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# Association between ambient air pollution and proliferation of umbilical cord blood cells☆

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ABSTRACT

It has been established that ambient air pollution (AAP) has an adverse effect on human health. The pathophysiological mechanism of this impact is likely to be related to the oxidative stress. In the current study we estimate the association between AAP and cell proliferation (CP) of umbilical cord blood cells, representing maternal organism most proximal to the fetal body.

Blood samples were tested for proliferation in 292 enrolled Arab-Bedouin women at delivery (July 2012–March 2013). The estimates of AAP were defined by a hybrid satellite based model predicting both $PM_{2.5}$ (particles < 2.5 µm in diameter) and $PM_{10}$ (particles < 10 µm in diameter) as well as monitoring stations for gaseous air pollutants. Risk estimates of pollution exposure were adjusted to medical history, household risk factors and meteorological factors on the day of delivery or one week prior. Ambient ozone ($O_3$) levels on 1, 2, 3and 4 days prior to delivery were associated with lower CP (Prevalence ratio (PR)=0.92, 0.92, 0.93, 0.93, respectively). Increase in inter-quartile range (IOR) of $PM_{2.5}$ one day before delivery was associated with 9% increase in CP levels (PR=1.09). The positive direction in association was changed to negative association with CP for $PM_{2.5}$ levels measured at more distant time periods (PR=0.90 and 0.93 for lags 5 and 6 days, respectively). Investigation of $PM_{10}$ levels indicated a similar pattern (PR=1.05 for pollution values recorded one day before delivery and 0.93 and 0.95 for lags of 5 and 6 days, respectively). Carbon monoxide (CO) levels were associated with lower CP on the day of delivery and 1 day prior (PR=0.92 and PR=0.94).

To conclude, the levels of cell proliferation of umbilical cord blood cells appear to be associated with the AAP. More studies are needed to support our findings.

## 1. Background

Despite of a growing evidence of adverse effects of ambient air pollutants (AAP) on human morbidity and mortality, the exact pathophysiological mechanisms have not yet been elucidated.

In a recent review by Møller et al. (2014) the authors concluded that air pollution particles generate oxidatively damaged DNA by promoting a milieu of oxidative stress and inflammation. This appears to be the prevailing theory in the field, which was suggested already decades ago (Gradecka et al., 2001; Tao et al., 1999; Burdon et al., 1990), and has been supported by various studies ever since (Nemmar et al., 2013; Niu et al., 2015; Saint-Georges et al., 2008; Beerman et al., 2013). Nemmar et al. (2013) in their recent review explored physiological, cellular and molecular mechanisms of causing adverse effects by exposure to particulate matter (PM). The review was based on in vivo and in vitro studies and largely confirmed the existing theory. The authors, however, pointed out the lack of in vivo studies that closely resemble the human disease pattern which would align with the in-vitro based findings. Ultimately, epidemiological studies involving testing of relevant human biomarkers could provide a crucial support to the theory.

Biomarker, like cell proliferation (CP), appears to be involved in the pathophysiological mechanism. For instance, cell proliferation may reflect an inflammation process (Nemmar et al., 2013), possibly relevant for short-term exposures. It is also a process believed to be affected by DNA damage (Nemmar et al., 2013) and therefore might indicate such damage, e.g. following a long-term exposure. Variability

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of cell proliferation in relation to the main pollutants has been investigated in a number of laboratory studies (Nadeau et al., 2010; Garçon et al., 2006; Liu et al., 2014; Abbas et al., 2016). Liu et al. (2014) investigated the role of endogenous sulfur dioxide ($SO_2$) in proliferation of vascular smooth muscle cells in an in-vitro study and found that $SO_2$ inhibited proliferation of these cells by reducing DNA synthesis. Abbas et al. (2016) showed that PMs can affect CP levels. Wang et al. (2015) suggested possible cellular mechanisms explaining the effect of pollutants, specifically $SO_2$, on cell proliferation.

Nevertheless, the above findings have not been tested in humans so far, specifically how ambient air pollution is associated with proliferation of cells in a human organism.

In the current study we focused on the proliferation of umbilical cord blood cells, representing maternal organism most proximal to the developing body of a fetus. We aimed to estimate the association of AAP with fetus health via cell proliferation (CP) of umbilical cord blood cells. The exposure window at study was therefore the gestational period of the fetus. Most covariates were verified for the entire period of pregnancy and the main AAP levels were considered relevant and valid for one week prior to delivery. The focus on the last week of pregnancy for the ambient exposure was primarily due to the physiological cycle of a cell usually measured in days.

