013, 246 for breast cancer. Risk of BC death was significantly higher for all three upper quartiles

of PM exposure compared to the lowest, with HRs of death: 1.82 (95%CI 1.15-2.89), 1.73 (95%CI 1.12-2.67), and 1.72 (95%CI 1.08-2.75).

Conclusions

Our study indicates that the risk of BC mortality increases with $PM_{2.5}$ exposure. Although further studies are required to confirm these findings, they are further evidence that $PM_{2.5}$ exposure increases mortality and indicate an urgent need to improve global air quality.

STRENGTHS AND LIMITATIONS OF THE STUDY

- These is one of few studies to address the relation between atmospheric $PM_{2.5}$ and breast cancer mortality.
- $PM_{2.5}$ exposure was assessed using a new but validated method based on satellite data, overcoming the major limitation of the usual method of measuring $PM_{2.5}$ at thinly and irregularly distributed ground stations.
- We controlled for the usual confounding factors and also for participation in screening that may have introduced length-time and lead-time biases.
- We used high quality population-based cancer registry data to identify all breast cancer cases in the study area over the study period, and assess patient mortality.
- A limitation is that exposure was assessed for the 10x10 km square containing the woman's residence at diagnosis: this is an imperfect assessment of exposure since time spent outside this square is unknown.

INTRODUCTION

Atmospheric particulate matter (PM) may be natural or anthropogenic. In industrial and urban areas, PM is mainly anthropogenic in origin.[1] PM of diameter up to 10 μm (PM_{10}) and fine PM, up to 2.5 μm ($PM_{2.5}$), are documented to have multiple adverse effects on human health[2], and are classified by the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC) as group 1 carcinogens (carcinogenic to humans).[3]

A recent prospective meta-analysis of 17 cohort studies from nine European countries found a significant association between increasing levels of both PM_{10} and $PM_{2.5}$ and increasing lung cancer risk. The study concluded that PM air pollution contributed to lung cancer incidence in Europe.[4]

Notwithstanding the known toxicity of $PM_{2.5}$, global population-weighted concentrations increased by 0.55 μg/m3/year (2.1%/year) from 1998 through 2012.[5] It is noteworthy that the incidence of breast cancer is also increasing worldwide: It is the most now common female cancer worldwide.[6] In 2012 an estimated 1.67 million new cases were diagnosed across the globe: 749,000 in developed countries and 883,000 in developing countries.[6] Reasonable hypotheses are that the global increase in breast cancer incidence might be linked to increasing in PM concentrations, and that high PM might also worsen breast cancer survival. This is supported by the findings of a population-based study in California[7] which found that exposure to higher PM_{10} (HR 1.13, 95%CI 1.02-1.25, per 10 $\mu g/m^3$) and $PM_{2.5}$ (HR 1.86, 95%CI 1.12-3.10, per 5 $\mu g/m^3$) was significantly associated with early

mortality among women with breast cancer after adjusting for numerous covariates.[7]

There are other reasons to suspect an association between breast cancer survival and PM levels in the atmosphere. A Canadian study which assessed NO_2 levels as a proxy of traffic-related air pollution found that breast cancer incidence increased with increasing NO_2 exposure.[8] A Japanese study found that $PM_{2.5}$ levels estimated from measured PM_{10} levels were significantly associated with mortality for breast, endometrial and ovarian cancers after adjusting for smoking, population density, and hormone-related factors.[9] A 2007 cohort study in Western New York State also found that high exposure to traffic emissions at the time of menarche was associated with increased risk of pre-menopausal breast cancer (OR 2.05, 95% CI 0.92–4.54, p trend 0.03); and that high exposure at time of first birth increased the risk of postmenopausal disease (OR 2.57, 95% CI 1.16–5.69, p trend 0.19).[10]

To further investigate the association of atmospheric PM with breast cancer mortality, we carried out a study in Varese Province, northern Italy. This area is characterized by high breast cancer incidence (world age standardized rate 89.3/100,000)[6], the highest $PM_{2.5}$ levels in Europe[5], and a high quality cancer registry that is likely to have registered essentially all breast cancer cases occurring over any relatively recent period.[11-12]

To estimate $PM_{2.5}$ exposure we used satellite-based data that infers near-surface $PM_{2.5}$ concentrations from the satellite-observed total column aerosol loading using a chemical transport model.[5] This dataset has been shown to represent and correlate well with levels determined with ground-based $PM_{2.5}$

detectors.[5] Data from satellite observations have the advantage that they make available data at 10x10 km resolution, while ground-based observation stations are generally few and irregularly spaced. In Varese Province only four ground-based sites measure $PM_{2.5}$.

MATERIALS AND METHODS

Breast cancer cases

We performed a retrospective study on a cohort of women diagnosed with primary breast cancer. The cases were archived by the Varese Province section of the Lombardy Cancer Registry. A search using site code C50 and malignant epithelial morphology codes M8010-M8575 of the International Classification of Disease for Oncology (ICDO-3) retrieved a total of 2021 primary breast cancers cases diagnosed in the predetermined study period (2003-2009) and conforming to our selection criteria (50-69 years at diagnosis, no other cancer diagnosed previously).[13] Disease stage was as specified by TNM (6th edition, 2002).[14]

Study endpoint

Study endpoint was breast cancer mortality. Mortality data are routinely collected by the cancer registry by linkage to the Varese Province mortality database. Other sources of mortality information are used routinely to ensure completeness.

Estimation of $PM_{2.5}$

The procedure for estimating $PM_{2.5}$ exposure involved first retrieving each woman's address at diagnosis from the cancer registry (obtained from

electronic sources and manual checks) and then determining the geographic coordinates (latitude and longitude) of each address using the ArcGis 10.0 software.[15] Ground level $PM_{2.5}$ exposure at each address was then estimated from satellite observations and was considered a proxy of total exposure, so that exposure variations arising from daily or periodic movements away from home were not considered. The method described by van Donkelaar et al.[5] was used to estimate ground level $PM_{2.5}$ exposure. This approach combined total column aerosol optical depth retrievals from the NASA Moderate Resolution Imaging Spectroradiometer (MODIS), Multiangle Imaging Spectroradiometer (MISR), and Sea-viewing Wide Field-of-view Sensor (SeaWIFS) satellite instruments, with vertical aerosol profile and scattering properties as estimated by the GEOS-Chem chemical transport model.[5] Total column aerosol optical depth is a measure of the total light extinction due to scattering and absorption by atmospheric aerosols.

A three-year running median of $PM_{2.5}$ concentration was used to reduce noise in the annual satellite-derived values. The ground-level $PM_{2.5}$ estimates were available at a resolution of 10x10 km, and breast cancer case exposure was estimated as the $PM_{2.5}$ concentration in the 10x10 km area containing each case's residence.

