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BIOSTATISTICAL MODELS TO ASSESS THE EFFECTS OF AIR POLLUTION ON VULNERABLE POPULATIONS

Modelli biostatistici per lo studio degli effetti dell'inquinamento atmosferico su popolazioni fragili

Coordinator: Ch.ma Prof.ssa Annalisa Angelini

Supervisor: Ch.mo Prof. Dario Gregori

Ph. D. student: Elisa Gallo

To my parents, Lorenza and Mario

ABSTRACT

The impact of air pollution on human health has become a hot topic in the last decades. Air pollution can mainly damage the respiratory, cardiovascular and neurological systems with short- and long-term effects. The harmful effects of exposure are observed in the general population as well as in vulnerable populations such as children and subjects with underlying pathologies.
Numerous study designs and methods have been developed to assess the effect of exposure to air pollution on several outcomes; the choice of the best one mainly depends on the research question at hand and on the available data.
The focus of this dissertation is the study of the effect of air pollution on vulnerable populations living in the Po valley, Italy, the most polluted area of Europe.

In the first contribution, a cross-sectional study is conducted to assess the acute effect of Nitrogen Dioxide (NO_2) and Particulate Matter of less than 10 $\mu g/m^3$ (PM_{10}) on the innate immune response of bronchial epithelial cells of children. Bronchial epithelial cells have been collected through a bronchoscopy and infected with rhinovirus, the expression of immune cells in response to the infection has been regressed against the short-term exposure to PM and NO_2. A compromised ability of cells to face a viral infection was found in association with both the considered pollutants.

A further contribution focuses on the Pediatric Emergency Department (PED) presentations for bronchiolitis in infants. A time-stratified case-crossover analysis is applied and combined with distributed lag non-linear models to evaluate if the exposure to air pollution could increase the risk of PED presentation and its time trend. PM has been found to impact at shorter lags, whereas NO_2 increases the risk of PED presentations at longer lags. The overall cumulative effect of exposure has also been evaluated, showing a positive result for NO_2.

The last part of the thesis deals with the study of a cohort of children and adolescents discharged from the Pediatric Intensive Care Unit (PICU). Subjects are followed for one year and the first readmission for cardiovascular, respiratory or neurological diseases is regressed against the exposure to NO_2, PM_{10} and Particulate Matter of less than 2.5 $\mu g/m^3$ ($PM_{2.5}$) subjects were monthly exposed to. This particular high-risk population shows an increased risk of readmission in association with all the considered pollutants, highlighting a risk that is not often considered in literature. Indeed, it is rare to find results of a cohort of high-risk patients followed over time to assess the risk of readmission associated with air pollution.

By showing different results of the air pollution effects on vulnerable populations, this dissertation aims at evaluating the impact of NO_2 and PM on different health outcomes in children living in the Po valley through different study designs and biostatistical methods.

LIST OF PUBLICATIONS

WITHIN THE FIRST THREE AUTHORS' NAMES

1. Gallo, E., Folino, F., Buja, G., Zanotto, G., Bottigliengo, D., Comoretto, R., Marras, E., Allocca, G., Vaccari, D., Gasparini, G., Bertaglia, E., Zoppo, F., Calzolari, V., Nangah Suh, R., Ignatiuk, B., Lanera, C., Benassi, A., Gregori, D., Iliceto, S., 2020. **Daily Exposure to Air Pollution Particulate Matter Is Associated with Atrial Fibrillation in High-Risk Patients**. International Journal of Environmental Research and Public Health 17. https://doi.org/10.3390/ijerph17176017

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*These authors share first authorship

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1. Rosato, I., Zare Jeddi, M., Ledda, C., Gallo, E., Fletcher, T., Pitter, G., Batzella, E., Canova, C., 2021. **How to investigate human health effects related to exposure to mixtures of per- and polyfluoroalkyl substances: A systematic review of statistical methods**. Environ Res 205, 112565. https://doi.org/10.1016/j.envres.2021.112565

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LIST OF ABBREVIATIONS

AHR: Aryl hydrocarbon receptor

ARPAV: Environmental Protection and Prevention Agency of Veneto Region

BAL: Bronchoalveolar lavage

BEBM: bronchial epithelial basal medium

BEGM: bronchial epithelial growth medium

CVD: cardiovascular disease

EEA: European environment agency

EPA: environmental protection agency

HBEC: primary bronchial epithelial cells

ICS: inhaled corticosteroids

IgE: immunoglobulin E

IFN: interferon

NO_2: nitrogen dioxide

mRNA: messenger RNA

PBS: phosphate buffered saline

PCR: polymerase chain reaction

PED: pediatric emergency department

PFAS: perfluoroalchil substances

PICU: pediatric intensive care unit

PM_{10}: particulate matter less than 10 µm in aerodynamic diameter

$PM_{2.5}$: particulate matter less than 2.5 µm in aerodynamic diameter

RNA: ribonucleic acid

RSV: respiratory syncytial virus

RTI: respiratory tract infections

RV16: rhinovirus type 16

SO_2: sulfur dioxide

TIPARP: TCDD-inducible poly-ADP-ribose polymerase

vRNA: viral RNA

WHO: world health organization

CHAPTER 1

INTRODUCTION

***Epidemiology**: the study of the occurrence and distribution on health-related states or events in specified populations, including the study of the determinants influencing such states, and the application of this knowledge to control health problems*

---Porta M., A Dictionary of Epidemiology

***Environmental Epidemiology**: epidemiologic studies on the effects of environmental exposures of human populations.*

---International Society for Environmental Epidemiology, 1988

***Air pollution**: The contamination of the indoor or outdoor environment by any chemical, physical or biological agent that modifies the natural characteristics of the atmosphere*

--- World Health Organization

1.1 WHEN PEOPLE RECOGNIZED AIR POLLUTION AS A RISK FOR HEALTH

The association between air pollution and human health is well established but it was not always the case. It is the mid of the twentieth century when in Europe and in the US extreme environmental events spurred scientific research, public awareness, and government action about the health impact of air pollution. At the time, smoke was considered a sign of progress and prosperity, whereas clear skies stood for economic depression and unemployment [1].

In Europe, in 1930, the first dramatic event recognized as a consequence of high levels of air pollution happened in the Meuse Valley, Belgium, when a thick fog arose at the beginning of December [2]. The valley was one of the leading steel industry sites, with a high concentration of iron mills and smelters. A set of circumstances including the lack of wind, the morphological conformation of the valley surrounded by hills, and a temperature inversion led to the stagnation of the air pollution produced in the highly industrialized area [2]. The severity of the event was stressed by the 60 sudden deaths observed during the 4 days of fog, and the amount of reported respiratory problems [3]. Some years later, a similar scenario occurred in the US and was publicly recognized as the first extreme air pollution episode in the country. Donora, Pennsylvania, was a small town with two major industrial plants, milling manufacturing steel, wire, and zinc products. At the end of October 1948, the city was covered by a very thick fog. Citizens tried to keep living their life without renouncing to Halloween parade or football matches but after such events, the health conditions of the population began to worsen. The

main symptoms included respiratory distress, chest pain, nausea, and vomiting. Visibility was so bad that ambulances had to be guided by a walking man in front of them and rapidly this was neither a feasible option, so firemen had to go door-by-door to provide oxygen to Donora residents [1]. After four days, the fog faded thanks to rain leaving behind 20 sudden deaths, 1440 people affected by serious illness, and 4470 people with mild or moderate symptoms. Almost 50% of the population were affected [4]. The US Public Health Service started to investigate the event leading to the first large-scale epidemiological study of environmental health disasters even conducted in the US [4]. The report drawn up by The US Public Health Service concluded that the concomitant pollution emitted by Donora factories and the temperature inversion that happened in that days led to the disastrous event. Donora is indeed backed by hills that form a natural bowl in which air can be trapped on the valley floor. The report did not identify a single pollutant responsible for the disaster, but hydrogen fluoride, carbon monoxide, nitrogen dioxide (NO_2), multiple sulfur compounds, and heavy metals within Particulate Matter (PM) were identified [4]. A study conducted by a university professor highlighted the long-term effects that could be observed among individuals in the community in the future [5]. A further study showed that for people who had reported having respiratory or heart disease in 1948, there was a higher death rate from cardiovascular diseases (CVD) than expected in the decade following the fog [6, 7].

The public understanding of the hazards of air pollution had just started to be spurred by the publicized episodes of lethal fog, when maybe the biggest event in terms of deaths occurred in London. The situation in London was critical since late '800, some episodes of thick fog lasting some days already happened, and many famous early nineteenth-century artists from Turner to Monet represented several pictures of the situation in London, also receiving damning criticism from art journals "*The city seems as a vast pandemonium. The picture is undoubtedly clever, and yet disagreeable*" [8].
As an insight, discussing the Meuse fog disaster that happened some months before, Professor J.S. Haldane wrote in *The British Medical Journal* "*it appears that the disaster was caused by the ordinary products of combustion from the chimneys of factories and other industrial undertakings scattered about in the sparsely populated neighbourhood of the district near Liege where the disaster occurred; and the possibility of a similar disaster happening in this country is a matter of great public interest. If a similar concentration of similar products of combustion were to occur in the air of any large town in this country, the deaths would be numbered not in tens but in thousands.*" [9]
The foresight of Prof. Haldane became reality at the beginning of December 1952, when London experienced a sudden drop in wind speed and low temperatures, and these meteorological conditions produced a temperature inversion with a complete absence of air movement at ground level [10, 11]. The concentration of pollution increased by 10-fold (particulate matter less than 10 µm in aerodynamic diameter (PM_{10}) peak was around 3000 µg/m^3) [12]. This so-called lethal *smog* (= smoke + fog) caused more than 4000 deaths in the first two weeks of December [13] and unusually high death rates have been observed for the following 3 months for respiratory or cardiovascular diseases [14].

It was only with the high number of deaths derived from the "Great London Smog" of 1952 that politicians and the whole population realized the severe health consequences of the smog. Europeans and US governments were thus prompted to enact legislation and establish organs aimed at studying and improving air quality.
The British government passed the Clean Air Act four years after the Great London Smog event, in order to decrease the use of coal both in domestic fires and in industrial furnaces [15, 16]. At that time, a big change on the type of heating sources used started with an even stronger increase in

natural gas employment [15]. The European Environmental Agency (EEA) came into force in 1993 with the objective "*to make informed decisions about improving the environment, integrating environmental considerations into economic policies and moving towards sustainability*" [17].
In the US, Congress passed the Clean Air Act in 1970 and simultaneously created the Environmental Protection Agency (EPA), an agency aimed at carrying out the law as a first purpose [18]. The Clean Air Act underwent subsequent amendments in the following year and different agencies worldwide started cooperating even more. The World Health Organization (WHO) has also a section about air pollution and periodically publishes reports on the effects of air pollution and on the new guidelines and maximum concentration of air pollution not to exceed [19].
Alongside agencies and legislation implementation to contrast air pollution, several researchers started to investigate the health effects of air pollution. The topic reached an exponential relevance during the years as proved by a quick PubMed search of the "air pollution" term that gives 1166 results if we restrict the search to 1950-1960, it reaches 20,192 records for the last 50 years of the twentieth century, and suddenly grows to 62,665 for the period 1950-2020. The study designs and the complexity of the statistical models have been constantly implemented to answer the even more detailed research questions that scientists tried to investigate and will be discussed in the following sections.

1.2 THE PECULIAR SETTING AND POPULATION OF THE PRESENTED STUDIES

In the decades following the events described in the previous section, a set of legislative actions were passed to promote a reduction in emission of air pollutants from house heating as well as from industrial facilities. Actually, levels of pollution are more than 10-fold lower than the ones recorded during the industrial revolution, but in some regions the levels of pollution observed are still critical and above the WHO guidelines.
One of these areas is the Po valley, in the north of Italy, considered the most polluted region in Europe [20]. The valley is an area of 47,820km^2 hosting almost 15 million inhabitants (1/4 of the Italian population) with a high prevalence of industries, and a topographical conformation that is quite adverse in terms of air circulation [21]. The presence of high mountain surrounding three sides of the valley and a shallow sea on the fourth make wind speed to be very low (average wind speed < 1.5m/s [22] and promotes the temperature inversion [23].
In details, when a temperature inversion occurs, the cold air full of pollutants in contact with the ground during the cold winter nights becomes trapped by the warmer air above that acts as a lid. The absence of wind promotes the stagnation of polluted air that could last for several days [24]. During the winter, it often happens that sun is not even able to disrupt the thermal inversion and the population lives in a steady foggy condition.
Despite the improvements in the industrial production process, heating, and vehicle emissions, the situation remains critic and the population is often exposed to high levels of air pollution.

Air pollution may have a stronger impact on vulnerable populations such as children, older people or subjects with underlying pathologies [25].
In this dissertation, the focus is on vulnerable populations, in particular, children and high-risk adolescents. The effect of air pollution on children is of great concern because their lungs, brain and organs are still developing and they breathe rapidly, thus absorbing more pollutants. Moreover, their height

makes them even closer to the ground, where some pollutants tend to reach their peak concentrations. They may also spend a lot of time outside playing, thus physically engaging in a pollutant environment [26].
The last WHO report on children and air pollution highlights as the main adverse outcomes an impairment of lung function, an increased risk of acute lower respiratory tract infections including pneumonia, the development of asthma and the exacerbation of asthma symptoms.
The same report underlines the growing number of studies on the negative influence of air pollution exposure on children's neurodevelopment. Lower cognitive test outcomes and behavioral disorders are the main evidence so far.
A potential harmful effect of air pollution has also been identified in association with adverse metabolic outcomes, including obesity and insulin resistance.
Exposure to air pollution during childhood is also associated with the occurrence of other health outcomes later in life, such as an impairment in lung function, reduced lung function, and an increased risk of chronic lung diseases and cardiovascular diseases [27].

The effect of air pollution on these populations living in this particular environmental setting will be presented in chapter 2, 3, and 4.

1.3 STUDY DESIGNS AND STATISTICAL MODELS FOR ENVIRONMENTAL EPIDEMIOLOGY

When the first epidemiological studies were conducted, it was often observed the correlation between exposure and health outcomes, usually mortality or morbidity [10]. Over the years, more complex study designs and methods were applied to evaluate the association between air pollution and the outcome of interest, making it possible to also adjust for possible confounders and evaluate the effects along time. Nowadays, the number of study designs and models has considerably grown letting researchers to choose the best approach depending on the research question and the data available.
Study designs can divide into many categories: time series studies; cohort studies; case-control studies; case-crossover studies; case time series studies; panel studies and cross-sectional studies.
In the further section of the dissertation, the different designs will be discussed in details.

1.3.1 TIME SERIES STUDIES

Time series design is one of the first designs applied in environmental epidemiology to assess the short-term effect of exposure on health outcomes.
Usually, there is a time-series of data (i.e. a sequence of data collected at equally-spaced and ordered time points) where the outcome is a count variable. Observation can be on a daily basis as well as hourly, annual, and so on, depending on the research question. Since the unit of the analysis is the unit of the time series, events are aggregated on that unit and, subjects' characteristics are lost.
Time series can cover years, hence it is fundamental to control for long-term patterns and seasonality to avoid bias in the estimates. This is usually assessed by smoothing the time term using a spline. Since the count structure of the outcome, the model usually performed is a Poisson regression [28, 29].
The effect of exposure is not always immediate, it can happen that there is a delay between the exposure and the outcome occurrence, thus the risk can be delayed in time.