The previous investigation by the study team was focused on the household factors located in the immediate proximity to women during their pregnancy (Novack et al., 2015). Our earlier findings indicated the possibility of CP being affected by surrounding factors. Specifically, CP was independently and negatively associated with complains on environmental noise, and positively associated with residence in the area of prevailing winds from the local industrial park and delivery during the cold season.

In the current study we aimed to explore the AAP, routinely estimated and collected in most environmental health studies, i.e. particulate matter (PM10 and PM2.5), nitrogen dioxide ($NO_2$), sulfur dioxide ($SO_2$), carbon monoxide (CO) and ozone ($O_3$), in relation to CP.

## 2. Methods

Enrollment and other study procedures are described elsewhere in detail (Novack et al., 2015). The current study focused on women of Arab-Bedouin origin characterized by low socio-economic status, young age, frequent paternal smoking, frequent consanguineous marriages and poor household conditions, e.g. living in temporary shacks or tents and cooking on open fire. This specific population has been undergoing a major change in life style, from semi-nomadic to sedentary dwellings in close proximity to urban and industrial activities. Every eligible woman of Arab-Bedouin origin admitted to the obstetric emergency room for a delivery during the 8 working hours 5 days a week in Jul 2012–Mar 2013 was invited to participate in the study. The enrollment was conducted in the Soroka University Medical Center, a local hospital providing free tertiary care to the population of over 700,000 residents and located in the southern part of Israel (Beer-Sheva, Israel). The umbilical cord blood sample was taken at delivery and a detailed questionnaire was administered during the women's hospitalization. The questionnaire included information on demographical factors of the participants, medical history and possible household hazards. The electronic records of medical charts provided information on medical history and clinical characteristics of the current delivery.

### 2.1. Testing for cell proliferation

We used the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) *Cell Proliferation Assay* (*Yellow MTT*) for measuring proliferation of cells in umbilical cord (Byun et al., 2013; Mosmann 1983; Novack et al., 2015). The cells were isolated from blood by Ficoll-Histopaque density gradient. Specifically, the optical density (OD) values of MTT were assessed by ELISA. Furthermore, the CP ratio was calculated as a ratio of the T cells stimulated by phyto-hemagglutinin (PHA) over an estimate of a not-stimulated proliferation of the same type of cells within the same individual, to neutralize (in-vitro) conditions of each subject.

### 2.2. AAP levels assessment

The residential addresses during pregnancy were geocoded using the Arcinfo 10 (ESRI, Redlands, CA, USA) geographic information system (GIS). As half of the women reside in small or temporary settlements, exact home address geocoding was unavailable. Therefore the spatial resolution of the address used in the study corresponded to the center of the town/village localities (all with radius less than 2 km) and were further linked to one of the 13 permanent monitoring stations in the study area for information on air pollutants' daily concentrations of $NO_2$, $SO_2$, $O_3$ and CO, and meteorological records. The information was considered available if the distance to the closest monitoring station did not exceed 20 km to the participants' residing address, except for precipitation records which were imputed from the Beer-Sheva station located in the center of the exposure area. These pollutant measurements were interpolated to the study areas using the inverse distance weighting method as described by Hwang and Jaakkola (2008). The methodology of AAP levels assessment in current study has been described in details elsewhere (Landau et al., 2015).

Levels of ambient $PM_{10}$ and $PM_{2.5}$ in the current study were assigned using satellite-based models at 1×1 km spatial resolution which were further averaged over each locality (Kloog et al., 2016; 2015). The model used an algorithm developed by NASA – MAIAC (Multi-Angle Implementation to Atmospheric Correction), which provides aerosol optical depth (AOD) data at a very high spatial resolution. For more in depth description please refer to Lyapustin et al. (2011). All AAP measurements were averaged over each day (24-h mean).

Due to the 20 km limitation between residential address and a monitoring station, the study population in the current analysis was limited to 292 subjects, which represents 84% of 346 subjects described earlier (Novack et al., 2015).

### 2.3. Statistical analysis

All continuous variables were presented as mean ± standard deviation (SD), median, minimal (min) and maximal (max) value. Pollutant values were also described by intra-quartile range (IQR). All nominal variables were presented as percent out of available cases.

CP ratio—the main outcome variable—was log-transformed and compared between subgroups using a log-normal regression model, providing the estimates of prevalence ratios (PR) representing the multiplicative difference in CP ratio from the reference category. CP ratio was compared between subgroups using a log-ratio *t*-test or univariable log-normal regression. For the multivariable analysis of CP ratio readings we used the same log-normal regression model along with the list of the relevant covariates verified at the univariate analysis stage. The patterns of association between CP ratio levels and pollution concentrations as well as other continuous factors (e.g. gestational age or birth weight) were investigated using a loess function. The significance level in the multivariable analysis was set to 10% due to the relatively small sample size.