Statistical methods

First, factors known or thought to influence breast cancer prognosis were analyzed by univariate Cox proportional hazard modeling to verify their effect on breast cancer mortality in our cohort. Factors analyzed were diagnosis period (2003-2006; 2007-2009), stage (I-IV) , grade (I-III, unknown), age

at diagnosis (two categories,50-59 years and 60-69 years), and participation in a breast cancer screening program (Yes, No). Year of diagnosis was included since, over time, treatment may have improved and diagnosis may have occurred earlier. Cancers diagnosed in the screening context are affected by length-time and lead-time bias and may also be less aggressive than those diagnosed outside of screening.[16]

We next ran univariate and multivariate Cox proportional hazard models to estimate hazard ratios (HRs) with 95% confidence intervals (CI) of breast cancer death according to quartiles of $PM_{2.5}$ exposure. The multivariate model was stratified (separate baseline hazard functions for each variable category within the Cox model) by age, grade, stage, diagnosis period and participation in screening.

Time to event or end of follow-up was calculated from date of diagnosis. Cases that died causes other than breast cancer were censored at date of death. Patients alive at study end were censored at that time (31/12/2013). Patients lost to follow-up were censored at date of loss to follow-up. The proportional hazards assumption was tested by analysis of scaled Schoenfeld residuals, estimating P values for each variable, adopting the method suggested by Therneau et al.[17]

Missing data were handled using a separate "not specified" category for unavailable disease stage and a separate "not specified" category for unavailable tumor grade when performing data analysis.

We also used the Kaplan–Meier method to produce survival curves for quartiles of $PM_{2.5}$ exposure, testing the significance of differences between

curves with the stratified log-rank test. We also used the Nelson-Aalen estimator to plot the cumulative hazard of breast cancer death for $PM_{2.5}$ exposure categories. The analyses were performed using the R statistical package.[18]

Italian legislation identifies cancer registries as collectors of personal data for research and public health purposes and does not consider that specific approval by an ethics committee is required to use this data for research and public health purposes. Although our study was an observational one based on individual data, all such data were anonymized prior to analysis.

RESULTS

Disease and other characteristics of the 2021 breast cancer patients are shown in Table 1. Eleven women moved outside the study area during follow-up and were censored at the date of leaving.

A total of 325 (16.1%) women died in the period up to 31 December 2013, 246 (12.2%) of these of breast cancer. Table 1 also shows HRs for breast cancer death according to categories of prognostic variables: HR of death increased significantly with advancing stage and grade, while participation in screening was associated with considerably reduced risk of death, at least over the study period. These findings are as expected.

Table 1. Patient (n=2021) and disease characteristics with univariate hazard ratios (HR) and 95% confidence intervals (CI) for breast cancer death

Variable		Breast cancer cases (N)	Breast cancer deaths (N)	HR (95% CI) for breast cancer death
Period of diagnosis				
	2003-2006	1199	163	1
	2007-2009	822	83	1.08 (0.82 1.42)
Disease stage				
	I	887	25	1
	II	550	48	3.21 (1.98-5.21)
	III	292	93	13.31 (8.56-20.70)
	IV	35	27	75.94 (43.94-131.24)
	Not specified	257	53	8.26 (5.14-13.3)
Participation in screening				
	No	1341	213	1
	Yes	680	33	0.29 (0.20-0.41)
Tumor grade				
	I	193	3	1
	II	1132	97	5.43 (1.72-17.13)
	III	513	104	14.06 (4.46-44.33)
	Not specified	183	42	16.93 (5.25-54.63)
Age at diagnosis				
	50-59	923	110	1
	60-69	1098	136	1.05 (0.82-1.35)

Table 2 shows univariate and multivariate HRs with 95% CIs for breast cancer death according to quartiles of $PM_{2.5}$ exposure. By the univariate model, breast cancer patients living in an area with $PM_{2.5}$ levels above the lowest quartile had significantly greater risk of breast cancer death than those living in areas with lowest quartile of $PM_{2.5}$ (<21.10 μg/m^3). The increased risk of death ranged from 72% (fourth quartile) to 82% (second quartile). In the multivariate model, which controlled for confounding factors, risks of breast cancer death were numerically greater and still significant for all exposure quartiles above the lowest.

Table 2. Hazard ratios (HR) and 95% confidence intervals (CI) for breast cancer death in relation to $PM_{2.5}$ exposure.

$PM_{2.5}$ quartiles (μg/m^3)	Cases (N)	Deaths (N)	HR (95%CI), breast cancer death	
			Univariate	Multivariate *
I (<21.10)	504	40	1	1
II (21.10-24.20)	462	56	1.56 (1.04-2.34)	1.82 (1.15-2.89)
III (24.20-26.50)	530	71	1.55 (1.06-2.29)	1.73 (1.12-2.67)
IV (≥26.50)	525	79	1.49 (1.02-2.19)	1.72 (1.08-2.75)

* Multivariate stratified by age, stage, grade, diagnosis and participation in screening.

Analysis of scaled Schoenfeld residuals showed that P values for increasing $PM_{2.5}$ quartiles were 0.93, 0.40, 0.38 and 0.32, indicating that the null

hypothesis of no variation of hazard with time could not be rejected, suggesting that the prognostic effect $PM_{2.5}$ remained constant over the entire follow-up.

Fig 1 shows Kaplan-Meir survival curves by $PM_{2.5}$ quartiles. Fig 2 shows Nelson-Aalen estimates of the cumulative hazard of breast cancer death by $PM_{2.5}$ quartiles. Figs 1 and 2 both indicate that breast cancer patients exposed to the three upper $PM_{2.5}$ levels (≥21.100 μg/m^3) had a significantly (P=0.04, stratified log-rank test) greater risk of breast cancer death than those living in an area with the lowest quartile of $PM_{2.5}$.

Yearly (2003-2009) averages of $PM_{2.5}$ exposure for all study women were: 26.57, 26.65, 26.43, 23.73, 21.78, 21.44, and 20.71 μg/m^3.

DISCUSSION

We have shown that high exposure to $PM_{2.5}$ is associated with increased mortality for breast cancer after correcting for a range of factors considered to influence breast cancer survival. As regards possible mechanisms mediating this association, little evidence is available. A recent study collected airborne particles in Taiwan and investigated their effects on breast cancer cell lines.[19] The particles themselves and their solvent extracts had a variety of effects on the cell lines, including in particular increased generation of reactive oxygen species (ROS), increased numbers of DNA strand breaks, and both estrogenic and anti-estrogenic activity (concentration dependent). It is noteworthy that particle-induced ROS generation was blocked by treatment with aryl hydrocarbon receptor antagonist suggesting that the aryl

hydrocarbon receptor mediated the particle-induced toxicity. This is consistent with the positive association between exposure to polycyclic aromatic hydrocarbons from car traffic and breast cancer incidence, reported by the Long Island Breast Cancer Study.[20] The authors of the Taiwan study concluded that particle-induced ROS formation contributed to oxidative DNA damage that may mediate particle-induced carcinogenesis.[19]

The finding that particles have both estrogenic and DNA-damaging effects suggests a potential mechanism for an effect on breast cancer: if inhaled PM entered the circulatory system from the lungs, estrogenic particles might find their way to breast tissue. However to our knowledge no data are available to indicate whether PM can reach breast tissue and further research is required in this area.[21] Most of the toxic effects of PM have been attributed either to direct damage to lung tissue or release of inflammatory mediators from airway cells into the circulatory system.[22] Notwithstanding these considerations the biological mechanisms by which $PM_{2.5}$ exposure increases to breast cancer mortality remain unknown.