The unit of time is the lag, defined as the period elapsed between the exposure and the event. Hence the exposure on lag 0 represents the exposure on the day of the event, exposure on lag 1 represents the exposure in the day before the event and so on.
In a backward perspective, the risk at time *t* is explained in terms of contributions by multiple exposures events at time *t-*ℓ in the past where ℓ stands for the lag.
On the opposite, in a forward perspective, if a subject is exposed to a high level of pollution today, the health effect associated may last some days before occurring, thus an exposure at time *t* determines increases in risk at multiple times *t+*ℓ in the future [30].
In the last decades, different approaches have been developed to assess the delayed effects in time, and one of the first solution employed was to perform a model for each lag. Hence, if the hypothesis was PM_{10} to have an impact on a particular health outcome with a delay of six days, seven different models would have been performed, each one evaluating the association between one of the seven lags and the outcome. The formula (1) represents a very general model, the risk is explained by a baseline hazard lambda, a function modeling the exposure of interest *f(x)* plus other functions adjusting for confounders $h_p(z_p)$. The main limitation is that the risk depends only on the exposure and confounders at time *t*.

$$\boldsymbol{\lambda_t = \lambda + f(x_t) + \sum_{p=1}^{P} h_p(z_{pt})} \qquad 1)$$

By applying several times the (1) to check the association for each lag, a problem would be that the lag effects are not adjusted for each other, whereas the aim is to model the exposure in terms of an exposure history.
The further step, was to enter in the model all the lagged variables simultaneously, this is the distributed lag model (DLM) framework, firstly developed in econometrics by Almon in 1965 [31] and then applied in environmental epidemiology for linear models starting from Schwartz in early '00 [32]. In this unconstrained model we overcome the issue arisen with single lag models, but the high correlation between exposure in adjacent days would result in collinearity in the model and subsequent imprecise estimates. To reduce the collinearity some constraints can be imposed on the effect estimates for the different lag. Constraints usually applied can be smooth terms such as polynomials and splines or the assumption of a constant effect within lag intervals [28].
DLM develops a lag-response function *w(*ℓ*)* for the new dimension of lag with $\ell \in [\ell_0, L]$. This function is used to weight the previous exposures and model the shape, expressing the association in terms of the full exposure history as in (2). The *w(*ℓ*)* can be any function, such as splines or polynomials, that uses a set of coefficients θ.

$$\boldsymbol{f(x_t, \ell; \theta) = \int_{\ell_0}^{L} x_{t-\ell} \cdot w(\ell; \theta)\ d\ell \ \approx \sum_{\ell=\ell_0}^{L} x_{t-\ell} \cdot w(\ell; \theta)} \qquad 2)$$

In (2) the exposure-response is always modelled as linear, but this is a strong assumption. Therefore, a further development first introduced by Armstrong [33] and further extended by Gasparrini and colleagues [30, 34, 35] called Distributed Lag Non-Linear Models (DLNM) opened to the possibility of describing non-linear relationships in the space of the predictor and along lags simultaneously. Here, *w(ℓ)* models the effect along lags and another function *g(x)* is added *to* relax the linearity assumption of exposure-response.
The combination of these two functions to a bivariate function brings to:

$$\boldsymbol{f(x_t, \ell; \theta) = \int_{\ell=\ell_0}^{L} g \cdot w(x_{t-\ell}, \ell; \theta) \approx \sum_{\ell=\ell_0}^{L} g \cdot w(x_{t-\ell}, \ell; \theta)} \qquad \mathbf{3)}$$

A bidimensional exposure-lag-response is thus obtained in (3), meaning that the association changes depending on the intensity of the model and on the lag.
In this framework, a cross-basis concept is introduced as a bi-dimensional space of functions describing simultaneously the shape of the relationship along *x* and its distributed lag effect [34].
Therefore, it is possible to have a three-dimensional plot showing how the risk varies both along lags and along different exposure values. By cutting the 3D plot on a certain exposure concentration (or difference of exposures) it is possible to evaluate how the risk develops in time in association with that specific exposure (or change in the exposure), whereas by cutting the plot on a specific lag it is possible to observe the risk for all the exposure values on that lag. Finally, the overall cumulative exposure response can be obtained by cumulating the contribution at each lag. Hence, this effect represents the total effect accounting for the delayed effects.
DLNM have been fully developed providing a flexible way to model complex time dependencies in association with time-varying exposures. They can be used in several study design and can be fitted with standard regression routines [35].
The fundamental concepts of lags and delayed effects in time will be recurrent topics in the further chapters of the dissertation.

1.3.2 COHORT STUDIES

In environmental epidemiology, cohort studies are usually performed to evaluate the associations between long-term exposure and a health outcome. Patients are followed for a long time, usually over time of mortality or other health events. The health outcome is usually categorical but could also be a continuous value representing for instance a physiological indicator. Cohort studies are performed through survival analysis methods typically involving the Cox proportional-hazard model.
The exposure variable included is often represented by the cumulative or average air pollution concentration [36].
The time-to-first event approach is very common, and the aim is to evaluate the association between exposure and the time at which the first health event occurs. Implementations of this model have

been proposed to evaluate recurrent events. The Andersen-Gill counting process evaluates repeated events assuming recurrent events to be independent. It means that the risk of an event at time *t* since study entry does not change irrespective of the occurrence or not of previous events [37].
If the risk of an event can be swayed by a previous one, the Prentice, Williams and Peterson (PWP) approach can be applied. The time can be considered from two perspectives: the *total time scale* considers the time since entry, whereas the *gap time scale* considers the time since the previous event. Independently from the time scale adopted, a separate hazard function is modeled with its own baseline hazard and regression parameter for each recurrent event [38].
The Wei-Lin-Weissfeld (WLW) model has the same modeling of the hazard function as in PWP, but individuals are considered at risk for every event, hence the subject is considered at risk of a subsequent event even though he has not experienced any event yet [39].
If recurrent events are considered in a study, the choice of the more suitable approach depends on the research question [40].

1.3.3 CASE-CONTROL STUDIES

Case-control studies are usually applied to assess associations between medium- and long-term exposures and rare health outcomes. The group of cases includes all the subjects that develop the outcome and is compared with a group of controls that are sampled from the source population from which the cases were identified. Controls should have similar risk characteristics to cases. The prior exposure to air pollution can be easily collected through the exposure records retrieved over time and regression models are usually applied. The main disadvantage is the identification of an appropriate control population. Since the exposure is determined after the development of the outcome, an exposure classification bias could be introduced in classifying the exposure status [41].

1.3.4 CASE-CROSSOVER STUDIES

The case-crossover design was developed by Maclure as a modification of a matched case-control study to evaluate the acute transient effects of exposure [42]. The idea is to compare the exposure on the event day (index time) to a set of exposure for separate time periods in the same subject (referent times).
One of the advantages of this design is that a control sample is not required, indeed the within-subject comparison allowed by design makes the time-invariant confounders controlled by design. Moreover, the choice of the referent scheme, (i.e. how to sample the control times for a given event in a given subject) is crucial due to the opportunity of matching the index time with the referent times in a way that allows to control for time-dependent confounders such as seasonality. Before analyzing the different schemes, the first partition is between localizable and non-localizable designs. A design is classified as localizable if the likelihood of the index time conditioned on the referent window yields information about β. On the opposite, the conditional likelihood in a non-localizable design is uninformative for β. Localizable designs are usually preferred because they allow time-dependent confounders to be controlled by matching [43].
Different approaches to the referent selection have been evaluated over the years.

In the unidirectional referent selection, control days are chosen only before the event time, usually on the same day of the week to control for it. A time trend bias could be present, (e.g. if there is an increasing trend in the exposure) and this design is non-localizable [43].
The full stratum bidirectional referent selection is an alternative in which control days are all the days in the exposure series both before and after the index time [44]. This solution overcomes the trend bias but introduces the need of adjusting for time-dependent variables because of the large referent window that makes confounding not controlled. Moreover, the 6 days before and after the index time should not be considered in the control days since it has been demonstrated an autocorrelation in time series of PM and it is comparable to overmatching in case-control studies [45]. This design is localizable.
In the symmetric bidirectional referent selection, few controls are chosen on the same day of the weak as the event time symmetrically in a forward and backward direction. In this way, the time trend is still controlled and there is also a control by design for the day of the week and seasonality. The design is non-localizable because the index time is in the middle of the referent window and this location does not provide information about β.
The time-stratified referent selection has referents chosen on the same day of the weak of the same month of the index time. Hence, it overcomes the time trend bias and can be considered localizable because the choice of the control times is in both directions, and there is no pattern in the placement of control times relative to index time.
Janes and colleagues suggest applying localizable referent schemes to obtain an unbiased estimation using a conditional logistic regression. Time-stratified referent selection is indeed one of the most unbiased referent schemes and quickly became one of the most used in epidemiological studies involving air pollution [43].

1.3.5 CASE TIME SERIES DESIGN

The case time series is a recent design proposed by Gasparrini in 2021 that combines the advantages of different well-known study designs such as case-crossover, self-controlled case series, and time series designs [46].
The case-crossover compares the event times with control times in the same subject, so the potential biases due to structural differences between subjects are excluded by self-matching.
The self-controlled case series [47] is based on a cohort logic. In this design, instead of taking an event and few controls, the whole follow-up period for each subject is considered and divided into exposed and unexposed periods. This creates a contrast of rates of occurrences of particular events in exposed versus non-exposed times within the same subject, thus the study is still self-matched and keeps the advantages of self-matched time-invariant factors of the case-crossover design, but involves the whole follow-up period, and includes the individual-level information. The disadvantage is that it is not applicable very easily with continuous exposures.
In the time series design, population data are aggregated to recreate a single series of events count and an average exposure throughout the population can be measured. An advantage is the temporal structure of the data that allows the application of complex statistical models to control for trends and look at temporal relationships.

The combination of the self-matched contrast and individual level setting of the first and second designs with the temporal structure and modeling flexibility of the third one, allows to obtain a design extension called by Gasparrini as a case time series design.
Therefore, data are at the individual level but with a time series structure. The framework is the one of a self-control case series but each of the subjects' follow-up is stratified in a separate individual series, hence there will be subject-specific series, and these series are repeated for all the subjects. The estimators are based on conditional likelihoods in which within subjects' differences are absorbed on the subject-specific terms. This design can be applied to all types of counting, binary or continuous outcomes using the extended exponential family of GLMs. In particular, for binary and counting outcomes conditional logistic and Poisson regressions can be respectively performed whereas the estimation for continuous outcomes involves mean-centring and a correction of the degrees of freedom [46].

1.3.6 PANEL STUDIES

In panel studies, a cohort is followed prospectively with frequent observations for each subject, usually recorded at a daily level. They are employed to evaluate the short-term effect of air pollution on patients' parameters that can be both continuous and binomial. Regression models can be used to estimate the association accounting for time-varying confounders. Because of the repeated measurement recorded for each patient, the variance-covariance matrix of a model is adjusted to correct for heterogeneity (i.e. multiple observations for each subject) and autocorrelation (i.e. the dependency among responses on consecutive days) [48, 49].

1.3.7 CROSS-SECTIONAL STUDIES

Cross-sectional studies usually describe the prevalence of disease at a particular point in time in a population. The outcome and the exposures in the study participants are measured at the same time and the individuals are the unit of the study.
Since exposure and outcome are measured at the same time, it is not possible to establish their temporal sequence [50].
This design is thus often avoided by researchers because of its limited possibility of implementation and because it precludes the evaluation of a temporal relationship, but in some particular cases, it is still applied.
For instance, if the exposure to a substance is measured in blood, it is not feasible to produce several repeated measures on the same patients. This is, for instance, the case of the study on perfluoroalkyl substances (PFAS) contamination in the Veneto region. For decades the population was exposed to PFAS through drinking water and nowadays there is an ongoing project aimed at evaluating the association between the prevalence of certain diseases and the exposure levels that can be measured in the blood. Therefore, researchers do not know the trend of exposure in the past and they cannot reconstruct it, but they can only evaluate the amount of chemicals that are still present at blood level [51].

1.4 MAIN CONTRIBUTIONS

Air pollution has a wide range of harmful effects on human health. In the last decades, several studies focused on its effects on different populations. In this dissertation, the focus is on vulnerable populations, in particular on children and high-risk adolescents.
The studies presented in the further chapters aim to evaluate the impact of outdoor air pollution on children living in the most polluted area of Europe.

The first contribution considered children that underwent a bronchoscopy, an invasive procedure performed only in case of need. For ethical reasons, the procedure cannot be repeated several times and the number of subjects undergoing this procedure is very small.
The aim of the study was to evaluate the association between exposure to PM_{10} and NO_2 and certain parameters of bronchial cells immune response expressed as a result of a viral infection.
Bronchial epithelial cells were collected during the bronchoscopy and subsequently infected with rhinovirus. The expression of mRNA coding for different types of interferons, proteins produced to face viral infection, were then collected.
Hence, the outcome was a continuous variable representing the number of mRNA copies collected from infected cells. Given the very low number of patients and the continuous outcome, a cross-sectional design was applied, considering only the exposure on lag 01, the average exposure between the day of the bronchoscopy and the day before. The choice of the lag depended on the clinical hypothesis regarding the timing of the effect air pollution can have on lung immune response. The findings of this study showed a decreased ability of bronchial epithelial cells to produce interferon to face viral infections when the level of exposure to air pollutants increases. This study represents a novel finding since it is rare to have the opportunity to study the effects of air pollution on human cells obtained from bronchoscopies.

In the further contribution, a time-stratified case-crossover design was applied to evaluate whether air pollution could increase the risk of pediatric emergency department (PED) presentations for bronchiolitis in infants. The population was thus made of infants aged less than 1 year living in Padova, in the Veneto region of Italy. Through the hospital database of daily PED presentations, we could retrieve all the cases of bronchiolitis over 11 years, but not other subjects' characteristics other than sex and age. The advantages of the design helped us to overcome the problem of unknown personal information, providing a self-matching of cases and controls as described in the previous section. This let to adjust by matching not only patients' characteristics but also the family habits like smoking parents or the use of wood stoves that could have an impact on the infants' baseline conditions. Moreover, time trends and seasonality were adjusted by design. The combination of the time-stratified case-crossover with DLNM allowed the great opportunity to evaluate the delayed effects in time. Hence, it was possible to study the effect of each pollutant on each lag considered and develop medical hypotheses on the timing and ways in which air pollution affects infants' lungs. Both PM and NO_2 were found to have an impact on the risk of PED presentation and hospitalization, but with a different timing. Along with the effect on single lag, it was also possible to evaluate the overall cumulative impact of air pollution on bronchiolitis onset which we found to be positive and significant for NO_2.

In the last chapter of the dissertation, a cohort study of children and adolescents will be presented. A cohort of subjects aged less than 16 years was followed after their discharge from the Pediatric Intensive Care Unit (PICU) to evaluate whether air pollution could increase the risk of hospital readmission within one year. The readmissions considered were the ones for cardiovascular, respiratory, or neurological diseases, since it has been demonstrated that air pollution mainly acts on these human body systems. The time between the discharge and the event (or end of follow-up) was divided into months and the average concentration of each air pollutant was calculated for each month. The outcome was the time-to-first event and a Cox proportional-hazard model was applied. The main effects of PM_{10}, $PM_{2.5}$ and NO_2 were evaluated in association to the risk of readmission finding a positive and significant increased risk in association to increased levels of exposure. The effect of pollutants was also considered as interacting with temperature, hence the results show the main impact of each pollutant at different temperatures giving the opportunity to assess the different effects at different temperatures. This is one of the first studies to follow a cohort of high-risk subjects over time after discharge and aiming to evaluate the impact of air pollution on the risk of readmission.

