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Relationships between mild PM10 and ozone urban air levels and spontaneous abortion: clues for primary prevention

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The effects of environmental pollution on spontaneous abortion (SAB) are still unclear. Records of SAB were collected from five cities (514,996 residents) and correlated with PM10, NO_2 and ozone levels. Median pollutant concentrations were below legal limits. Monthly SABs positively correlated with PM10 and ozone levels but not with NO_2 levels. The mean monthly SAB rate increase was estimated equal to 19.7 and 33.6 % per 10 μg/m^3 increase in PM10 or ozone concentration, respectively. Higher values of PM10 and SABs were evident in cities with- than in those without pollutant industries, with a number of SABs twofolds higher in the former group. In conclusion, SAB occurrence is affected by PM10 (particularly if industrial areas are present) and ozone concentrations, also at levels below the legal limits. Thus, SAB might be considered, at least in part, a preventable condition.

Keywords: spontaneous abortion; PM10; ozone; nitrogen dioxide; pollution

Introduction

Air pollution is a heterogeneous mixture of gases and solid particles, each component having potential effect on the human body (World Health Organization 2013).

Of note, the foetal period is a vulnerable period for toxic substances, in particular considering the immune system development, which might be strongly affected by maternal exposure (Hertz-Picciotto et al. 2008).

Previous observations reported adverse effects of all particles equal to and less than 10 microns in aerodynamic diameter (PM10) on pregnancy, mainly in terms of birth defects, intrauterine growth retardation, premature birth and low birth weight (Kim et al. 2007; Patelarou & Kelly 2014). Similar effects were noticed in the case of nitric oxides (Brauer et al. 2008; Wu et al. 2009, 2011; Ghosh et al. 2012) and ozone (O_3) (Morello-Frosch et al. 2010; Olsson et al. 2012; Lee et al. 2013; Olsson et al. 2013).

Besides post-birth outcomes, spontaneous abortion (SAB) is considered the most common complication of early pregnancy (Regan & Rai 2000) (occurs 20 weeks or earlier into gestation, according to the definition of the Centers for Disease Control and Prevention, and the World Health Organization (Schorge et al. 2008)), with an incidence of about 17–22 % of all pregnancies (Garcia-Enguidanos et al. 2002).

A detrimental effect of environmental pollution on SAB has been previously suggested in terms of magnetic fields (Juutilainen et al. 1993; Lindbohm & Hietanen 1995;

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Lee et al. 2002; Li et al. 2002), parental smoking and environmental tobacco smoke (Hyland et al. 2014; Pineles et al. 2014). However, the existence of possible relationships between SAB occurrence and exposure to outdoor pollutants during pregnancy is still under investigation, with few previous studies leading to incomplete, inconclusive and/or conflicting results (Hemminki & Niemi 1982; Hansteen et al. 1996; Green et al. 2009).

Thus, the aim of this study was to examine whether maternal exposure to specific air pollutants is associated with the occurrence of SABs in a large group of free-living women.

Methods

A retrospective review of the medical records of SAB (all hospital admissions between 01 January 2013 and 31 December 2013, data provided by the Regional Epidemiological Observatory) was performed in eight district hospitals located in Apulia, Southern Italy. All hospitals (public and private) present in the study areas were considered in the analysis. The examined hospitals are in five different cities, provide medical services for a total of 514,996 inhabitants (Taranto, 198,728; Brindisi, 88,611; Grottaglie, 32,544; Barletta, 94,681; Andria, 100,432) and are the major providers of medical services for residents in their respective areas.

SAB was defined as the spontaneous loss of a foetus within 180 days of gestation, according to the definition of the ISTAT-D11 survey form, which was employed to collect data.

The total number of SABs, the birth rate (the number of live births per 1000 residents), the SAB ratio (number of SAB/number of live births) and the SAB rate (the number of SAB per 1000 fertile women [15–49 years]) were determined in the examined areas and compared with regional and national figures. The monthly number of live births, residents, fertile women and SABs at national and regional level were derived from the National Institute of Statistics (ISTAT).

All examined cities have pollutant emission by vehicular traffic. As reported by the European Pollutant Release and Transfer Register, industrial plants with large pollutant emissions (very close to urban area or enclosed in it) are present in three of the observed cities: Taranto (steel industry, cement plant, waste incinerators, two power plants and oil refinery), Brindisi (three power plants and oil refinery) and Barletta (large cement plant with co-combustion of pet-coke and waste-derived fuel).

No individual information for patients was used, since data were analysed at the aggregate level. Thus, the study had a waiver of informed consent.

The medical records were stratified according to the time of the event (month).

The monthly number of SABs was linked to average monthly air levels of PM10, nitrogen dioxide (NO_2) and ozone in the same geographical area, obtained through specific daily air monitoring networks managed by the Regional Environmental Agency (ARPA Puglia).

The monitoring stations (Taranto, $n = 6$; Brindisi, $n = 6$; Barletta, $n = 3$; Andria, $n = 1$; Grottaglie, $n = 1$) were positioned and managed by the Regional Environmental Agency according to technical criteria depicted by national and regional laws, in order to adequately and specifically assess the air pollutant concentrations and the dispersion of pollutants in each geographical area.