Our study has several strengths. We used a population-based cancer registry to identify virtually all the breast cancer cases in the study area over the study period, in turn linking them to local and regional mortality databases to obtain accurate and complete survival information. Another strength is our use of satellite derived $PM_{2.5}$ data produced by van Donkelaar et al.[5] Traditional ground-based PM measurement methods may be accurate, but because measurement sites are thinly and irregularly distributed, their data cannot be used to assess the exposure of individuals over a wide geographic

area. The satellite data made it possible to estimate exposure in the 10x10 km area that included each woman's home. We consider that this 10x10 km area is particularly apt for our study purposes as it comprises the area where the woman is likely to have walked, visited friends, done her shopping, worked, and carried out other daily activities. Of course some women may have spent a considerable fraction of their time outside this area, perhaps at work, and this is a study weakness. Importantly, none of the women had missing values for $PM_{2.5}$ exposure.

Another study strength is that we controlled for factors (e.g. stage, grade, and participation in screening) known or suspected to influence breast cancer mortality. However we did not control for lifestyle factors, including diet and alcohol consumption, that may also influence breast cancer mortality.[23-24]

The Californian study[7] – the only other published study on breast cancer mortality in relation to $PM_{2.5}$ exposure – also found a strong association between breast cancer mortality and $PM_{2.5}$ exposure. However $PM_{2.5}$ exposure for people living in California was much lower than in Varese Province – which is among the highest in the world.[5] The lowest exposure category for California was $PM_{2.5}$ <11.64 μg/m^3: only three patients in our dataset had such a low exposure. However, the California researchers reported an HR for the upper category of 1.76 that is similar to the HRs for our three upper categories (1.82, 1.73 and 1.72 respectively).

A report of ongoing research in northern China on the link between PM_{10} and breast cancer survival also indicated increased risk with increasing PM exposure, and also that survival was lower in women with estrogen receptor

positive disease.[26] The authors suggested that PM may act as a xenoestrogen, in line with the data from the study on effects of PM on breast cancer cell lines.[25]

It is important to emphasize that the first quartile of exposure in our study is not a risk zero category, but only the reference category for the other quartiles.

CONCLUSIONS

Although our study has limitations, its findings are consistent with those of the California study and the report of a study in China indicating a strong association between breast cancer death and atmospheric PM exposure.

Clearly further studies are justified to further explore this association, particularly in view of the increasing worldwide incidence of breast cancer and increasing worldwide PM concentrations.[5-6] Our data add to the wealth of evidence that atmospheric PM has multiple adverse effects on human health, and increase pressure to lower PM levels worldwide.

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Competing interests

All authors declare they have no competing interests.

Data sharing statement

No additional data are available

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Authors' contributions

P. Contiero contributed to study conception, designed the study, performed the statistical analysis and wrote the first draft of the paper.

G. Tagliabue contributed to study conception, coordinated the clinical section of the study and contributed to writing the paper.

R. Tessandori and G. Barigelletti contributed to practical aspects of study design.

A. Borgini, M. Bertoldi and A Tittarelli were responsible for the exposure assessment.

A. van Donkelaar and R. V. Martin were responsible for developing the model deriving PM data from satellite data.

A Tittarelli, S Fabiano, A Scaburri and G Barigelletti developed the information system to archive and manage the data and performed the descriptive statistics.

A Maghini and T Codazzi retrieved the clinical information and performed the record linkage between the sources.

I Favia and A Cau transferred the clinical data into the study database.

All authors read and approved the final manuscript.

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Fig 1. Survival of breast cancer cases, diagnosed 2003-2009 and resident in Varese Province, northern Italy according to exposure to $PM_{2.5}$ (quartiles)

Fig 2. Cumulative hazard of breast cancer death in cases diagnosed 2003-2009 and resident in Varese Province, according to exposure to $PM_{2.5}$ (quartiles)

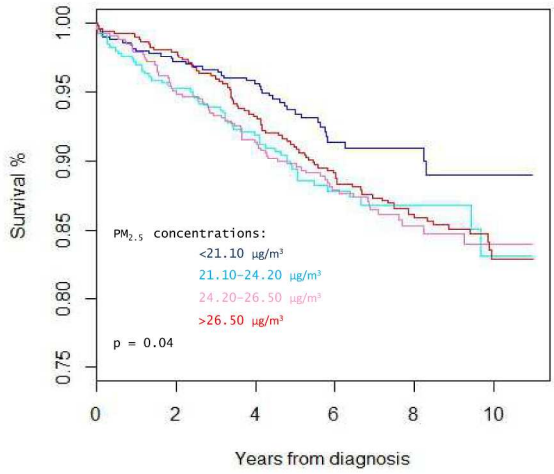

Fig 1. Survival of breast cancer cases, diagnosed 2003-2009 and resident in Varese Province, northern Italy according to exposure to PM2.5 (quartiles)

297x209mm (300 x 300 DPI)

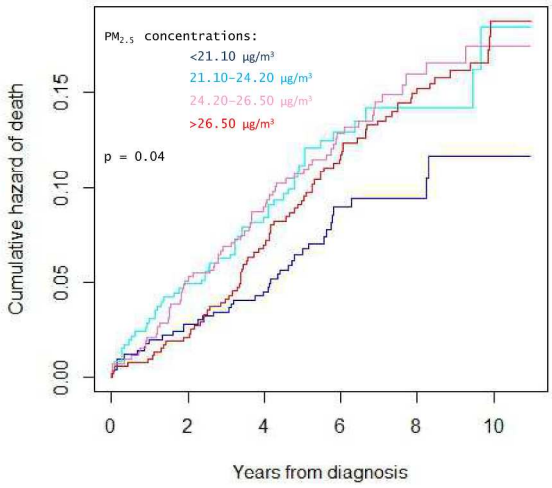

Fig 2. Cumulative hazard of breast cancer death in cases diagnosed 2003-2009 and resident in Varese Province, according to exposure to PM2.5 (quartiles)

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page 1: Title: "A population-based cohort study"
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Pages 2-3**: "**Our study indicates that the risk of BC mortality increases with $PM_{2.5}$ exposure."
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Pages 4-5: "PM2.5 [has] multiple adverse effects on human health, and is classified by WHO and IARC as carcinogenic to humans." "global PM2.5 concentrations increased by 2.1%/year from 1998 through 2012." "breast cancer incidence is also increasing worldwide".
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 5: "To investigate the association of atmospheric PM with breast cancer mortality ." Hypothesis:"the global increase in breast cancer incidence might be linked to increasing in PM concentrations and high PM might also worsen breast cancer survival ".
Methods			
Study design	4	Present key elements of study design early in the paper	Pages 6, Methods: "a retrospective study [of breast cancer mortality] on a cohort of women diagnosed with primary breast cancer".
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Pages 6-7, Methods: "...-cases archived by Lombardy Cancer Registry.[...] A total of 2021 primary breast cancers cases diagnosed in predetermined study period (2003-2009)". Page 2: "Of 2021 BC cases, 325 died during follow-up