1.5 DISSERTATION OUTLINE

In the further chapters of the dissertation, three different studies assessing the health effects of outdoor air pollution will be presented each of which will face a different design to answer different research questions and difficulties intrinsic to the database. All the populations included live in the Po valley and are considered vulnerable because of their young age or previous pathologies.

Chapter 2 starts with a relatively simple cross-sectional design, where the acute effect of air pollution is evaluated in association with the immune response to viral infection retrieved from the bronchial cells of children. The low number of subjects and the lack of feasibility of repeated measurements, restrict the range of suitable designs. A generalized linear model is thus applied using a Gamma family to account for the skewed distribution of the outcome.

Moving on to more complex designs, the third chapter presents a time-stratified case-crossover design combined with a conditional logistic regression model to assess the effect of air pollution on infants' ED presentations for bronchiolitis. Beyond the classical design, DLNM is applied to evaluate the delayed effect in time lasting 14 days. The number of lags to include is decided based on the infection length. The contribution of exposure of each day preceding the ED presentation as well as the overall cumulative effect of each pollutant can thus be easily computed allowing speculations on the physiology behind the effect of air pollution on infants' lungs.

In the last chapter, a cohort of high-risk children is followed after their discharge from the PICU till the first readmission for cardiovascular, respiratory, or neurological diseases, or the end of the follow-up lasting one year. The research question is thus whether these children could be affected by air pollution in the critical first year after discharge. A Cox proportional-hazard model is applied to evaluate the association between the first readmission and the monthly average exposure to air pollution.

All these studies provide a picture of some of the possible risks associated with the exposure to quite high levels of air pollution, such as the ones recorded in the Po valley, in the young population.

CHAPTER 2

AIR POLLUTION EXPOSURE IMPAIRS AIRWAY EPITHELIUM IFN-β EXPRESSION IN PRE-SCHOOL CHILDREN

SUMMARY

Introduction: air pollution is a risk factor for respiratory infections and asthma exacerbations. We previously reported impaired Type-I and Type-III interferons (IFN-β/λ) from airway epithelial cells of preschool children with asthma and/or atopy. In this study we analyzed the association between rhinovirus-induced IFN-β/λ epithelial expression and acute exposure to the principal outdoor air pollutants in the same cohort.

Methods: we studied 34 children (17asthmatics/17non-asthmatics) undergoing fiberoptic bronchoscopy for clinical indications. Bronchial epithelial cells were harvested by brushing, cultured and experimentally infected with Rhinovirus Type 16 (RV16). RV16-induced IFN-β and λ expression was measured by quantitative real time PCR, as was RV16vRNA. The association between IFNs and the mean exposure to PM_{10}, SO_2 and NO_2 in the day preceding bronchoscopy was evaluated using a Generalized Linear Model (GLM) with Gamma distribution.

Results: acute exposure to PM_{10} and NO_2 was negatively associated to RV16-induced IFN-β mRNA. For each increase of 1 μg/m^3 of NO_2 we found a significative decrease of 2.3x10^3 IFN-β mRNA copies and for each increase of 1 μg/m^3 of PM_{10} a significative decrease of 1x10^3 IFN-β mRNA copies. No significant associations were detected between IFN-λ mRNA and NO_2 nor PM_{10}. Increasing levels of NO_2 (but not PM_{10}) were found to be associated to increased RV16 replication.

Conclusions: short-term exposure to high levels of NO_2 and PM_{10} is associated to a reduced IFN-β expression by the airway epithelium, which may lead to increased viral replication. These findings suggest a potential mechanism underlying the link between air pollution, viral infections and asthma exacerbations.

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2.1 INTRODUCTION

Air pollution is increasingly recognized as an important cause of asthma exacerbations and, possibly, as a cofactor for asthma origins [52]. Many studies have shown that the exposure to pollutants is associated to increased risk of respiratory viral infections, i.e. the most frequent triggers of asthma exacerbations, including rhinovirus, influenza and respiratory syncytial virus. In asthma patients there is evidence that the exposure to pollutants in the week preceding a respiratory viral infection increases the severity of the associated asthma exacerbations [53]. The mechanisms by which air pollution may increase susceptibility to infections and favor viral-induced exacerbations in asthma patients are still largely unknown.

Previous studies- though not unanimously- have identified impaired antiviral immune response as a possible mechanism for increased susceptibility to infections in asthmatic patients. In particular, we previously reported, impaired type I (IFN-β) and type III interferon (IFN-λ) production following rhinovirus-16 (RV16) infection in bronchial epithelial cells of pre-school asthmatic and/or atopic children compared to non-asthmatic non-atopic children [54]. Furthermore, we reported that such impaired IFN response was associated to persistence of asthma symptoms up to school-age [55] in line with the epidemiological data suggesting that respiratory tract infections in childhood increase the risk of asthma.

To the best of our knowledge, no study has ever investigated the association between viral-induced IFN responses in bronchial epithelial cells and exposure to air pollution in children at risk for asthma.

In this study we sought to investigate the relationship between exposure to air pollutants (nitrogen dioxide- NO_2, particulate matter less than 10 µm in aerodynamic diameter- PM_{10}, sulfur dioxide-SO_2) and RV16-induced IFN-β and λ expression in primary bronchial epithelial cells obtained from a cohort of pre-school children [54]. Aim of this study is to evaluate whether RV16 induced IFN-β and λ mRNA levels were affected by the ambient air concentration of: i) nitrogen dioxide-(NO_2), ii) particulate matter less than 10 µm in aerodynamic diameter (PM_{10}) and iii) sulfur dioxide (SO_2).

2.2 METHODS

2.2.1 STUDY POPULATION

The study design and the population of the children cohort considered in this study has been previously described in details [54]. The study was performed according to the Declaration of Helsinki and was approved by the Ethics Committee of the Padova City Hospital. Children underwent bronchoscopy, with bronchoalveolar lavage, for appropriate clinical indications according to current guidelines [56]. Bronchial biopsy and brushing were harvested for research purposes during bronchoscopy upon approval from the children's parents. A detailed medical history was collected by a respiratory pediatrician before bronchoscopy. The physician administered parental interviews investigating the pattern of respiratory symptoms, the frequency of respiratory tract infections (RTI) in the previous year and on-course treatment. Presence of wheezing with a pattern suggestive of asthma was based on the report of repeated episodes of wheezing in the previous year, often associated to cough and dyspnea,

particularly at night or in the early morning, that was responsive to bronchodilators. None of the children in the non-asthmatic control group complained of episodes of wheezing, breathlessness or cough that were responsive to bronchodilators. All children underwent routine blood tests, including complete white blood cell count (total leukocytes, neutrophils, lymphocytes, monocytes, eosinophils and basophils) and total/specific IgE. The presence of atopy was defined by an increase of total (higher than the age-related normal levels) and specific IgE (>0.35KU/L; IMMunoCAp, Phadia, Sweden). In particular, specific IgE for the following aeroallergens were investigated in all children: house dust mite (*Dermatophagoides pteronyssinus and Dermatophagoides farinae*), moulds (*Alternaria alternate*), cat dander and grass pollens (*Lolium perenne, Poa pratensis, Phleum pratense, Dactylis glomerata and Cynodon dactylon*).

2.2.2 AIR POLLUTION EXPOSURE EVALUATION

Daily levels of PM_{10}, NO_2 and SO_2 were retrieved from the monitoring stations of the Environmental Protection and Prevention Agency of Veneto Region (ARPAV) [57, 58]. The concentrations of the pollutant the day of the bronchoscopy and the day before were collected. Each child was linked to the data of the monitoring station nearest to his/her residence. Schools were in proximity of the residential address for all children. Air pollution concentrations were compared with the 2005 WHO air quality guidelines, which set cut-offs not to be exceeded at 50 $\mu g/m^3$ for PM_{10} and 40 $\mu g/m^3$ for NO_2 [59].

2.2.3 PRIMARY BRONCHIAL EPITHELIAL CELL CULTURES FOR DETECTION OF IFN-β, IFN-λ AND VIRAL RNA

Primary bronchial epithelial cells (HBEC) were harvested by bronchial brushing, handled and cultured as previously detailed [54]. Briefly, HBEC cultures were set up into hormonally supplemented bronchial epithelial growth medium (BEGM, Clonetics, San Diego, USA) containing 50U/ml penicillin and 50 mg/ml streptomycin. At 80% confluency, cells were resuspended, counted (1.8 $x10^5$ cells/ml) and immediately seeded in 12-well plates (2 ml in each well) for the infection. Cells were starved in bronchial epithelial basal medium (BEBM with no supplements) overnight before exposure to RV16. Rhinovirus type 16 (RV16; a major group rhinovirus) was obtained from the Health Protection Agency Culture Collections, Health Protection Agency Microbiology Services, Salisbury, United Kingdom. The virus was used at a multiplicity of infection of 5 for all experiments. Cells were exposed to rhinovirus at room temperature for 1 hour, washed with phosphate buffered saline (PBS) to eliminate unbound virus and grown in BEGM (for 8hrs or 48hrs). Samples from each donor were performed in duplicate. As internal control of rhinovirus infection, we exposed the cell cultures to inocula from which virus particles were removed by ultrafiltration (Amikon, London, UK) or inactivated by UV-irradiation.

Quantitative real-time PCR was carried out for rhinovirus, IFN-β, IFN-λ, viral RNA (vRNA) and18S rRNA (a housekeeping gene) at 8 hrs post infection, as previously described [54]. Interferons and viral RNA (vRNA) expressions were normalized to 18S rRNA levels, compared with standard curves, and expressed as copy numbers per microgram of RNA. Furthermore, detection of IFN-β and IFN-λ protein (and IL-8 as internal control) was performed at 48hrs after infection as previously described [54]. Since 8 hrs cultures for mRNA detection were prioritized, and only when enough cells were available plates

for the 48 hrs time point were set-up, IFN-β and IFN-λ protein levels were available in a subset of children in our cohort (17 subjects for IFN-β, 9 subjects for IFN-λ). Due to the smallness of the sample we failed to obtain a reliable association model between pollution exposure and protein production, so we did not consider it for further analyses.

2.2.4 STATISTICAL ANALYSIS

Children's characteristics were expressed as median and IQR for continuous variables, and counts and percentages for categorical variables. The difference in explanatory variables was assessed using a Chi-squared test or Fisher test for dichotomous and categorical variables, or Mann–Whitney U test for continuous variables.

The acute effect of air pollution on RV16 vRNA copies, IFN-β and IFN-λ mRNA copies was modeled using a Generalized Linear Model (GLM) assuming a Gamma distribution with an identity link function to account for the skewness of the outcomes [60]. Single pollutant models were performed including the acute exposure to the pollutant and the presence of asthma as covariates in the linear predictor. For what concerns acute exposure, the average concentration of the pollutant between the day of the bronchoscopy and the day before (lag 01) was used.

In view of the limited sample size and the high degree of skewness in the covariates, to evaluate stability of the results, a sensitivity analysis was conducted by replacing the variable indicating presence of asthma with the one representing the presence of atopy [61, 62]. All analyses were performed using R statistical software [63, 64].

2.3 RESULTS

Patients' characteristics were previously detailed [54]. Briefly, the cohort included 17 asthmatics (either atopic: n=8 or non-atopic: n=9) and 17 controls (8 atopic; 9 non-atopic). The bronchoscopic procedure was well tolerated by all children, and no complications were encountered. Clinical indications for bronchoscopy did not differ significantly between asthmatic and non-asthmatic children. Asthmatic and non-asthmatic children had a similar age and gender distribution (table 2.1).

Table 2.1. Description of the whole cohort and comparison between asthmatic and non-asthmatic children.

	WHOLE CHORT	**ASTHMATICS**	**NON-ASTHMATICS**	**p**
Subjects	34 (100%)	17 (50%)	17 (50%)	-
Male (n; %)	18 (52.9%)	11 (64.7%)	7 (41.2%)	0.169
Age (y)	5 [4-6]	5 [3.5-6.5]	5 [4-5.5]	0.973

Symptoms onset (y)	1 [0.5-3.5]	1 [0.5-3.5]	n.a.	-
RTI (ep/month)	1 [0-2]	2 [0.5-2.5]	2 [1-2]	0.786
ICS (n; %)	11 (32%)	10 (58.8%)	1 (5.9%)	0.001
Air pollution exposure	N=30	N=14	N=16	
PM_{10} ($\mu g/m^3$)	40.5 [23.0-53.0]	40.5[27.5-59.1]	34.5[22.6-46.2]	0.381
NO_2 ($\mu g/m^3$)	39.8 [28.9-47.5]	40.7[32.2-50.4]	36.0[24.0-46.9]	0.270
Serum IgE (kU/L)	47.5 [13.9-221.5]	89 [29-504]	32 [12.2-136]	0.079
Blood Eosinophils (cell/µL)	269 [142-539]	443 [220-710]	260 [74.5-361]	0.024
BAL Eosinophils (%)	0 [0-2.25]	0 [0-1.5]	0 [0-3.5]	0.483
Biopsy Eosinophils (cell/mm^2)	14 [6.7-126.5]	63 [13-161]	12 [0-81]	0.217
Basement membrane thickness (µm)	4.66 [3.83-5.19]	5.19 [4.36-5.96]	4.05 [3.49-4.79]	0.032
IFN-β mRNA (x10^3 copies/µg)	3.7 [1.0-77.6]	2.1 [0.8-16.8]	32.2 [2.9-213.3]	0.049
IFN-λ mRNA (x10^3 copies/µg)	3.8 [0.8-21.3]	1.2 [0.4-6.5]	14.2 [1.4-142.5]	0.011
RV16 vRNA (x10^3 copies/µg)	44.4 [12.3-542.1]	224.3 [35.5-772.6]	25.5 [9.8-47.5]	0.009

The p-value is referred to the Mann-Whitney-U test or Chi-square/Fisher's exact comparison between asthmatic and non-asthmatic children. Data are expressed as median [interquartile range]. RTI: respiratory tract infections during previous year ICS: inhaled corticosteroid therapy at bronchoscopy n.a.: not applicable;

No children were chronically treated with oral or high-dose inhaled corticosteroids. Asthmatic children showed higher levels of peripheral blood eosinophils (p=0.02), BAL eosinophils were not different in the two groups. The two groups of children had a comparable history of respiratory tract infections (RTI). Though asthmatic and non-asthmatic children were matched for atopic status (as per design of the original study), a non-significant trend for increased levels of total serum IgE was observed in asthmatic children compared to non-asthmatics (p=0.074).

RV16-induced IFN-β induction in asthmatic children was significantly reduced compared to non-asthmatic children (p=0.049); a similar reduction was also observed for IFN-λ (p=0.011). The reduction in IFNs levels was mirrored by a significant increase in RV16 vRNA levels in asthmatic children compared to non-asthmatics (p=0.009) as previously described (Table 2.1, Figure 2.1a-c).