Each hospital was in the same urban area included in the radius of influence of the monitoring stations. The concentrations of pollutants used are averages of all stations in each city. It can be assumed that they are representative for use in the study populations, since the positioning of the monitoring stations was specifically selected by ARPA Puglia to explore the full dispersion of pollutants in the whole geographical area of interest. In the case of Taranto, Brindisi and Barletta, the concentrations of the same pollutant between the different monitoring stations were correlated each other ($0.60 < \rho < 0.99$; $0.0000001 < p < 0.01$, data not shown).

To allow for adjustment for the potential confounding effects of weather, data on meteorological variables (air temperature and humidity) were collected from the Italian Air Force Meteorological Service.

Frequencies of categorical variables and medians and range, or means and standard errors of continuous variables were calculated, as appropriate. Correlations were assessed by Spearman's rank correlation coefficient. A generalized additive model (GAM) for Poisson count data with penalized splines has been used to model relationships among SABs and exposure variables, while at the same time controlling for the above mentioned non-linear confounding effects of weather (Hastie & Tibshirani 1990). Models were fitted using R software version 3.1.1 (The R Foundation for Statistical Computing, Vienna, Austria) with the "mgcv" package.

The full model used for data analysis has symbolic expression log(mean monthly SAB rate) ~ intercept + s(Temperature, δ_1) + s(Relative humidity, δ_2) + PM10 + NO_2 + O_3, where $s(\ldots, \delta)$ indicates a smooth cubic spline term controlling for the corresponding confounding variable, the roughness being function of the smoothing parameter δ. Conversely, exposure variables were entered into the model on a linear scale, in order to retain standard parameter interpretation of the Poisson regression model (see below). Simultaneous exposure variable parameter estimation and smoothing parameter selection were based on a suitable model choice criterion (UBRE, UnBiased Risk Estimation) minimization, grounding on recently introduced ad hoc numerical methods based on penalized likelihood estimation (Wood 2004). In order to assess global significance of exposure variables, the full model and all the submodels nested within the full model were compared, by analysis of deviance, with the baseline model in which only smooth confounders entered the model predictor, log(SAB rate) ~ s(Temperature, δ_1) + s (Relative humidity, δ_2). At the same time, the UBRE score was estimated for all the eight nested models, with a view to provide a ranking of estimated models in terms of their goodness of fit after adjusting for increasing complexity. The full model turned out to be the most satisfying explanation for the SAB data (Table 1).

Numerical routines for parameter estimation provided by the "mgcv" package are fast and stable. However, as the iterative method by which GAMs are fitted can fail to converge in some circumstances, we used a slower "outer" version of the algorithm in which the numerical scheme is iterated to convergence for a suitable trial set of smoothing parameters, and UBRE scores are only evaluated after convergence has been diagnosed. We also varied in a sensible range the maximum number of iterations to perform, ascertaining that the impact on parameter estimates was irrelevant.

Given that a change in the level of exposure variable has a multiplicative effect on the mean SAB rate, interpretation of parameters is standard. For example, $\exp(10^* \beta_{PM10})$ is the relative risk (or, more precisely, rate ratio [RR]) for an increase of 10 μg/m^3 in the outdoor level of PM10, with other exposure variables held fixed and after having controlled for the non-linear smooth effect of weather. Alternatively, we can say that average SAB rate per cent variation (after a 10 μg/m^3 increase) is equal to $[100^*(1 - \exp(10^* \beta_{PM10}))]\%$. If the standard error of the estimates is s.e.(β_{PM10}), the standard error of the RR is calculated accordingly as $\exp[10^*\text{s.e.}(\beta_{PM10})]$.

Table 1. Descriptive statistics and analysis of variance between baseline model and all alternative models considering smoothing of confounding variables and linear predictors for air pollutant concentrations.

Symbolic model formulae (for brevity, intercept is omitted)	R^2 (adj.)**	Deviance explained (%)	UBRE	P
Log(mean SAB rate) ~ *s*(temperature) + *s*(humidity) [*Baseline*]	−0.0596	20.8	6.07	
1 *Baseline* + PM10	−0.0187	27.9	5.52	<0.01
2 *Baseline* + NO_2	0.0465	30.2	5.35	<0.01
3 *Baseline* + O_3	0.36	49.1	3.74	NA*
4 *Baseline* + PM10 + NO_2	0.0506	35	4.99	<0.01
5 *Baseline* + PM10 + O_3	0.355	51.5	3.59	<0.01
6 *Baseline* + NO_2 + O_3	0.345	49.4	3.76	<0.01
7 *Baseline* + PM10 + NO_2 + O_3	0.34	51.8	3.61	<0.01

*NA, not available. The simpler model (baseline) has the lower residual deviance, so it is impossible to conduct this test. This can happen with penalized estimators, even though the models are nested (especially if the smoothing parameters are selected automatically).
**For GAMs models, adjusted R-squared is defined as the proportion of the variance explained on the scale of the response variable (counts), where original variance and residual variance are both estimated using a suitable unbiased estimator (adjusted on the number of observations and the fitted degrees of freedom). Unlike the proportion of the null deviance explained by the model, the presence of multiple non-linear terms in the linear predictor makes it possible that this quantity can be negative if the estimated model is worse than a one-parameter constant model (and hence, the estimated model can be safely discarded from the search space).

Kruskal–Wallis analysis of variance by ranks followed by multiple-comparison *Z*-value test or Mann–Whitney *U*-test was employed to compare differences among groups, as appropriate. p-values < 0.05 were considered statistically significant.