The main association at study was relationship between the levels of CP ratio on the day of delivery and the AAP on that same day or within a week prior to that. The time lags used in the analysis, were adjusted to the fact that not all women gave birth on the day of their hospitalization. Thus for example, a woman giving birth on the 3rd day after hospitalization could be considered only for pollutants levels recorded 3–6 days before the delivery.

In models involving multiple days of exposure, the standard errors were estimated using the robust method, correcting for the clustered

**Table 1**
Demographic, medical and household characteristics of the study population (n=292).

| Maternal Characteristics | Description in the study population (N=292) | Effect on CP, Prevalence Ratio (90%CI) | p-value |
|---|---|---|---|
| Maternal age, years | | | |
| Mean ± SD (n) | 27.4 ± 5.9 (292) | 1.01(1.00; 1.01) | 0.199 |
| Median | 26.0 | | |
| Min; Max | 18.9; 47.0 | | |
| Maternal age > 35, % (n/N) | 13.0 (38/292) | 1.16 (1.00; 1.33) | 0.094 |
| Parity, % (n/N) | | | |
| 1st delivery | 31.2 (91/292) | 1.00 | |
| 2nd–5th delivery | 46.9 (137/292) | 1.03 (0.92; 1.15) | 0.669 |
| 6th+delivery | 21.9 (64/292) | 1.00 (0.88; 1.13) | 0.999 |
| Consanguineous marriage, % (n/N) | 53.7 (125/233)[a,b] | 1.11 (0.95; 1.31) | 0.281 |
| History of repeated abortions, % (n/N) | 4.8 (14/292) | 0.75 (0.60; 0.93) | 0.032 |
| Living in a temporary shack or tent, % (n/N) | 20.8 (50/241)[a] | 0.97 (0.85; 1.11) | 0.740 |
| Cooking on open fire, % (n/N) | | | |
| Never | 21.8 (50/229)[a] | 1.00 | – |
| Sometimes | 65.5 (150/229) | 1.10 (0.18; 6.65) | 0.267 |
| All the time | 12.7 (29/229) | 1.04 (0.19; 5.79) | 0.447 |
| Husband smoking, % (n/N) | 94.4 (217/230)[a] | 0.79 (0.63; 1.00) | 0.105 |
| Birth in a cold season, % (n/N) | 72.3 (211/292) | 1.06 (0.95; 1.18) | 0.357 |
| Environmental noise, % (n/N) | 6.6 (16/241)[a] | 0.89 (0.72; 1.10) | 0.373 |
| Living in the area of prevailing winds from a local industrial park, % (n/N) | 12.3 (36/292) | 1.17 (1.01; 1.36) | 0.074 |

[a] based on questionnaires, which were filled out for 241 participants (82.5% of the study population). The rest of the variables were retrieved from the hospital medical charts.
[b] 77.5% (93/292) are marriages of cousins.

structure of the data. Multivariable models with single pollutants included only one pollutant at a time, and adjustment was made to all other necessary covariates. Likewise, models with multiple pollutants included all pollutants as the main exposure at study and were adjusted to a list of covariates. All pollutants were analyzed in IQR units in the final models, i.e. divided by IQR. All analyses were performed using SAS 9.4 software.

## 3. Results

Mothers participating in the study were on average 27.4 years old (minimal age 18.9 years and maximal 47.0 years), and the majority of them (68.8%) gave birth in the past. More than half of the study population reported being in a consanguineous marriage (53.7%) and frequently they were married to a cousin (77.5% of women in consanguineous marriages). Seventy-eight percent (78%) of women reported cooking on open fire and husbands of 94.4% of the study participants smoked. About 5% of the study population had a history of repeated abortions. One fifth of the study population reported living in a temporary shack or tent (Table 1). In relation to the CP ratio values, the analysis showed that the proliferation increased in women above 35 years old and those who resided in the area of the prevailing wind, while complaints on environmental noise, the history of repeated abortions and second-hand smoking were associated with lower CP ratio levels.

Table 2 presents the newborn characteristics. The gestational age of the newborns was 39.3 ± 1.5 weeks on average, with the earliest delivery at 33 weeks. Being born before 37 weeks or after 40 weeks had a tendency of having lower CP ratio values, however it was not supported statistically in the univariate analysis. Birth weight was 3207 ± 466 gr and was not associated with CP ratio.