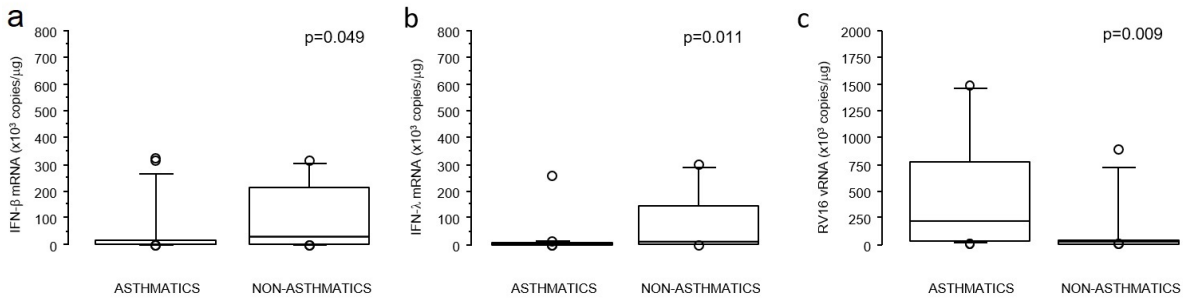

Figure 2.1 (a-c). Boxplots reporting values of IFN-λ mRNA (a), IFN-β mRNA (b), and RV16 vRNA (c) in asthmatics and non-asthmatics children. Bottom and top of the box-plot: 25th and 75th percentiles; solid line: median; brackets: 10° and 90° percentiles.

Data on air pollution were available for 30 out of 34 children. Data on median levels of outdoor air pollution on lag01 are summarized in Table 2.1. SO_2 levels were virtually undetectable across the whole region so we did not consider this pollutant for further analyses. As shown in the table children in our cohort were exposed to high levels of air pollutants. The median exposure values for NO_2 and PM_{10} were very close to the corresponding WHO recommended highest limit. Of note, we did not observe significant different levels of exposure to PM_{10} and NO_2 among districts, nor between asthmatic and non-asthmatic children.

When considering the whole cohort of preschool children, a negative association between acute (lag01) exposure to PM_{10} or NO_2 and the levels of RV16-induced IFNβ mRNA in HBEC was observed (Figure 2.2, Table 2.2). For each increase of 1 µg/m^3 of NO_2 we found a significative decrease of 2.3 x10^3 in RV16-induced IFN-β mRNA copy number. For each increase of 1µg/m^3 of PM_{10} a significative decrease of 1 x10^3 in RV16-induced IFN-β mRNA copies was observed (Figure 2.2a-b). No significant associations were detected between RV16-induced IFN-λ mRNA and NO_2 nor PM_{10}. Exposure to increasing levels of NO_2 (but not PM_{10}) was positively associated to increased RV16 replication in HBEC (Table 2.2).

Table 2.2 β coefficients and relative 95% Confidence Interval (CI) of the multivariable models

Outcome	Pollutant	β	95% CI
IFN- β mRNA (x10^3 copies/µg)	PM_{10}	-1.05	-1.97; -0.14
	NO_2	-2.36	-3.70; -1.01
IFN-λ mRNA (x10^3 copies/µg)	PM_{10}	1.04	-0.71; 2.79
	NO_2	0.80	-0.99; 2.58
RV16 vRNA (x10^3 copies/µg)	PM_{10}	8.06	-13.10; 29.21
	NO_2	31.73	6.59; 56.88

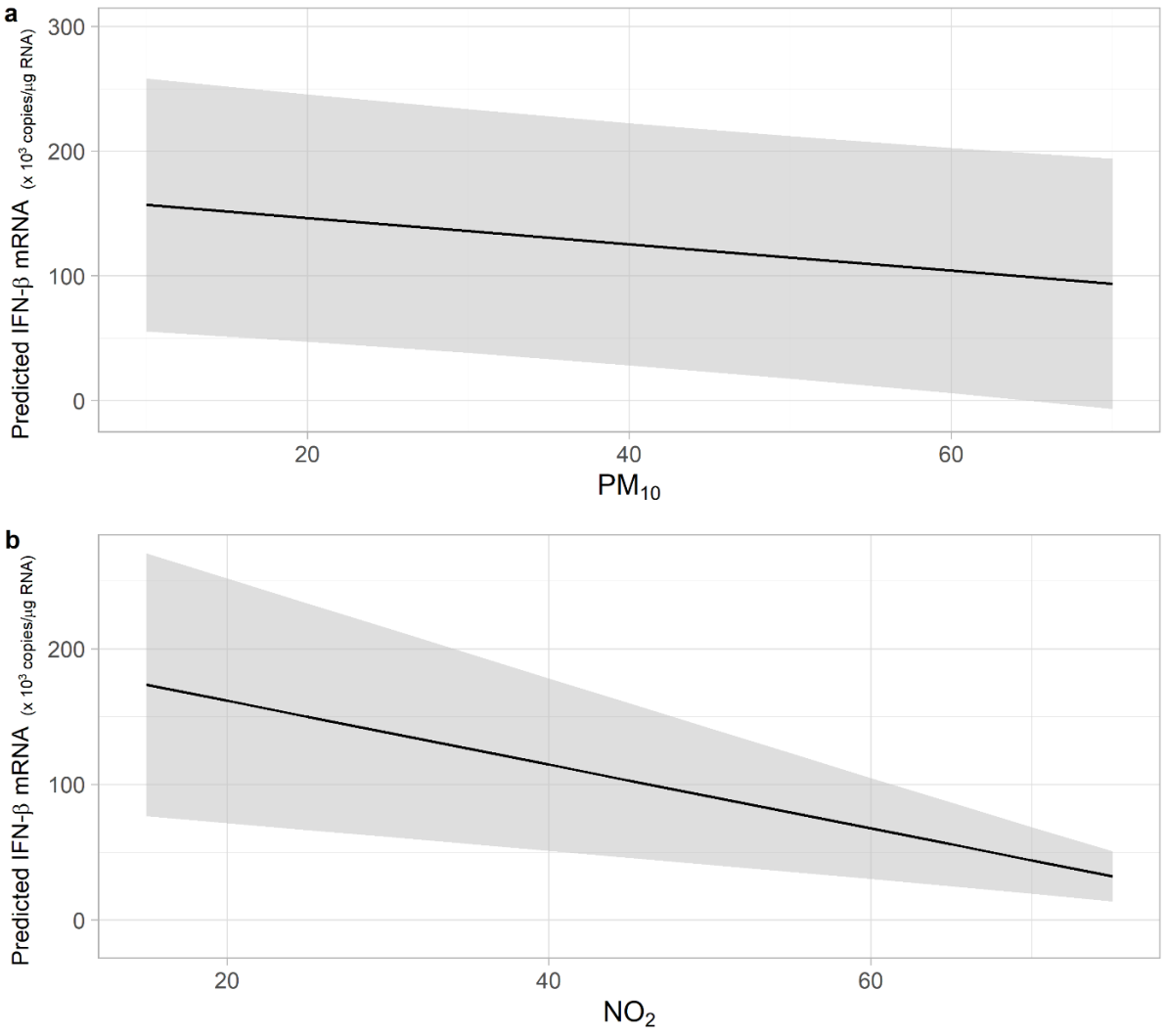

Figure 2.2 (a-b). The figures show the predicted values of IFN- β mRNA in association with short-term exposure to PM_{10} (a) and NO_2 (b). Shaded area indicates the confidence interval (CI). Predicted values of IFN- β mRNA were obtained from Generalized Linear Model (GLM).

Since we have previously shown that both asthma and atopy were associated to reduced IFN expression by epithelial cells [54], we performed a sensitivity analysis in which the presence of asthma was replaced with the presence of atopy. Consistently, the analyses confirmed the significant decrease of IFN-β mRNA copies in association with both PM_{10} and NO_2. However, the significance of the association between RV16 vRNA copies and NO_2 was lost in this sensitivity analysis. We also confirmed null associations between RV16 replication and PM_{10}, or between IFN-λ mRNA copies and PM_{10} / NO_2 (data not shown).

2.4 DISCUSSION

In this study we explored the relationship between exposure to air pollutants and innate immune response to viral infection in primary epithelial cells in a cohort of preschool asthmatic children and controls. We found that acute exposure to either NO_2 and PM_{10} was associated to impaired antiviral immune response, in terms of RV16-induced IFN-β expression. Furthermore, exposure to NO_2, but not PM_{10}, was associated to increased levels of viral replication.

In our previous study performed in this cohort, we have demonstrated that the deficient expression of interferons by epithelial cells in response to rhinovirus, which was previously documented only in adults with atopic asthma, was present even in preschool children and that both asthma and atopy were independent factors associated to this reduced IFN induction. In this study we have considered the effect of air pollution on the innate immune response to viral infection.

An epidemiological link between air pollution and lower tract respiratory infections has been well described in multiple cohorts, either in developed and in emerging countries [65, 66]. Nevertheless, the association between antiviral immune response and air pollutants has been investigated mainly systemically, correlating the concentration of air pollutants to serum levels of IFN-γ [66, 67]. So far, the evidence relating air pollution to impairment of innate immune responses directly on the lung has been scarce. Our results indicate that exposure to increasing levels of air pollutants, was related to an impaired IFN-β expression from the airway epithelium, which resulted in increased viral replication. No such effect of PM_{10} and NO_2 was observed on IFN-λ in our study, which points out the different regulation of type I and type III IFNs [68]. To our knowledge, this is the first study that investigates *ex-vivo* the influence of air pollution on interferon responses from the airway epithelium in children.

Our findings would point to the mechanisms through which air pollution can negatively affect the biological integrity of airway epithelial barrier predisposing to respiratory infections. It is widely recognised that air pollutants may influence the expression of multiple genes through epigenetic modifications, such as gene methylation, particularly during intrauterine life and early infancy [69]. Earlier studies have focussed on the regulation of IFNγ production by PBMC [70], while few data are available on the effect of pollution on IFN expression in the lung. In line with our results, Tao and coworkers reported in vitro that exposure to particulate matter ($PM_{2.5}$) deteriorated influenza virus infection by reducing the production of IFN-β by alveolar macrophages in vitro [71]. Mechanistically, PM 2.5 exposure did suppress the NLRP3 inflammasome and IL-1β through the AHR-TIPARP signaling pathway. The Aryl hydrocarbon receptor (AHR) is a transcription factor that mediates the toxic activity of many environmental xenobiotics and it has been shown that AHR, acting on the TCDD-inducible poly-ADP-ribose polymerase (TIPARP), is a negative regulator of type I interferon expression. Activation of AHR is able to upregulate expression of the ADP-ribosylase TIPARP, which in turn causes down-regulation of type I interferon responses [72].

The mechanisms regulating the interaction between the airway epithelium and air pollutants is a current gap in asthma research [73]. Indeed, despite a recent statement from the American Thoracic Society concluded that there is enough epidemiologic evidence indicating a causal link between exposure to outdoor air pollution and asthma, the underlying pathophysiology has not been thoroughly studied [74]. The appraisal of the role of air pollution should be addressed in the short term, as a cause of asthma exacerbations, but also in the long term as a possible cofactor for asthma persistence.

Indeed, in a longitudinal follow-up of our cohort we were able to demonstrate that the abnormal IFN response in early childhood (mean age 5yrs) correlated with the persistence of asthma symptoms throughout adolescence [55]. It is thus conceivable that air pollution, by downregulating innate immune responses in early life, may track its effect throughout adulthood.

Of interest, we recently observed a dual effect of long-term pollutants exposure on cellular inflammation in airway tissue: while in asthmatic children air pollution enhances eosinophilic inflammation in bronchial mucosa, in non-asthmatic ones it associates with reduced numbers of both eosinophils and neutrophils [75]. Altogether the results of the two studies suggest that air pollution can have a pro-inflammatory effect in susceptible subjects (asthmatics) while, at the same time, impairing innate immune responses. We can envisage the existence of one or multiple immunological checkpoints (e.g. AHR) able to heighten or desensitize innate responses by integrating several inputs, either endogenous or exogenous (e.g. air pollution or the Type-2 cytokine environment) [76].

A major strength of our study is the well characterized cohort of children undergoing bronchoscopy with brushing collection. The study, however, has limitations: first, analyses are based on regionally available pollutants measurements, not on personal exposure, and are limited to RV16 stimulation. Second, the limited sample size of our cohort and the lack of extended temporal data on pollutants exposure, hampered the possibility to perform more complete analyses which could account for the high degree of heterogeneity and manage potential confounding covariates. Furthermore, since protein measurements were available only in a subset of subjects, we could not investigate the association between air pollution and IFNs proteins. Finally, we are conscious that our patient sample is not representative of the general population due to the clinical conditions for which bronchoscopy was indicated either in asthmatic and non-asthmatic children. However, lower airway sampling during bronchoscopy in a pediatric population only for research purposes would be ethically unacceptable in our Hospital setting. More readily available biological samples such as nasal epithelial cells could be explored in future studies as a possible alternative to airway biopsy specimens since they may reflect, at least in part, innate immune responses from lower airways [77].

In conclusion, we demonstrated that acute exposure to high levels of NO_2 and PM_{10} is associated to a reduced expression of IFN-β by airway epithelium and, consequently, to increased viral replication. Our results suggest that such impaired IFN-β response could be a pivotal element of the mechanism underlying the epidemiological link between air pollution, respiratory infections susceptibility, and asthma development that deserves further investigation.

CHAPTER 3

INCREASED RISK OF EMERGENCY DEPARTMENT PRESENTATIONS FOR BRONCHIOLITIS IN INFANTS EXPOSED TO AIR POLLUTION

SUMMARY

Air pollution has been linked to an increased risk of several respiratory diseases in children, especially respiratory tract infections. The present study aims to evaluate the association between Pediatric Emergency Department (PED) presentations for bronchiolitis and air pollution. PED presentations due to bronchiolitis in children aged less than 1 year were retrospectively collected from 2007 to 2018 in Padova, Italy, together with daily environmental data. A conditional logistic regression based on a time-stratified case-crossover design was performed to evaluate the association between PED presentations and exposure to NO_2, $PM_{2.5}$ and PM_{10}. Models were adjusted for temperature, relative humidity, atmospheric pressure and public holidays. Delayed effects in time were evaluated using Distributed Lag Non-Linear Models. Odds Ratio for lagged exposure from 0 to 14 days were obtained. Overall, 2251 children presented to the PED for bronchiolitis. Infants' exposure to higher concentrations of PM_{10} and $PM_{2.5}$ in the five days before the presentation to the PED increased the risk of accessing the PED by more than 10%, whereas high concentrations of NO_2 between 2 to 12 days before the PED presentation were associated with an increased risk of up to 30%. The association between pollutants and infants who required hospitalization was even greater. A cumulative effect of NO_2 among the two weeks preceding the presentation was also observed. In summary, PM and NO_2 concentrations are associated with PED and hospital presentations for bronchiolitis. Exposure of infants to air pollution could damage the respiratory tract mucosa, facilitating viral infections and exacerbating symptoms.

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3.1 BACKGROUND

Bronchiolitis is the first reason of hospitalization in children aged less than 1 year in Europe [78]. It consists of a lower-respiratory tract viral infection, usually due to Respiratory Syncytial Virus (RSV), that leads to expiratory wheezing and respiratory distress [79, 80]. The smaller airways, the developing lungs and immune system of children make them more vulnerable to the infection [81]. Furthermore, the development of bronchiolitis in infancy has been linked to an increased risk of asthma or lifetime wheezing later in life [82].
The time course of the disease illustrated by Karr and colleagues shows the beginning of symptoms 6-7 days after the infection and a peak in severity, compatible with the period of PED visits, on days 8-9 since infection [83].
Moreover the disease has a typical seasonality, with a highest number of cases between November and March in the northern hemisphere [84].