Results

Globally were recorded 984 cases of SAB. The average birth rate in the examined cities was comparable to regional and national figures. However, all the examined cities excluding Andria showed a trend towards increased SAB ratio and rate as compared with the regional and national values, with the highest indices in the case of Brindisi, Barletta and Grottaglie. The highest absolute number of SABs was recorded in the three industrial cities (Taranto, Brindisi and Barletta) (Table 2).

Median air levels of PM10 (20.3 μg/m^3, range 9–41.5 μg/m^3), NO_2 (41.6 μg/m^3, range 19.6–81.3 μg/m^3) and ozone (97.2 μg/m^3, range 57.3–141.7 μg/m^3) were below the limits set by the WHO Air Quality Guidelines for Europe (50, 200 and 100 μg/m^3, respectively) (World Health Organization 2005) in the whole group of cities, with isolate exceeding levels only in the case of ozone during summer. The annual median temperature and humidity were, respectively, 19 °C (range 8.1–30.8) and 72 % (range 38.2–90.7), reflecting the typical local and Mediterranean climate.

Table 3 shows correlations between levels of air pollutants, meteorological variables and SABs. Levels of NO_2 and ozone (but not PM10) resulted to be correlated with air temperature and humidity.

The smoothed effect of temperature and relative humidity on SABs (baseline GAM) is shown in Figure 1. Diagnostic information about the fitting and the results of the best model are shown in Figure 2. The normal probability plot of deviance residuals looks close to a straight line, not showing substantive departures from normality (Shapiro–Wilk test of normality: $p = 0.81$: see also the histogram of residuals). In the same way,

Table 2. Comparison of average birth rate and epidemiological indices of SAB in the examined cities and with regional (Apulia) and national (Italy) figures during the year 2013.

Area	Birth rate (× 1000)	SAB (n.)	SAB ratio	SAB rate (× 1000)
Taranto	0.66 ± 0.04	21.3 ± 2.5[b,c]	0.17 ± 0.05[c,f]	0.46 ± 0.1[c,f]
Brindisi	0.68 ± 0.04	27.3 ± 2.5[a,b,c]	0.47 ± 0.05[b,d,e]	1.32 ± 0.1[b,d,e]
Barletta	0.69 ± 0.04	15.9 ± 2.5[b]	0.26 ± 0.05[d,e]	0.70 ± 0.1[d]
Grottaglie	0.69 ± 0.04	10.0 ± 2.5	0.5 ± 0.05[b,d,e]	1.29 ± 0.1[b,d,e]
Andria	0.82 ± 0.04	7.5 ± 2.5	0.10 ± 0.05[a,c]	0.30 ± 0.1[a,c,f]
Apulia	0.69 ± 0.04	327.6 ± 32.8*	0.11 ± 0.05	0.34 ± 0.1
Italy	0.72 ± 0.04	5743 ± 149*	0.14 ± 0.05	0.43 ± 0.1
ANOVA (*P*)	NS	<0.0005	<0.0005	<0.0005

Notes: Values are means ± SE. Differences were tested by Kruskal–Wallis analysis of variance by ranks followed by multiple-comparison *Z*-value test.
NS, not significant.
[a]$p < 0.05$ vs. Barletta; [b]$p < 0.05$ vs. Andria; [c]$p < 0.05$ vs. Grottaglie; [d]$p < 0.05$ vs. Apulia; [e]$p < 0.05$ vs. Italy; [f]$p < 0.05$ vs. Brindisi.
*Values excluded from the ANOVA.

Table 3. Correlations between levels of air pollutants, meteorological variables and SABs.

	PM10 (μg/m^3)	NO_2 (μg/m^3)	Ozone (μg/m^3)	Temperature (°C)	Humidity (%)
PM10 (μg/m^3)	–	NS	NS		
NO_2 (μg/m^3)	NS	–	NS		
Ozone (μg/m^3)	NS	NS	–		
Temperature (°C)	NS	$\rho = -0.53$ $p < 0.0001$	$\rho = 0.75$ $p < 0.002$	–	
Humidity (%)	NS	$\rho = 0.49$ $p < 0.001$	$\rho = -0.64$ $p < 0.001$	$\rho = -0.72$ $p < 0.001$	–
SAB (n.)	$\rho = 0.36$ $p < 0.01$	NS	$\rho = 0.44$ $p < 0.005$	NS	NS

Note: NS, not significant.

the plot of deviance residuals vs. linear predictor does not exhibit any apparent systematic patterns, which might be attributable to omitted terms or variables in the linear predictor and/or incorrect relationship between mean rate and explanatory variables (e.g. wrong link function).

Table 4 shows RRs and related 95 % approximate confidence intervals, effect estimates and approximate *p*-values for each exposure variable entering the best model. Significant effects were evident in the case of PM10 and O_3, but not of NO_2 air concentration.

The (mean) monthly SAB rate increase was estimated equal to 19.7 % per 10 μg/m^3 increase of PM10 and to 33.6 % per 10 μg/m^3 increase of ozone concentration.

Table 5 shows pollutant concentrations in each city, with significant differences for PM10 and NO_2, but not ozone. As for the absolute number of SABs, the highest levels of PM10 and NO_2 were recorded in the three industrial cities (Taranto, Brindisi and Barletta).