			to 31/12/2013."
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Page 6 Methods – Breast cancer cases: "conforming to selection criteria (50-69 years at diagnosis, no other cancer diagnosed previously)"[were selected] Disease stage was as specified by TNM. "Mortality data are routinely collected by the cancer registry by linkage to the Varese Province mortality database. Other sources of mortality information are used routinely to ensure completeness."
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A, it is not a matched study.
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 6 : "Study endpoint was breast cancer mortality." Pages 7-8 Statistical methods: [potential confounders] "were diagnosis, stage, grade, age at diagnosis and participation in a breast cancer screening program. [and] year of diagnosis."
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Pages 6-8 Methods - Breast cancer cases from Varese cancer registry. " Mortality data [..] collected by the cancer registry by linkage to the Varese Province mortality database. Other sources of mortality information used to ensure completeness", Estimation of PM2.5, Statistical methods: "To estimate $PM_{2.5}$ exposure we used satellite-based data that infers near-surface $PM_{2.5}$ concentrations from the satellite-observed total column aerosol loading using a chemical transport model."
Bias	9	Describe any efforts to address potential sources of bias	Page 8 Methods – Statistical methods: "Cancers diagnosed in the screening context are affected by length-time and lead-time bias and may also be less aggressive than those diagnosed outside of screening"

Study size	10	Explain how the study size was arrived at	Page 6 Methods – Breast cancer cases: The cancer database was searched "using site code C50 and malignant epithelial morphology codes M8010-M8575 of the ICDO-3." "A total of 2021 primary breast cancers cases diagnosed in the predetermined study period (2003-2009) and conforming to selection criteria (50-69 years at diagnosis, no other cancer diagnosed previously)" were used.
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Pages 7-8 Methods – Statistical methods: "Factors analyzed were diagnosis period (2003-2006; 2007-2009), stage (I-IV) , grade (I-III, unknown), age at diagnosis (two categories,50-59 years and 60-69 years), and participation in a breast cancer screening program (Yes, No)"
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page 8 Methods – Statistical methods: "We ran univariate and multivariate Cox proportional hazard models to estimate HRs with 95% CI of breast cancer death according to quartiles of PM2.5 exposure. The multivariate model was stratified by age, grade, stage, diagnosis period and participation in screening."
		(*b*) Describe any methods used to examine subgroups and interactions	We did not analyse subgroup and we did not study interactions.
		(*c*) Explain how missing data were addressed	Page 8 - Missing data were handled using a separate "not specified" category for unavailable disease stage and a separate "not specified" category for unavailable tumor grade when performing data analysis.
		(*d*) If applicable, explain how loss to follow-up was addressed	Page 8 – loss of follow-up was addressed by censoring at date of loss of follow-up
		(*e*) Describe any sensitivity analyses	Not performed
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing	N/A

		follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Pages 10-11 Results – Table1
		(b) Indicate number of participants with missing data for each variable of interest	Pages 10-11. Table 1 and above
		(c) Summarise follow-up time (eg, average and total amount)	Page 9, Results: Followed up to 31 December 2013
Outcome data	15*	Report numbers of outcome events or summary measures over time	Page 9 – "A total of 325 (16.1%) women died in the period up to 31 December 2013, 246 (12.2%) of these of breast cancer." Pages 10-11 Results – Table1
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 11 Results – Table2 Page 11: "By the univariate model, breast cancer patients living in an area with $PM_{2.5}$ levels above the lowest quartile had significantly greater risk of breast cancer death than those living in areas with lowest quartile of $PM_{2.5}$ (<21.10 μg/m^3). The increased risk of death ranged from 72% (fourth quartile) to 82% (second quartile). In the multivariate model, which controlled for confounding factors, risks of breast cancer death were numerically greater and still significant for all exposure quartiles above the lowest."
		(*b*) Report category boundaries when continuous variables were categorized	Pages 10-11 Results – Table1; Table2 $PM_{2.5}$ was categorised into: I (<21.10); II (21.10-24.20), III(24.20-26.50) , IV (≥26.50) Page 11 Results – Table2
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 12 Discussion: "We have shown that high

			exposure to PM2.5 is associated with increased mortality for breast cancer after correcting for a range of factors considered to influence breast cancer survival."
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 14 Discussion: "...some women may have spent a considerable fraction of their time outside this area, perhaps at work, and this is a study weakness". "...we did not control for lifestyle factors, including diet and alcohol consumption, that may also influence breast cancer mortality."
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Pages 13-14 Discussion Page 15 Conclusions: "Our data add to the wealth of evidence that atmospheric PM has multiple adverse effects on human health".
Generalisability	21	Discuss the generalisability (external validity) of the study results	This is a preliminary study.
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 16: "This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors."

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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ATMOSPHERIC FINE PARTICULATE MATTER AND BREAST CANCER MORTALITY: A POPULATION-BASED COHORT STUDY

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ATMOSPHERIC FINE PARTICULATE MATTER AND BREAST CANCER MORTALITY: A POPULATION-BASED COHORT STUDY

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ABSTRACT

Objectives

Atmospheric fine particulate matter ($PM_{2.5}$) has multiple adverse effects on human health. Global atmospheric levels of $PM_{2.5}$ increased by 0.55 $\mu g/m^3$/year (2.1%/year) from 1998 through 2012. There is evidence of a causal relationship between atmospheric $PM_{2.5}$ and breast cancer (BC) incidence but few studies have investigated BC mortality and atmospheric $PM_{2.5}$. We investigated BC mortality in relation to atmospheric $PM_{2.5}$ levels among patients living in Varese Province, northern Italy.

Methods

We selected female BC cases, archived in the local population-based cancer registry, diagnosed at age 50-69 years, between 2003 and 2009. The geographic coordinates of each woman's place of residence were identified and individual $PM_{2.5}$ exposures were assessed from satellite data. Grade, stage, age at diagnosis, period of diagnosis, and participation in BC screening were potential confounders. Kaplan-Meir and Nelson-Aalen methods were used to test for mortality differences in relation to $PM_{2.5}$ quartiles. Multivariable Cox proportional hazards modeling estimated hazard ratios (HR) and 95% confidence intervals (CI) of BC death in relation to $PM_{2.5}$ exposure.

Results

Of 2021 BC cases, 325 died during follow-up to 31/12/2013, 246 for breast cancer. Risk of BC death was significantly higher for all three upper quartiles

of PM exposure compared to the lowest, with HRs of death: 1.82 (95%CI 1.15-2.89), 1.73 (95%CI 1.12-2.67), and 1.72 (95%CI 1.08-2.75).

Conclusions

Our study indicates that the risk of BC mortality increases with $PM_{2.5}$ exposure. Although additional research is required to confirm these findings, they are further evidence that $PM_{2.5}$ exposure is harmful and indicate an urgent need to improve global air quality.