Several studies have shown an association between air pollution and respiratory morbidity in children [85–87]. Studies on the chronic and long-term exposure to air pollution showed that children living closer to the main roads have an increased risk of developing bronchiolitis [88, 89].
The short-term effect of air pollution on the risk of developing bronchiolitis has been mostly evaluated on single lags or on the moving average concentration of various pollutants during the days before the PED visit or the hospitalization. Hospitalization for bronchiolitis has been shown to be associated with the moving average concentration of PM_{10}, $PM_{2.5}$ and NO_2 in the week before [90–92]. However, lack of consistency has emerged for restricted time windows [92–94].

Studies on single lag reported on one side a non-significant association between hospitalization for bronchiolitis and $PM_{2.5}$ on day 1 and 4 preceding the event [83], on the other side a significant association between hospitalizations and the levels of PM_{10} in all the 11 days before the hospitalization [90].

Recently, some researchers started to focus also on the cumulative effect of air pollution on children's lungs diseases. Leung and colleagues found a positive effect of NO_2 and a null overall effect of PM_{10} on bronchiolitis hospitalizations in the subtropical region of Hong Kong [95], whereas a positive 7-days cumulative effect was observed on lower respiratory obstructive diseases in children aged less than 4 years old [96].

The present study aims to investigate the association between PED presentations for bronchiolitis and air pollution concentrations in the two weeks preceding the visit, with a particular focus on non-linear and delayed effects in time.

3.2 METHODS

3.2.1 DATA COLLECTION

Data on children PED presentations from January 1st, 2007 to December 31st, 2018 in Padova, Italy, were retrospectively collected from the PED database. To more accurately identify all the children with a diagnosis of bronchiolitis, we reviewed the free text descriptive diagnosis field searching for

the keyword root “bronchiol*” in order to maximize the sensitivity of our search. The PED of the University Hospital of Padova is a tertiary-care academic centre, providing a 24/7 service to a catchment area of approximately 20 km, from the hospital.

Data of children younger than one year of age, who received a diagnosis of bronchiolitis in the PED and lived within 20 km from the hospital were abstracted from the database. Patient disposition data (discharge from the PED or hospitalization) were also retrieved.

Air quality and meteorological data were collected thanks to the Regional Agency for Environmental Prevention and Protection of the Veneto region (ARPAV). An urban background air quality monitoring station (located 5 km away from the hospital) (Figure A1) retrieves daily data on temperature, relative humidity, atmospheric pressure and concentrations of PM_{10}, $PM_{2.5}$,and NO_2 [57].
The distribution of pollutants in this region has been evaluated by comparing levels of pollution of Padova with the ones of two other urban background monitoring stations, located in Treviso and Vicenza, at 46 and 31 km away from Padova hospital respectively.

3.2.2 STATISTICAL ANALYSIS

Continuous variables are represented as 1st quartile, median and 3rd quartile. Categorical variables are shown as percentages (relative frequencies).
The pair-wise relations between NO_2, PM_{10} and $PM_{2.5}$ were assessed using the Spearman's rank correlation (ρ).
The association between air pollution concentration and PED presentations for bronchiolitis has been investigated using a multivariate conditional logistic regression based on a time-stratified case-crossover design [42, 45].
This approach allows to account for the possibility of confounding by seasonal patterns by selecting controls on the same day of the week of the same month of the same year of cases. For example, a case on the 8th of November 2017 will have the 1st, 15th, 22nd and 29th of November 2017 as control days. In this way every case will be matched with 3 or 4 controls.
Therefore, subjects serve as their own control and known and unknown time invariant characteristics of all patients (eg. parental smoking, wood burning habits that could produce indoor air pollution, preterm birth) are controlled by design.

The potential delayed effects in time of air pollution have been evaluated combining the conditional logistic regression with a Distributed Lag Non-Linear Model (DLNM) [30]. In this way, through a “cross-basis” function, it will be taken into account not only the potential non-linear association between the exposure and the outcome, but also the non-linear effect across lags.
Lags from 0 (the exposure on the day of PED presentation) to 14 (the exposure 14 days before the presentation) were considered for every pollutant to assess both the acute and the delayed effect of air pollution. The number of lags was chosen to evaluate the role of air pollution during the development of the disease and in the days preceding the infection.

Because of the strong correlation between the pollutants, three different models were performed, one for each pollutant. The pollutant entered into the model as a cross-basis matrix in which the exposure-response association has been linearly modelled, whereas the lag structure has been modelled with a natural cubic spline with 5 degrees of freedom.

Moreover, the potential strong and delayed effect of temperature on the development of bronchiolitis [97] was taken into account by means of a cross-basis matrix for lags 0 to 14 of temperature. Natural cubic splines with 4 and 5 degrees of freedom were used to model the temperature-response association and the relationship between outcome and lag structure, respectively.
Bayesian Information Criterion (BIC) score was used to select the best cross-basis matrix.

The three multivariate models were adjusted for the abovementioned matrix of lagged temperature, public holidays and the 3 days moving average values of atmospheric pressure and relative humidity.

A sensitivity analysis has been performed by temporally splitting the dataset into two parts (from 2007 to 2012 ad from 2013 to 2018). The analyses have been performed in both datasets in order to check the Bradford Hill criteria of strength [98].

Models were also applied only on children that were hospitalized, aiming at evaluating how the risk changes in a subcohort with more severe conditions.

Besides the daily effect of air pollution, the cumulative effect among the two weeks has also been assessed.

Environmental missing data (2.1%) were imputed with univariate imputation using the robust non-linear method implemented in the transcan function of Hmisc R package with default settings [99].
Results are shown as the odds ratio (OR) associated to a 10 unit increase in the selected pollutant.
All the analyses were performed using R statistical software [100] and dlnm [35] package.

3.3 RESULTS

Overall, 42 543 infants in the first year of life presented to the PED between the 1st of January 2007 and the 31st of December 2018 and 2215 (5.2%) received a final diagnosis of bronchiolitis. Overall 62% of infants with bronchiolitis were male and the median age was 3.6 months (Table 3.1).

Table 3.1. Presentations characteristics.
Data are median [IQR] or n (%). $PM_{2.5}$ and PM_{10} = Particulate Metter of less than 2.5 and less than 10 um in aerodynamic diameter respectively. NO_2 = nitrogen dioxide. WHO = World Health Organization.

	Presentations (n = 2215)
Age (months)	3.6 [2-6.3]
Gender (m)	1368 (62%)
Exposure to $PM_{2.5}$	
< WHO threshold (25µg/m^3)	649 (29%)
>WHO threshold (25 µg/m^3)	1566 (71%)

Exposure to PM_{10}	
< WHO threshold (50 µg/m^3)	1134 (51%)
>WHO threshold (50 µg/m^3)	1081 (49%)
Exposure to NO_2	
< WHO threshold (40 µg/m^3)	1083(49%)
>WHO threshold (40 µg/m^3)	1132 (51%)

Air pollution concentrations in the period of the admissions, mostly between November and March, are shown in Table 3.2. Levels were high, especially for $PM_{2.5}$, which exceeded the World Health Organization (WHO) thresholds in 63% of days, whereas for PM_{10} and NO_2 exceeding occurred in 43% and 48% of days respectively [101].

Table 3.2 Median concentrations and Interquartile Range [IQR] for Particulate Matter with aerodynamic diameter of less than 2.5 µm ($PM_{2.5}$) and less than 10 µm (PM_{10}) and Nitrogen dioxide (NO_2) in the days of PED visits for bronchiolitis in Padova, Italy. World Health Organization (WHO) daily threshold.

Pollutants	**median [IQR] µg/m^3**	**WHO threshold µg/m^3**
$PM_{2.5}$	36.00 [20.44, 58.00]	25
PM_{10}	44.79 [28.25; 69.00]	50
NO_2	40.00 [29.00, 51.00]	40

As an example of the distribution of air pollution across the Veneto Region, Fig A2 shows the pollutants concentrations in Padova, Treviso and Venezia during the cold months of 2014/2015. Comparable trends of concentrations were observed for the three monitoring stations considered in the present study, suggesting a homogeneous distribution of pollutant levels across the plain area of the Veneto Region.

As expected, PM_{10} and $PM_{2.5}$ were the most strongly correlated pollutants ($\rho = 0.95$), whereas the correlation between NO_2 and particulate matter was slightly weaker ($\rho = 0.70$ with PM_{10} and $\rho = 0.73$ with $PM_{2.5}$). A detailed description of correlations by year is presented in supplementary material (Table A1).

The association between presentations for bronchiolitis and 10 µg/m^3 increase in both $PM_{2.5}$ and PM_{10} is statistically significant from lag 0 to lag 4 (Figure 3.1).

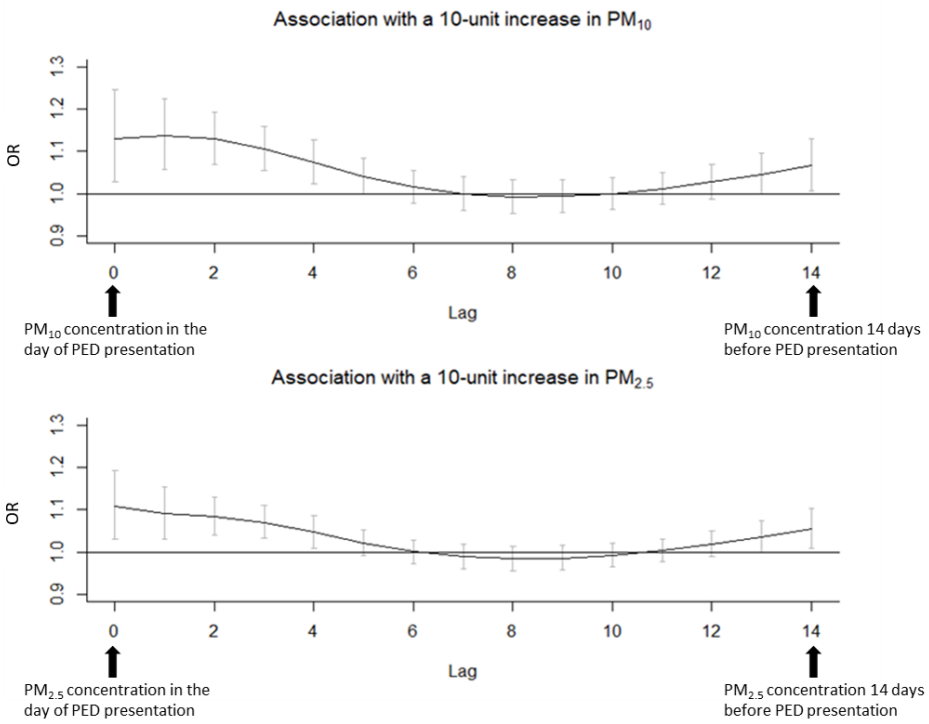

Figure 3.1. Odds Ratio (OR) and 95% confidence interval for the association between Pediatric Emergency Department (PED) presentations for bronchiolitis and Particulate Matter of less than 10 µm (PM_{10}) and less than 2.5 µm ($PM_{2.5}$) in aerodynamic diameter resulted from the multivariate model. The OR was calculated for a 10 unit increase in PM_{10} and $PM_{2.5}$ on lags from 0 to 14.

The highest risk due to PM_{10} is on lag 0, lag 1 and lag 2 with an OR (95% CI) of 1.13 (1.03-1.24), 1.15 (1.07-1.23) and 1.13 (1.07-1.20) respectively. Moreover, from lag 2 the risk starts decreasing until it becomes non-significant from lag 5. The risk of PED presentation associated with $PM_{2.5}$ shows a similar descending trend, in this case starting from the first lag and becoming non-significant from lag 5.

The risk associated with NO_2 starts to increase from lag 2, has a peek on lag 4 with an OR (95% CI) of 1.34 (1.21-1.49) and decreases progressively. The association is significant from lag 2 to lag 12 for a 10 µg/m^3 increase of NO_2 (Figure 3.2). The OR for each lag is reported in the supplementary material (Table A2).

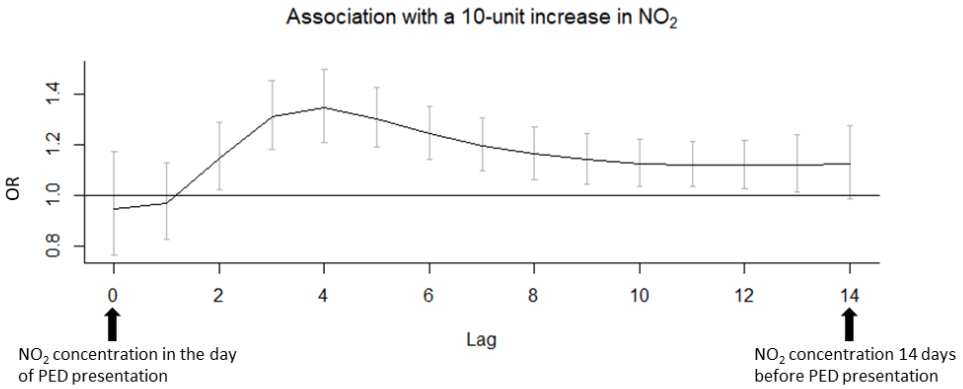

Figure 3.2. Odds Ratio (OR) and 95% confidence interval for the association between nitrogen oxide (NO_2) and PED presentations for bronchiolitis resulted from the multivariate model. The OR was calculated for a 10 unit increase in NO_2 on lags from 0 to 14.

The three-dimensional plot in Figure 3.3a-b shows the joint relationship between OR, lags and pollutant concentrations. The exposure to higher concentrations of all the pollutants is associated with a higher risk of presenting to PED for bronchiolitis and the trend along lags remains constant.

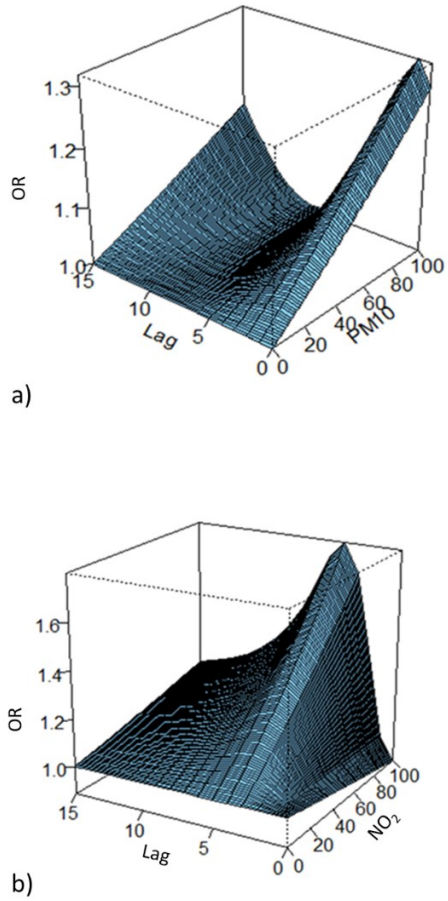

Figure 3.3 a) 3D graph of PM_{10} effect. b) 3D graph of NO_2 effect. Odds Ratio (OR) of presentations by daily levels of particulate matter of less than 10 um in aerodynamic diameter (PM_{10}) and nitrogen dioxide (NO_2).