Cities were thereafter grouped according to the presence/absence of industrial pollution (Table 6), and higher values of PM10 (not NO_2 and ozone), higher SAB counts (but not SAB ratio and rate) and a lower birth rate were evident in the group with- as

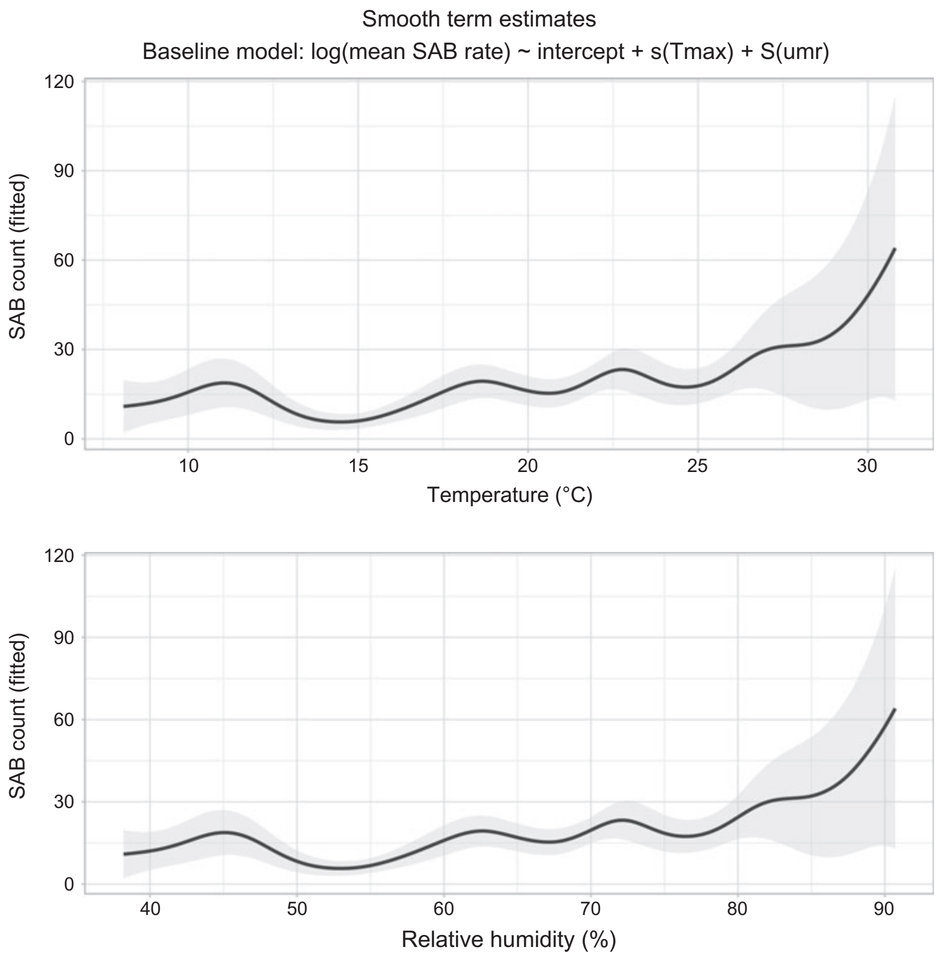

Figure 1. Smoothed effect of temperature (°C) and relative humidity (%) on the monthly number of SABs (GAM analysis, baseline model).

compared to that without industries. Absolute number of SABs was about twofolds higher in the group of cities with industrial pollution.

Levels of NO_2 (but not PM10 and ozone) positively correlated with the number of residents ($\rho = 0.39$, $p = 0.001$).

A trend towards comparable time variations was evident between SABs and the corresponding PM10 concentrations (Figure 3) but neither O_3 (Figure 4) nor NO_2 (data not shown) monthly air levels. PM10, NO_2 and O_3 levels were not correlated each other (Table 3).

Interestingly, ozone air concentration levels showed a positive linear correlation with both SAB rate and SAB ratio and a negative linear correlation with birth rate (Figure 5).

Discussion

SAB is a frequent complication of the early pregnancy (Regan & Rai 2000; Garcia-Enguidanos et al. 2002), and the present study demonstrates that exposure to specific air