**Table 2**
Neonatal characteristics in the study population (n=292).

| Neonatal Characteristics | Description in the study population (N=292) | Effect on CP, Prevalence Ratio (90%CI) | p-value |
|---|---|---|---|
| Gestational age, weeks | | | |
| Mean ± SD (n) | 39.3 ± 1.5 (292) | 0.98 (0.95; 1.02) | 0.423 |
| Median | 39.4 | | |
| Min; Max | 33.0; 43.0 | | |
| Gestational age, % (n/N) | | | |
| < 37 weeks | 4.8 (14/292) | 0.89 (0.70; 1.11) | 0.385 |
| 37–40 weeks | 47.6 (139/292) | 1.00 | |
| 40 weeks | 47.6 (139/292) | 0.93 (0.84; 1.03) | 0.238 |
| Male gender, % (n/N) | 52.1 (152/292) | 1.00 (1.00; 1.00) | 0.411 |
| Birth weight, grams | | | |
| Mean ± SD (n) | 3207.1 ± 465.5 (292) | 1.04 (0.95; 1.15) | 0.480 |
| Median | 3240.0 | | |
| Min; Max | 1700.0; 4505.0 | | |

Distribution of ambient air pollutants on the day of delivery is described in Table 3. The study area is characterized with relatively high levels of PM of both sizes captured in the table, i.e. $PM_{10}$ was estimated at 48.9 ± 32.2 μg/m$^3$ on average and $PM_{2.5}$ at 23.1 ± 16.9 μg/m$^3$ on the day of delivery. Temperatures averaged over 24 h were 19.9 ± 4.6 °C, with the maximal reached 27.7 °C during the study period. Featuring the desert climate of the study population, precipitations occurred only on 8.3% of the days.

Many of the pollutants were statistically associated with each other, as shown in Table 4, specifically $PM_{10}$ with CO and $NO_2$, $PM_{2.5}$ with $O_3$ and $SO_2$, $O_3$ with $SO_2$. Temperature and rain were correlated with the values of certain pollutants, i.e. $NO_2$ and $O_3$.

The exploratory analysis of the short-time association of pollutants with CP ratio, after adjustment to major demographic, medical and household factors previously shown to influence the proliferation, is described in Fig. 1. The analysis revealed few patterns.

Increase in IOR of *$PM_{2.5}$* a day before delivery was associated with 9% increase in CP levels (PR=1.09, p-value 0.012). The positive direction in association was changed to negative association with CP for $PM_{2.5}$ levels measured at more distant time periods. Specifically, PR=0.90 (p-value=0.002) and 0.93 (p-value=0.030) were obtained for lags 5 and 6 days, respectively. Investigation of $PM_{10}$ levels indicated a similar pattern, i.e. PR=1.05 (p-value=0.017) for $PM_{10}$ values recorded one day before delivery and 0.93 (p-value=0.077) and 0.95 (p-value=0.004) for lags of 5 and 6 days, respectively.

Increase in IQR of *$O_3$* was associated with lower CP one day, 2-days, 3-days and 4-days prior to delivery (PR=0.92 (p-value=0.046), PR=0.92 (p-value=0.078), PR=0.93 (p-value=0.078) and PR=0.93, p-value=0.094, respectively). No association was found with $O_3$ values estimated at more distant times.

*CO* levels were negatively associated with CP ratio on the day of delivery and 1 day prior to it (PR=0.92, p-value=0.049 and PR=0.94, p-value=0.079, respectively).

*$SO_2$* was shown to be associated only 5 days prior to delivery (PR=0.95, p-value=0.064). *$NO_2$ levels* were not statistically associated with the CP ratio values.

Analysis of simultaneous effect of the three main pollutants, $PM_{2.5}$, $O_3$ and $SO_2$, over the 7 days of exposure (data not shown) indicated that only $O_3$ was independently and negatively associated with CP adjusted to two other pollutants (PR=0.93% and 90%CI: 0.88; 0.99).

**Table 3**
Pollutants distribution and meteorological factors and during the week preceding delivery in the study population – 292 women.

| Environmental factors[a] | On the day of delivery[b] | | | | | |
|---|---|---|---|---|---|---|
| | Mean ± SD (n) | Median | Min | Max | 25th–75th percentiles | IQR |
| $PM_{10}$, μg/m$^3$ | 48.88 ± 32.20 (198) | 38.38 | 17.81 | 197.02 | 33.25–48.28 | 15.04 |
| $PM_{2.5}$, μg/m$^3$ | 23.14 ± 16.94 (198) | 18.34 | 10.81 | 136.95 | 15.86–23.27 | 7.41 |
| CO, ppm | 0.05 ± 0.04 (150) | 0.03 | 0.00 | 0.22 | 0.02–0.06 | 0.04 |
| $O_3$, ppb | 15.97 ± 8.30 (202) | 16.15 | 0.02 | 41.52 | 11.93–21.14 | 9.21 |
| $SO_2$, ppb | 0.63 ± 1.20 (200) | 0.51 | 0.00 | 16.15 | 0.25–0.73 | 0.48 |
| $NO_2$, ppb | 9.10 ± 3.73 (200) | 8.34 | 2.74 | 27.75 | 6.61–11.72 | 5.21 |
| Temperature, °C | 18.93 ± 4.64 (200) | 18.96 | 18.19 | 27.71 | 16.44–23.08 | 6.73 |
| Rain, mm | 0.000 ± 0.003 (144) | 0.00 | 0.00 | 0.030 | 0.00–0.00 | 0.00 |

[a] All environmental factors are presented as an average over one day.
[b] Estimated within women who gave birth on the day of their arrival to the hospital.