STRENGTHS AND LIMITATIONS OF THE STUDY

- These is one of few studies to address the relation between atmospheric $PM_{2.5}$ and breast cancer mortality.
- $PM_{2.5}$ exposure was assessed using a new but validated method based on satellite data, overcoming the major limitation of measuring $PM_{2.5}$ at thinly and irregularly distributed ground stations.
- We used high quality population-based cancer registry data to identify breast cancer cases, and assess patient mortality.
- We controlled for confounding factors and also for participation in screening that may have introduced length-time and lead-time biases.
- Limitations are that lifestyle factors and comorbidities were not considered, and that exposure was assessed in the 10x10 km square containing the woman's residence, while time spent outside this square is unknown.

INTRODUCTION

Atmospheric particulate matter (PM) may be emitted or formed from natural or anthropogenic sources. In industrial and urban areas, PM is mainly anthropogenic.[1] PM of diameter up to 10 μm (PM_{10}) and fine PM, up to 2.5 μm ($PM_{2.5}$), are documented to have multiple adverse effects on human health[2,3], and are classified by the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC) as group 1 carcinogens (carcinogenic to humans).[4]

A recent prospective meta-analysis of 17 cohort studies from nine European countries found a significant association between increasing levels of both PM_{10} and $PM_{2.5}$ and increasing lung cancer risk. The study concluded that PM air pollution contributed to lung cancer incidence in Europe.[5]

Notwithstanding the known toxicity of $PM_{2.5}$, global population-weighted concentrations increased by 0.55 μg/m3/year (2.1%/year) from 1998 through 2012, largely driven by increases in developing countries such as China and India.[6] It is noteworthy that the incidence of breast cancer is also increasing worldwide and it is now the most common female cancer worldwide.[7] In 2012 an estimated 1.67 million new cases were diagnosed across the globe: 749,000 in developed countries and 883,000 in developing countries.[7]

Reasonable hypotheses are that the global increase in breast cancer incidence might be linked to increasing in PM concentrations, and that high PM might also worsen breast cancer survival. This is supported by the findings of a population-based study in California[8] which found that exposure

to higher PM_{10} (HR 1.13, 95%CI 1.02-1.25, per 10 μg/m^3) and $PM_{2.5}$ (HR 1.86, 95%CI 1.12-3.10, per 5 μg/m^3) was significantly associated with early mortality among women with breast cancer after adjusting for numerous covariates.

There are other reasons to suspect an association between breast cancer survival and PM levels in the atmosphere. A Canadian study which assessed NO_2 levels as a proxy of traffic-related air pollution found that breast cancer incidence increased with increasing NO_2 exposure.[9] A Japanese study found that $PM_{2.5}$ levels estimated from measured PM_{10} levels were significantly associated with mortality for breast, endometrial and ovarian cancers after adjusting for smoking, population density, and hormone-related factors.[10] A 2007 cohort study in Western New York State also found that high exposure to traffic emissions at the time of menarche was associated with increased risk of pre-menopausal breast cancer (OR 2.05, 95% CI 0.92–4.54, p trend 0.03); and that high exposure at time of first birth increased the risk of postmenopausal disease (OR 2.57, 95% CI 1.16–5.69, p trend 0.19).[11]

To further probe the association of atmospheric PM with breast cancer, we carried out the present study in Varese Province, northern Italy. We investigated breast cancer mortality (primary study endpoint) in relation to residential exposure to atmospheric $PM_{2.5}$ as determined by a satellite-based method.

MATERIALS AND METHODS

Study area

Varese Province, Region of Lombardy, northern Italy (Fig 1) has a population of 877 000 and population density of 731.4/km^2. $PM_{2.5}$ comes from mainly from non-industrial emissions (e.g. heating) (30-42%) and road traffic (30-32%).[12] As Fig. 1 shows, Varese Province is situated in an area (the plain of the river Po) enclosed by mountains which block atmospheric circulation. As a result atmospheric pollution tends to build up. In fact atmospheric $PM_{2.5}$ levels in the plain of the Po are among the highest in the world.[6]

Varese Province is also characterized by high breast cancer incidence (world age standardized rate 89.3/100,000)[7] and a high quality cancer registry that is likely to have registered essentially all breast cancer cases occurring over any relatively recent period. [13-14]

Breast cancer cases

We performed a retrospective study on a cohort of women diagnosed with primary breast cancer. The cases were archived by the Varese section of the Lombardy Cancer Registry. A search using site code C50 and malignant epithelial morphology codes M8010-M8575 of the International Classification of Disease for Oncology (ICDO-3) retrieved a total of 2021 primary breast cancer cases diagnosed in the predetermined study period (2003-2009) and conforming to our selection criteria (50-69 years at diagnosis, no other cancer diagnosed previously).[15] All 2021 breast cancer cases were used in the analysis. Disease stage was as specified by TNM (6th edition, 2002).[16]

Mortality ascertainment

Mortality data from the Varese Province mortality database are routinely collected by the cancer registry and linked to cancer cases by the Epilink

software, which achieved 98.8% specificity and 96.5% sensitivity for linking in a published study.[17] Epilink flags problematic cases for manual checking to enhance linkage accuracy. Each cancer case identified as deceased is checked against the Social Security List of all persons who receive health caser in the Region of Lombardy (essentially the entire population). The vital status field for each person in the Social Security List is updated frequently and serves as an independent check of the vital status of cancer cases archived by the registry.

Estimation of $PM_{2.5}$

The procedure for estimating $PM_{2.5}$ exposure involved first retrieving each patient's address at the date of diagnosis (reference date) from the cancer registry and then determining the geographic coordinates (latitude and longitude) of each address using the ArcGis 10.0 software.[18] Ground level $PM_{2.5}$ exposure at each address was then estimated from satellite observations. The actual $PM_{2.5}$ value used as exposure proxy was the median of ground level $PM_{2.5}$ concentrations over the three years around the diagnosis date (so as to reduce noise in the annual satellite-derived values). Thus if a woman was diagnosed in 2006, the $PM_{2.5}$ concentration used was the median of annual concentrations for the years 2005, 2006 and 2007. The method described by van Donkelaar et al.[6] was used to estimate ground level $PM_{2.5}$ exposure. This approach combined daily total column aerosol optical depth data from the NASA Moderate Resolution Imaging Spectroradiometer (MODIS), Multiangle Imaging Spectroradiometer (MISR), and Sea-viewing Wide Field-of-view Sensor (SeaWIFS) satellite instruments, with coincident

vertical aerosol profile and scattering properties estimated by the GEOS-Chem chemical transport model, so as to produce longer-term means. [6] Total column aerosol optical depth is a measure of the total light extinction due to scattering and absorption by atmospheric aerosols.

The ground-level $PM_{2.5}$ estimates were available at a resolution of 10x10 km, and breast cancer case exposure was estimated as median $PM_{2.5}$ concentration over three years in the 10x10 km area containing each case's residence. Exposure variations arising from daily or periodic movements away from home are not considered by this method.

Data produced by this method have been shown to correlate well with levels determined by ground-based $PM_{2.5}$ detectors,[6] and have the advantage that they are available over an entire territory, while ground-based observation stations are generally few and irregularly spaced. In Varese Province only four ground-based sites measure $PM_{2.5}$.

