Air pollution exposure induces a decrease in type II interferon response: A paired cohort study

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Summary

Background While air pollution is a major issue due to its harmful effects on human health, few studies focus on its impact on the immune system and vulnerability to viral infections. The lockdown declared following the COVID-19 pandemic represents a unique opportunity to study the large-scale impact of variations in air pollutants in real life. We hypothesized that variations in air pollutants modify Th1 response represented by interferon (IFN) γ production.

Methods We conducted a single center paired pilot cohort study of 58 participants, and a confirmation cohort of 320 participants in Nice (France), with for each cohort two samplings at six months intervals. We correlated the variations in the production of IFNγ after non-specific stimulation of participants' immune cells with variations in key regulated pollutants: NO_2, O_3, $PM_{2.5}$, and PM_{10} and climate variables. Using linear regression, we studied the effects of variations of each pollutant on the immune response.

Findings In the pilot cohort, IFNγ production significantly decreased by 25.7% post-lockdown compared to during lockdown, while NO_2 increased significantly by 46.0%. After the adjustment for climate variations during the study period (sunshine and temperature), we observed a significant effect of NO_2 variation on IFNγ production (P=0.03). In the confirmation cohort IFNγ decreased significantly by 47.8% and after adjustment for environmental factors and intrinsic characteristics we observed a significant effect of environmental factors: NO_2, PM_{10}, O_3, climatic conditions (sunshine exposure, relative humidity) on variation in IFNγ production (P=0.005, P<0.001, P=0.001, P=0.002 and P<0.001 respectively) but not independently from the BMI at inclusion and the workplace P=0.007 and P<0.001 respectively).

Interpretation We show a weakening of the antiviral cellular response in correlation with an increase of pollutants exposition.

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Keywords: Air pollution; Lockdown; Nitrogen dioxide; Interferon gamma; Immune response

Introduction

Background

Air pollution is considered to be the leading environmental cause of premature death worldwide with nearly four million deaths attributable annually according to the WHO.[1] In France nearly 40,000 deaths were attributable to fine particles ($PM_{2.5}$) and 7000 to nitrogen dioxides (NO_2) between 2016 and 2019.[2] At the beginning of this study, fine particles ($PM_{2.5}$ and PM_{10}) and nitrogen dioxides (NO_2) are regulated in France and in Europe with a limited annual average value in accordance with the WHO recommendations (i.e. 40 µg/m³ for NO_2 and 10 µg/m³ for $PM_{2.5}$).[3–5] Fine

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Research in context

Evidence before this study

Air pollution is a major environmental determinant of health. According to the latest WHO report, the burden attributable to ambient air pollution on lower respiratory infections exceeds 8% (Disability Adjusted Life-Years). Currently, more than 4 million deaths in the world are linked each year to a pathology impacted by this environmental risk factor. In France, a gain of 51.8% on the reduction of all- cause mortality in large cities (more than 100,000 inhabitants), has been observed by the reduction of NO_2 emissions alone over the period of the first population lockdown (from March 2020 until the total removal of sanitary restrictions in June 2020). While outdoor air pollution has been shown in several studies to increase pro-inflammatory cytokines secreted by innate immune cells, few data are available on its impact on the adaptive response and in particular on the antiviral response. Interferon gamma or type II (IFNγ) is the main mediator of the specific lymphocyte response (Th1), allowing the elimination of pathogens. As population lockdown represents a unique opportunity through the reduction of pollutant emissions, we propose to describe the impact of air pollutant variations on vulnerability to viral infections through IFNγ production.

Added value of this study

After collecting matched pollution and meteorological data for each participant before and after the end of the restrictive sanitary measures and exposing the blood samples to an *in vitro* lymphocyte stimulation test, we obtain interesting results in two different cohorts: a pilot cohort of 58 participants and a confirmation cohort of 320 participants. Firstly, we show a 25.7% and 47.8% average decrease in IFNγ concentration in the two cohorts and at the same time 46% and 35% variations in NO_2 exposure at the sampling dates in the two cohort. We have highlighted a negative correlation between IFNγ and NO_2, PM_{10} exposure and a positive correlation with O_3, sunshine and humidity exposure in multivariate analysis. Our hypothesis is reinforced by a quartile analysis showing that the IFN response is weaker as the amplitude of the exposure variations is higher. Animal models have confirmed our data by demonstrating a decrease in the Th1 pathway (antiviral immunity pathway) in favor of the Th2 pathway (exacerbated in allergic diseases). However, our results need to be confirmed on a larger representative sample size, with finer variations of pollution exposure outside the lockdown period.

Implications of all the available evidence

These results combined with the data already available suggest an increased vulnerability to viral infections linked to our urban environment: increased production of pro-inflammatory cytokines and decreased production of antiviral cytokines. Our results reinforce the importance of reducing emissions of pollutants, particularly from road traffic (nitrogen oxides) and, by extension, compliance with the new WHO standards for outdoor air pollution.

particles are mainly generated by domestic heating, while NO_2 is generated by transport (exhaust fumes) and industry.[6–8]

Over the last 20 years, the impact of air pollution on cardiovascular and respiratory mortality and morbidity has been well documented.[1] Various studies showed an increase in the relative risk of morbidity and mortality with in particular an additional 8.6% risk of death per 10 $\mu g/m^3$ increase in NO_2.[9–14] Moreover, several studies have suggested a link between pollution peaks and exacerbations of chronic diseases such as heart failure, asthma or chronic obstructive pulmonary disease (COPD),[15–20] as well as hospital admissions for respiratory symptoms in the context of viral infections.[21]

Immune response to a virus induces two types of immunity. Firstly, innate immunity, through the stimulation of Toll-like receptors (TLR) 3, 7 and 8, triggers inflammation (the secretion of IL1β and IL6) and produces antiviral cytokines [Type I interferon (IFN)]. Then, the adaptative immune response selects antigen-specific T-cells (Th1 response) producing type II IFN (IFNγ).[22–27]

IFNγ is mainly produced by natural killer (NK) cells and in minor proportion by T cells and macrophages. Both type I and type II IFNs have a plethora of antiviral effects such as inducing apoptosis and HLA expression of infected cells and activating macrophages, NK cells and T effector and regulatory cells, making it a central cytokine of the Th1 response.[28,29] In COVID-19 patients, several studies have shown a dysregulation of IFNs production[30,31] with an inverse correlation between the severity of COVID-19-related respiratory symptoms and the level of circulating IFNγ after *in vitro* stimulation.[32,33] We have found a decrease of IFNγ production in immunocompromised patients with chronic diseases,[34–36] but we do not know the environmental factors that modify its production in general population. Moreover, IFN are pleiotropic cytokines with various functions (i.e, antiviral, antibacterial, antitumor, and immunomodulatory properties), being central coordinators of the immune response.[37–39]

In humans, air pollution induces pro-inflammatory cytokine production,[40,41] and seems to exacerbate COVID19 evolution. In rats, NO_2 exposure causes an imbalance of Th1/Th2 differentiation and decreases IFNγ production.[42] In humans, exposure to long-term ambient air pollution leads to the alterations in DNA methylation favoring the Th2 immuno-allergic pathway known to have an inhibitory effect on the activity of the Th1 antiviral pathway.[43]

The emergence of the COVID-19 pandemic and the subsequent restrictive sanitary measures, such as lockdown, caused important decrease in the activity of service, transport and industrial sectors,[44] representing a unique opportunity to evaluate the impact of pollution variations on human health. Santé Publique France estimated that the total lockdown in France (March 2020 to

June 2020) avoided 51.8% of all-causes deaths by reducing NO_2 emissions in urban areas with more than 100,000 inhabitants. This result is more pronounced in urbanized and densely populated areas with a strong decrease of road traffic related NO_2.[2] The impact of these air pollutants on the quality of immune response is not well known.

Objectives

We aimed to evaluate the impact of air pollution on the responsiveness of the immune system. We hypothesized that variations in air pollutants generated by the lockdown influenced the Th1 immune response by decreasing the IFNγ production. Through a pilot cohort of 58 subjects monitored during a period of strict lockdown and post-lockdown and a confirmation cohort of 320 subjects monitored at two times when the variations of exposure to air pollutants are less important, we aimed to measure the impact of these exposures on the individual's capacity to produce type II interferon based on functional assay.

Methods

Study design

We conducted a single center prospective analytical matched cohort study at Nice University Hospital in Nice, Alpes-Maritimes Departmental Administrative Center (CADAM), La Trinité municipality, France. Inclusion criteria were workers from Archet Hospital, Pasteur Hospital, Alpes-Maritimes Departmental Administrative Center (CADAM), and La Trinité municipality which represent the four inclusion centers. From these four inclusion centers we established 3 exposure areas (or workplaces) entitled Nice Airport for CADAM workers, Nice West for Archet workers, and Nice East for Pasteur and La Trinité workers. The sensors used for these zones are: Airport sensor for Nice Airport, Botanique and Promenade des Anglais sensors for Nice West and Arson sensor for Nice East. Non-inclusion criteria were a current infection, any symptoms of COVID-19 or a history of COVID-19 within the last month as proven by a positive SARS-CoV-2 RT-PCR test, immunosuppressive treatment, pregnancy, and inability to give an informed consent. Exclusion criteria were withdrawal of informed consent and inability to follow up for geographical, social, or psychological reasons.

Study setting and definition of exposure

For the pilot cohort, participants were recruited between 27 March 2020 and 26 June 2020 at two sites of Nice University Hospital: Nice-West (Archet hospital) and Nice-East (Pasteur hospital). During the first sampling time (Time 1 or T1), we considered them to exposed to low levels of air pollutants due to the strict national lockdown in place from March 2020 to June 2020. The follow-up visit took place between 23 September 2020 and 14 January 2021. During the second sampling time (Time 2 or T2), we considered that the participants were exposed to normal levels of air pollutants since all activities and travel have resumed on the French territory since July 2020. For the confirmation cohort, participants were recruited between 28 July 2020 and 21 July 2021 at four sites: Archet Hospital, Pasteur Hospital, Alpes-Maritimes Departmental Administrative Center (CADAM), and La Trinité municipality. A centralized anonymized database was created. Each participant was registered in the database of volunteers for biomedical research (VRB database), in compliance with national legislative requirements.

Power analysis

Based on previous work,[26] we estimate for the pilot cohort with a risk α at 5%, and a power β of 90%, a necessary number of subjects of 62 in matched conditions to show a difference of 45% in IFNγ between unexposed and exposed conditions. Finally, in a confirmation cohort we analyzed 320 participants to confirm these preliminary data.

Health questionnaire and follow-up visits

For each participant we collected demographic data: age, gender, interval between samples (months), occupation level (Merchants, executives, employees, manual workers, mid-level professions), education level ($<$ Licence, $\leq$ Master, PhD) degree of urbanization (City center ($>$10,000 inhabitants), city center ($<$10,000 inhabitants), outlying residential area, outlying industrial area, rural area) at its living place, proximity to a highway or expressway (road traffic) $<$1 km, anthropometric data (BMI). We collected medical history: smocking (yes/no, yes for active or weaned smoker), allergies (yes/no, yes if at least one chronic allergy), comorbidities (cardiovascular events (yes/no) yes if at least one of hypertension, stroke, myocardial infarction, lower limb arterial disease, heart failure, heart rhythm disorder, prosthetic valve, thrombosis or thrombophilia), history of cancer (yes/no), history of auto-immune disease (yes/no), weekly physical activity $>$30 min (yes/no), concomitant treatments (yes/no, i.e: at least one of antihypertensive, immunosuppressant, hypolipidemic, oral antidiabetic), and only for the confirmation cohort the stress level (between 0 and 10). The same data were collected at the follow-up six months after inclusion. A biological sample was collected during the inclusion and follow-up visit.