Gestational age appeared to be an important factor to account for, when analyzing CP ratio, which was the lowest for newborns born before 37 weeks and after 40 weeks of gestation. Among the household factors, smoking of a husband was associated with lower values of CP.

## 4. Discussion

The current study followed our investigation of the same study population, where we found that proliferation of cells can be sensitive to the hazardous household conditions (Novack et al., 2015). This analysis adds another, previously unaccounted factor of ambient air pollution frequently measured in the environmental epidemiology research. An association between the cell proliferation and monitored pollutants could bring us closer to an understanding of the "black box" between the pollution and human morbidity.

Firstly, the results indicated a possible short-term influence of ambient pollutants, whereas PMs were associated with increased cell proliferation and $O_3$ and CO with decreased proliferation, all within one day from delivery. The association with PM levels changed its direction to negative for more "distant" values recorded within a week before the delivery. Once adjusted to other pollutants, only $O_3$ was statistically associated with the CP ratio levels over one week before delivery. Secondly, the prevailing direction of the pollutants' association with CP was negative.

Our findings largely support the theory of oxidative stress induced by AAP (Beerman et al., 2013; Burdon et al., 1990), as the CP ratio was found related to the outdoor pollutants, which according to this theory may cause further pathophysiological changes.

### 4.1. Association with specific pollutants and exposure period on CP ratio

The particular direction of an association, depending on a specific pollutant and its timing requires a special investigation.

#### 4.1.1. Why the association with CP varies by pollutant?

The effect of different types of pollutants on CP is expected to vary depending on their chemical structure and their aerodynamic features, e.g. gaseous elements, like $NO_2$ or $O_3$, vs. particulate matter. This expectation is for the most part confirmed by our findings of higher CP values related to higher values of PM and lower CP values obtained for high gaseous $O_3$ pollution on the day of delivery. Worth noting that the prevalent direction of association with other gaseous pollutants, like $SO_2$ and CO, was also negative.

#### 4.1.2. Why the cell proliferation reaction varies by the time of exposure to PM?

While we cannot completely rule out the possibility of an error, the CP association with pollutants appears to change direction over time. Focusing on statistically significant results, PMs were related to higher CP on the day of delivery and with lower CP values for pollutants levels recorded at more extended days. The variability in direction of an association may correspond to different exposure windows whereas association estimated on the day of delivery possibly shows an immediate effect and estimations for more distant days could indicate a long-term exposure to general pollution levels. This distinction in time corresponding to varying direction of a possible effect implies different mechanisms. The current research of umbilical cord blood CP does not provide a clear evidence of high or low levels of proliferation

**Table 4**
Spearman correlation estimates between pollutants and meteorological factors on the day of delivery in the study population – 292 women.

| | $PM_{10}$, μg/m$^3$ | $PM_{2.5}$, μg/m$^3$ | CO, ppm | Ozone, ppb | $SO_2$, ppb | $NO_2$, ppb | Temperature, °C | Rain, mm |
|---|---|---|---|---|---|---|---|---|
| $PM_{10}$, μg/m$^3$ | | r=0.80<br>p < 0.001 | r=0.29<br>p < 0.001 | r=−0.07<br>p=0.328 | r=−0.04<br>p=0.565 | r=0.20<br>p=0.005 | r=−0.15<br>p=0.040 | r=0.07<br>p=0.312 |
| $PM_{2.5}$, μg/m$^3$ | | | r=0.09<br>p=0.268 | r=0.15<br>p=0.034 | r=0.17<br>p=0.021 | r=0.05<br>p=0.465 | r=0.11<br>p=0.119 | r=−0.02<br>p=0.790 |
| CO, ppm | | | | r=0.15<br>p=0.073 | r=−0.28<br>p < 0.001 | r=0.26<br>p=0.001 | r=−0.04<br>p=0.639 | r=0.15<br>p=0.070 |
| Ozone, ppb | | | | | r=0.50<br>p < 0.001 | r=−0.23<br>p=0.001 | r=0.19<br>p=0.007 | r=−0.12<br>p=0.096 |
| $SO_2$, ppb | | | | | | r=−0.07<br>p=0.342 | r=0.24<br>p < 0.001 | r=−0.14<br>p=0.047 |
| $NO_2$, ppb | | | | | | | r=0.05<br>p=0.528 | r=0.08<br>p=0.297 |
| Temperature, °C | | | | | | | | r=−0.17<br>p=0.016 |