Statistical methods

The analyses we performed are based on the Cox proportional hazard model which specifies the hazard as $\lambda(t) = \lambda_0(t)\exp(\beta X)$ where $\lambda(t)$ is the hazard function for the event in question (death). X is a vector of covariates, and β is a vector of coefficients to be estimated. The hazards for two subjects with fixed covariate vectors X_i e X_j are respectively $\lambda_i(t) = \lambda_0(t)\exp(\beta X_i)$ and $\lambda_j(t) = \lambda_0(t)\exp(\beta X_j)$. The hazard ratio (HR) is $\lambda_i(t)/\lambda_j(t) = \exp(\beta(X_i - X_j))$. To test the null hypothesis H_0 that $\beta = 0$ we used the likelihood ratio test. Because the Cox model assumes proportional hazards, this was tested by analysis of scaled Schoenfeld residuals, with associated p values. When the hazard was

suspected to be non-proportional over time, we performed additional analyses, substituting the conventional Cox β coefficient (for a given variable) with a time-dependent function β(t) obtained by adding the smoothed scaled Schoenfeld residuals to the conventional β coefficient.[19,20,21] Factors known or thought to influence breast cancer prognosis were initially analyzed by univariate Cox proportional hazard modeling to verify their effect on breast cancer mortality in our cohort. Factors analyzed were diagnosis period (2003-2006; 2007-2009), stage (I-IV) , grade (I-III, unknown), age at diagnosis (two categories,50-59 years and 60-69 years), and participation in a breast cancer screening program (Yes, No). Year of diagnosis was included since, over time, treatment may have improved and diagnosis may have occurred earlier. Cancers diagnosed in the screening context are affected by length-time and lead-time bias and may also be less aggressive than those diagnosed outside of screening.[22]

We next ran univariate and multivariate Cox proportional hazard models to estimate HRs with 95% confidence intervals (CI) of breast cancer death according to quartiles of $PM_{2.5}$ exposure. The multivariate model was stratified (separate baseline hazard functions for each variable category within the model) by age, grade, stage, diagnosis period and participation in screening.by age, grade, stage, diagnosis period and participation in screening to control for the possible confounding effects of these variables on mortality.

Time to event or end of follow-up was calculated from date of diagnosis. Cases that died of causes other than breast cancer were censored at the date

of death. Patients alive at study end were censored at that time (31/12/2013). Patients lost to follow-up were censored at the date of loss to follow-up. Cases with missing data (missing disease stage and tumor grade) were assigned to "not specified" categories in the analyses.

We also used the Kaplan–Meier method to produce survival curves for quartiles of $PM_{2.5}$ exposure, testing the significance of differences between curves with the stratified log-rank test. We also used the Nelson-Aalen estimator to plot the cumulative hazard of breast cancer death for $PM_{2.5}$ exposure categories. The analyses were performed using the R statistical package.[23]

Italian legislation identifies cancer registries as collectors of personal data for research and public health purposes and does not consider that specific approval by an ethics committee is required to use this data for research and public health purposes. Although our study was an observational one based on individual data, all such data were anonymized prior to analysis.

RESULTS

Disease and other characteristics of the 2021 breast cancer patients are shown in Table 1. One hundred and one (5%) women changed address during the study period: 90 of these moved from one part of the Province to another (and may have changed $PM_{2.5}$ exposure), while 11 moved outside the study area and were censored at the date of leaving. A total of 325 (16.1%) women died in the period up to 31 December 2013, 246 (12.2%) of these of breast cancer. Table 1 also shows HRs for breast cancer death

according to categories of prognostic variables: HR of death increased significantly with advancing stage and grade, while participation in screening was associated with considerably reduced risk of death, at least over the study period. These findings are as expected.

Table 1. Patient (n=2021) and disease characteristics with univariate hazard ratios (HR) and 95% confidence intervals (CI) for breast cancer death

Variable		Breast cancer cases (N)	Breast cancer deaths (N)	HR (95% CI) for breast cancer death
Period of diagnosis				
	2003-2006	1199	163	1
	2007-2009	822	83	1.08 (0.82 1.42)
Disease stage				
	I	887	25	1
	II	550	48	3.21 (1.98-5.21)
	III	292	93	13.31 (8.56-20.70)
	IV	35	27	75.94 (43.94-131.24)
	Not specified	257	53	8.26 (5.14-13.3)
Participation in screening				
	No	1341	213	1
	Yes	680	33	0.29 (0.20-0.41)
Tumor grade				
	I	193	3	1
	II	1132	97	5.43 (1.72-17.13)
	III	513	104	14.06 (4.46-44.33)
	Not specified	183	42	16.93 (5.25-54.63)
Age at diagnosis				
	50-59	923	110	1
	60-69	1098	136	1.05 (0.82-1.35)

Table 2 shows the results of the univariate and multivariate analyses, presented as HRs with 95% CIs for breast cancer death according to quartiles of $PM_{2.5}$ exposure. By the univariate model, breast cancer patients living in an area with $PM_{2.5}$ levels above the lowest quartile had significantly greater risk of breast cancer death than those living in areas with lowest quartile of $PM_{2.5}$ (<21.10 μg/m^3). The increased risk of death ranged from 72% (fourth quartile) to 82% (second quartile). For the multivariate model, which controlled for confounding factors, the likelihood ratio test gave p = 0.029, indicating that the null hypothesis of no association between breast cancer mortality and $PM_{2.5}$ could be rejected. In detail: HRs of breast cancer death were numerically greater than those produced by the univariate analyses and significant for all exposure quartiles above the lowest.

Table 2. Hazard ratios (HR) and 95% confidence intervals (CI) for breast cancer death in relation to $PM_{2.5}$ exposure.

$PM_{2.5}$ quartiles (μg/m^3)	Cases (N)	Deaths (N)	HR (95%CI), breast cancer death	
			Univariate	Multivariate *
I (<21.10)	504	40	1	1
II (21.10-24.20)	462	56	1.56 (1.04-2.34)	1.82 (1.15-2.89)
III (24.20-26.50)	530	71	1.55 (1.06-2.29)	1.73 (1.12-2.67)
IV (≥26.50)	525	79	1.49 (1.02-2.19)	1.72 (1.08-2.75)

* Multivariate stratified by age, stage, grade, diagnosis and participation in screening.

Analysis of scaled Schoenfeld residuals showed that P values for increasing $PM_{2.5}$ quartiles were 0.93, 0.40, 0.38 and 0.32, indicating that the null hypothesis of no variation of hazard with time could not be rejected, suggesting that the prognostic effect $PM_{2.5}$ remained constant over the entire follow-up.

Fig 2 shows Kaplan-Meir survival curves by $PM_{2.5}$ quartiles. Fig 2 shows Nelson-Aalen estimates of the cumulative hazard of breast cancer death by $PM_{2.5}$ quartiles. Figs 2 and 3 both indicate that breast cancer patients exposed to the three upper $PM_{2.5}$ levels (≥21.100 μg/m^3) had a significantly (P=0.04, stratified log-rank test) greater risk of breast cancer death than those living in an area with the lowest quartile of $PM_{2.5}$.

Yearly (2003-2009) averages of $PM_{2.5}$ exposure for all study women were: 26.57, 26.65, 26.43, 23.73, 21.78, 21.44, and 20.71 μg/006D^3.