The sensitivity analysis performed in the two different time periods shows that for both PM_{10} and $PM_{2.5}$ the trend and the estimated risks remain constant with higher OR observed in the 4 days before the presentation. Since the sample size halved, wider CI are observed (Figure A3 a-d).
The impact of NO_2 on both cohorts shows a similar trend with a peak on lag 4. (Figure A3 e-f)

Analysis on hospitalized children (707 infants) confirmed the results obtained on the whole cohort for all pollutants. Nevertheless, wider confidence intervals were observed because of the lower sample size. The OR of the models shows that the risk of hospitalization associated to air pollution is higher than the risk associated to PED presentation (Figure 3.4).

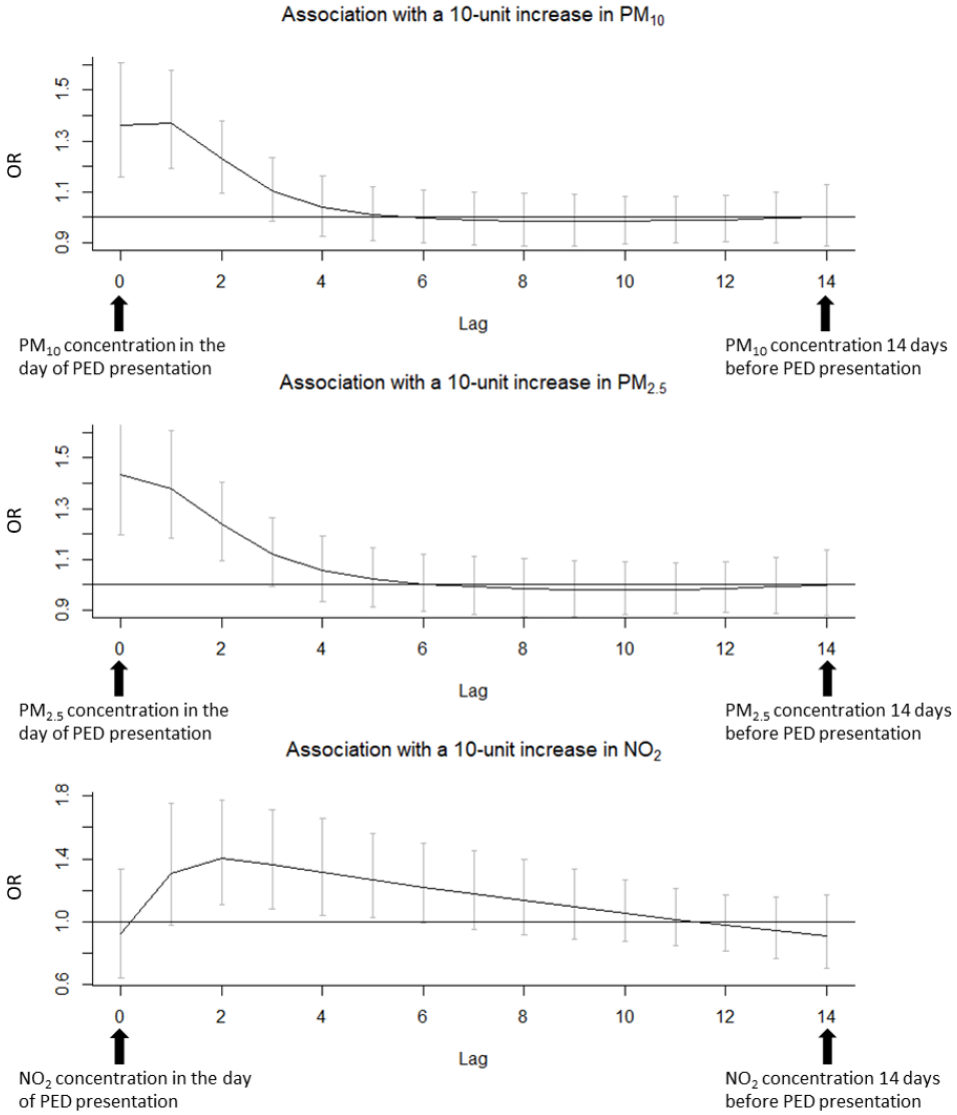

Figure 3.4. Odds Ratio (OR) and 95% confidence interval for the association between air pollutants and hospitalizations for bronchiolitis resulted from the multivariate model. The OR was calculated for a 10 unit increase in Particulate Matter of less than 10 µm (PM_{10}) and less than2.5 µm ($PM_{2.5}$) in aerodynamic diameter and nitrogen dioxide (NO_2) on lags from 0 to 14.

The overall cumulative effect of air pollution infants have been exposed to in the two weeks before the PED presentation shows a positive effect of NO_2, whereas PM_{10} and $PM_{2.5}$ do not seem to significantly affect the risk of being admitted (Table A2). A 10-unit increase in the overall exposure to NO_2 is associated to an OR of 1.47 (95%CI: 1.24-1.74) of being admitted to PED.

3.4 DISCUSSION

The present study found an association of PM_{10} and $PM_{2.5}$ concentrations with PED presentations for bronchiolitis in the four days before the presentation. According to what was published by Karr and colleagues, this period mostly corresponds to the first appearance of symptoms, thus suggesting PM to play a role in the onset of symptoms [83].

On the other hand, NO_2 is associated with presentations for bronchiolitis in a range of days consistent with the incubation period before symptoms onset. This finding suggests that NO_2 could impair the functional integrity of the respiratory mucosa, facilitating infection from viral agents and progression of the disease. Moreover, NO_2 also demonstrated a cumulative effect over the two weeks preceding presentation to the ED.

Evidence of the effects of air pollution on the immune system has been recently summarised by Glencross and colleagues [102], whereas the NO_2 role in the incubation period of the infection was studied by Becker and Soukup [103]. The exposure of RSV infected bronchial epithelial cells to different concentrations of NO_2 showed a decrease in Interleukin (IL)-6 and IL-9 production [103]. On the same line, increased exposure to NO_2 was associated to a deficient production of the type I interferon-(IFN-β) from bronchial epithelial cells exposed to other respiratory viruses, such as RV [104]. Activation of the interferon and inflammatory pathways in the airway epithelium represent the first line of defence to prevent the spread of the virus; thus, NO_2 by impairing innate responses at the epithelial surface may facilitate the progression of the infection.

Not only NO_2 may affect the innate immune response to viral infections, but it can also enhance the epithelial expression of the intracellular adhesion molecule-1 (ICAM-1) [105], a protein required for RSV adhesion to epithelial cells [106]. The observation of an effect of cumulative exposure to NO_2 in the few days before the infection and during the period of infection could be related to sustained upregulation of ICAM-1 expression, which facilitates entry of RSV into epithelial cells and its spread through the respiratory tract.

The exposure of human bronchial epithelial cells to $PM_{2.5}$ has been demonstrated to cause an increase in production of Reactive Oxygen Species (ROS) and cell apoptosis [97]. This damage to the epithelial cells of the airway mucosa could lead to a higher symptoms severity in case of concurrent viral infection. The risk of hospitalization in our sample was approximately 20% higher than the risk of presenting to the PED based on PM exposure. This was not true for NO_2, thus supporting the hypothesis that NO_2 plays a role in increasing the probability of infection, but not in increasing symptoms severity, which is instead more closely related to PM exposure.

Other studies focused on the association between air pollution and bronchiolitis. An association was found between hospitalization for bronchiolitis and the moving average concentration of NO_2, PM_{10}

and $PM_{2.5}$ one week before the hospitalization in the south of Israel [92]. A similar result was found on the five days moving average concentration of NO_2 and PM_{10} in Paris [94]. Moreover, Carugno and colleagues evaluated the weekly lags of PM_{10} and found an association with hospitalization for bronchiolitis in the two weeks before [90]. In the same study single lags were also analysed and results showed an association in the first 9 lags. Their findings are partially different from the results of the present study. One possible reason is that Carugno et colleagues did not model lag in a constrained framework, thus resulting in a possible collinearity in the model [28].

A correlation between the number of RSV positive patients that accessed the hospital and the mean PM_{10} concentration in the preceding week was also measured by Vandini and colleagues [97]. On the other side, the study published by Karr and colleagues did not find an association between $PM_{2.5}$ and hospitalization for bronchiolitis. However, the data retrieved by the monitoring stations in the study by Karr et al. were recorded every 3 days [83]. Focusing on the models proposed in the present study, different lags have been shown to variably impact on the risk of PED presentation, thus the effect of lag 1 could be quite different from the effect of lag 3 on the risk of hospitalization.

Another research conducted in the subtropical region of Hong Kong found a positive overall effect of NO_2 on hospitalization for bronchiolitis, whereas for PM_{10} they only observed an immediate but not long lasting effect, similarly to what was found in the present study [95].

Most of the published cited studies focused on the moving average concentration of pollutants in the days preceding the hospitalizations. Nevertheless, the moving average concentration approach does not allow one to estimate the single lag effect and it only focuses on the average atmospheric conditions the patients were exposed to.

Otherwise, DLNM can estimate the potential effect of air pollution on all days of the disease progression and can reveal the time at which air pollution can have a higher impact on favouring the onset or worsening the symptoms of bronchiolitis in infants.

3.4.1 LIMITATIONS

Linking individuals to the nearest monitoring station may arise some issues on the accuracy of the measured exposure. However, the uniform distribution of air pollution in the Veneto region partially reduces potential inconsistency of measured exposure.

The geological characteristics including a flat land with mountains on three sides and a shallow sea on the fourth with an average wind speed of 4 km/h in a year, favours stagnation of air pollutants in the study area. For this reason, concentrations provided by the monitoring station are representative of a quite wide area.

The correlation between the pollutants did not allow to perform a model with both NO_2 and PM simultaneously, so it was not possible to disentangle the effect of one single pollutant (adjusting for the other). As so, uncertainty remains as to whether there is confounding by correlated pollutants or NO_2 itself represents a pollution mixture (eg. from traffic vehicles).

3.5 CONCLUSIONS

Results shown in this study underline the great impact air pollution has on the respiratory system of infants. The higher the concentration of air pollutants they are exposed to, the higher the risk of presenting to the PED for bronchiolitis. Moreover, the novel approach used shows new insights: different air pollutants have a different time-related impact during the development of the disease. This could be very interesting to investigate also at a pathophysiological level and useful for implementing prevention strategies.

CHAPTER 4

LIFE AFTER DISCHARGE FROM PEDIATRIC ICU: AIR POLLUTION EFFECTS ON HOSPITAL READMISSIONS IN HIGH-RISK CHILDREN

SUMMARY

Rationale: Air pollution is a known risk factor for cardiovascular, respiratory and neurological hospitalizations in the general population. Few studies focused on subjects with a previous hospitalization in Intensive Care Unit (ICU).

Objectives: Evaluate the risk of hospital readmission for cardiovascular, respiratory or neurological diseases in association with air pollution in children discharged from the Pediatric ICU (PICU).

Methods: Children hospitalized in the PICU of Padova hospital (Italy) between 2013 and 2019 were followed for one year after the discharge.

A Cox proportional-hazard model adjusted for exposure and subject-related confounders was applied to evaluate the association between monthly levels of NO_2, PM_{10} and $PM_{2.5}$ and the risk of readmission within one year. In a second analysis, an interaction between each pollutant and the temperature was included in the model.

Results: Overall, 1331 children were admitted to the PICU of which 302 were readmitted for cardiovascular, respiratory or neurological diseases within one year. An association has been found between readmissions and NO_2 (HR:2.25, 95%CI:1.61 – 3.16), PM_{10} (HR:1.80, 95%CI:1.42-2.29) and $PM_{2.5}$ (HR:1.71, 95%CI:1.40-2-09). PM has an effect on cardiovascular, respiratory and nervous systems, whereas NO_2 shows a strong impact mainly on the nervous system. When an interaction with temperature is introduced, the risk associated with PM is high in cold months, whereas the risk associated with NO_2 is high both in cold and warm months.

Conclusions: The exposure of high-risk children to high levels of NO_2, PM_{10} and $PM_{2.5}$ could lead to an increased risk of readmission for several diseases.

This chapter was submitted and is currently under revision as:

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4.1 INTRODUCTION

Critically ill children admitted to Pediatric Intensive Care Units (PICU) (i.e., specific clinical environment providing highly specialized care) represent a population at risk of subsequent readmission. Even if hospital readmission after PICU discharge is known to be associated with poorer health outcomes [107, 108], the factors associated with this phenomenon have been scantly investigated. Only few studies assessed pediatric readmissions after a hospital stay, which included admission to PICU, overall or among specific diseases, and most of them focused on readmission within 48 h [108–111].
More in general, hospital readmissions rates have been widely used as indicators of quality of care [112] and are considered an important source of healthcare costs [113]. In children, researchers reported a readmission rate within 1 year of 20% [114]. Studies assessing pediatric readmissions have focused on either general and disease-specific patterns [115–117].
In the last decades, several studies tried to assess the role of exposure to air pollution on different clinical outcomes, but few of them focused on readmissions. The impact of air pollution on children's respiratory health is well documented [26], but its association with the risk of readmission for respiratory diseases has been studied only in the last years. In particular, in asthmatic children the exposure to high levels of air pollution has been associated to an increased risk of hospital readmission for symptoms exacerbation [118].
In the adult population, it has been demonstrated that air pollution has an impact also on the cardiovascular and neurological systems [119, 120]. In the last years, the harmful effect of air pollution was found also on the cognitive development of children [121]. Some studies on hospital readmission for cardiovascular outcomes and exposure to air pollution has been performed in myocardial survivors adults [122] and in patients with heart failure [123]. However, no studies evaluated the impact of air pollution exposure on hospital readmissions among pediatric patients after a first discharge from the PICU. To address this gap, this study explored the effect of outdoor air pollutants (particulate matter less than 10 µm (PM_{10}) and 2.5 µm ($PM_{2.5}$) in aerodynamic diameter, and nitrogen dioxide (NO_2)) on the risk of readmission for cardiovascular, respiratory, or neurological diseases within 1 year after the PICU discharge.

4.2METHODS

4.2.1 STUDY COHORT

Children under 16 years of age with at least one PICU hospitalization (index hospitalization) at the University Hospital of Padova between 1st of January 2013 and 30th of January 2019, and for whom environmental exposure data were available, were included in the cohort. Data were collected from the anonymized hospital discharge records, and for each patients subsequent hospitalizations were analysed. The following subjects' information have been collected: patient demographics at hospital admission and readmission, date of PICU admission and discharge, diagnosis, ICD-10 codes associated with each hospitalization, and deprivation index based on the municipality of residence.
Children readmitted within 30 days from the first PICU discharge (n=7) were excluded from the analyses because the worsening of their condition has been hypothesized to be mostly related to a non-complete recovery. Follow-up was terminated when the first readmission for respiratory,

cardiovascular, or neurological diseases occurred, when the study period reached the end point (365 days from the discharge, or December 31st, 2020), or death occurred, whichever came first.

4.2.2 ENVIRONMENTAL EXPOSURE

Daily NO_2, PM_{10} and $PM_{2.5}$ concentrations were retrieved from permanent monitoring stations of the Regional Agency for Environmental Prevention and Protection of the Veneto Region (ARPAV). In addition, hourly levels of temperature and humidity were collected from the meteorological centre of the same agency. Environmental data were aggregated to monthly average values to better meet the study design.
Patients were associated with the exposure data from the monitoring station that was nearest to their residence with a maximum distance allowed of 20 km.

