Original Research Paper

PM2.5 levels strongly associate with multiple sclerosis prevalence in the Province of Padua, Veneto Region, North-East Italy

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Abstract

Background: Incidence and prevalence trends of multiple sclerosis (MS) in the Province of Padua, North-East Italy, suggest that environmental factors may be associated with increased MS risk.

Objective: To investigate the association of PM2.5 with MS prevalence in one of the most polluted geographical area of Italy.

Methods: In total, 1435 Italian MS patients residing in the Province of Padua were enrolled. The province surface was classified into urban areas, isolated villages, industrialized places, and countryside. Satellite-derived dust-free and sea salt-free PM2.5 concentrations (annual average 1998–2015, $\mu g/m^3$) allowed the identification of 18 classes of territorial sections with statistically evaluable numbers of inhabitants. Possible correlations between residential locality types, PM2.5 concentrations, and MS prevalence were investigated.

Results: MS prevalence was significantly ($p < 0.0001$) higher in urban areas (ranging from 219 in Padua City to 169/100,000 in other urban areas) compared to isolated villages (116/100,000) or rural domains (109/100,000) and strongly correlated with the annual average concentration of PM2.5 ($r = 0.81$, $p < 0.001$). Regression analysis further associated MS cases with PM.2.5 average concentration ($\beta = 0.11$, $p < 0.001$).

Conclusion: In the Province of Padua, MS prevalence is strongly associated with PM2.5 exposure suggesting that air pollutants may be one of the possible environmental risk factors for MS.

Introduction

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) characterized by inflammation, demyelination, and neurodegeneration. Although the etiology of MS is still unknown, the disease has the features of a complex-trait disease, in which genetic, environmental, and behavioral factors likely form a complex puzzle of critical elements that contribute to disease susceptibility.[1]

Experimental and epidemiological studies have shown possible associations between exposure to airborne particulate matter (PM) and negative health effects,[2] including neuroinflammation[3] and neurodegeneration.[4] However, only a few studies have investigated the relationship between PM10 or PM2.5 and MS.[5–8] A fourfold higher risk of monthly MS relapses was observed when the concentration of PM10 (i.e. PM with aerodynamic diameter $\leqslant 10\,\mu m$) was in the highest quartile compared to the lowest one[5] and an increased relative risk of hospitalization ($>40\%$) for MS relapse was observed on the days which were preceded by 1 week of the highest PM10 levels.[6] A study on the potential role of long-term exposure to air pollutants, including PM10, showed a significant different incidence of MS compared to matched controls in Tehran, Iran.[7] Taken together, these findings suggest that PM10 may be a risk factor for MS.

Several studies indicated that PM2.5 can induce a variety of chronic pathologies, including pulmonary disorders, cardiovascular dysfunctions, diabetes mellitus, and neurodegenerative diseases. From an immunopathological point of view, PM2.5 is particularly interesting since these micro-particles are capable of carrying various toxic materials, passing through nasal filtration, reaching the end of the respiratory tract with airflow and accumulating there by diffusion, resulting in damage to other parts of the body through air exchange in the lungs. Most importantly, preliminary data suggest that variations in PM2.5 may influence the immunological network and the course of autoimmune diseases in humans.[9–11] Indeed, PM2.5 has been reported to act by increasing oxidative stress, inflammatory responses, and genotoxicity.[12]

The few studies available failed to find an association between exposure to PM2.5 and MS incidence.[13–15] However, these studies differ in the methodological approach, in the patient populations analyzed and in the average PM2.5 concentrations detected in the study territory. To date, no study has investigated the prevalence of MS in relation to the exposure to PM2.5 in a large industrialized and highly polluted area of Italy. MS incidence and prevalence in the Province of Padua, Veneto Region of Italy, have dramatically increased over the last five decades, from 0.9 to 6.5/100,000/year and from 16 to 182/100,000, respectively,[16] and these trends strongly point out the role of exogenous/environmental agents in determining the risk of MS in this geographical area. Considering that (1) the Veneto Region is one of the most industrialized areas of Italy, (2) the city of Padua (located in the center of the Region) has been recognized as one of the most ozone- and PM-polluted city in Europe by the European Environment Agency,[17] (3) air pollution is generally characterized by high concentrations of ultrafine particles, that is, particle size ≤2.5 μm (PM2.5),[18] and (4) PMs with an aerodynamic diameter smaller than 10 μm have a greater impact on human health, we investigated the association between increased PM2.5 exposure and higher MS prevalence in this Province.