DISCUSSION

We have shown that high exposure to $PM_{2.5}$ is associated with increased mortality for breast cancer after correcting for a range of factors considered to influence breast cancer survival. As regards possible mechanisms mediating this association, little evidence is available. A recent study collected airborne particles in Taiwan and investigated their effects on breast cancer cell lines.[24] The particles themselves and their solvent extracts had a variety of effects on the cell lines, including in particular increased generation of reactive oxygen species (ROS), increased numbers of DNA strand breaks,

and both estrogenic and anti-estrogenic activity (concentration dependent). It is noteworthy that particle-induced ROS generation was blocked by treatment with aryl hydrocarbon receptor antagonist suggesting that the aryl hydrocarbon receptor mediated the particle-induced toxicity. This is consistent with the positive association between exposure to polycyclic aromatic hydrocarbons from car traffic and breast cancer incidence, reported by the Long Island Breast Cancer Study.[25] The authors of the Taiwan study concluded that particle-induced ROS formation contributed to oxidative DNA damage that may mediate particle-induced carcinogenesis.[24]

The finding that particles have both estrogenic and DNA-damaging effects suggests a potential mechanism for an effect on breast cancer: if inhaled PM entered the circulatory system from the lungs, estrogenic particles might find their way to breast tissue. However to our knowledge no data are available to indicate whether PM can reach breast tissue and further research is required in this area.[26] Most of the toxic effects of PM have been attributed either to direct damage to lung tissue or release of inflammatory mediators from airway cells into the circulatory system.[27] Notwithstanding these considerations, the biological mechanisms by which $PM_{2.5}$ exposure increases to breast cancer mortality remain unknown.

Our study has several strengths. We used a population-based cancer registry to identify virtually all the breast cancer cases in the study area over the study period, in turn linking them to mortality databases to obtain accurate and complete survival information. Another strength is our use of satellite derived $PM_{2.5}$. Traditional ground-based PM measurement methods are

locally accurate, but are sparsely and irregularly distributed, adding considerable uncertainty to individual exposure assignments over a wide geographic area. The satellite data made it possible to estimate the exposure in each 10x10 km area containing each woman's home. We consider that this area is particularly apt for our purposes as it comprises the area where the woman is likely to have carried out most of her daily activities. Of course some women may have spent a considerable fraction of their time outside this area, perhaps at work, and this is a study weakness. Importantly, none of the women had missing values for $PM_{2.5}$ exposure.

Another study strength is that we controlled for factors (e.g. stage, grade, and participation in screening) known or suspected to influence breast cancer mortality. However we did not control for lifestyle factors, including diet and alcohol consumption, or comorbidities, that may also influence breast cancer mortality.[28,29,30]

The Californian study – the only other published study on breast cancer mortality in relation to $PM_{2.5}$ exposure – also found a strong association between breast cancer mortality and $PM_{2.5}$ exposure. However $PM_{2.5}$ exposure for people living in California was much lower than in Varese Province – which is among the highest in the world.[6] The lowest exposure category for California was $PM_{2.5}$ <11.64 μg/m^3: only three patients in our dataset had such a low exposure. However, the California researchers reported an HR for the upper category of 1.76 that is similar to the HRs for our three upper categories (1.82, 1.73 and 1.72 respectively).

A report of ongoing research in northern China on the link between PM_{10} and breast cancer survival also indicated increased risk with increasing PM exposure, and also that survival was lower in women with estrogen receptor positive disease.[31] The authors suggested that PM may act as a xenoestrogen, in line with the data from the study on effects of PM on breast cancer cell lines.[24]

It is important to emphasize that the first quartile of exposure in our study is not a risk zero category, but only the reference category for the other quartiles.

In conclusion, although our study has limitations, its findings are consistent with those of the California study. 5and the report of a study in China indicating a strong association between breast cancer death and atmospheric PM exposure. Clearly more research is justified to further explore this association, particularly in view of the increasing worldwide incidence of breast cancer and worldwide increases in PM concentrations.[6-7] Our data add to the wealth of evidence that atmospheric PM has multiple adverse effects on human health, and speak to the urgent need to lower atmospheric PM levels worldwide.

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Competing interests

All authors declare they have no competing interests.

Data sharing statement

No additional data are available

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Authors' contributions

P. Contiero contributed to study conception, designed the study, performed the statistical analysis and wrote the first draft of the paper.

G. Tagliabue contributed to study conception, coordinated the clinical section of the study and contributed to writing the paper.

R. Tessandori and G. Barigelletti contributed to practical aspects of study design.

A. Borgini, M. Bertoldi and A Tittarelli were responsible for the exposure assessment.

A. van Donkelaar and R. V. Martin were responsible for developing the model deriving PM data from satellite data.

A Tittarelli, S Fabiano, A Scaburri and G Barigelletti developed the information system to archive and manage the data and performed the descriptive statistics.

A Maghini and T Codazzi retrieved the clinical information and performed the record linkage between the sources.

I Favia and A Cau transferred the clinical data into the study database.

All authors read and approved the final manuscript.

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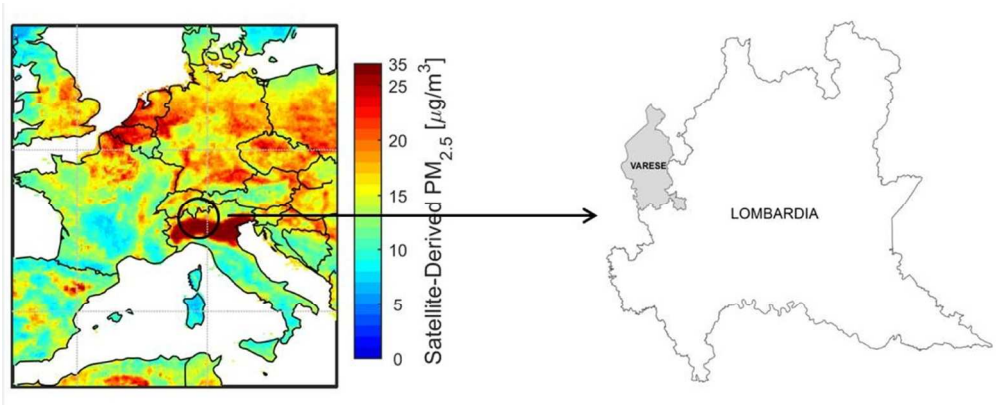
Fig 1. Map of the study area and satellite – derived $PM_{2.5}$

Fig 2. Survival of breast cancer cases, diagnosed 2003-2009 and resident in Varese Province, northern Italy according to exposure to $PM_{2.5}$ (quartiles)

Fig 3. Cumulative hazard of breast cancer death in cases diagnosed 2003-2009 and resident in Varese Province, according to exposure to $PM_{2.5}$ (quartiles)