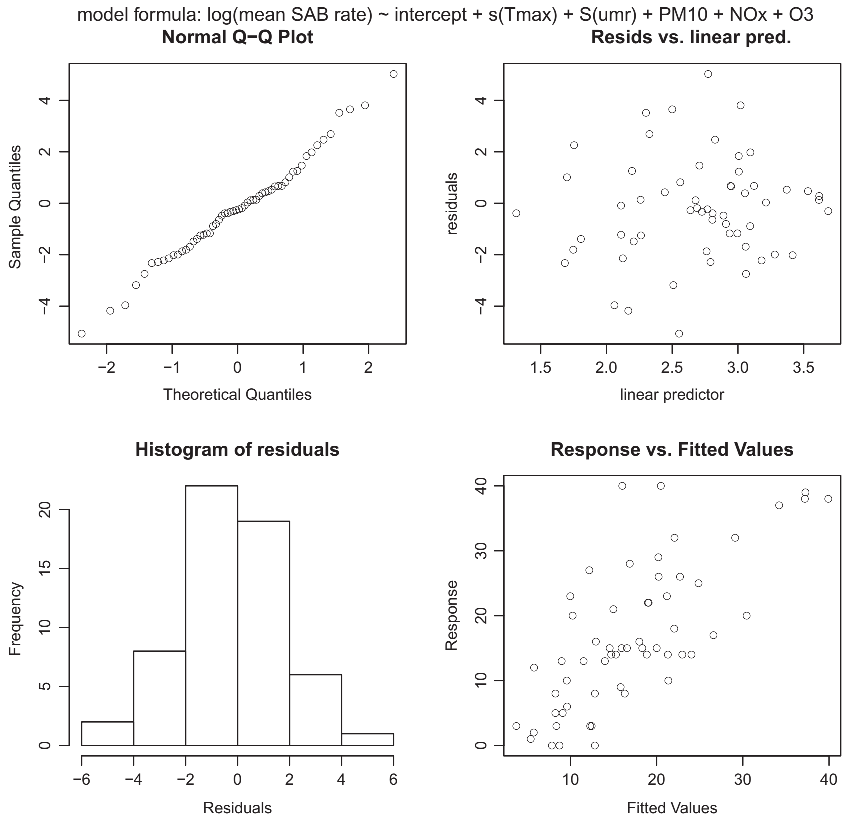

Figure 2. Diagnostic information about the fitting and results of the best model (deviance residuals are used). Correlation between fitted values on the scale of the response variable and observed counts (see "Response vs. Fitted Values") is $\rho = 0.75$ ($p < 0.0001$).

pollutants can play a critical role. In fact, a positive correlation has been shown between air levels of PM10 and ozone (but not NO_2, at least in the explored range of values), and the number of SABs in a large group of free-living women.

For the first time, it has been demonstrated that PM10 and ozone outdoor levels have detrimental effects on SAB occurrence also with pollutant concentrations below the legal limits.

A negative effect of environmental factors of human origin on early pregnancy loss has been previously suggested by some studies pointing at indoor toxics as magnetic fields (Juutilainen et al. 1993; Lindbohm & Hietanen 1995; Lee et al. 2002; Li et al. 2002) and smoke (Hyland et al. 2014; Pineles et al. 2014). Studies exploring the role of outdoor air pollutants on SAB occurrence have been limited to the effects of vehicular traffic (Green et al. 2009), did not fully consider the possibility of non-linear correlations and collected data in the presence of some pollutant levels above the limits (Enkhmaa et al. 2014) indicated in the WHO Air Quality Guidelines for Europe (World Health Organization 2005) or led to conflicting results (Hemminki & Niemi 1982; Hansteen et al. 1996).

Exposure to PM10 during pregnancy (also at relatively low levels (Dugandzic et al. 2006)) significantly affects late outcomes, since it has been linked to stillbirths, birth

Table 4. Adjusted risk rate, effect estimates, approximate significance of smooth terms and global model scores in the selected best model.

Approximate significance of parameter estimates					
	Adj. RR (CI)	Estimate	Std. Error	*Z*-value	*p*
(Intercept)		−0.317	0.450	−0.71	NS
PM10	1.20 (1.08–1.34)	0.018	0.006	3.26	<0.01
NO_2	0.96 (0.87–1.05)	−0.004	0.005	−0.82	NS
O_3	1.34 (1.26–1.42)	0.029	0.003	9.33	<0.001
Approximate significance of smooth terms					
	Edf*	X^2 value**		p**	
s (temperature, δ_1)	8.37	8.84		<0.001	
s (humidity, δ_2)	7.59	8.47		<0.001	
Global scores					
R^2 (adj)	Dev. explained			UBRE score	
0.34	51.8			3.61	
*Test on the basis dimension used for smooth terms****					
		k'		k-index	
s (temperature, δ_1)		9.00		1.14	
s (humidity, δ_2)		9.00		1.14	

Note: NS, not significant.
*Edf is the estimated degree of freedom of each smooth term. It is inversely related to the smoothing parameter estimate (δ), ranging between +1 (a straight line) and +∞ (a perfectly interpolating spline).
**A significance test for smooth terms assumes the null hypothesis that the spline being tested is identically null. The approximate null distribution is a χ^2 distribution with a suitable number of degrees of freedom (Wood 2004).
***The test of whether the basis dimension for a smooth is adequate has been obtained by simulation. Instead of reporting p-values, it is customary to report the k-index computed on each smooth term. The quantity k' is the maximum possible Edf for the term, and the further the k-index below 1 is, the more likely it is that there is missed pattern left in the residuals (and hence that the basis dimension in not adequate).

Table 5. Average levels of air pollutants in the examined cities.

Area	PM10 (μg/m^3)	NO_2 (μg/m^3)	O_3 (μg/m^3)
Taranto	23.0 ± 1.0[a,b]	42.0 ± 2.5[b,c]	98.5 ± 6.3
Brindisi	19.0 ± 1.0[a,b]	42.4 ± 2.5[b,c]	100.1 ± 6.3
Barletta	30.6 ± 1.0[b,c]	46.0 ± 2.5[c]	98.1 ± 6.3
Grottaglie	18.5 ± 1.1	29.6 ± 2.7	103.9 ± 6.8
Andria	13.3 ± 1.0	56.3 ± 2.5	81.5 ± 6.3
ANOVA (*P*)	<0.00005	<0.0005	NS

Notes: Values are means ± SE. NS, not significant. Differences were tested by Kruskal–Wallis analysis of variance by ranks followed by multiple-comparison Z-value test.
[a]$p < 0.05$ vs. Barletta; [b]$p < 0.05$ vs. Andria; [c]$p < 0.05$ vs. Grottaglie.

defects, intrauterine growth retardation, premature birth and low birth weight (Dugandzic et al. 2006; Kim et al. 2007; Brauer et al. 2008; Xu et al. 2011; Dadvand et al. 2013).