Meteorological data sources and management

The meteorological data for the study period, were obtained from the meteorological sensor located at Nice airport (43°38′56" North, 7°12′32" East, altitude 2 m) via open access sources. Based on these data and the distribution of each variable we chose temperature (in°C), wind speed (km/h), relative humidity (%) and the sunshine duration (in minutes) as meteorological variables

of interest. The daily mean temperature (calculated from the temperatures at each hour) was recorded over the seven days prior to sampling, and then averaged over the seven days for each sampling time. Relative humidity was also averaged over the seven days prior to sampling. The daily average was calculated from hourly data over the seven days for each sampling time. Wind speed was measured in km/h and averaged over seven days. The daily average was calculated from hourly data over the seven days for each sampling time. The sunshine duration was obtained in hours and then converted to minutes. The daily average was calculated from hourly data over the seven days for each sampling time. In order to better represent the influence of climatic variations and to have better quality distributions, deltas was calculated to show this difference (Delta=climatic variable at T2−climatic variable at T1). This choice is guided by our objective to compare variations between environmental variables and the IFNγ production between the two sampling times.

Air pollution data sources and management

The data concerning the air pollutants: nitrogen dioxide (NO_2), fine particles smaller than 2.5 µm ($PM_{2.5}$), fine particles smaller than 10 µm (PM_{10}), and ozone (O_3), were provided by Atmosud, a monitoring laboratory in air quality, certified for the analysis of air pollution in the PACA (Provence-Alpes-Côte-d'Azur) region. We used measurements in µg/m^3 from four sensors: one located in Nice Airport (43°39′25.86″N, 7°12′11.88″E°), two located in the West of Nice (one in Promenade des Anglais (43°41′20.70″N, 7°14′30.10″E), and one in the West side of the city called Nice Botanique (43° 41′10.06″N°, 7°12′41.11″E), and one in the east of Nice (in Arson district: (43°42′7.45″N, 7°17′7.41″E). We chose these four sensors because they are the most representative for the workplaces studied. From daily averages we established weekly averages (in µg/m^3) for each sampling time, then deltas (Delta=average value of a pollutant at T2−average value of a pollutant at T1) were calculated for the exposure of each participant.

To verify our hypothesis without a sensor effect and to have a more accurate measure of the exposure of the participants (on the living place), we also collected the exposures on D-7 before each sampling for these same pollutants, using a multipollutant model directly adjusted on the meteorological data, validated, and used in air pollution forecasting.

AtmoSud is developing the local high-resolution model AZUR over the whole South PACA (Provence Alpes Côte-d'Azur) region in France. It allows the mapping at 25 m spatial resolution of NO_2, O_3, PM_{10} and $PM_{2.5}$ pollutants.[45] For ozone, a regional photochemical pollutant, it uses results from the CHIMERE transport chemistry model.[46]

For particulate matter PM_{10} and $PM_{2.5}$ and NO_2, the model uses annual concentrations modeled with the high-resolution model ADMS-URBAN.[47,48] The principle of the AZUR model of AtmoSud is the study of the relationship between annual values and daily values given by the measuring stations in nitrogen dioxide in ambient air. We found that for all pairs of measurement stations and for all ranges of values, the ratio of their daily values is a polynomial function of the ratio of their annual values. From this relation we propose a model allowing to estimate the daily values in the vicinity of a station measuring nitrogen dioxide, the annual values being known at all points. This model is applied to all the stations of a given domain and thus it allows the elaboration of daily values maps. It is these daily concentrations modeled at high resolution that are used for the link with health.

Blood sample collection and processing

We collected 3 mL of peripheral blood in lithium heparin tube, at inclusion (T1) and approximately six months later (T2), in the morning between 8am and 12pm. The samples were immediately prepared for analysis.

Cellular response (functional immune assay)

One milliliter of whole blood was stimulated with immune ligands (anti-CD3 as a T-cell stimulant, and R848 as a TLR 7/8 agonist) in QuantiFERON^{TM} Monitor blood collection tubes (from Qiagen catalogue 0650-0701 QuantiFERON Monitor Lyosphere, 0650-0201 QuantiFERON Monitor kit ELISA and 0650-0101 QuantiFERON Monitor Blood Collection Tubes, Qiagen GmbH, QIAGEN Strasse 1-40724 Hilden, Germany) within 8 h of blood collection. Stimulated blood samples were incubated for 16−24 h at 37 °C and then centrifuged at 2000−3000 xg for 15 min to harvest the stimulated plasma. Stimulated plasma was stored at −20 °C until analysis and freeze-thaw cycles were minimized to preserve sample quality. The levels of IFNγ after non-specific stimulation of immune cells were measured using the QuantiFERON-Monitor ELISA assay.

Outcomes

The primary endpoint of this study was to demonstrate a significant variation in Th1 immunity after stimulation (IFNγ levels) when activity restrictions are lifted, by measuring the impact of variations of the atmospheric pollutants in Nice.

The secondary endpoint was the demonstration of an impact of population lockdown on the level of atmospheric pollutants (NO_2, O_3, $PM_{2.5}$, PM_{10}), as well as the influence of climatic variables on these pollutants.

Statistical analyses

The data obtained via questionnaire are represented by the median and interquartile range (IQR: P25-P75) for quantitative non-parametric variables and by the number of events (percentage) for the qualitative variables. A

Shapiro-Wilk test was used to determine if a variable had a normal distribution. A Wilcoxon signed rank test was used to compare two non-parametric measurements with median and 95% confidence interval, of a quantitative variable performed on the same subjects. Chi-Squared test was used to compare 2 qualitative variables. Mann-Whitney test was used to compare a quantitative variable between two groups and Kruskall-Wallis test was used to compare a quantitative variable between more than two groups. Spearman's test was used to assess the correlation between two continuous variables. We conducted a quartile analysis of the variation in IFNγ, splitting the deltas or Δ (value at T2−value at T1) into four classes. After transformation of the variable (Δ/ IFNγ T1), we represented four quartiles from Q1 to Q4 which correspond to an increase of the variation of the T2 value compared to T1. For the variable tested (ΔNO_2) the variances of the quartiles were heterogeneous (data not shown). We therefore assume to use the one-way ANOVA test with unequal variances (Welch's test) with a slight risk of alpha risk inflation rather than the Kruskal-Wallis test, which is more unstable in this situation.[49] Then to compare the quartile pairs 2 by 2 we used a Games-Howell post-hoc test for unequal variances to specify which pairs are significant.

$$\Delta = \text{IFN}\gamma\ T2 - \text{IFN}\gamma\ T1\ (\text{IU/mL}),\ \frac{\Delta}{\mathbf{T1}} = \frac{\text{IFN}\gamma\ T2 - \text{IFN}\gamma\ T1}{\text{IFN}\gamma\ T1}(\%) \quad (1)$$

A *P*-value <0.05 (based on univariate analysis) was used to select variables that were entered into a multiple linear model using the backward procedure. In this multivariate linear model, we initially included all the significant variables ($P<0.05$) in the univariate analyses, then the selection of variables in the final model was done step by step by removing the least significant variable and using the adjusted R^2 and AIC coefficients until a *P* value <0.2. We considered a threshold of 0.4 for the Spearman's ρ coefficient, beyond which we considered a risk of multi-collinearity between the variables of the model. We considered separately the pollutants of interest to avoid multicollinearity phenomena (Variance Inflation Factor or VIF<2.5).

In the pilot cohort we analyzed in a multivariate model the impact of environmental factors that change between the two times in univariate analysis, on the variations of IFNγ. In the confirmation cohort we analyzed (i) the impact of environmental factors that change between the two times, and (ii) clinical characteristics factors that differ significantly with variations of IFNγ, (iii) clinical characteristics that differ significantly between groups according to workplaces (area of exposure) in univariate analysis. Statistical analyses were performed using Jamovi (Version 1.8.4.0), Excel (Version 2205 Build 16.0.15225.20278), and GraphPad Prism (Version 9.00).

Ethics and consent

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was reviewed and approved by institutional review committee (NCT04429594 and NCT04355351): Comité de protection des personnes (C.P.P.) Sud-Ouest et Outre-Mer I (RCB: 2020-A00908-31−CPP 1-20-027 / 7715) and comité de protection des personnes (C.P.P.) Sud-Ouest et Outre-Mer II (RCB: 2020-A01677-32). Written informed consent was obtained from participants prior to inclusion in the study. All collected data and samples were securely stored.

Role of funders

Grants of the Agence Nationale de la Recherche (Flash-COVID ANR-20-COVI-000),Conseil Départemental des Alpes-Maritimes (CD06) and Region Sud funded this study. Moreover, Conseil Départemental des Alpes-Maritimes (CD06) allowed us for data collection by providing locations for sampling, support in obtaining materials for sampling and sample processing but didn't have any role in study design, data analyses, interpretation, or writing of the report.

Results

Characteristics of the study population

In the pilot cohort, sixty-five participants were enrolled in the study (CovImmune 1, NCT: 04355351). Of these, 58 were included in the final analyses and seven were excluded: seven participants IFNγ measurement was impossible due to technical issues. All participants completed the two questionnaires, and none was lost to follow-up or withdrew their consent (Figure 1a). The median age of the cohort was 39 (IQR=33.3−49.0) years, the distribution of participants between the two sampling sites was equal (n=29, 50%). We did not observe any significant difference in the intrinsic profile: age, sex, BMI, comorbidities, allergies, professional category level, and physical activity level between the two sites (data not shown). The median time between the two samples was 5.93 months (IQR=5.1−7.2). The data are available in Table 1a.