Materials and methods

Study population
Detailed demographic data were obtained from the "15th Italian General Census of Population and Housing",[19] the "Italian Institute of Statistics",[20] and the "2016-updated Demographic and Epidemiology

Bureau of the Veneto Region".[21] This population-based cross-sectional study included 1435/1532 (94%) Italian MS patients living in the Province of Padua on 31 December 2015 (Figure 1). Indeed, 97 patients who moved to a different type of residential locality of the Province (see below) before developing MS were excluded. MS cases were collected from the following sources: clinical records of the six Neurology Units (two in Padua and one in each of the cities of Cittadella, Camposampiero, Monselice, and Piove di Sacco) present in the Province of Padua, first- and second-line drugs-dispensing records, disease-specific exemptions from co-payment to health care, outpatient records of Neurorehabilitation Services, Chronic Care Services, the membership rolls of the local branch of the Italian Society for Multiple Sclerosis (AISM), the archives of the National Pension Institute and the National Health Insurance, and the Epidemiologic Bureau of the Veneto Region (DRG Code). All patients were registered with their Fiscal Code that protected from double counting. The methodology used for prevalence analysis has been previously described in detail.[22,23]

The Province of Padua encompasses an area of 2144 and a total populations of 936,887 inhabitants (481,359 females; 455,528 males), of whom 209,899 (111,205 females; 98,694 males) living in Padua City, while the others are distributed fairly uniformly in smaller towns/villages. However, 10.1% (95,083 inhabitants) of the Province's population is composed of immigrants from Eastern Europe, Northern Africa, China, and Middle Eastern Countries with 32,891 of them (17,530 females; 15,361 males) living in Padua City. Therefore, 841,804 subjects constituted the local Venetian population.