Results from the present study also indicate the possibility of an early risk, since a positive correlation between outdoor levels of PM10 and the absolute number of SABs was demonstrated, and this adverse effect seems to be particularly evident in geographical areas harvesting industrial plants with large pollutant emissions. In fact, the highest

Table 6. Average levels of air pollutants, birth rate and epidemiological indices of SABs in cities grouped according to the presence/absence of pollutant producing industries.

	With pollutant producing industries	Without pollutant producing industries
PM10 (μg/m^3)	24.2 ± 1.0[a]	15.7 ± 0.9
NO_2 (μg/m^3)	43.5 ± 1.2	44.2 ± 3.6
O_3 (μg/m^3)	98.9 ± 3.7	91.7 ± 4.7
SABs (n.)	21.5 ± 1.9[a]	8.8 ± 1.6
Birth rate (× 1000)	0.68 ± 0.02[a]	0.75 ± 0.03
SAB ratio	0.30 ± 0.03	0.31 ± 0.07
SAB rate (× 1000)	0.83 ± 0.08	0.80 ± 0.1

Note: Values are means ± SE. Differences were tested by Mann–Whitney *U*-test.
[a]$p < 0.05$.

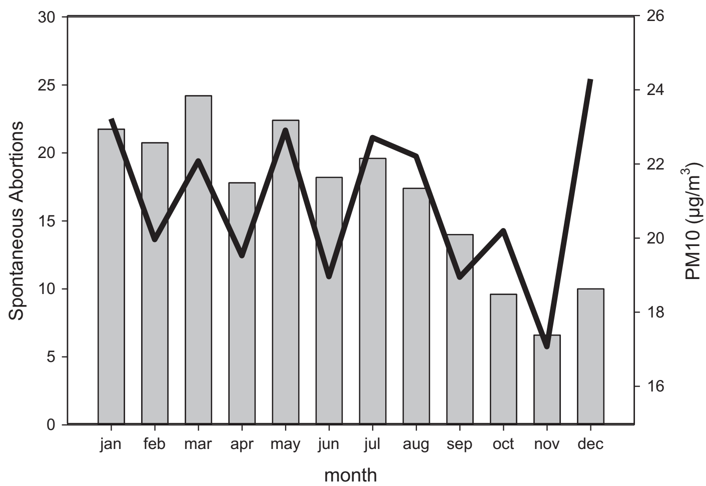

Figure 3. Time variations of monthly average SABs (vertical bars) and PM10 levels (black line) during a 12-month period (year 2013).

PM10 concentrations and number of SABs were recorded in the three industrial cities, as compared with towns characterized by air pollution mainly generated by vehicular traffic.

Despite this evidence, the other epidemiological indices of SAB occurrence (i.e. SAB ratio and rate) were statistically similar, in the present series, between cities with or without industrial pollution. This discrepancy was probably due to the high SAB rate and ratio in the city of Grottaglie, where a role for different environmental factors (in particular, a landfill with the largest regional disposal of special waste, about 220,000 tons/year (ISPRA 2013)) on SAB cannot be excluded (Dummer et al. 2003; Palmiotto et al. 2014).

Furthermore, it has to be underlined the presence of a significantly lower birth rate in the group of cities with- as compared to that without industrial pollution. This finding parallels previous results pointing to a harmful role of environmental pollution on human fertility (Nieuwenhuijsen et al. 2014).

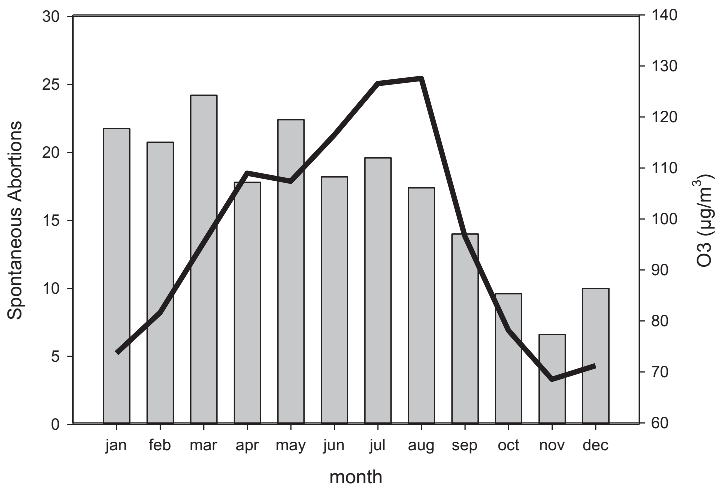

Figure 4. Time variations of monthly average SABs (vertical bars) and ozone (O_3) levels (black line) during a 12-months period (year 2013). A typical increment in ozone concentrations during summer was evident.