Five hundred fifty-eight participants were eligible for the confirmation cohort (cohort CovImmune 2 NCT: 04429594). Of these, 408 were included and 150 were excluded: 2 participants did not live in the Alpes-Maritimes, 3 had a non-fixed workplace, 4 failed to take their first sample (T1) and 141 had incomplete pollution exposure data on 7 days before T1 sampling. Finally, 320 participants were included in the final analysis after 6 months follow-up and two samples (3 participants had missing IFN assay for technical reasons, 65 participants were lost to follow-up and 20 participants had incomplete pollution exposure data on 7 days before T2

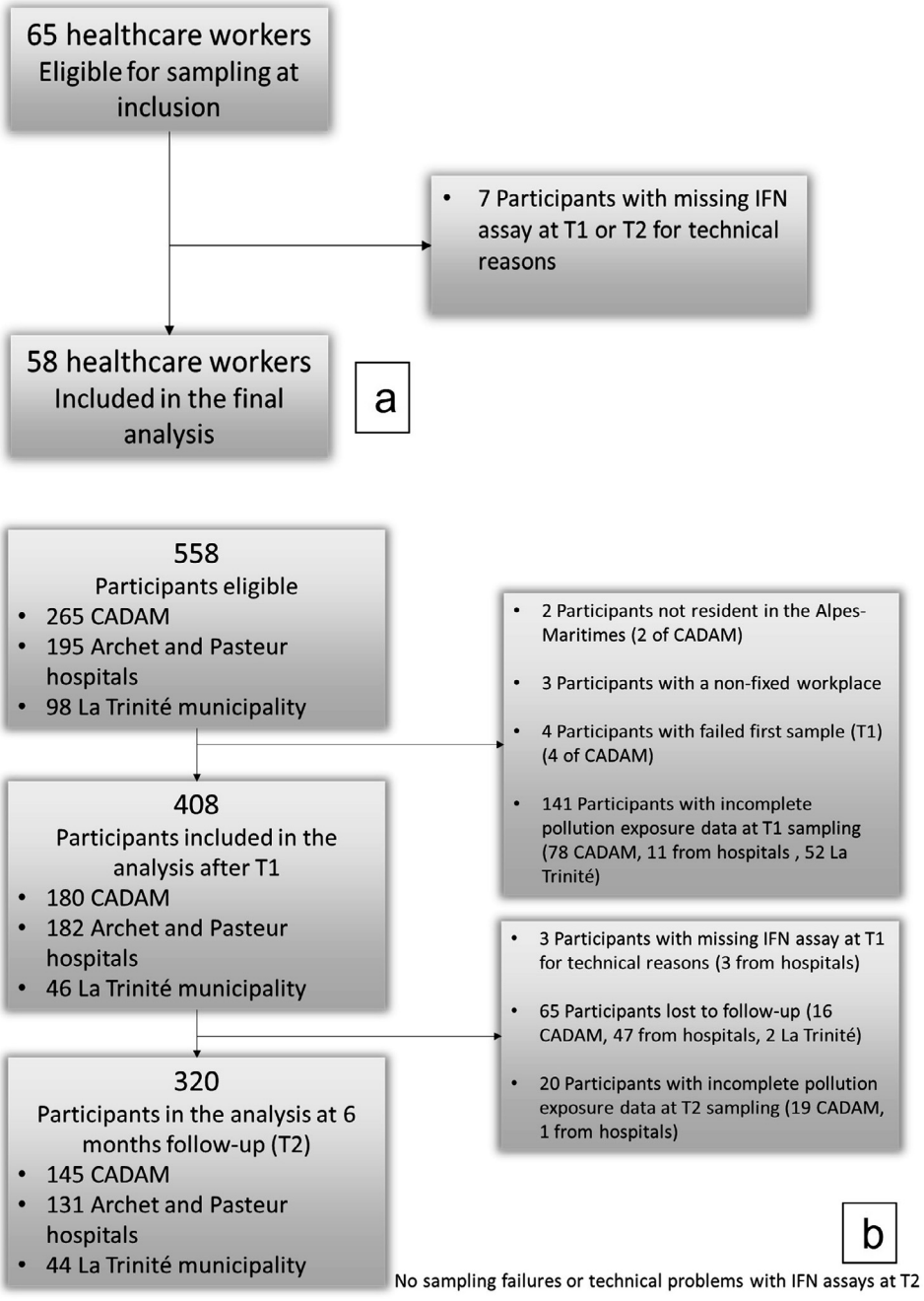

Figure 1. Flowchart of participants: for the pilot cohort (a) and the confirmation cohort (b).
Note: CADAM is Alpes-Maritimes Departmental Administrative Center.

sampling (Figure 1b). All participants completed the two questionnaires, and none withdrew their consent. The median age of the confirmation cohort was 47 (IQR = 38–55) years, the distribution of participants between the three sampling sites was in favor of Nice airport (n = 145, 45%) vs Nice West (n = 81, 25%) and Nice East (n = 94, 29%). The median time between the two samples was 6.1 months (IQR = 6.0–6.3). The data are available in Table 1b and compared according to their workplace (Table 1c): we measured a significant difference for age (P<0.001), education level (P<0.001), BMI (P = 0.014), smoking status (P = 0.035), comorbidities (P = 0.012), long-term treatment (P = 0.012), and variation in IFNγ (P = 0.015).

a. Pilot Cohort

Characteristics	All participants (*N*=58) Median (IQR) or n (%)	
Age	39 (33.3; 49.0)	
BMI at inclusion[a]	23.6 (21.4; 26.0)	
Interval between samples (months)	5.9 (5.1; 7.2)	
Gender	Female	36 (62%)
	Male	22 (38%)
Workplace	Nice West	29 (50%)
	Nice East	29 (50%)
Mid-level professionals and executives[a]	Yes	34 (62%)
	No	21 (38%)
Road traffic <1 km[b]	Yes	34 (62%)
	No	21 (38%)
Living in the city centre	Yes	39 (67%)
	No	19 (33%)
Comorbidities	Yes	20 (34%)
	No	38 (66%)
Allergies	Yes	9 (16%)
	No	49 (84%)
Weekly physical activity > 30 min at inclusion[c]	Yes	38 (70%)
	No	16 (30%)

b. Confirmation Cohort

Characteristics	All participants (*N*=320) Median (IQR) or n (%)	
Age	47 (38; 55)	
BMI at inclusion[d]	23.5 (21.5; 26.9)	
Stress at inclusion[e] (/10)	5 (3; 7)	
Interval between samples (months)	6.1 (6.0; 6.3)	
Gender	Female	256 (80%)
	Male	64 (20%)
Workplace	Nice West	81 (25%)
	Nice East	94 (29%)
	Nice Airport	145 (45%)
Mid-level professionals and executives	Yes	198 (62%)
	No	122 (38%)
Road traffic <1 km[d]	Yes	198 (62%)
	No	120 (38%)
Living in the city centre (>10,000 inhabitants)	Yes	124 (39%)
	No	196 (61%)
Comorbidities*	Yes	71 (22%)
	No	249 (78%)
Allergies	Yes	88 (28%)
	No	232 (72%)
Weekly physical activity > 30 min at inclusion[f]	Yes	176 (56%)
	No	141 (44%)

c. Clinical characteristics in the confirmation cohort according to the workplace (area of exposure)

	Nice Airport *n*=145	Nice West *n*=81	Nice East *n*=94	*P*-value
Age	52 (44; 57)	39 (30; 49)	42 (34; 55)	<0.001[†]
Sex				
F	120 (83%)	64 (79%)	72 (77%)	0.49[‡]
M	25 (17%)	17 (21%)	22 (23%)	

Table 1 *(Continued)*

c. Clinical characteristics in the confirmation cohort according to the workplace (area of exposure)

	Nice Airport *n*=145	Nice West *n*=81	Nice East *n*=94	*P*-value
Education level				
< Licence	53 (37%)	14 (17%)	31 (33%)	<0.001‡
≤Master	79 (54%)	39 (48%)	42 (45%)	
PhD	13 (9%)	28 (35%)	21 (22%)	
BMI at inclusion	24.5 (21.7; 27.1)	22.4 (20.8; 24.6)	23.7 (21.4; 27.1)	0.014†
Smocking[g]				
Yes	37 (26%)	20 (25%)	38 (40%)	0.035‡
No	104 (74%)	59 (75%)	56 (60%)	
Comorbidities				
No	115 (79%)	70 (86%)	64 (68%)	0.012‡
Yes	30 (21%)	11 (14%)	30 (32%)	
Allergies				
No	102 (70%)	60 (74%)	70 (74%)	0.73‡
Yes	43 (30%)	21 (26%)	24 (26%)	
Concomitant Treatments				
No	127 (88%)	76 (94%)	74 (79%)	0.012‡
Yes	18 (12%)	5 (6%)	20 (21%)	
Mid-level professionals and executives				
No	58 (40%)	25 (31%)	39 (41%)	0.29‡
Yes	87 (60%)	56 (69%)	55 (59%)	
Road traffic <1 km[h]				
No	139 (43%)	29 (36%)	29 (31%)	0.16‡
Yes	82 (57%)	51 (64%)	65 (69%)	
Living in the city centre (>10,000 inhabitants)				
No	94 (65%)	41 (51%)	61 (65%)	0.076‡
Yes	51 (35%)	40 (49%)	33 (35%)	
Stress variation[i]	5 (4; 6)	5 (3; 7)	5 (3; 8)	0.78†
Weekly physical activity > 30 min at inclusion[j]				
Yes	85 (59%)	41 (51%)	50 (54%)	0.49‡
No	59 (41%)	39 (49%)	43 (46%)	
ΔIFNγ (IU/mL)	−45 (−321; 1)	−59 (−193; 11)	−18 (−83; 2)	0.015†

***Table 1*: Description characteristics of the study population.**

Notes: Data are presented as the median (IQR for Inter Quartile Range) or n (%) of the total *N*=58 for the pilot cohort or N=320 for the confirmation cohort.
BMI: Body Mass Index.
[a]Data missing for three participants
[b]Data missing for three participants
[c]Data missing for four participants
[d]Data missing for two participants
[e]Data missing for five participants
[f]Data missing for three participants
*Autoimmune diseases, cancers, cardio-vascular diseases
[g]Data missing for five participants
[h]Data missing for two participants
[i]Data missing for six participants
[j]Data missing for three participants
†Kruskall-Wallis test
‡Chi-Squared test
The *P*-value is significant at $P<0.05$

Changes in the concentrations of the air pollutants

Monthly average of concentrations in µg/m³ of atmospheric pollutants, collected in Nice West (Promenade des Anglais sensor, Nice Botanique), Nice Airport (Airport sensor) and Nice East (Arson sensor) are presented in Figures 2a, b, and 3a, b and c.

For the pilot cohort, in Figure 2a and 2b, during lockdown the concentration of nitrogen dioxide (NO_2) dropped between February 2020 and April 2020 by 61.5% (33,1 µg/m³ versus 12,7 µg/m³) and 64,0% (38,7 µg/m³ vs. 13,9 µg/m³) for Nice East and Nice West stations, respectively. In contrast, ozone (O_3)