Territory classification and patients mapping
The Province is flat and belongs to the so-called Venetian-Friulian plain, apart from the Euganean hills located in the SouthWest area (Figure 1(a)–(c)). The study territory was classified into four different locality types, according with the Italian Institute of Statistics:[20] urban areas (i.e. aggregate of neighboring houses, strictly closed or with interposed streets and/or squares, characterized by the presence of public services—school, public office, pharmacy, shop, etc.—constituting an independent site of social life and a gathering place for the inhabitants of the surrounding areas), isolated villages (i.e. inhabited area constituted by a group of at least 15 adjacent and neighboring buildings, with at least 15 families, with interposed roads, paths, squares, farmyards, small gardens, uncultivated trees and the like, with the
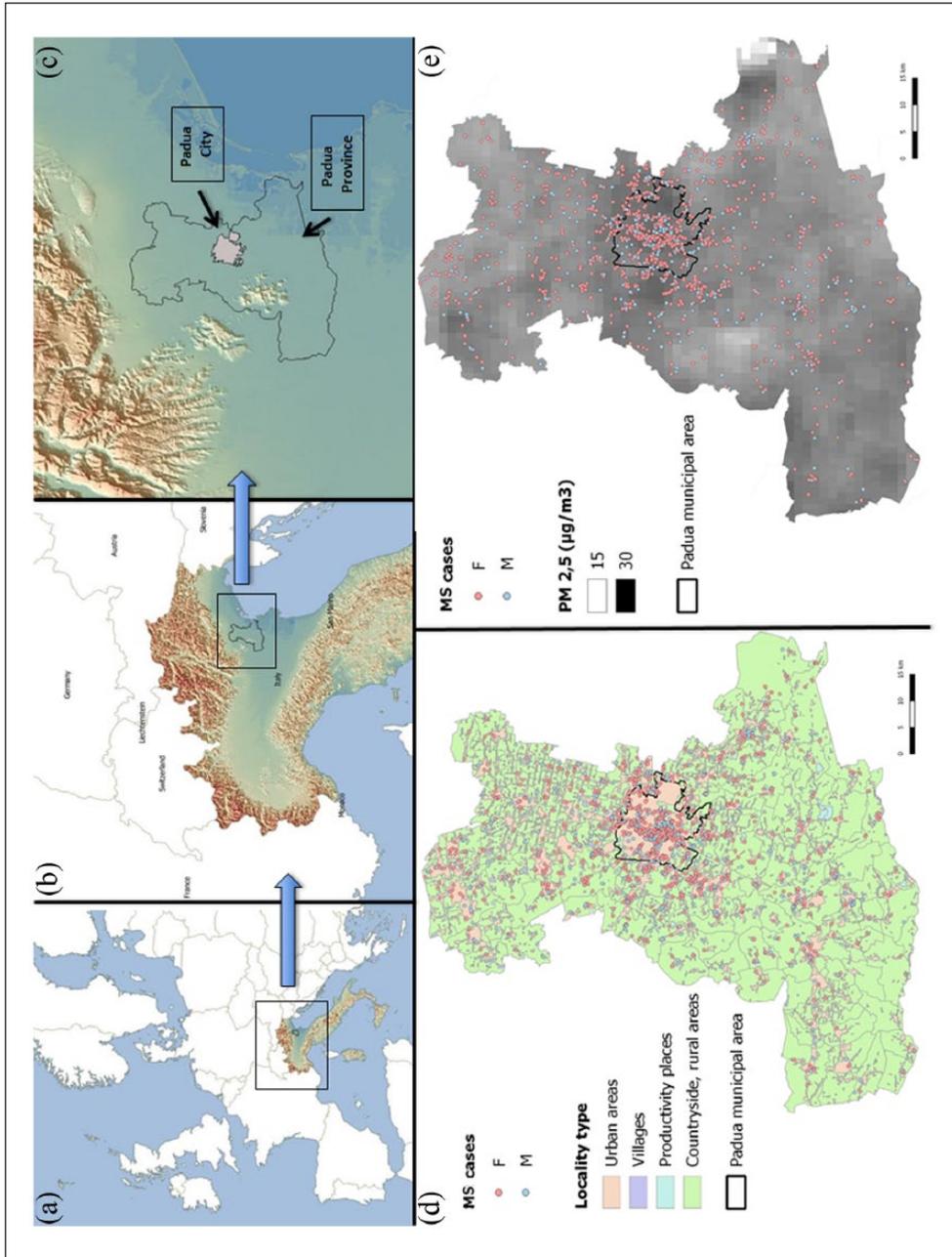
Figure 1. (a)–(c) The geographic location of the Province of Padua. (d) Distribution of MS patients in the Province (red dots: females; light blue dots: males). (e) PM2.5 concentrations (gray gradient) and patient distribution are merged.
F: female; M: male.

interval between house that does not exceed 30 m, and with no gathering place or public services), isolated industrialized places (i.e. extra-urban areas in which there are local units in numbers above 10, or whose total number of employees is more than 200, contiguous or closed with interposed streets and squares, and short solutions continuity not exceeding 200 m; the minimum area must correspond to 5 ha) and countryside (i.e. houses scattered in the municipal area at a distance that could not constitute a village and with interposed fields). Each locality was further categorized into sections that allowed a detailed and complete characterization (i.e. inhabitants, MS cases, PM2.5 concentration) of the study area.

Each patient was mapped according the above-mentioned territorial classification using Quantum Geographic Information System (QGIS) software[24] (Figure 1(d)). Furthermore, the urban domain of Padua was considered as an additional locality type because of its higher numbers of residents compared with all the other urban areas of the Province.

PM2.5 measurement

Since the ground-based measurements, achieved by means of Automatic Monitoring Stations, did not cover the entire area of the Province, the annual PM2.5 averages for the period 1998–2015 were obtained from satellite-derived estimations, after removing the relative contribution of mineral dust and sea salt, following the methodology described in detail elsewhere[25,26] (Figure 1(e)).

Statistical analysis

Overall, age- and sex-specific prevalence rates were calculated, and 95% confidence interval (95% CI) was computed assuming a Poisson distribution. The different prevalence rates observed within subgroups were tested with x^2, analysis of variance with the Bonferroni correction in order to compare PM2.5 concentrations in different locations. Since sections differed in the number of the resident population, the number of MS cases (and not the prevalence value) was explored by means of a regression model, considering the resident population as an additional independent variable. Moreover, to further evaluate this association, sections were clustered according to the mean PM2.5 concentration in 18 classes (each class was composed by 305 sections) having a statistically evaluable numbers of residents and then tested with the Spearman correlation and regression analysis. All tests were

two-tailed with a significant level of 0.05. All the analyses were performed using IBM SPSS Statistics (24.0).