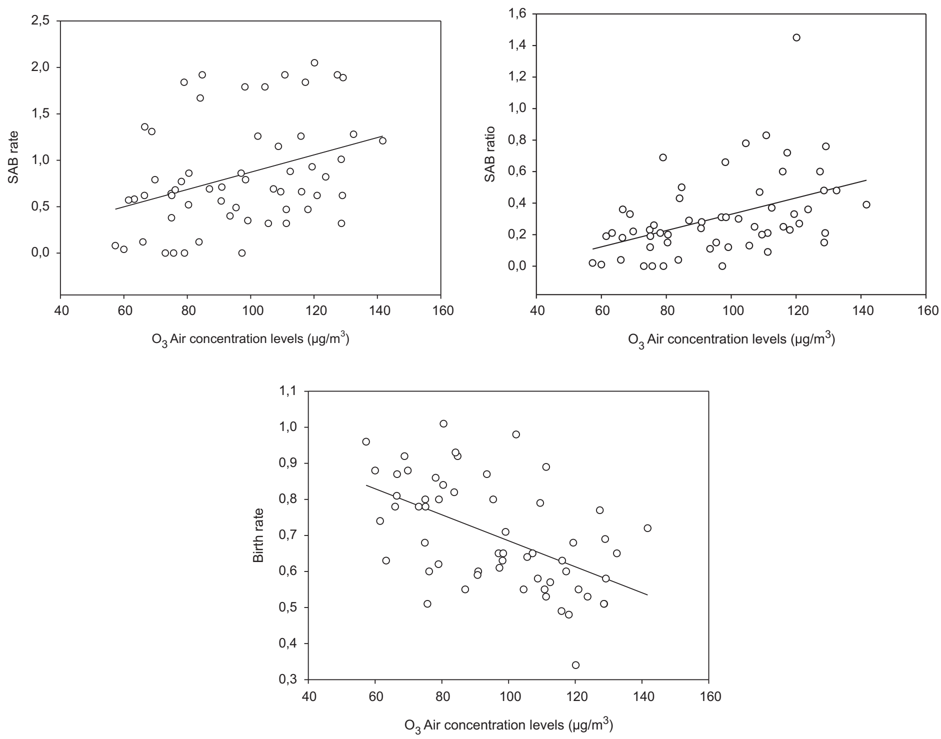

Figure 5. Correlations between SAB rate ($\rho = 0.43$, $p = 0.0007$), SAB ratio ($\rho = 0.50$, $p = 0.00007$), birth rate ($\rho = -0.42$, $p = 0.0009$) and mean outdoor concentrations of ozone (O_3). Values were calculated for each city in the same months.

Of note, a critical indication deriving from the analysis of results is that the effect of PM10 on SAB was also measurable at air concentrations about twofolds lower than that indicated in the WHO Air Quality Guidelines for Europe (50 μg/m^3), with a calculated monthly increment in the mean rate of SAB of 19.7 % per 10 μg/m^3 increase in the air concentration of PM10.

Interestingly, the presence of a dangerous effect of outdoor pollutants (PM10, PM2.5) also at concentrations lower than the European Union limits has been previously reported by a large European cohort study focusing on the risk of low birth weight at term (Pedersen et al. 2013).

As far as particulate-induced adult mortality is concerned, it has been previously observed that no apparent threshold exists below which the link between particulate air pollution and adverse health effects no longer applies (Ware 2000). It has also been reported a linear relationship between PM10 and PM2.5 and various health indictors for concentration levels from 0 to up to 200 μg/m^3 (World Health Organization 1999). Our data suggest that this might also be the case for the relationship between PM10 air levels and SAB.

Several studies demonstrated a direct link between particulate matter and human health (World Health Organization 2013), with injuries starting at cellular level (Donaldson et al. 1997; Dick et al. 2003; Eiguren-Fernandez et al. 2010; Laing et al. 2010; Osornio-Vargas et al. 2011; Velasco 2010). In particular, the main pathogenic mechanisms driven by acute and chronic exposure to particulate matter classically include oxidative stress (Miller et al. 2012), vasoconstriction (Mills et al. 2005), prothrombotic (Lucking et al. 2008) and anti-fibrinolytic (Mills et al. 2005) activity, ischaemic damage induction (Mills et al. 2007), genotoxicity (Rittinghausen et al. 2013) and insufficient DNA repair (Hartwig 2002).

Environmental exposure to PM10 is able to alter markers of placental growth and function (van den Hooven, Pierik, et al. 2012), and at least theoretically, almost all the pathogenic factors producing PM10-induced systemic toxicity may be potentially involved in suboptimal placentation, foetal toxicity, altered foetal development and, therefore, SAB, as suggested by observations linking this adverse and early pregnancy outcome with oxidative stress (Agarwal et al. 2012), vasoconstriction-related genes (Su et al. 2011), thrombophilia (de Jong et al. 2013) and thrombotic/inflammatory processes (Kwak-Kim et al. 2009) and sperm DNA damage (Evenson & Wixon 2005; Robinson et al. 2012).

The link between particulate exposure and SAB may also be mediated by a genetic damage, since chromosomal abnormalities have been frequently described in early SAB (Byrne et al. 1985), and PM10 from both urban and industrial areas has a strong genotoxic activity (Brits et al. 2004; Coronas et al. 2009). Interestingly, a study exploring biomarkers of genetic damage in venous blood, cord blood and placenta and showing higher DNA adduct levels in polluted vs. control districts concluded that relatively low air pollution can significantly increase the adverse reproductive outcomes (Sram et al. 1999).