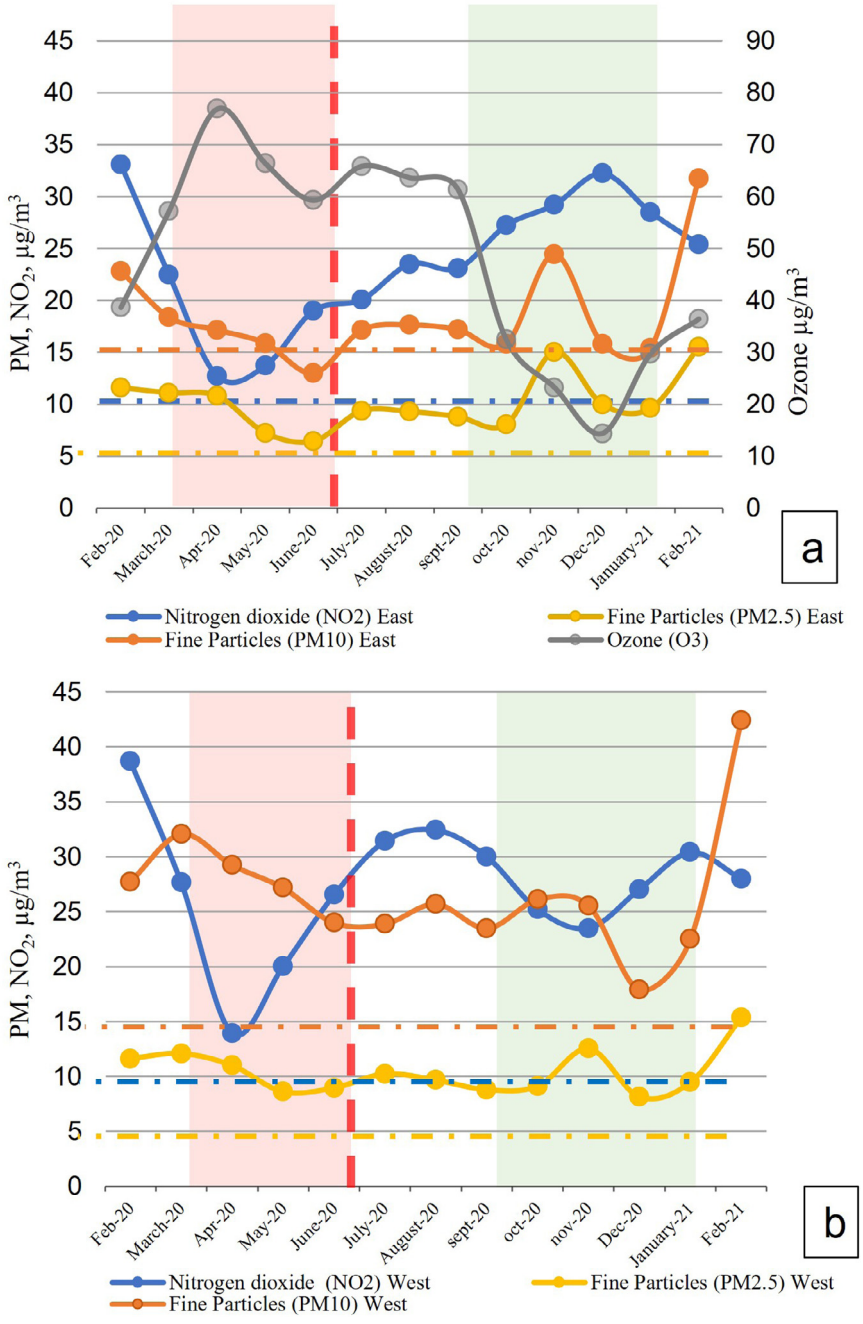

Figure 2. Evolution of the concentrations of the air pollutants at Nice East station (a) and West station (b): pilot cohort.

The main and secondary vertical axis show the concentrations of pollutants in µg/m^3. The horizontal axis show the average values between February 2020 and February 2021 for each pollutant, with colour areas corresponding to the sampling periods (red for the lockdown = T1, from 27/03/2020 to 26/06/2020 and green for the resumption of activities = T2, from 23/09/2020 to 14/01/2021). A vertical red dotted line represents the end of lockdown (30/06/2020). Horizontal blue, orange and yellow dotted lines are the annual average regulatory values (2021 guidelines) for NO_2 (10 µg/m^3), PM10 (15 µg/m^3), and PM2.5 (5 µg/m^3).

concentration was increased by 99,0% during the same period (38.6 µg/m³ vs. 76.7 µg/m³) at the Nice East station. When sanitary measures were lifted in July 2020, the level of nitrogen dioxide progressively returned to the initial concentration pre-lockdown. Ozone (O3) decreased after the normal activities were resumed, while the levels of fine particles ($PM_{2.5}$ and PM_{10}) remained stable.

Figure 3. Evolution of the concentrations of the air pollutants at Nice East station (a) and West station (b) and Airport station (c) confirmation cohort.

The main and secondary vertical axis show the concentrations of pollutants in µg/m3. The horizontal axis show the average values between July 2020 and July 2021 for each pollutant. Horizontal blue, orange and yellow dotted lines are the annual average regulatory values (2021 guidelines) for NO_2 (10 µg/m^3), PM10 (15 µg/m^3), and PM2.5 (5 µg/m^3).

In the confirmation cohort, in Figure 3a, b and c, we were able to observe these variations over one year after the lifting of the health measures between July 2020 and July 2021 in the 3 exposure zones. For NO_2 we have an increase over the T1 period (July 2020 to January 2021) of 41.8% (20.1 vs 28.5 µg/m^3) and 136.6% (9.3 vs 22.0 µg/m^3) for Nice East and Nice Airport respectively. In Nice West exposure remains stable at higher values (31.4 vs 30.2 µg/m^3). Ozone over the same period decreased by 54.3% (65.8 vs 30.1 µg/m^3), 28.6% (72.7 vs 51.9 µg/m^3) and 41.2% (62.8 vs 36.9 µg/m^3) respectively in Nice East, Nice West and Nice Airport.

For the second period T2 (February 2021 to July 2021) we observe for NO_2 a decrease of 33.0% (25.4 vs 19.1 µg/m^3), and 23.1% (16 vs 12.3 µg/m^3) in Nice East and Nice Airport respectively. In Nice West exposure remains stable at higher values (28 vs 29.2 µg/m^3). At the same time, in T2 we have an increase in ozone of 81.3% (36.4 vs 66.0 µg/m^3), 34.3% (54.3 vs 72.9 µg/m^3) and 59.6% (42.8 vs 68.3 µg/m^3) respectively for Nice East, Nice West and Nice Airport. Fine particles $PM_{2.5}$ and PM_{10} show two peaks in November and February for the three exposure zones. The pollutants regulated annually (NO_2, $PM_{2.5}$ and PM_{10}) are practically constantly above the regulatory threshold values for the 3 workplaces.

To represent the entire exposure period of the confirmation cohort, we performed an exposure mapping of nitrogen dioxide over the study period for the confirmation cohort in Figure 4. We noted that the highest exposure values are located along the main transport routes (highways, expressways, and national roads), and in the city center in the east of the map where the urban density is higher. It is clear that the 3 workplaces were not exposed to the same levels of nitrogen dioxide concentration.

Comparison of the median difference in IFNγ produced between the two sampling times

In the pilot cohort, the concentration of IFNγ after *in vitro* stimulation was significantly decreased after the end of restrictive measures in comparison to the lockdown period: 297.50 UI/ml (IQR 116.75–661.00) during lockdown (T1) versus 221.00 IU/mL (IQR: 73.25–420.00) after the lockdown (T2); median variation was -25.7% and median difference was -105.00(IU/mL (95% CI = (−224.00, −69.00), P<0.001, Table 2a and Figure 5a, Table S1a in supplementary material).

In the confirmation cohort, the concentration of IFNγ after *in vitro* stimulation was significantly decreased between T1 and T2: 150.50 IU/mL (IQR 35.00–388.50) during T1 versus 78.50 IU/mL (IQR

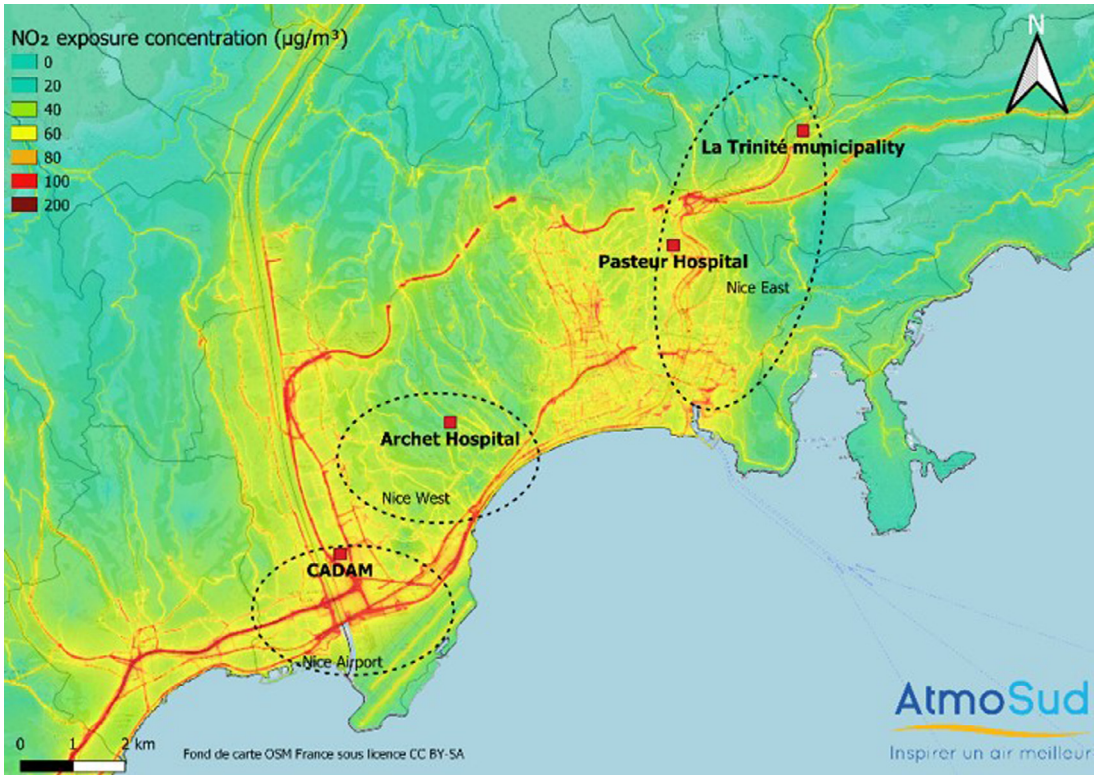

Figure 4. Exposure mapping of nitrogen dioxide over the study period for the confirmation cohort.

The background map shows the exposure concentration in µg/m^3 (between 28 July 2020 and 21 July 2021) for nitrogen dioxide (average of daily maximums). The 3 dotted ellipses represent the 3 exposure zones (or workplaces) in the analysis, according to the location of the participants and the sensor considered. The 4 inclusion centers are marked with a red square.

CADAM is Alpes-Maritimes Departmental Administrative Center. The white arrow points north.

15.75–197.00) at T2; median variation was −47.8% and median difference was −40.50 IU/mL (95% CI = (−57, −26), P<0.001, Table 2b and Figure 5b and Table S1b in supplementary material).

To specify the significant univariate associations between ΔIFNγ and all the variables collected in the questionnaire, we performed comparative tests adapted to the type of explanatory variable. Finally, we found a significant difference for sex (P = 0.027) and workplace (P = 0.015). BMI at inclusion was almost significant (P = 0.056) (Table S2).

Comparison of the median differences in climate data between the two sampling times

Among the climatic variables, in the pilot cohort only the temperature and the sunshine duration differed between the two sampling times. Temperature decreased by 20.9% between T1 and T2: 18.77 °C (17.40–18.77) at T1 and 14.84 °C (8.50–15.73) at T2 (P<0.001). The sunshine duration decreased by 24.7% between T1 and T2: 372 min (198.00–618.00) at T1 and 280 min (125.00–459.75) at T2 (P = 0.008). No significant difference between T1 and T2 was measured for the wind (P = 0.321) and humidity (P = 0.327) (Table 2a, Figure 5a). In the confirmation cohort, only the relative humidity and the sunshine duration differed between the two sampling times. Relative humidity decreased by 3.0% between T1 and T2: 73.5% (70.80–76.80) at T1 and 71.3% (68.27–75.29) at T2 (P<0.001). The sunshine duration increased by 40.7% between T1 and T2: 300.86 min (200.29–396.71) at T1 and 423.43 min (357.86–526.29) at T2 (P<0.001). No significant difference between T1 and T2 was measured for the temperature (P = 0.583) and wind speed (P = 0.166) (Table 2b, Figure 5b).