4.2.3 STATISTICAL ANALYSIS

Continuous variables are expressed as median (1st and 3rd quartile), and categorical variables are expressed as frequencies (percentages).
The main outcome was the first hospital readmission occurred within 1 year from the PICU discharge. Two different analytical approaches have been adopted to evaluate the association between the outcome and the monthly average of NO_2, PM_{10} and $PM_{2.5}$ children have been exposed to.
In the first analysis, the first hospital readmission was regressed against monthly average concentration of each pollutant through a Cox proportional-hazard model adjusted for covariates to assess the main effect of the pollutants. Only readmissions related to diseases of the circulatory system (ICD-10 I00-I99), nervous system (ICD-10 G00-G99) and respiratory system (ICD-10 J00-J99) were considered, as these three human systems are the most affected by air pollution [120].
Considering the variation of exposure with time, multiple monthly records have been created for each subject until the outcome of interest occurs. Models were thus adjusted for age, sex, monthly average temperature, deprivation index, and month to account for seasonality. Since the effect of temperature has often a U- or a J-shape effect, with higher risk for human health at low and high values, it was modelled with a natural spline with two knots placed at the 25th and 75th percentile. The analysis has been first conducted on the overall number of readmissions, and then on the three different groups of outcomes separately, thus providing three different models assessing whether the three different human body systems were differently affected by each pollutant.

In the second approach, an interaction term between the exposure and the temperature was included in the models to assess if air pollution could have a different impact on the risk of readmission at different temperatures.

Many sensitivity analyses were performed. We first introduced a frailty term on the cause of the baseline PICU admission in order to evaluate if there is a different hazard based on the different system involved in the primary health problems of the children.
Secondarily, the analyses were restricted to subjects with the same discharge and readmission ICD-10 codes to evaluate if the association changed when only children with cardiovascular, respiratory or

neurological diseases were considered. Consequently, the baseline population was also restricted to subjects with an ICD-10 equal to G00-G99, I00-I99, or J00-G99.
Lastly, the knots in the spline of temperature have been moved to higher values (10th and 90th percentile) to better model the flexibility at extreme temperatures.

The main effects of air pollutants are reported as the Hazard Ratio (HR) of readmission for a 10-unit increase in the exposure.
Results of the interaction models are presented as the HR for a 10-unit increase in the exposure to the pollutant at three different temperatures (5°C, 15°C, and 24°C) which correspond to the 10th, 50th and 90th percentile of the distribution of the temperature observed. In this way, the change in the hazard of being readmitted linked to air pollution can be studied at low, median and high temperature.
All the analyses were performed using R statistical software [124].

4.3 RESULTS

Overall, 1331 children have been discharged from the PICU of Padova between 2013 and 2019. The 23% of them (302 patients) were subsequently readmitted within 1 year in the hospital of Padova, for cardiovascular, respiratory or neurological problems. Subjects' characteristics and a detailed description of the first PICU admission are presented in table 4.1 stratified by readmission occurrence.

Table 4.1. Patients' characteristics stratified by readmission for cardiovascular, respiratory or neurological reasons within 1 year from the first Paediatric Intensive Care Unit (PICU) discharge

Patients' characteristics	Readmitted within 1 year (n = 302)	Not readmitted within 1 year (n= 1029)
Age (days)	207 [1 – 1793]	119 [0 – 2142]
Age at readmission (days)	359 [159 – 1894]	/
Days elapsed between discharge and readmission	119 [81 – 189]	/
Sex (m)	166 (55%)	586 (57%)
Diagnosis of PICU hospitalization:		
G00-G99 Diseases of the nervous system	84 (28%)	138 (13%)
I00 – I99 Diseases of the circulatory system	85 (28%)	156 (15%)
J00 – J99 Diseases of the respiratory system	53 (18%)	151 (15%)
P00 – P96 Certain conditions originating in the perinatal period	19 (6%)	221 (21%)
K00 – K95 Diseases of the digestive system	13 (4%)	91 (9%)
H00 – H59 Diseases of the eye and adnexa	7 (2%)	45 (4%)
E00-E89 Endocrine, nutritional and metabolic diseases	4 (1%)	21 (2%)

N00 – N99 Diseases of the genitourinary system	4 (1%)	44 (4%)
D50 – D89 Diseases of the blood and blood-forming organs and certain disorders involving the immune mechanism	1 (0%)	8 (1%)
C00 – D49 Neoplasms	4 (1%)	30 (3%)
A00 – B99 Certain infectious and parasitic diseases	4 (1%)	34 (3%)
F01 – F99 Mental, Behavioural and Neurodevelopmental disorders	2 (1%)	3 (0%)
S00 – T88 Injury, poisoning and certain other consequences of external causes	2 (1%)	26 (3%)
M00 – M99 Diseases of the musculoskeletal system and connective tissue	1 (0%)	19 (2%)
L00 – L99 Diseases of the skin and subcutaneous tissue	0	4 (0%)
Not recorded	19 (7%)	38 (4%)

The median age of the overall population is 4 months (IQR: 0-5 years), and the 57% is male.
The most recurrent causes for PICU admission were related to diseases of the circulatory system (18%), to certain conditions originating in the perinatal period (18%), to diseases of the nervous, respiratory and digestive systems (17%, 15% and 6%, respectively).
The diagnoses of readmission were neurological for the 41.4% of cases, respiratory in 25.4% of subjects and cardiovascular in 33.1% of cases. These diagnoses have been further divided in subcategories to better describe the population (Table 4.2).

Table 4.2. Diagnosis of readmission

	N (%)
Respiratory outcomes	
Respiratory infection	26(8.6%)
Asthmatic	11 (3.6%)
Other	40 (13.2%)
Cardiovascular outcomes	
Heart failure	15 (5%)
Congenit cardiopathy	68 (22.5%)
Arrithmias	9 (3%)
Other	8 (2.6%)
Neurological outcomes	
Epilepsy	14 (4.6%)
Oncological	28 (9.3%)
Cerebrovascular	15 (5%)
Infective	13 (4.3%)
Other	55 (18.2%)

Beneath the children that experienced a second admission for cardiovascular, respiratory or neurological reasons, the same ICD was assigned to 182 (60%) of them.
The exposure to air pollution in the year after the first PICU discharge is quite high, as it often happens to people living in the high polluted Po valley. Mean PM_{10} and $PM_{2.5}$ concentration recorded are 35 and 26 (IQR 17-43 and 12-33) $\mu g/m^3$ respectively. NO_2 shows a mean concentration of 32 (IQR 20-40) $\mu g/m^3$.

When considering the main effects of air pollutants, we observed an increased risk of readmission within 1 year in association with a 10-unit increase in monthly exposure to NO_2 (HR 2.25, 95%CI: 1.61 – 3.16) as well as PM_{10} and $PM_{2.5}$ (HR 1.80, 95%CI: 1.42 – 2.29 and HR 1.71, 95%CI: 1.40 – 2.09 respectively).
When the cardiovascular, respiratory and neurological outcomes were considered separately, the models showed increased risks of readmission for cardiovascular diseases in association with exposure to higher levels of PM_{10} and $PM_{2.5}$, whereas NO_2 remains positively associated but with a wide confidence interval. A similar pattern is observed for respiratory admissions, with PM_{10} and $PM_{2.5}$ as the only pollutants who have a significant impact on the risk of readmission. Otherwise, NO_2, PM_{10}, and $PM_{2.5}$ are all associated with an increased risk of readmission for neurological diseases (Table 4.3).

Table 4.3. Hazard ratio and 95% Confidence Interval (CI) of the association between a 10-unit increase in each pollutant and the first hospital readmission for cardiovascular, respiratory or neurological diseases considered separately.

	HR (95%CI) cardiovascular diseases n = 95	HR (95%CI) respiratory diseases n = 77	HR (95%CI) neurological diseases n = 125
NO_2	1.87 (0.98 – 3.59)	1.19 (0.58 – 2.44)	4.00 (2.06 – 7.78)
PM_{10}	1.77 (1.24 – 2.54)	1.64 (1.08 – 2.49)	1.99 (1.37 – 2.90)
$PM_{2.5}$	1.64 (1.15 – 2.35)	1.62 (1.06 – 2.48)	1.90 (1.45 – 2.51)

In the secondary analysis, since the non-linear effect of temperature is considered to interact with the pollutant, the association between exposure to air pollution and the risk of readmission has been studied at different temperatures. Figure 4.1A illustrates how NO_2 increased the risk of readmission in high-risk children at low as well as at high temperatures with an HR for a 10-unit increase of 1.83 (95%CI: 1.11 – 3.04) when the external temperature is 5°C and a HR of 2.75 (95%CI: 1.04 – 7.27) when temperature reaches 24°C (Table 4.4).

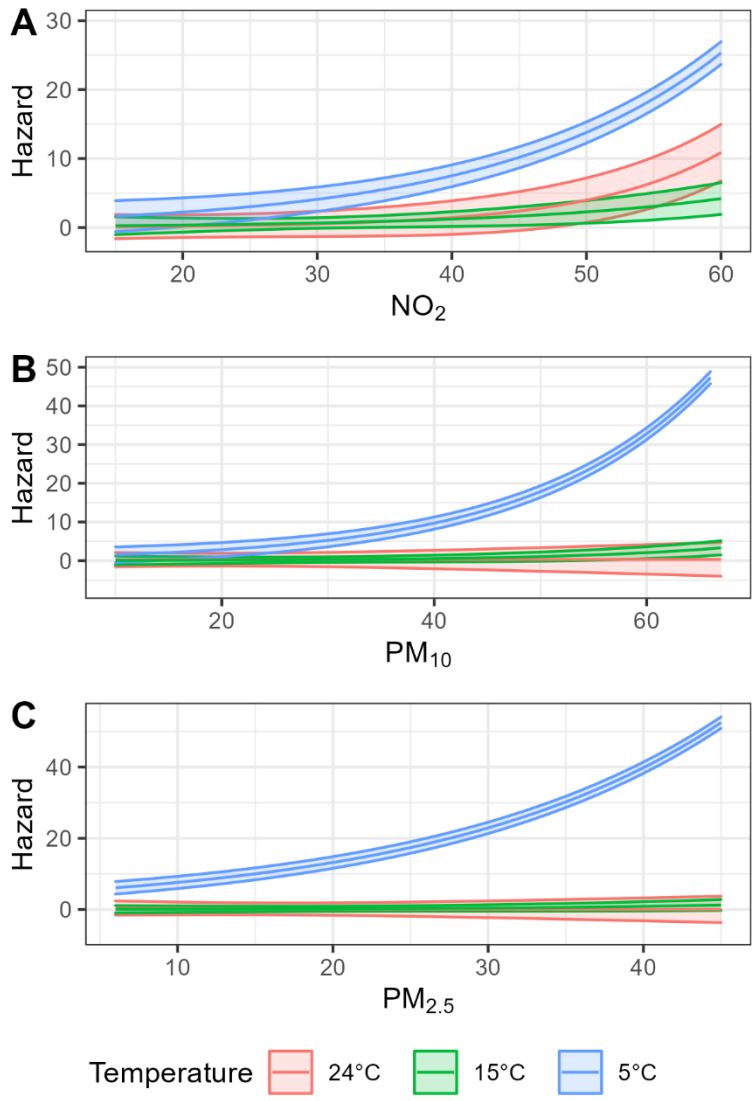

Figure 4.1. Hazard of readmission in association with (A) NO_2, (B) PM_{10} and (C) $PM_{2.5}$ evaluated at three different temperatures

Table 4.4 Hazard and relative 95% Confidence Interval (CI) of the association between the first hospital readmission and a 10unit increase in the selected pollutant at selected temperatures

	HR	95% CI
NO_2 (5°C)	1.84	1.11 – 3.05
NO_2 (15°C)	1.85	0.91 – 3.78
NO_2 (24°C)	2.75	1.04 – 7.27
PM_{10} (5°C)	1.84	1.36 – 2.49
PM_{10} (15°C)	1.94	1.24 – 3.03
PM_{10} (24°C)	1.06	0.45 – 2.50
$PM_{2.5}$ (5°C)	1.74	1.36 – 2.21
$PM_{2.5}$ (15°C)	2.13	1.26 – 3.60
$PM_{2.5}$ (24°C)	0.41	0.14 - 1.26

PM show a slightly different behaviour with an increased risk of readmission only when the external temperature is low. For an increase of 10-units of PM_{10} we found a HR of readmission of 1.83 (95%CI: 1.36 – 2.48) when the external temperature is low (5°C), whereas no effect was observed at higher temperatures (Figure 4.1B, Table 4.4). $PM_{2.5}$ shows a similar trend to PM_{10}, with an increased hazard at low temperature and a non-significant one at higher temperatures (Figure 4.1C, Table 4.4).

4.3.1 SENSITIVITY ANALYSIS

In the first sensitivity analysis a frailty is set on the ICD-10 of the first PICU admission in order to evaluate if the system involved in the primary hospitalization can bring to different results in terms of exposure impact. Results obtained confirm what was previously observed, with an increased hazard of readmission associated to NO_2, PM_{10}, and $PM_{2.5}$ (Table B1).
When the cohort is restricted to children discharged for cardiovascular, respiratory or neurological diseases and readmitted only for the same ICD-10 codes, the association between all the air pollutants and the readmissions is still positive and even greater than that already observed, with an HR for a 10-unit increase in NO_2 of 2.99 (95%CI 1.78 – 5.01) and an HR for a 10-unit increase in PM_{10} and $PM_{2.5}$ of 2.71 (95%CI 2.00 – 3.68) and 2.34 (95%CI 1.66 – 3.32), respectively.
In the sensitivity analyses (performed changing the knots in the temperature series), the increased hazard of readmission associated to NO_2, PM_{10} and $PM_{2.5}$ remains high and positive (Table B2).

4.4 DISCUSSION

The present study showed the impact of air pollution on high-risk children through a comparison between subjects. An increased risk of readmission for cardiovascular, respiratory and neurological diseases has indeed been found in association with PM_{10}, $PM_{2.5}$ and NO_2 in children previously discharged from the PICU.
The strong effect of PM_{10} and $PM_{2.5}$ is observed mainly in cold months, whereas in warm months the risk associated to PM is null. Hence temperature seems to play an important role on the way PM impact on human body. The cold temperature could be a synergetic factor affecting the epithelial bronchial cells that are more vulnerable to the damage caused by particles.
The risk associated to NO_2 is high in warm as well as in cold months, showing the typical U-shape of temperature.
To our knowledge, no previous studies followed a cohort of high-risk children to assess the impact air pollution can have on subsequent readmissions.