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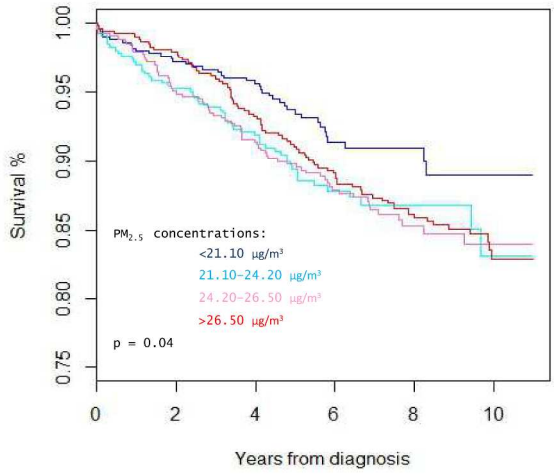

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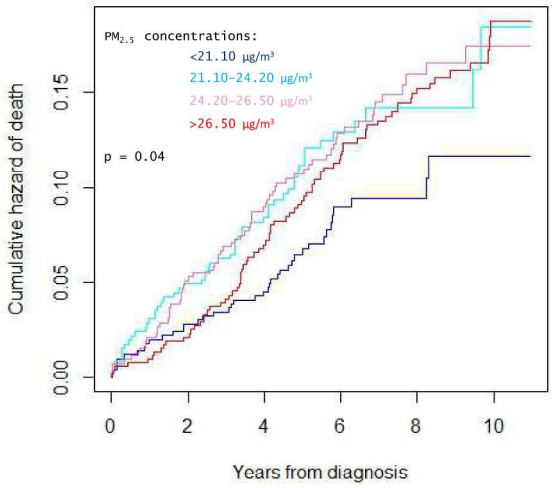

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page 1: Title: "A population-based cohort study"
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Pages 2-3**: "**Our study indicates that the risk of BC mortality increases with $PM_{2.5}$ exposure."
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Pages 4-5: "PM2.5 [has] multiple adverse effects on human health, and is classified by WHO and IARC as carcinogenic to humans." "global PM2.5 concentrations increased by 2.1%/year from 1998 through 2012." "breast cancer incidence is also increasing worldwide".
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 5: "To investigate the association of atmospheric PM with breast cancer mortality ." Hypothesis:"the global increase in breast cancer incidence might be linked to increasing in PM concentrations and high PM might also worsen breast cancer survival ".
Methods			
Study design	4	Present key elements of study design early in the paper	Pages 6, Methods: "a retrospective study [of breast cancer mortality] on a cohort of women diagnosed with primary breast cancer".
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Pages 6-7, Methods: "...-cases archived by Lombardy Cancer Registry.[...] A total of 2021 primary breast cancers cases diagnosed in predetermined study period (2003-2009)". Page 2: "Of 2021 BC cases, 325 died during follow-up

			to 31/12/2013."
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Page 6 Methods – Breast cancer cases: "conforming to selection criteria (50-69 years at diagnosis, no other cancer diagnosed previously)"[were selected] Disease stage was as specified by TNM. "Mortality data are routinely collected by the cancer registry by linkage to the Varese Province mortality database. Other sources of mortality information are used routinely to ensure completeness."
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A, it is not a matched study.
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 6 : "Study endpoint was breast cancer mortality." Pages 7-8 Statistical methods: [potential confounders] "were diagnosis, stage, grade, age at diagnosis and participation in a breast cancer screening program. [and] year of diagnosis."
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Pages 6-8 Methods - Breast cancer cases from Varese cancer registry. " Mortality data [..] collected by the cancer registry by linkage to the Varese Province mortality database. Other sources of mortality information used to ensure completeness", Estimation of PM2.5, Statistical methods: "To estimate $PM_{2.5}$ exposure we used satellite-based data that infers near-surface $PM_{2.5}$ concentrations from the satellite-observed total column aerosol loading using a chemical transport model."
Bias	9	Describe any efforts to address potential sources of bias	Page 8 Methods – Statistical methods: "Cancers diagnosed in the screening context are affected by length-time and lead-time bias and may also be less aggressive than those diagnosed outside of screening"

Study size	10	Explain how the study size was arrived at	Page 6 Methods – Breast cancer cases: The cancer database was searched "using site code C50 and malignant epithelial morphology codes M8010-M8575 of the ICDO-3." "A total of 2021 primary breast cancers cases diagnosed in the predetermined study period (2003-2009) and conforming to selection criteria (50-69 years at diagnosis, no other cancer diagnosed previously)" were used.
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Pages 7-8 Methods – Statistical methods: "Factors analyzed were diagnosis period (2003-2006; 2007-2009), stage (I-IV) , grade (I-III, unknown), age at diagnosis (two categories,50-59 years and 60-69 years), and participation in a breast cancer screening program (Yes, No)"
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page 8 Methods – Statistical methods: "We ran univariate and multivariate Cox proportional hazard models to estimate HRs with 95% CI of breast cancer death according to quartiles of PM2.5 exposure. The multivariate model was stratified by age, grade, stage, diagnosis period and participation in screening."
		(*b*) Describe any methods used to examine subgroups and interactions	We did not analyse subgroup and we did not study interactions.
		(*c*) Explain how missing data were addressed	Page 8 - Missing data were handled using a separate "not specified" category for unavailable disease stage and a separate "not specified" category for unavailable tumor grade when performing data analysis.
		(*d*) If applicable, explain how loss to follow-up was addressed	Page 8 – loss of follow-up was addressed by censoring at date of loss of follow-up
		(*e*) Describe any sensitivity analyses	Not performed
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing	N/A

		follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Pages 10-11 Results – Table1
		(b) Indicate number of participants with missing data for each variable of interest	Pages 10-11. Table 1 and above
		(c) Summarise follow-up time (eg, average and total amount)	Page 9, Results: Followed up to 31 December 2013
Outcome data	15*	Report numbers of outcome events or summary measures over time	Page 9 – "A total of 325 (16.1%) women died in the period up to 31 December 2013, 246 (12.2%) of these of breast cancer." Pages 10-11 Results – Table1
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 11 Results – Table2 Page 11: "By the univariate model, breast cancer patients living in an area with $PM_{2.5}$ levels above the lowest quartile had significantly greater risk of breast cancer death than those living in areas with lowest quartile of $PM_{2.5}$ (<21.10 μg/m^3). The increased risk of death ranged from 72% (fourth quartile) to 82% (second quartile). In the multivariate model, which controlled for confounding factors, risks of breast cancer death were numerically greater and still significant for all exposure quartiles above the lowest."
		(*b*) Report category boundaries when continuous variables were categorized	Pages 10-11 Results – Table1; Table2 $PM_{2.5}$ was categorised into: I (<21.10); II (21.10-24.20), III(24.20-26.50) , IV (≥26.50) Page 11 Results – Table2
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 12 Discussion: "We have shown that high

			exposure to PM2.5 is associated with increased mortality for breast cancer after correcting for a range of factors considered to influence breast cancer survival."
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 14 Discussion: "...some women may have spent a considerable fraction of their time outside this area, perhaps at work, and this is a study weakness". "...we did not control for lifestyle factors, including diet and alcohol consumption, that may also influence breast cancer mortality."
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Pages 13-14 Discussion Page 15 Conclusions: "Our data add to the wealth of evidence that atmospheric PM has multiple adverse effects on human health".
Generalisability	21	Discuss the generalisability (external validity) of the study results	This is a preliminary study.
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 16: "This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors."

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.