Comparison of the median differences in pollutant concentrations between the two sampling times

In the pilot cohort, the concentrations of the ozone precursor pollutants (NO_2) were significantly higher after lockdown (T2) in comparison to during lockdown (T1). The concentration of NO_2 increased by 46,0% between

a. Pilot Cohort Δ (T2-T1)	Median	95% CI	*P*-values
ΔIFNγ (IU/mL)	−105.00	(−224.00; −69.00)	<0.001
ΔNO_2 (µg/m^3)	8.26	(3.61; 10.97)	<0.001
ΔO_3 (µg/m^3)	−33.74	(−45.24;−28.91)	<0.001
$\Delta PM_{2.5}$ (µg/m^3)	−0.37	(−2.13; 2.39)	0.846
ΔPM_{10} (µg/m^3)	−1.77	(−6.93; 5.16)	0.205
ΔTemperature (°C)	−3.94	(−5.83; −3.93)	<0.001
ΔRelative humidity (%)	−0.39	(−2.97; 1.87)	0.327
ΔWind speed (km/h)	0.32	(−0.69; 0.83)	0.321
ΔSunshine duration (minutes)	−154.00	(−247.00; 82.00)	0.008

b. Confirmation Cohort Δ (T2-T1)	Median	95% CI	*P*-values
ΔIFNγ (IU/mL)	−40.50	(−57; −26)	<0.001
ΔNO_2 (µg/m^3)	−4.73	(−5.24; −3.59)	<0.001
ΔO_3 (µg/m^3)	31.15	(27.31; 35.33)	<0.001
$\Delta PM_{2.5}$ (µg/m^3)	−0.20	(−0.46; 0.09)	0.240
ΔPM_{10} (µg/m^3)	1.45	(0.09; 2.21)	0.012
ΔTemperature (°C)	−0.94	(−1.20; −0.56)	0.583
ΔRelative humidity (%)	−1.73	(−2.6; −0.71)	<0.001
ΔWind speed (km/h)	−0.63	(−0.92; −0.11)	0.166
ΔSunshine duration (minutes)	122.60	(112.40; 129.60)	<0.001
Δ Stress	-0.18	(−0.46; 0.10)	0.207

***Table 2*: Paired samples tests.**
Notes.
P-values comparing the median values T1 to T2 using the delta (T2-T1) or Δ with Wilcoxon test.
The *P*-value is significant at $P<0.05$. CI is 95% confidence interval
IFNγ: gamma interferon
$PM_{2.5}$: fine particulate matter with an aerodynamic diameter of <2.5 µm,
PM_{10}: fine particulate matter with an aerodynamic diameter of <10 µm.
NO_2: nitrogen dioxide,
O_3: ozone
T1 is the first sample collected/during lockdown, T2 is the second sample collected/after lockdown
Pollutants are measured in micrograms per cubic meter (µg/m^3), temperature in degrees Celsius (°C), wind in kilometres per hour (km/h)

T1 and T2: 16.31 (9.70−21.93) vs 23.81 (20.88−25.95) µg/m^3, respectively ($P<0.001$). On the other hand, the concentration of O_3 decreased by 58.4% between T1 and T2: 59.61 (54.34−60.34) vs. 24.81(14.37−33.00) µg/m3 respectively (P<0.001). There was no significant difference in the concentrations of fine particles $PM_{2.5}$ and PM_{10} (Table 2a, Figure 5a). In the confirmation cohort, the concentrations of the ozone precursor pollutants (NO_2) were significantly lower at T2 in comparison to T1. The concentration of NO_2 decreased by 35.3% between T1 and T2: 23.81 (16.81−25.66) vs 15.40 (12.37−24.34) µg/m^3, respectively ($P<0.001$). On the other hand, the concentration of O_3 increased by 99,3% between T1 and T2: 33.66 (24.03−56.31) vs. 67.07 (59.36−75.51) µg/m^3 respectively, ($P<0.001$). There was no significant difference in the concentrations of fine particles $PM_{2.5}$ while PM_{10} decreased by 1.1% between T1 and T2: 18.94 (17.27−24.92) vs. 18.73(16.10−24.53) µg/m^3 respectively ($P = 0.012$) (Table 2b, Figure 5b).

Correlations between variations in cellular immunity, pollution, and climatic conditions

In the pilot cohort, ΔIFNγ was inversely correlated $\Delta PM_{2.5}$ ($r = -0.338$, $P = 0.009$), ΔPM_{10} ($r = -0.295$, $P = 0.025$). There was a tendency with NO_2 ($r = -0.250$, $P = 0.059$). There was no significant correlation with climatic conditions (Table 3a). We also performed assays on a few samples ($n = 37$) from pilot cohort to measure other markers characteristic of T cell pathways: Th1 (IFNγ and IL-12), Th2(IL-4 and IL-5) and Th17(IL-17A) and pro-inflammatory cytokines (IL-1β, IL-6, IL-8 and TNF-α). Using a principal component analysis, we showed: an inverse association between variation in exposure to air pollutants and Th1 response, and a positive association between variation in exposure to air pollutants Th2 and Th17 response and pro-inflammatory cytokines production as described in other studies.[41] These preliminary results are presented in the Figure S1 and Table S3.

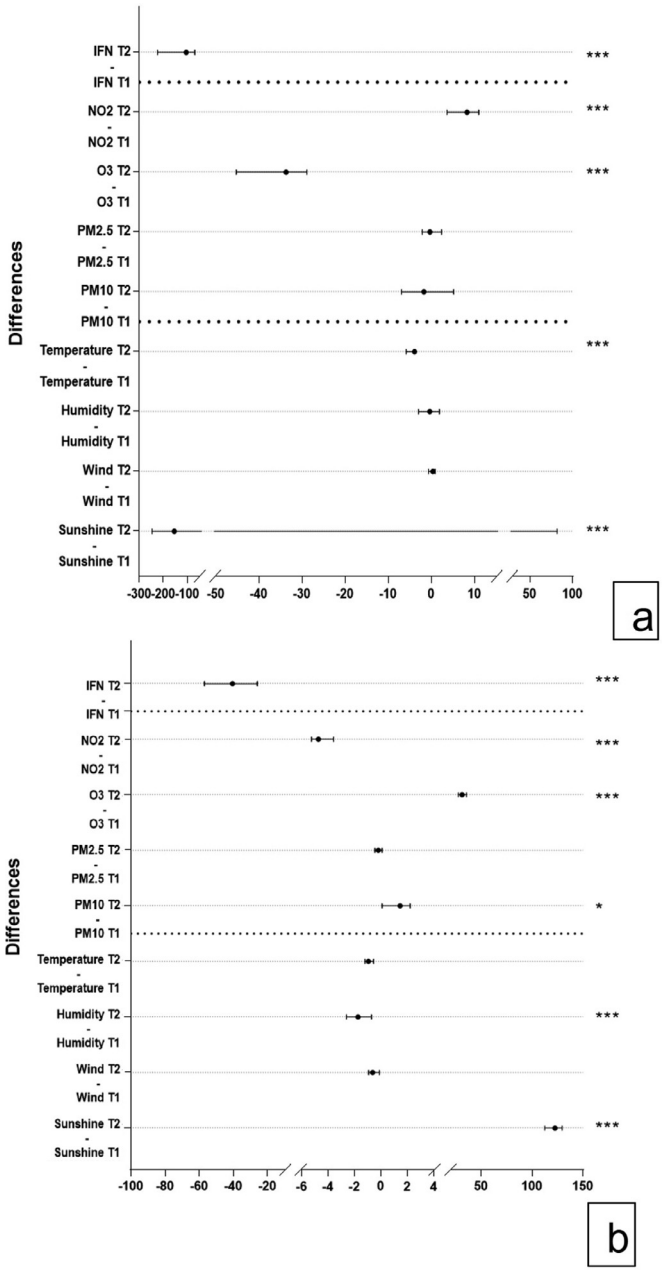

Figure 5. Comparison of medians of immunity, pollution and climate variables between T1 and T2 for the pilot (a on left) and confirmation (b on right) cohorts.

Each boxplot represents the median of variation (median of differences) and its 95% confidence interval for each variable between T1 and T2. Test de Wilcoxon pour données appariées. The horizontal axis represents values in units (IU/mL for IFNγ, µg/m^3 for pollutants, °C for temperature, % for relative humidity, km/h for wind speed and minutes for sunshine). For the pilot cohort N=58 and for the confirmation cohort N=320. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$.

In the confirmation cohort, ΔIFNγ was inversely correlated with ΔNO_2 ($r = -0321$, $P<0.001$), $\Delta PM_{2.5}$ ($r = -0.183$, $P<0.01$). There was no significant correlation between ΔIFNγ and ΔPM10 ($r = -0.050$, $P = 0.374$, Table 3b). There was no significant correlation with relative humidity but there were a significantly and positively correlation with variations in temperature ($r = 0.379$, $P = <0.001$), and sunshine duration ($r = 0.306$, $P = <0.001$), and negatively correlation with wind speed ($r = -0.332$, $P = <0.001$), (Table 3b). Stress variation

a. Pilot Cohort Δ (T2-T1)	ΔIFNγ	*P*-value
ΔNO_2	−0.250	0.059
ΔO_3	0.143	0.284
$\Delta PM_{2.5}$	−0.338	0.009
ΔPM_{10}	−0.295	0.025
ΔTemperature (°C)	0.137	0.303
ΔRelative humidity (%)	−0.109	0.415
ΔWind speed (km/h)	0.013	0.920
ΔSunshine duration (minutes)	0.172	0.197

b. Confirmation Cohort Δ (T2-T1)	ΔIFNγ	*P*-value
ΔNO_2	−0.321	<0.001
ΔO_3	0.270	<0.001
$\Delta PM_{2.5}$	−0.183	0.001
ΔPM_{10}	−0.050	0.374
ΔTemperature (°C)	0.379	<0.001
ΔRelative humidity (%)	0.105	0.061
ΔWind speed (km/h)	−0.332	<0.001
ΔSunshine duration (minutes)	0.306	<0.001
ΔStress	0.037	0.514

***Table 3*: Spearman correlation coefficient between IFNγ and climatic data.**
Notes.
Correlation matrix using spearman's rho coefficient Delta (T2-T1) or Δ
IFNγ: interferon gamma
$PM_{2.5}$: fine particulate matter with an aerodynamic diameter of <2.5 μm,
PM_{10}: fine particulate matter with an aerodynamic diameter of <10 μm.
NO_2: nitrogen dioxide,
O_3: ozone
T1 is the first sample collected/during lockdown T2 is the second sample collected/after lockdown
The *P*-value is significant at $P<0.05$

was not correlated with IFNγ variations. Correlations between climate and pollution data are available in the supplementary material (Table S4a and S4b).

Comparisons between quartiles of cellular immune response and exposure to nitrogen oxides

In the pilot cohort, participants were divided into four quartiles (Q1−Q4) depending on the variation of the cellular immune response (ΔIFNγ / IFNγ T1)): Q1 corresponds to the group of participants with the highest IFNγ decrease of 83,2% corresponding to a mean variation of −238.53±200.01 IU/mL, compared to Q4 which corresponds to participants with a mean increase of 56.9% of IFNγ (99.51±65.72 IU/mL).