Furthermore, it has been previously shown that exposure to high PM10 levels is associated with elevated maternal CRP levels in the first trimester and with elevated foetal CRP levels at delivery, indicating maternal and foetal inflammatory responses secondary to exposure to this air pollutant (van den Hooven, de Kluizenaar, et al. 2012).

Further studies are needed, in the next future, to better explore the effects of these different factors on SAB occurrence in PM10-exposed pregnant women.

Environmental exposure to ozone has been previously linked with preterm delivery (in particular during the first trimester of pregnancy) (Olsson et al. 2012; Lee et al. 2013; Olsson et al. 2013) and low birth weight (Morello-Frosch et al. 2010). Besides these previous observations on late pregnancy, results from the present study demonstrate a positive correlation between outdoor ozone levels and the number of SABs, indicating a potential and critical early damage in pregnant women.

Air ozone concentrations were also positively related with both SAB rate and ratio and negatively related with birth rate, further confirming the major role of this pollutant in the environmental stress leading to both SAB occurrence and reduced nativity.

Again, as for the PM10, the effect of ozone on SAB occurrence was also evident at air concentrations of this pollutant lower than that indicated in the WHO Air Quality Guidelines for Europe (100 μg/m^3 (World Health Organization 2005)), with a calculated monthly increment in the mean rate of SAB of 33.6 % per 10 μg/m^3 increase in the air concentration of ozone. It is worth noting that the effect of ozone on SABs seems to be, at least in part, independent from both PM10 levels (the air concentrations of the two pollutants were not correlated), and from the presence of air pollution of industrial origin (comparable ozone levels were recorded in all the examined cities), confirming the high sensitivity of ambient ozone concentrations to meteorological conditions, as demonstrated by a direct and positive correlation between O_3 levels and temperature.

At variance from data on PM10 and ozone, results from the present study showed that air concentrations of NO_2 were not correlated with the number of SABs.

Conflicting results derive from a previous study that recently demonstrated a strong correlation between NO_2 levels and SAB occurrence (Enkhmaa et al. 2014). This discrepancy might be explained, at least in part, by the presence, in the geographical areas examined in the cited work, of an average NO_2 level (110 μg/m^3) much higher than that recorded in the present series (43.7 μg/m^3).

Thus, it is conceivable that, contrarily from PM10 and O_3, measurable effects of NO_2 air levels on SAB occurrence only emerge above a specific threshold level.

NO_2 has been previously shown to be a good marker for traffic-related air pollution (Jerrett et al. 2005). This information might be indirectly supported by results from the present study, since a significant positive correlation was demonstrated between NO_2 levels and the number of residents. Furthermore, the highest average level of NO_2 was recorded in one of the two cities without pollutant emissions of industrial origin.

In the present series, the possibility also exists that NO_2 emissions have been strongly influenced by domestic heating, since a negative correlation between temperature and NO_2 air concentrations was noticed.

Considering the relationships between outdoor pollutant concentrations and SAB occurrence at urban level, it might be argued that a large amount of people can generate high pollution levels and, in turn, more SABs. Of note, however, results from the present study did not support this hypothesis, since the population size in each of the examined cities was not related to PM10 and ozone air levels.

Previous studies demonstrated a negative effect of other environmental factors as parental smoking and environmental tobacco smoke (Hyland et al. 2014; Pineles et al. 2014), magnetic fields (Juutilainen et al. 1993; Lindbohm & Hietanen 1995; Lee et al. 2002; Li et al. 2002), and further pollutants as SO_2 and CO (Enkhmaa et al. 2014) on SAB occurrence. In addition to these previous evidences, our data showed a critical and independent effect of specific air pollutants (PM10 and O_3, but not NO_2, at least in the observed range of values) and, although the present work was not designed to analyse the influence of all the mentioned factors, it might be hypothesized the possibility of a complex interplay among all involved toxic agents, with a cumulative effect (Koppe et al. 2006). Further studies should deeply explore this topic.

Although the adjusted RR for PM10 and ozone were relatively low in the present series, it has to be underlined that, at variance with the indoor pollutants, the entire population is exposed to these toxic agents (even during early *in utero* life) and, thus, the public health effect might be quite large.

In conclusion, the occurrence of SAB is unfavourably influenced by urban PM10 (particularly in the presence of industrial areas) and ozone air concentrations, this latter also affecting birth rate. These harmful effects are also present if levels of these pollutants are below the legal limits.

Further studies are needed in order to assess a threshold risk level for NO_2 (apparently not influencing SAB occurrence at low concentrations) and to better explore the cumulative effects of all environmental factors involved in the increased risk of SAB.

A limit of the present study is that only one year of data has been examined, excluding the possibility to fully explore temporal trends, potential seasonal effects and the effects of delay (lags). However, it has to be considered that the period of maternal and foetal exposure before SAB occurrence is limited, by definition, to a maximum of 180 days of gestation and, although monthly variations in SAB occurrence seem to exist, in our series, the concentration of air toxics was constantly ranged below the limits set by the WHO Air Quality Guidelines for Europe in all the explored areas and throughout the examined period. Further specific studies on a larger period are certainly needed to better clarify these aspects.

Finally, our evidences support the hypothesis that SAB may be considered, at least in part, a preventable condition. Thus, primary prevention policies acting through a marked abatement of pollutant emissions might attenuate future SAB incidence at urban level.

Conflict of interest

The authors have no actual or potential competing financial interests involving people or organizations that might reasonably be perceived as relevant.

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