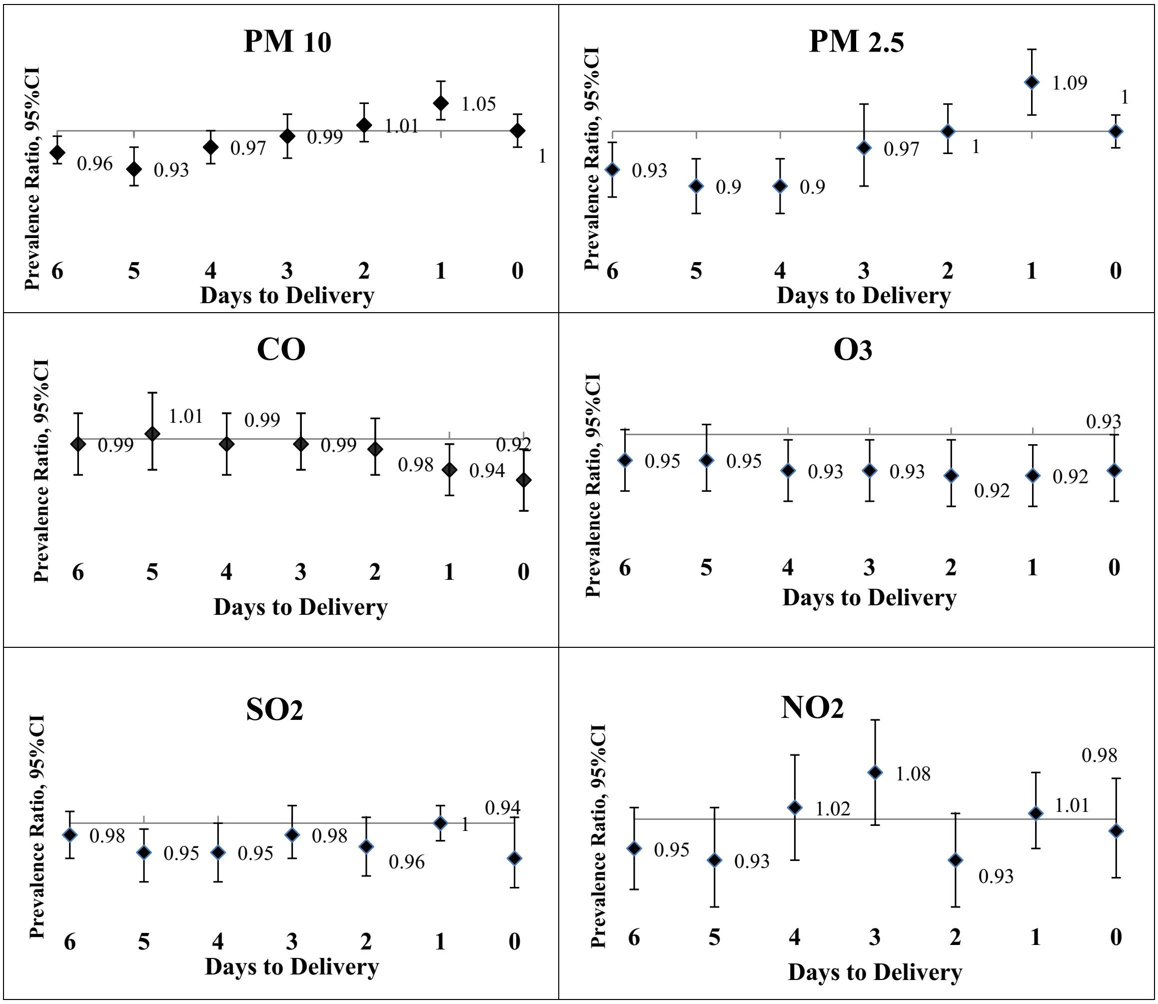


**Fig. 1.** Independent effects of pollutants on cell proliferation on the day of and during the week preceding delivery, expressed in Prevalence Ratio (PR) for increase in a unit of intra-quartile range. All estimates are adjusted to age above 35, gestational age at the time of delivery (before 37 weeks or after 40 weeks), complaint of a woman on an environmental noise, husband smoking, cooking on open fire and meteorological factors, i.e. rain (yes/no) and temperature. The x axis ("Days to delivery") crosses the y axis at the value of "1".

reflecting any pathological process. In general, increased proliferation of cells usually indicates an immediate inflammation process (Kiraly et al., 2015), and therefore we can speculate that the positive associations with PMs measured on the day close to delivery essentially show an inflammatory process. The decreased proliferation may probably reflect a cumulative effect of inflammation, known as compensatory anti-inflammatory response (Ward et al., 2008). We also know that cell proliferation can be a sign of DNA damage (Nemmar et al., 2013) presumably relevant for long-term exposures. This perspective is even more intriguing in view of the general negative association of pollutants with CP observed in our study. More studies are vital to speak of the pathophysiological mechanism with more certainty.