No difference was found for NO_2 variations between quartiles and pairwise comparison (Figure 6a, Table S5a and S6a in supplementary material). In the confirmation cohort, participants were divided into four quartiles (Q1−Q4) depending on the variation of the cellular immune response (ΔIFNγ / IFNγ T1)): Q1 corresponds to the group of participants with the highest IFNγ decrease of 86.3% corresponding to a mean variation of −362.07±305.67 IU/mL. Q1 exhibited the smallest decrease between T1 and T2 of ΔNO_2: −2.18±5.53 μg/m^3. Q4 which corresponds to the group of participants with a mean increase of 70.0% of IFNγ (91.56±103.24 IU/mL), exhibited the highest decrease between T1 and T2 of ΔNO_2: −5.36±4.88 μg/m^3. The difference of ΔNO_2 exposure was different between the four quartiles ($P = 0.001$), and by pairwise comparison we found a difference between Q1 and Q2 ($P = 0.015$) and Q1 and Q4 ($P = 0.001$) (Figure 6b,Table S5b and S6b in supplementary material).

Multiple linear regressions on the association between IFNγ variation and pollutants

We performed a multivariate model that analyzes the impact of environmental factors that change between the two times, on the changes in IFNγ in the pilot cohort. We found a significant association with NO_2 variations ($P = 0.030$). A non-significant association was observed with mean temperature change ($P = 0.077$), sunshine duration ($P = 0.303$) and O_3 variations ($P = 0.444$). The adjusted R^2 coefficient of the model is estimated at 0.077 (Table 4a).

We then performed a multivariate model on the confirmation cohort that analyzes the impact of environmental factors that change between the two times,

a. Pilot Cohort Predictor Pollutant of ΔIFNγ variation	β	95% IC β	P-value	VIF	Adjusted R^2
ΔNO_2	−11.21	(−21.27; −1.15)	0.030	1.264	0.077
ΔO_3	−1.46	(−5.25; 2.34)	0.444	1.254	
ΔTemperature (°C)	11.99	(−1.34; 25.32)	0.077	1.086	
ΔSunshine duration (minutes)	0.09	(−0.08; 0.26)	0.303	1.018	

b. Confirmation Cohort: final model after backward selection Predictor Pollutant of ΔIFNγ variation	Univariate Analysis (p value)*	Level	β	95% IC β	*P*	VIF	Adjusted R^2
Age			2.20	(−0.04; 4.45)	0.054	1.115	0.387
Sex	0.027	Male vs Female	−44.25	(−105.45; 13.95)	0.136	1.026	
BMI at inclusion			−7.71	(−13.26; −2.17)	0.007	1.050	
Workplace	0.015	Airport vs Nice West	−218.52	(−289.00; −148.06)	<0.001	1.490	
	(overall)	Nice East vs Nice West	−334.32	(−447.03; −221.61)	<0.001		
ΔNO_2			−13.26	(−22.41, −4.11)	0.005	2.165	
ΔO_3			4.17	(1.62; 6.72)	0.001	2.186	
ΔPM_{10}			−14.46	(−19.62; −9.31)	<0.001	1.740	
ΔSunshine duration (minutes)			0.32	(0.12; 0;52)	0.002	1.861	
ΔRelative humidity (%)			15.33	(2.55; 10.31)	<0.001	1.399	

***Table 4*: Coefficient of multiple linear regressions on the association between IFNγ variation and pollutants.**
Notes.
β: Non-standardized effect, IC *β*: Confidence interval of *β*, P: *P*-value, *β*: Standardized effect, IC *β*: Confidence interval of *β*, R^2: Coefficient of determination, SD: Standard deviation VIF: Variance Influence Factor. The *P*-value is significant at $P<0.05$
Δ: Delta (T2-T1) where T1 is the first sample collected/during lockdown and T2 is the second sample collected/after lockdown
IFN*γ*: interferon gamma NO_2: nitrogen dioxide
* Univariate *p*-values correspond to variables with a $P<0.05$ for comparative tests according to the variation of IFN gamma.
Variables that differ significantly by workplaces were removed by the backward method ($P>0.2$) and are not shown here.

intrinsic factors associated with variations in IFN*γ* production, and clinical characteristics that differ significantly between groups according to workplaces (area of exposure) in univariate analysis (Table S7).

We found a significant association with NO_2 variations ($P = 0.005$). A significant association was observed for ΔPM_{10} ($P<0.001$) and ΔO_3 ($P = 0.001$). Variations of sunshine and humidity remain significant ($P = 0.002$ and $P<0.001$ respectively). We found an effect of BMI ($P = 0.007$) but the effect of age and sex are no longer significant. It is important to note that we demonstrate a strong influence of the workplace on the variations of the interferon response ($P<0.001$) although this influence cannot be explained by the different clinical characteristics of the subjects included in our confirmation cohort. Indeed, all these variables statistically different between the exposure areas: comorbidities ($P = 0.946$), concomitant treatments ($P = 0.835$), education level ($P = 0.635$ and 0.595) and smocking status ($P = 0.471$); were excluded from our multivariate model. The adjusted R^2 coefficient of the model is estimated at 0.387 (Table 4b, Figure 7a, b and c), showing that our model around 39% of the variations of IFN*γ* between T1 and T2.

The analysis of the impact of these pollutants (measured on living place) on the interferon response using the multipollutant model (AZUR) which already includes meteorological variations in the measurement of pollutant concentrations, confirmed these results: a significant inverse association between IFN*γ* variations and NO_2 variations ($P<0.001$), PM_{10} variations ($P = 0.03$), and a positive association with O_3 variations ($P<0.001$) (Table 5).

Discussion

Our pilot study suggests a significant decrease of IFN*γ* (pivotal in antiviral response) with increasing NO_2 concentrations. Moreover, our study shows that the IFN response is weaker as the amplitude of the exposure variations is higher. We observe a more pronounced decrease in NO_2 at the Nice West site than at the Nice East site due to a greater dependence of the West side sensor on road traffic, the main source of NO_2. After the adjustment for mean temperature and sunshine, we confirm a non-negligible effect of NO_2 on IFN*γ*. Our data on the confirmation cohort confirm and precise

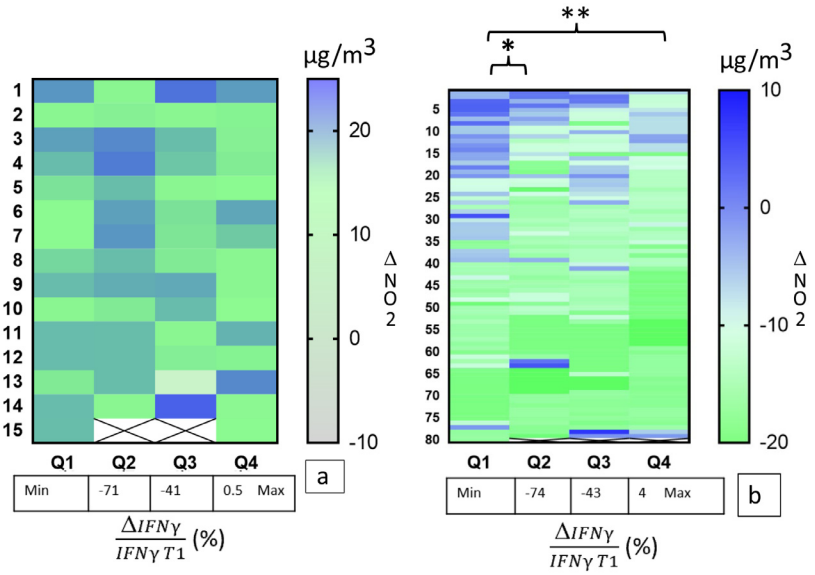

Figure 6. Comparison of the quartiles of IFNγ variation as a function of the nitrogen dioxide variation for the pilot cohort (a on left) and confirmation cohort (b on right).

Each panel represents a heatmap for ΔNO_2. Each column represents a quartile (Q1 to Q4): quartiles of IFNγ change in % (T2-T1/T1). The colours represent the intensity of exposure variations for each participant in each quartile. For the pilot cohort *N*=58 and for the confirmation cohort *n*=320.*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$.

these results: as demonstrated by the multivariate analysis, exposure to different pollutants (NO_2, O_3 and PM_{10} and various climatic conditions (sunshine duration and relative humidity) modify our immune response and induce modifications in Th1 response.

Interestingly, similar results were obtained in an animal model: rats were exposed to 5 mg/m^3 NO_2 for seven days. The results showed that NO_2 exposure caused (i) pulmonary pathological alteration, and significantly stimulated MUC5AC expression (ii) up-regulated changes of pro-inflammatory cytokines (IL-1β, IL-6, and ICAM-1) and (iii) imbalance in the ratio of Th1/Th2 differentiation (IL-4, IFN-γ, GATA-3 and T-bet) by the activation of following JAK-STAT pathway (JAK1, JAK3 and STAT6).[42]

Our results also highlight an important factor that modify immune response. Our confirmation cohort includes different workers from different communities: Nice University Hospital, Alpes-Maritimes Departmental Administrative Center (CADAM), La Trinité municipality. We showed that these workers had different clinical characteristics between the different groups. In multivariate analysis we were able to demonstrate an extremely important role on the interferon response of the workplace with no explanation for this decrease in interferon response by age, sex, comorbidities or other clinical variables which showed no impact on the interferon response. This role of the workplace on the immune response raises questions about the exposure of these workers during their work. The Nice Airport area seems to be more impacted by these variations, it is important to note that this area has undergone an urban requalification program with numerous constructions and destruction of buildings during the period of our study. This work may have had an impact on our results and should be the subject of further study. This model explains more than 38% of variations in IFN production.