Results

Province partition

The Province was partitioned in 1400 localities and further divided into 5864 sections. Three hundred sixty-nine uninhabited sections (6.3%) were excluded from the analysis. Of the remaining 5495 sections, 3823 (69.6%) were defined as urban (711,088 inhabitants), 1008 (18.3%) as villages (48,098 inhabitants), 40 (0.7%) as industrial areas (1707 inhabitants) and 624 (11.4%) as countryside (77,045 inhabitants). Since sections of the urban areas had significantly higher inhabitant numbers (mean $\pm$ SD: 182.0 ± 227.3) compared to those of villages (47.3 ± 46.4, $p < 0.001$), industrial areas (37.9 ± 42.3, $p < 0.001$) and countryside (122.1 ± 123.3, $p < 0.001$), the variance of MS prevalence could not be analyzed.

MS prevalence is significantly higher in urban areas

The distribution of MS cases in the whole territory of the Province is shown in Figure 1(d). Significant differences in MS prevalence were found among the four types of residential areas. Indeed, the prevalence in Padua City and in the other urban areas was 218.6/100,000 ($CI_{95\%}$: 196.6–240.6) and 168.8/100,000 ($CI_{95\%}$: 157.8–179.8, $p < 0.0001$), respectively, while in isolated villages and in the countryside the values were significantly lower, namely 116.4/100,000 ($CI_{95\%}$: 86.4–146.4) and 109.0/100,000 ($CI_{95\%}$: 86.0–132.0 $p < 0.0001$), respectively (Table 1). The highest prevalence observed in the industrial areas (351.5/100,000; $CI_{95\%}$: 70.5–632.5; $p = 0.09$ compare to urban areas) was not further investigated given the very low number of MS cases (6 patients) and residents (1707 inhabitants) registered in this locality.

Mean PM2.5 annual levels are increased in urban areas

As depicted in Figure 2, people living in urban areas had a significantly higher PM 2.5 exposure ($22.4 \pm 1.2 \, \mu g/m^3$) compared to those living in villages ($21.5 \pm 0.8 \, \mu g/m^3$, $p < 0.001$), industrial areas ($21.6 \pm 0.5 \, \mu g/m^3$, $p < 0.001$), and countryside ($21.6 \pm 0.8 \, \mu g/m^3$, $p < 0.001$). Notably, in each locality type, the section with the lowest mean annual

Table 1. MS prevalence in the Province of Padua according to gender and territorial subtypes.

Residential types	Overall				Female				Male			
	Cases	LP	Prevalence	95% CI	Cases	LP	Prevalence	95% CI	Cases	LP	Prevalence	95% CI
Urban areas	1289	711,088	181.3	±10	882	367,154	240.2	±16	407	343,934	118.3	±11
Padua city only	389	177,926	218.6	±22	265	94,955	279.1	±34	124	82,971	149.4	±26
Other urban areas	900	533,162	168.8	±11	617	272,199	226.7	±18	283	260,963	108.4	±13
Villages	56	48,098	116.4	±30	34	24,004	141.6	±48	22	24,094	91.3	±38
Industrial areas	6	1707	351.5	±281	5	857	583.4	±510	1	850	117.6	±230
Countryside	84	77,045	109.0	±23	59	38,138	154.7	±39	25	38,907	64.3	±25

Local population (LP) numbers refer only to Italy borne residents. Prevalence rates are expressed on 100,000 inhabitants.
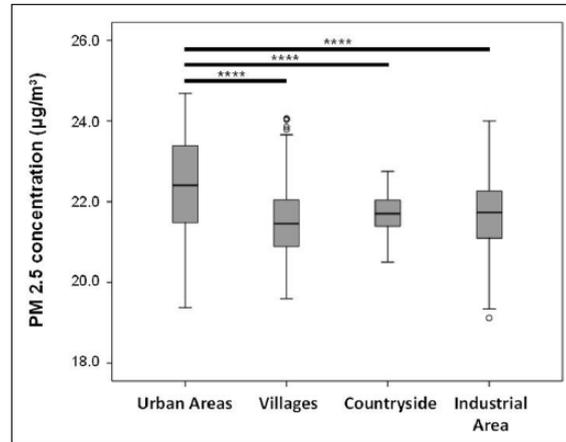
Figure 2. Boxes indicate PM2.5 concentrations (95% error bars are reported) in the different territorial types. Each box represents second and third quartile, reporting the median value. The highest PM2.5 concentrations were observed in urban areas.
****$p < 0.001$.