### 4.2. Other factors associated with CP ratio

In our previous investigation (Novack et al., 2015) we noticed that high levels of CP featured women with chronic hypertension (PR=2.23, p-value=0.023) and low levels were recorded in women with history of repeated abortions (PR=0.81, p-value=0.092) and those who received a recommendation for abortion (PR=0.65, p-value=0.016), based on a univariate analysis. Our current analysis also showed that levels of CP of umbilical cord blood might be regulated by gestational age, with the highest proliferation levels between 37 and 40 weeks of pregnancy and lowest before 37 and after 40. A deeper understanding of the meaning of CP levels might come from a long-term follow-up during the pregnancy and after delivery, for both women and their newborns.

### 4.3. Strengths and limitations of the study

The current analysis has benefited from the information collected in the questionnaires and the hospital charts on the baseline clinical and demographical status of the women, as well as a detailed description of their household environment. These data enabled a more valid estimation of the pollutants' potential effect with minimal residual confounding, as opposed to ecological studies which cannot account for obviously important covariates.

The apparent limitation of the current research is its relatively small sample size, restricted by the budget and laboratory logistics. In addition, the study population is somewhat biased to low income strata prone to morbidity, and cannot fully represent the populations with higher socio-economic strata due to a possibly different set of exposures in their household. A larger sample including populations of higher socio-economical status is needed in future studies.

Additionally, in view of a relatively short exposure period (one week) defined as relevant for an effect of air pollution on cell proliferation, the current study can be seen as a cross-sectional analysis. Furthermore, there is a chance of non-differential misclassification bias in our estimation of air pollution values for CO, $O_3$, $SO_2$ and $NO_2$ based on monitoring stations sometimes located 20 km away from the women's residence. In addition, we had to use coordinates of a village/town center for half of the participants who did not have an exact home address. Even so, once AAP estimates are obtained, their assignment to the study subjects should be less problematic. The misclassification bias of AAP is expected to be minimal due to specific household environment of the study population frequently residing in temporary housing. In particular, with the high air ventilation in temporary tents and shacks, the outdoor pollution can be safely assumed to approximate the indoor levels.

## 5. Conclusions

To conclude, the levels of cell proliferation of umbilical cord blood cells appear to be related to the AAP. Assuming that proliferation is involved in the oxidative stress, our findings may indicate an existence of a possible pathophysiological path from ambient pollutants to future childhood morbidity. Longer follow-up and more studies in humans are required to interpret our results with more certainty.