Lockdown caused by the outbreak of COVID-19 generated unique environmental conditions were met to measure these changes in humans. The levels of nitrogen oxides, such as NO_2, which are linked to transport, decreased significantly during lockdown, as observed in the UK,[50] India,[51] China,[52] and Spain.[53] The same studies also showed a clear increase in O_3 during lockdown. O_3 is produced by photodissociation (UV) of NO_2 or NO_x, but is then reformed via anthropogenic NO.[6,7] The concentration of fine particles ($PM_{2.5}$ and PM_{10}) remained stable or exhibited a slight increase throughout the observed period and did not seem to be impacted by the lockdown, which can be partly explained by the increase in their production by heating and wood combustion during lockdown (which is confirmed in the confirmation cohort with two peaks in November and February).[54] We can nevertheless note a correlation between the exposure to fine particles and IFN variation possibly because of secondary aerosol formation, under favorable weather conditions (NO_2 proxy for PM).[55]

The concentration levels of the atmospheric pollutants are directly affected by climatic variables. As

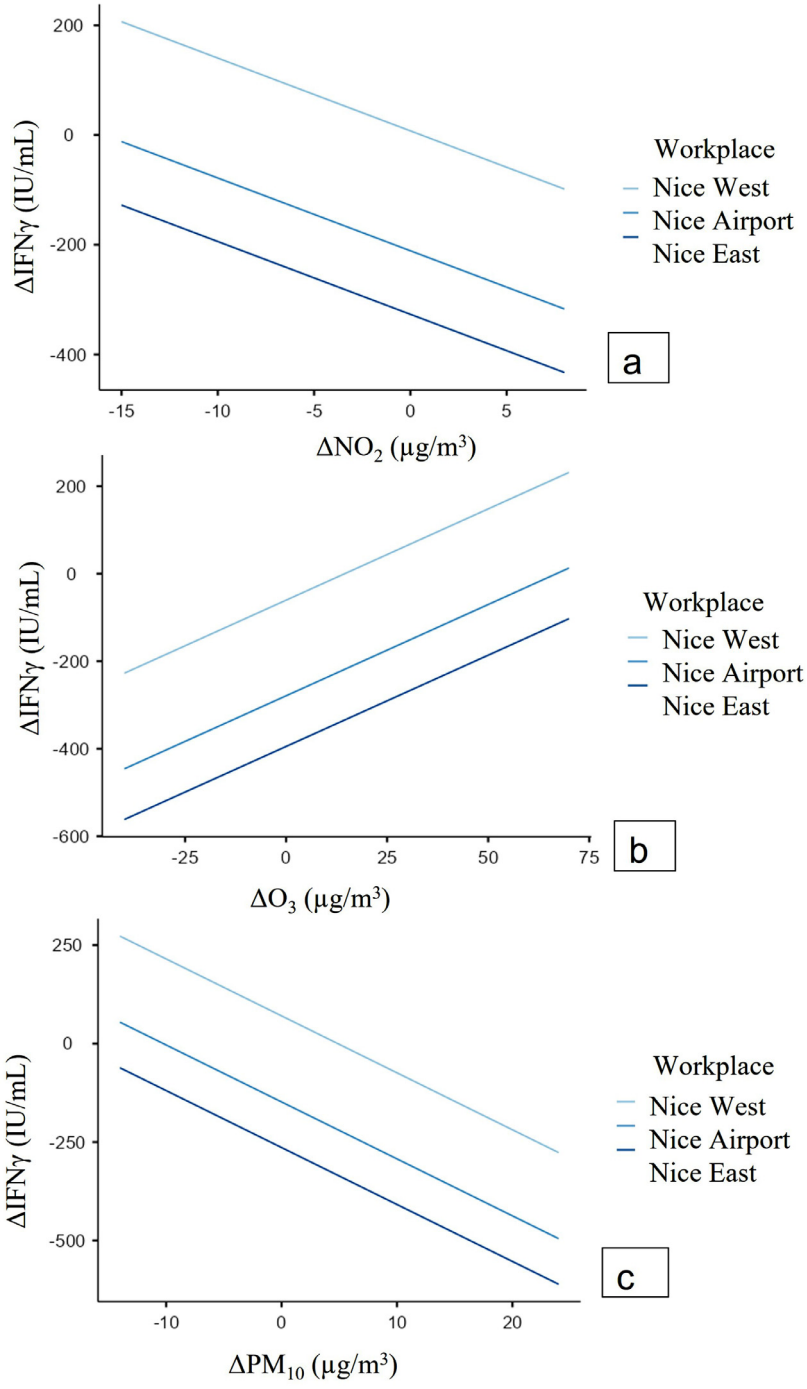

Figure 7. Multiple linear regression curve between IFNγ variation and pollutants variation.

The curves represent the estimated marginal means after fitting the multivariate model for the variation of IFNγ as a function of workplace and the variation in NO_2 (a), O_3 (b) and PM_{10} (c)

Predictor Pollutant of ΔIFNγ variation	Univariate Analysis (*P* value)*	Level	β	95% IC β	*P*-value	VIF	Adjusted R^2
Age			2.59	(0.21; 4.97)	0.033	1.106	0.318[¥]
Sex	0.027	Male vs Female	−27.04	(-89.04; 34.97)	0.392	1.023	
BMI at inclusion			−8.19	(−14.12; −2.26)	0.007	1.055	
Workplace	0.015 (overall)	Airport vs Nice West	−77.69	(−144.61; −10.76)	0.023	1.140	
		Nice East vs Nice West	−48.90	(−119.37; 21.56)	0.173		
ΔNO_2 Multipollutant model[T]			−7.02	(−10.43; −3.61)	<0.001	1.032	
ΔO_3 Multipollutant model[T]			4.77	(3.72; 5.81)	<0.001	1.043	
ΔPM_{10} Multipollutant model[T]			−5.73	(−10.88; −0.57)	0.030	1.168	
Comorbibdities		Yes vs No	−6.26	(−81.94; 69.42)	0.871 [α]	1.291	0.311
Concomitant Treatments		Yes vs No	7.46	(−70.46; 85.38)	0.851 [β]	1.088	0.313
Smoking status		Yes vs No	10.06	(−44.09; 64.22)	0.715 [γ]	1.023	0.315
Education Level		≤ Master vs PhD	−37.33	(−105.04; 30.38)	0.279 [δ]	1.043	0.317
		< Licence vs PhD	−49.63	(−123.85; 24.60)	0.189 [δ]		

***Table 5*: Coefficient of multiple linear regressions on the association between IFNγ variation and pollutants using a multipollutant model (AZUR), with potential confounders related to the workplace.**

Notes.
β: Non-standardized effect, IC *β*: Confidence interval of *β*, P: *P*-value, *β*: Standardized effect, IC *β*: Confidence interval of *β*, R^2: Coefficient of determination, SD: Standard deviation VIF: Variance Influence Factor
The P-value is significant at $P<0.05$
Δ: Delta (T2-T1) where T1 is the first sample collected/during lockdown and T2 is the second sample collected / after lockdown
IFN*γ*: interferon gamma NO_2: nitrogen dioxide
* Univariate *p*-values correspond to variables with a $P<0.05$ for comparative tests according to the variation of IFN gamma.
¥: Final model with sex, age, BMI at inclusion, workplace, ΔNO_2, ΔO_3, ΔPM_{10}, ΔSunshine duration and ΔRelative humidity
α: Model with final model, plus comorbities, concomitant treatments, Education level and Smoking status
β: Model with final model, plus concomitant treatments, Education level and Smoking status
γ: Model with final model, plus Education level and Smoking status
δ: Model with final model, plus Education level
T: Pollutant data from the exposure extraction calculated by the multi-pollutant model (Azur) which already includes meteorological variations in the measurement of pollutant concentrations

described in our study, lower temperature and less sunshine and higher humidity are associated with an increase of NO_2 concentrations (the opposite for O_3). Similar variations were observed in China[7] by separating meteorological and anthropogenic effects.

Numerous studies established a relationship between pollutant levels (NO_2, and PM) and cases of COVID-19 or deaths.[56–61] Low temperatures and high nitrogen oxide pollution are considered as risk factors for respiratory viral infections. Our results could thus explain a high vulnerability to viral infections in polluted areas associated with a poorer antiviral Th1 immune response.[57,62–65] Our study has several limits. First, the post lockdown period is not really representative of the pre-pandemic period (before March 2020) although the levels of pollution are close to it, due to large population movements, rapid economic recovery and low adherence to subsequent lockdowns. Second, participants in our study were recruited on a voluntary basis that could introduce a bias on IFN measurement related to living conditions (stress, physical activity etc). Nevertheless, the matched design of our study was able to limit the effect of these biases. Third, our cohort is not representative of the general population but of a French worker. Lastly, we did not identify factors associated with workplace explaining a decrease of interferon production over then pollutant exposure.

Conclusion

Our study showed a clear and significant correlation between the increase of pollutants exposure and the concomitant decrease of Th1 cellular immunity as measured by the production of IFN*γ*. This result suggests a possible environmental component associated with vulnerability to viral infections, which should be highlighted in the current context. More broadly, these data are in line with several studies pushing for a lowering of the WHO recommended thresholds for air pollution, as they have been lowered in September 2021.[66]

Contributors

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Data sharing statement

Data are available upon request to the corresponding author.

Declaration of interests

The authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2022.104291.

References

1 Chan M, Solheim E, Taalas P. Working as one UN to address the root environmental causes of ill health. *Bull World Health Org*. 2017;95(1). 2–2.
2 Medina S, Wagner V. Impact de pollution de l'air ambiant sur la mortalité en France métropolitaine. Réduction en lien avec le confinement du printemps 2020 et nouvelles données sur le poids total pour la période 2016-2019. 2021 April; Available from: https://www.santepubliquefrance.fr/import/impact-de-pollution-de-l-air-ambiant-sur-la-mortalite-en-france-metropolitaine.-reduction-en-lien-avec-le-confinement-du-printemps-2020-et-nouvelle [cited 2021 July 27].
3 Lignes directrices OMS relatives à la qualité de l'air : particules, ozone, dioxyde d'azote et dioxyde de soufre.pdf. Available from: https://apps.who.int/iris/bitstream/handle/10665/69476/WHO_SDE_PHE_OEH_06.02_fre.pdf;jsessionid=69ED7A9FCD7C6F65FBAB18F6FE64E08B?sequence=1 [cited 2022 January 24].
4 Directive 2008/50/CE du Parlement européen et du Conseil du 21 mai 2008 concernant la qualité de l'air ambiant et un air pur pour l'Europe.
5 Evaluation du Plan de Protection de l'Atmosphère des Alpes-Maritimes: 2013-2018. AtmoSud. 2018 Available from: https://www.atmosud.org/publications/evaluation-du-plan-de-protection-de-latmosphere-des-alpes-maritimes-2013-2018-0 [cited 2021 August 30].
6 Lu X, Zhang L, Shen L. Meteorology and climate influences on tropospheric ozone: a review of natural sources, chemistry, and transport patterns. *Curr Pollut Rep*. 2019;5(4):238–260.
7 Lin C, Lau AKH, Fung JCH, et al. Removing the effects of meteorological factors on changes in nitrogen dioxide and ozone concentrations in China from 2013 to 2020. *Sci Total Environ*. 2021;793:148575.
8 Les principaux polluants surveillés. Atmo Nouvelle-Aquitaine. 2017 Available from: https://www.atmo-nouvelleaquitaine.org/article/les-principaux-polluants-surveilles [cited 2021 August 30].
9 Strak M, Weinmayr G, Rodopoulou S, et al. Long term exposure to low level air pollution and mortality in eight European cohorts within the ELAPSE project: pooled analysis. *BMJ*. 2021;374:n1904.
10 Corso M, Blanchard M, Medina S, Wagner V. Short-term associations of nitrogen dioxide (NO_2) on mortality in 18 French Cities, 2010–2014. *Atmosphere*. 2020;11(11):1198.
11 SPF. Evaluation de l'impact sanitaire de la pollution atmosphérique urbaine. Agglomérations de Cannes et de Nice. Impact à court et long terme. Available from: https://www.santepubliquefrance.fr/provence-alpes-cote-d-azur-et-corse/evaluation-de-l-impact-sanitaire-de-la-pollution-atmospherique-urbaine.-agglomerations-de-cannes-et-de-nice.-impact-a-court-et-long-terme [cited 2021 October 15].
12 SPF. Evaluation de l'impact sanitaire de la pollution atmosphérique urbaine dans six agglomérations de la région Provence-Alpes-Côte d'Azur, 2004-2006. Available from: https://www.santepubliquefrance.fr/provence-alpes-cote-d-azur-et-corse/evaluation-de-l-impact-sanitaire-de-la-pollution-atmospherique-urbaine-dans-six-agglomerations-de-la-region-provence-alpes-cote-d-azur-2004-2006 [cited 2021 October 15].
13 Hayes RB, Lim C, Zhang Y, et al. PM2.5 air pollution and cause-specific cardiovascular disease mortality. *Int J Epidemiol*. 2020;49(1):25–35.
14 Fuks KB, Weinmayr G, Foraster M, et al. Arterial blood pressure and long-term exposure to traffic-related air pollution: an analysis in the european study of cohorts for air pollution effects (ESCAPE). *Environ Health Perspect*. 2014;122(9):896–905.
15 Pfeffer PE, Donaldson GC, Mackay AJ, Wedzicha JA. Increased chronic obstructive pulmonary disease exacerbations of likely viral etiology follow elevated ambient nitrogen oxides. *Am J Respir Crit Care Med*. 2019;199(5):581–591.
16 Kowalska M, Skrzypek M, Kowalski M, Cyrys J. Effect of NO_x and NO_2 concentration increase in ambient air to daily bronchitis and asthma exacerbation, Silesian Voivodeship in Poland. *Int J Environ Res Public Health*. 2020;17(3):754.
17 Chen J, Zeng J, Shi C, et al. Associations between short-term exposure to gaseous pollutants and pulmonary heart disease-related mortality among elderly people in Chengdu, China. *Environ Health*. 2019;18(1):64.
18 To T, Zhu J, Stieb D, et al. Early life exposure to air pollution and incidence of childhood asthma, allergic rhinitis and eczema. *Eur Respir J*. 2020;55(2). [cited 2022 Sep 8]Available from: https://erj.ersjournals.com/content/55/2/1900913.
19 Andersen ZJ, Bønnelykke K, Hvidberg M, et al. Long-term exposure to air pollution and asthma hospitalisations in older adults: a cohort study. *Thorax*. 2012;67(1):6–11.
20 Ling SH, van Eeden SF. Particulate matter air pollution exposure: role in the development and exacerbation of chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis*. 2009;4:233–243.