The few studies on readmission rate in children often focused on asthmatic young subjects and the association between exposure to air pollution and the readmission due to an exacerbation of symptoms [118, 125], or to readmissions related to a particular health condition in children, regardless the level of air pollution exposure [109, 126].
The three human body systems considered in the present study are the ones that are the most affected to air pollution.
The pro-inflammatory effects of air pollution on the respiratory system in both adults and children are well known. The respiratory tract represents the first interface between the immune system and the pollutants inhaled. The interaction between pollutants and the epithelial cells generates local

inflammation and triggers intracellular signaling pathways [127]. The main outcomes studied in pediatric cohorts relate to the effect of air pollution on asthma exacerbations or on hospital admissions for respiratory infections [118, 128, 129]. Air pollutants can induce airway inflammation and hyperresponsiveness in asthma, thus favouring subsequent exacerbations [52]. The studies that focused on hospital admissions for different respiratory viruses such as respiratory syncytial virus or rhinovirus observed increased rates of hospitalization in association with increased levels of exposure [128, 129]. From a cellular point of view, epithelial bronchial cells of children showed a decreased production of innate immune mediators, particularly in the interferon pathway, in response to rhinovirus infection when the previous expose to air pollution was high. Hence, the exposure to air pollution seems to reduce the ability of epithelial cells to face viral infections [104].

Concerning the effects on the cardiovascular system, air pollution acts in many different ways. The short-term exposure to PM, usually in terms of hours, seems to cause an impairment of the autonomic nervous system that leads to a decrease in the parasympathetic and an increase in the sympathetic response that can cause arrhythmias [130]. Otherwise, a little bit longer effects of air pollution include the induction of oxidative stress that starts at lung level and later propagate systemically with a production of Reactive Oxygen Spices (ROS), and the systemic inflammation. Hence, it has been observed an increased number of C-reactive protein, IL-6, fibrinogen and tissue necrosis factor in the blood in association with exposure to air pollution [131]. A secondary pattern through which air pollution could impact on the cardiovascular system, is the signal transduction via biological intermediates. The formation of reactive biological intermediates caused by the systemic effects of exposure could affect other tissues [131]. Another hypothesis still under evaluation is the possibility of air pollution to directly translocate to the blood and damages further organs [131].
Most of the studies on the cardiovascular adverse outcome associated to air pollution were conducted on the adult population which had inevitably been exposed to several contaminants during life. Results showed how air pollution has a negative impact on the risk of cardiovascular mortality [132], atrial and ventricular fibrillation[58, 133], hypertension [134], thrombosis [135] and heart failure [136]. Studies on children are few, in an adolescent cohort an increased risk of arrythmia was observed in association with PM [137], whereas a longitudinal study conducted in China on children and adolescents found increased systolic and diastolic blood pressure in association with exposure to high levels of pollution [138].

The systemic inflammation that damages the cardiovascular system also impact on the central nervous system. Circulating cytokines can activate cerebral immune response through receptors located in the brain endothelial cells. Moreover, the microglia innate response can also be directly activated from PM that cross the olfactory tract and access the brain [139].
If the different outcomes of the present study are analyzed separately we observe an impact of PM that is constant in all the three human system considered, whereas NO_2 has its major effect on the nervous system. Different studies found a positive effect of PM and NO_2 on the risk of hospital admission for epilepsy and migraine [140–142]. In particular, NO_2 was found to have a stronger impact on epilepsy in young populations [143] and on neurodevelopment in children [121].
Here we demonstrate for the first time how high-risk children, independently from the reason of the first PICU admission, are strongly affected by air pollution. The baseline risk of readmission of high-risk children is higher than the one of healthy children, but here we assessed if the concomitant exposure to air pollution could even increase or anticipate the readmission. We can speculate that their compromised health condition put them in a sensitive situation as respect to air pollution.

4.4.1 LIMITATIONS

The study has some limitations. First the air pollution exposure was measured using fixed monitoring stations, thus allowing a possible exposure misclassification. The particular geographical setting of the study makes the air pollution to be quite constant in big areas because of the low wind speed and geological conformation [21] thus reducing this bias.
Second, the time structure of the design does not allow a deep study of the delayed effects in time, since the moving average of each month is used.
Third, information about children were limited, thus in we could not take into account all the subjects' characteristics as confounders in the model.

4.5 CONCLUSIONS

This study demonstrates the impact of air pollution on high-risk children. The risk of a readmission for cardiovascular, neurological or respiratory issues following a PICU discharge is increased if the children are exposed to high levels of PM or NO_2. This risk is higher especially in cold and warm months. Further studies on wider populations of high-risk children would be of great interest, in particular to address if there is a specific subgroup of children with a specific pathology or condition that can be more affected by air pollution.

APPENDIX A

INCREASED RISK OF EMERGENCY DEPARTMENT PRESENTATIONS FOR BRONCHIOLITIS IN INFANTS EXPOSED TO AIR POLLUTION: SUPPLEMENTARY MATERIAL

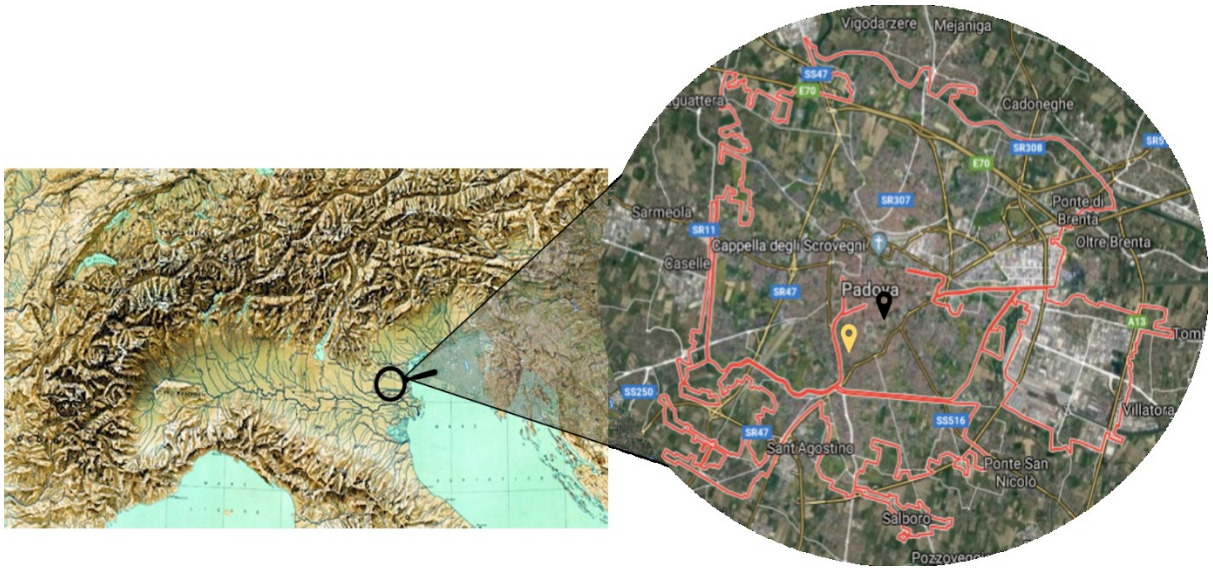

Figure A1 The north of Italy and Padova. Hospital is black and monitoring station is yellow.

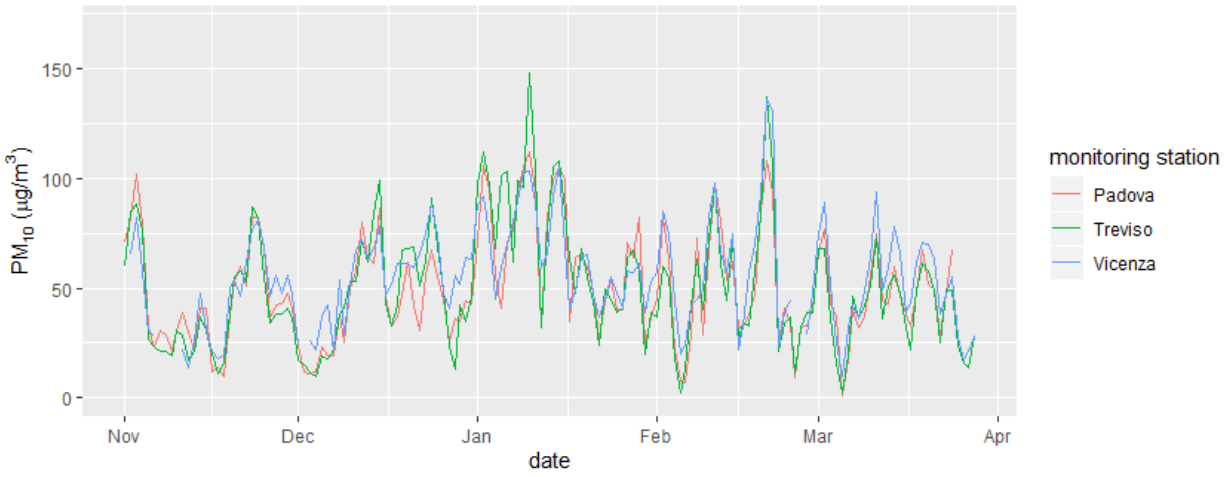

Figure A2 Nitrogen dioxide (NO_2) and Particulate Matter of less than 10 µm in aerodynamic diameter (PM_{10}) levels in Padova, Treviso and Vicenza between November 2014 and March 2015

Table A1 Concentration of PM_{10} and NO_2 and Spearman's correlation between the pollutants in the cold months (November to March)

year	median	IQR	ρ	pollutant
2007	65	46-90	0.61	PM_{10}
	62	51-90		NO_2
2008	47	32-72	0.74	PM_{10}
	47	37-57		NO_2
2009	50	33-76	0.63	PM_{10}
	44	36-54		NO_2
2010	53	33-75	0.69	PM_{10}
	39	32-50		NO_2
2011	58	38-79	0.60	PM_{10}
	37	31-45		NO_2
2012	54	36-76	0.63	PM_{10}
	45	35-54		NO_2
2013	43	26-69	0.68	PM_{10}
	45	34-55		NO_2
2014	40	26-57	0.61	PM_{10}
	40	33-47		NO_2
2015	55	38-78	0.66	PM_{10}
	46	37-57		NO_2
2016	47	32-72	0.72	PM_{10}
	37	30-46		NO_2
2017	55	39-82	0.68	PM_{10}
	43	37-51		NO_2
2018	45	31-57	0.73	PM_{10}
	39	30-46		NO_2

Table A2 Odds Ratio (OR) of the association between bronchiolitis presentations and pollutants for each lag obtained from multivariate models.

	Lag	**OR (95% CI)**
PM10	0	1.13 (1.03 – 1.24)
	1	1.15 (1.07 – 1.23)
	2	1.13 (1.07 – 1.20)
	3	1.10 (1.05 – 1.16)
	4	1.07 (1.02 – 1.13)
	5	1.05 (1.00 – 1.09)
	6	1.03 (0.99 – 1.06)
	7	1.01 (0.97 – 1.05)
	8	1.00 (0.96 – 1.05)
	9	1.00 (0.96 – 1.04)
	10	1.01 (0.97 – 1.05)
	11	1.01 (0.98 – 1.05)
	12	1.02 (0.99 – 1.06)

	13	1.04 (1.00 – 1.08)
	14	1.05 (1.01 – 1.05)
	Overall	1.12 (1.00 – 1.23)
PM2.5	0	1.11 (1.03 – 1.19)
	1	1.10 (1.04 – 1.15)
	2	1.09 (1.04 – 1.13)
	3	1.07 (1.03 – 1.11)
	4	1.05 (1.01 – 1.09)
	5	1.03 (1.00 – 1.06)
	6	1.01 (0.98 – 1.04)
	7	1.00 (0.97 – 1.03)
	8	0.99 (0.96 – 1.02)
	9	0.99 (0.97 – 1.02)
	10	1.00 (0.97 – 1.03)
	11	1.01 (0.98 – 1.04)
	12	1.02 (0.99 – 1.05)
	13	1.03 (1.01 – 1.07)
	14	1.05 (1.01 – 1.09)
	Overall	1.12 (1.00 – 1.22)
NO_2	0	0.94 (0.76 – 1.16)
	1	0.99 (0.85 – 1.14)
	2	1.15 (1.02 – 1.29)
	3	1.29 (1.17 – 1.43)
	4	1.34 (1.21 – 1.49)
	5	1.32 (1.20 – 1.44)
	6	1.26 (1.16 – 1.37)
	7	1.22 (1.12 – 1.33)
	8	1.18 (1.08 – 1.29)
	9	1.15 (1.06 – 1.26)
	10	1.13 (1.04 – 1.23)
	11	1.11 (1.03 – 1.21)
	12	1.10 (1.02 – 1.19)
	13	1.09 (1.00 - 1.19)
	14	1.08 (0.98 – 1.99)
	overall	1.47 (1.24 – 1.74)

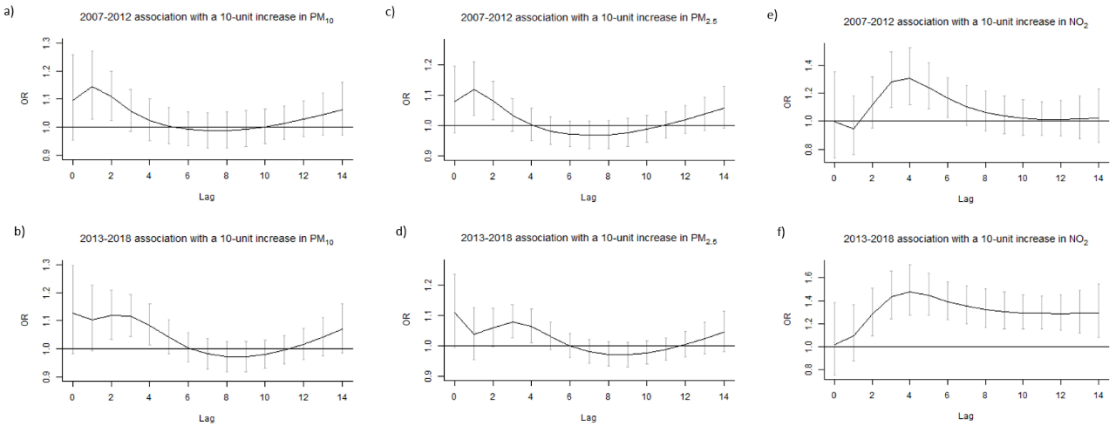

Figure A3 Sensitivity analysis showing the Odds Ratio (OR) of the association between bronchiolitis presentations and a 10-unit increase in PM_{10}, $PM_{2.5}$ and NO_2 performed on different years. a) PM_{10} from 2007 to 2012; b) PM_{10} from 2013 to 2018; c) $PM_{2.5}$ from 2007 to 2012; d) $PM_{2.5}$ from 2013 to 2018; e) NO_2 from 2007 to 2012; f) NO_2 from 2013 to 2018

APPENDIX B

LIFE AFTER DISCHARGE FROM PAEDIATRIC INTENSIVE CARE UNITSICU: AIR POLLUTION EFFECTS ON HOSPITAL READMISSIONS IN HIGH-RISK CHILDREN SUPPLEMENTARY MATERIAL

Table B1. Hazard ratio and relative 95% Confidence Interval (CI) of the association between the first hospital readmission and a 10-unit increase in the selected pollutant when a frailty term is included in the model.

Pollutant	HR	95% CI
NO_2	2.17	1.47 – 3.23
PM_{10}	1.99	1.56 – 2.52
$PM_{2.5}$	1.82	1.48 – 2.23

Table B2. Hazardratio and relative 95% Confidence Interval (CI) of the association between the first hospital readmission and a 10-unit increase in the selected pollutant when the knots of temperature spline are moved.

Pollutant	HR	95%CI
NO_2	2.22	1.49 – 3.31
PM_{10}	1.82	1.43 – 2.31
$PM_{2.5}$	1.72	1.40 – 2.10

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