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## References

Abbas, I., Verdin, A., Escande, F., Saint-Georges, F., Cazier, F., Mulliez, P., Courcot, D., Shirali, P., Gosset, P., Garçon, G., 2016. In vitro short-term exposure to air pollution PM2.5-0.3 induced cell cycle alterations and genetic instability in a human lung cell coculture model. Environ. Res., 146–158.
Beerman, I., Bock, C., Garrison, B.S., Smith, Z.D., Gu, H., Meissner, A., Rossi, D.J., 2013. Proliferation-dependent alterations of the DNA methylation landscape underlie hematopoietic stem cell aging. Cell. Stem Cell. 12 (4), 413–425.
Burdon, R.H., Gill, V., Rice-Evans, C., 1990. Oxidative stress and tumour cell proliferation. Free Radic. Res Commun. 11 (1–3), 65–76.
Byun, H.M., Panni, T., Motta, V., Hou, L., Nordio, F., Apostoli, P., Bertazzi, P.A., Baccarelli, A.A., 2013. Effects of airborne pollutants on mitochondrial DNA methylation. Part Fibre Toxicol. 10, 18.
Garçon, G., Dagher, Z., Zerimech, F., Ledoux, F., Courcot, D., Zerimech, F., Ledoux, F., Courcot, D., Aboukais, A., Puskaric, E., Shirali, P., 2006. Dunkerque City air pollution particulate matter-induced cytotoxicity, oxidative stress and inflammation in human epithelial lung cells (L132) in culture. Toxicol. In-Vitro 20 (4), 519–528.
Gradecka, D., Palus, J., Wasowicz, W., 2001. Selected mechanisms of genotoxic effects of inorganic arsenic compounds. Int. J. Occup. Med. Environ. Health 14 (4), 317–328.
Hwang, B., Jaakkola, J.J., 2008. Ozone and other air pollutants and the risk of oral clefts. Environ. Health Perspect. 116, 1411–1415.
Kiraly, O., Gong, G., Olipitz, W., Muthupalani, S., Engelward, B.P., 2015. Inflammation-induced cell proliferation potentiates DNA damage-induced mutations in vivo. PLoS Genet. 11 (2), e1004901.
Kloog, I., Sorek-Hamer, M., Lyapustin, A., Coull, B.A., Wang, Y., Just, A.C., Schwartz, J.D., Broday, D.M., 2016. Estimating daily PM2.5 and PM10 across the complex geo-climate region of Israel using MAIAC satellite-based AOD data. Atmos. Environ. 122, 409e416.
Kloog, I., Melly, S.J., Coull, B.A., Nordio, F., Schwartz, J.D., 2015. Using satellite-based spatiotemporal resolved air temperature exposure to study the association between ambient air temperature and birth outcomes in Massachusetts. Environ. Health Prospect. 123 (10), 1053–1058.
Landau, D., Novack, L., Yitshak-Sade, M., Sarov, B., Kloog, I., Hershkovitz, R., Grotto, I., Karakis, I., 2015. Nitrogen dioxide pollution and hazardous household environment: what impacts more congenital malformations. Chemosphere 139, 340–348.
Liu, D., Huang, Y., Bu, D., Liu, A.D., Holmberg, L., Jia, Y., Tang, C., Du, J., Jin, H., 2014. Sulfur dioxide inhibits vascular smooth muscle cell proliferation via suppressing the Erk/MAP kinase pathway mediated by cAMP/PKA signaling. Cell Death Dis. 5, e1251.
Lyapustin, A., Wang, Y., Laszlo, I., Kahn, R., Korkin, S., Remer, L., Levy, R., Reid, J.S., 2011. Multiangle implementation of atmospheric correction (MAIAC): 2. Aerosol algorithm. J. Geophys. Res.: Atmos. 116.
Møller, P., Danielsen, P.H., Karottki, D.G., Jantzen, K., Roursgaard, M., Klingberg, H., Jensen, D.M., Christophers, D.V., Hemmingsen, J.G., Cao, Y., Loft, S., 2014. Oxidative stress and inflammation generated DNA damage by exposure to air pollution particles. Mutat. Res. Rev. Mutat. Res. 762, 133–166.
Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65 (1–2), 55–63.
Nadeau, K., McDonald-Hyman, C., Noth, E.M., Pratt, B., Hammond, S.K., Balmesx, J., Tager, I., 2010. Ambient air pollution impairs regulatory T-cell function in asthma. J. Allergy Clin. Immunol. 126 (4), 845–852.
Nemmar, A., Holme, J.A., Rosas, I., Schwarze, P.E., Alfaro-Moreno, E., 2013. Recent advances in particulate matter and nanoparticle toxicology: a review of the in vivo and in vitro studies. Biomed. Res. Int. 2013, 279371.
Niu, Y., DesMarais, T.L., Tong, Z., Yao, Y., Costa, M., 2015. Oxidative stress alters global histone modification and DNA methylation. Free Radic. Biol. Med. 82, 22–28.
Novack, L., Manor, E., Gurevich, E., Yitshak-Sade, M., Landau, D., Sarov, B., Hershkovitz, R., Vodonos, T., Karakis, I., 2015. Can cell proliferation of umbilical cord blood cells reflect environmental exposures? SpringerPlus 4, 372.
Saint-Georges, F., Abbas, I., Billet, S., Verdin, A., Gosset, P., Mulliez, P., Shirali, P., Garçon, G., 2008. Gene expression induction of volatile organic compound and/or polycyclic aromatic hydrocarbon-metabolizing enzymes in isolated human alveolar macrophages in response to airborne particulate matter (PM2.5). Toxicology 244 (2–3), 220–230.
Tao, L., Ge, R., Xie, M., Kramer, P.M., Pereira, M.A., 1999. Effect of trichloroethylene on DNA methylation and expression of early-intermediate protooncogenes in the liver of B6C3F1 mice. J. Biochem. Mol. Toxicol. 13 (5), 231–237.
Wang, X.B., Du, J.B., Cui, H., 2015. Signal pathways involved in the biological effects of sulfur dioxide. Eur. J. Pharmacol. 764, 94–99.
Ward, N.S., Casserly, B., Ayala, A., 2008. The compensatory anti-inflammatory response syndrome (CARS) in critically ill patients. Clin. Chest Med. 29 (4), 617–625.