21 Domingo JL, Rovira J. Effects of air pollutants on the transmission and severity of respiratory viral infections. *Environ Res*. 2020;187:109650.
22 Bonilla FA, Oettgen HC. Adaptive immunity. *J Allergy Clin Immunol*. 2010;125(2):S33–S40.
23 Annunziato F, Cosmi L, Liotta F, Maggi E, Romagnani S. Defining the human T helper 17 cell phenotype. *Trends Immunol*. 2012;33(10):505–512.
24 Xu X, Wang G, Chen N, et al. Long-term exposure to air pollution and increased risk of membranous nephropathy in China. *J Am Soc Nephrol*. 2016;27(12):3739–3746.
25 Aguilera J, Han X, Cao S, et al. Increases in ambient air pollutants during pregnancy are linked to increases in methylation of IL4, IL10, and IFNγ. *Clin Epigenet*. 2022;14(1):40.
26 Cremoni M, Brglez V, Perez S, et al. Th17-immune response in patients with membranous nephropathy is associated with thrombosis and relapses. *Front Immunol*. 2020:11.. cited 2022 Mar 12] Available from: https://www.frontiersin.org/article/10.3389/fimmu.2020.574997.
27 Robert M, Miossec P. Effects of Interleukin 17 on the cardiovascular system. *Autoimmunity Rev*. 2017;16(9):984–991.
28 Knop J. Immunologic effects of interferon. *J Invest Dermatol*. 1990;95(6 Suppl):72S–74S.
29 Pestka S, Krause CD, Walter MR. Interferons, interferon-like cytokines, and their receptors. *Immunol Rev*. 2004;202:8–32.
30 Hadjadj J, Yatim N, Barnabei L, et al. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. *Science*. 2020;369(6504):718–724.
31 Hu ZJ, Xu J, Yin JM, et al. Lower circulating interferon-gamma is a risk factor for lung fibrosis in COVID-19 patients. *Front Immunol*. 2020;11:2348.
32 Ruetsch C, Brglez V, Crémoni M, et al. Functional exhaustion of Type I and II interferons production in severe COVID-19 patients. *Front Med*. 2021;7:1106.
33 Cremoni M, Ruetsch C, Zorzi K, et al. Humoral and cellular response of frontline health care workers infected by SARS-CoV-2 in Nice, France: a prospective single-center cohort study. *Front Med*. 2021;7:1128.
34 Boyer-Suavet S, Cremoni M, Dupeyrat T, et al. Functional immune assay using interferon-gamma could predict infectious events in end-stage kidney disease. *Clin Chim Acta*. 2020;502:287–292.
35 Massa F, Cremoni M, Gérard A, et al. Safety and cross-variant immunogenicity of a three-dose COVID-19 mRNA vaccine regimen in kidney transplant recipients. *EBioMedicine [Internet]*. 2021;73. [cited 2022 Jan 24]. Available from: https://www.thelancet.com/journals/ebiom/article/PIIS2352-3964(21)00473-4/fulltext.
36 Gérard A, Doyen J, Cremoni M, et al. Baseline and early functional immune response is associated with subsequent clinical outcomes of PD-1 inhibition therapy in metastatic melanoma patients. *J Immunother Cancer*. 2021;9(6):e002512.
37 Jorgovanovic D, Song M, Wang L, Zhang Y. Roles of IFN-γ in tumor progression and regression: a review. *Biomarker Res*. 2020;8(1):49.
38 Fenton SE, Saleiro D, Platanias LC. Type I and II interferons in the anti-tumor immune response. *Cancers (Basel)*. 2021;13(5):1037.
39 Müller E, Speth M, Christopoulos PF, et al. Both Type I and Type II interferons can activate antitumor M1 macrophages when combined with TLR stimulation. *Front Immunol*. 2018;9:2520.
40 Prunicki M, Cauwenberghs N, Ataam JA, et al. Immune biomarkers link air pollution exposure to blood pressure in adolescents. *Environ Health*. 2020;19(1):108.
41 Chen R, Li H, Cai J, et al. Fine particulate air pollution and the expression of microRNAs and circulating cytokines relevant to inflammation, coagulation, and vasoconstriction. *Environ Health Perspect*. 2018;126(1).
42 Ji X, Han M, Yun Y, Li G, Sang N. Acute nitrogen dioxide (NO2) exposure enhances airway inflammation via modulating Th1/Th2 differentiation and activating JAK-STAT pathway. *Chemosphere*. 2015;120:722–728.
43 Wang M, Zhao J, Wang Y, et al. Genome-wide DNA methylation analysis reveals significant impact of long-term ambient air pollution exposure on biological functions related to mitochondria and immune response. *Environ Pollut*. 2020;264:114707.
44 Arrêté du 14 mars 2020 portant diverses mesures relatives à la lutte contre la propagation du virus covid-19.
45 Gutman L, Pauly V, Orleans V, et al. Long-term exposure to ambient air pollution is associated with an increased incidence and mortality of acute respiratory distress syndrome in a large French region. *Environ Res*. 2022;212:113383.
46 Menut L, Bessagnet B, Briant R, et al. The CHIMERE v2020r1 online chemistry-transport model. *Geosci Model Dev*. 2021;14(11):6781–6811.
47 Seaton M, O'Neill J, Bien B, et al. A multi-model air quality system for health research: road model development and evaluation. *Environ Model Softw*. 2022;155:105455.
48 Stocker J, Hood C, Carruthers D, McHugh C. ADMS-Urban: developments in modelling dispersion from the city scale to the local scale. *Int J Environ Pollut*. 2012;50(1–4):308–316.
49 Liu H. *Comparing Welch's ANOVA, a Kruskal-Wallis test and traditional ANOVA in case of Heterogeneity of Variance*. 2015.
50 Higham JE, Ramírez CA, Green MA, Morse AP. UK COVID-19 lockdown: 100 days of air pollution reduction? *Air Qual Atmos Health*. 2020:1–8.
51 Srivastava S, Kumar A, Bauddh K, Gautam AS, Kumar S. 21-day lockdown in india dramatically reduced air pollution indices in Lucknow and New Delhi, India. *Bull Environ Contam Toxicol*. 2020;105(1):9–17.
52 Bao R, Zhang A. Does lockdown reduce air pollution? Evidence from 44 cities in northern China. *Sci Total Environ*. 2020 Aug 20;731:139052.
53 Briz-Redón Á, Belenguer-Sapiña C, Serrano-Aroca Á. Changes in air pollution during COVID-19 lockdown in Spain: a multi-city study. *J Environ Sci (China)*. 2021;101:16–26.
54 [COVID-19] Impact du confinement sur la qualité de l'air des grandes villes de la région Sud [Internet]. AtmoSud. 2020 [cited 2021 Aug 30]. Available from: https://www.atmosud.org/publications/covid-19-impact-du-confinement-sur-la-qualite-de-lair-des-grandes-villes-de-la-region.
55 Grundström M, Hak C, Chen D, Hallquist M, Pleijel H. Variation and co-variation of PM10, particle number concentration, NOx and NO2 in the urban air – relationships with wind speed, vertical temperature gradient and weather type. *Atmosp Environ*. 2015;120:317–327.
56 Zhu Y, Xie J, Huang F, Cao L. Association between short-term exposure to air pollution and COVID-19 infection: evidence from China. *Sci Total Environ*. 2020;727:138704.
57 Jiang Y, Wu XJ, Guan YJ. Effect of ambient air pollutants and meteorological variables on COVID-19 incidence. *Infect Control Hospital Epidemiol*. 2020;41(9):1011–1015.
58 Li H, Xu XL, Dai DW, Huang ZY, Ma Z, Guan YJ. Air pollution and temperature are associated with increased COVID-19 incidence: a time series study. *Int J Infect Dis*. 2020;97:278–282.
59 Ogen Y. Assessing nitrogen dioxide (NO2) levels as a contributing factor to coronavirus (COVID-19) fatality. *Sci Total Environ*. 2020;726:138605.
60 Fattorini D, Regoli F. Role of the chronic air pollution levels in the Covid-19 outbreak risk in Italy. *Environ Pollut*. 2020;264:114732.
61 Copat C, Cristaldi A, Fiore M, et al. The role of air pollution (PM and NO2) in COVID-19 spread and lethality: a systematic review. *Environ Res*. 2020;191:110129.
62 Sarmadi M, Rahimi S, Evensen D, Kazemi Moghaddam V. Interaction between meteorological parameters and COVID-19: an ecological study on 406 authorities of the UK. *Environ Sci Pollut Res*. 2021;28:67082–67097. https://doi.org/10.1007/s11356-021-15279-2.
63 Ma Y, Zhao Y, Liu J, et al. Effects of temperature variation and humidity on the death of COVID-19 in Wuhan, China. *Sci Total Environ*. 2020;724:138226.
64 Qi H, Xiao S, Shi R, et al. COVID-19 transmission in Mainland China is associated with temperature and humidity: a time-series analysis. *Sci Total Environ*. 2020;728:138778.
65 Wu Y, Jing W, Liu J, et al. Effects of temperature and humidity on the daily new cases and new deaths of COVID-19 in 166 countries. *Sci Total Environ*. 2020;729:139051.
66 World Health Organization. *WHO Global Air Quality Guidelines: Particulate Matter (PM2.5 and PM10), Ozone, Nitrogen Dioxide, Sulfur Dioxide and Carbon Monoxide [Internet]*. World Health Organization; 2021:.273 p.. [cited 2022 Mar 31]. xxi,. Available from: https://apps.who.int/iris/handle/10665/345329.