PM2.5 concentration (urban area: 19.4 µg/m³; villages: 19.6 µg/m³; industrial areas: 20.5 µg/m³; countryside: 19.1 µg/m³) had values that were invariably much higher than the annual average value (10 µg/m³) indicated as the quality limit by the most recent World Health Organization (WHO) guidelines.[20]

Mean PM2.5 annual levels associates with MS prevalence

Regression analysis revealed that the number of MS patients in a given territorial section would be predicted ($r = 0.48$) by both exposed resident population ($\beta = 0.48$, $p < 0.001$) and PM2.5 concentrations ($\beta = 0.11$, $p < 0.001$). The latter association was further confirmed when sections were divided on the basis of PM2.5 average concentrations in 18 groups with statistically evaluable numbers of inhabitants (see Supplementary Materials). Indeed, a strong association between MS prevalence and PM2.5 groups was observed ($r = 0.81$, $p < 0.001$) (Figure 3). No association between PM2.5 levels and gender was found.

Due to the low number of inhabitants and MS cases in villages (56 MS cases), countryside (84 MS cases) and industrial area (6 MS cases) compared to urban areas (1289 MS cases), the effect of the localities could not be explored. When analyzing exclusively urban areas, the association between number of patients ($r = 0.48$) and both the exposed resident population ($\beta = 0.49$, $p < 0.001$) and the PM2.5 concentrations ($\beta = 0.11$, $p < 0.001$) was confirmed.
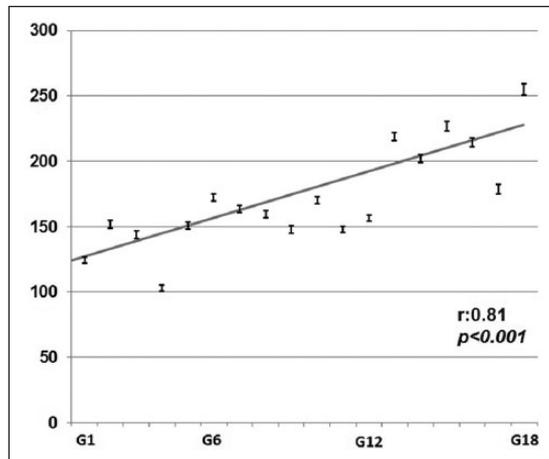
Figure 3. PM2.5 levels associates with MS prevalence in urban areas ($r = 0.81$, $p < 0.001$). Based on mean annual (1998–2015) PM2.5 air concentration, territory sections were divided into 18 groups (G1–G18). Dots indicate MS prevalence rates in each group (error bars report lowest and highest values) on 31 December 2015.

Discussion

MS is a multi-factorial disease probably determined by a complex interaction between susceptible genes and environmental triggers.[27] The geographic distribution of MS has been intensively studied and several epidemiological survey carried out in the Mediterranean area have questioned the north-to-south latitudinal gradient of MS and indicated a more complex geographic/territorial distribution. The dramatic temporal changes in incidence and prevalence observed in the Province of Padua over the last 50 years[16,22,23] strongly focused our attention on environmental risk factors.

Particulate air pollution is a global public health threat.[28] Both acute and chronic exposures to PM, a type of air pollution that comprises a heterogeneous mixture of different particle sizes and chemical compositions, have been associated with premature death from multiple causes.[29–31] In addition to the respiratory and cardiovascular effects of air pollution, emerging evidence indicates that neurological dysfunctions may be correlated with airborne PM exposure.[32–34] Moreover, poor air quality has been suggested to be associated with autoimmune disease exacerbation, including type 1 diabetes, rheumatic disease, and systemic lupus erythematous.[4] Finally, a growing body of epidemiological research indicates that air pollution affects neurological function, as pointed out in a recent review based on 66 articles[35] and in a Canadian study on three common neurodegenerative diseases, that is, dementia, Parkinson, and MS.[36]

In MS patients, an association between the risk of relapse and high PM10 concentration was observed in South-western Finland.[5] Indeed, when the concentration of inhalable PM10 was at the highest quartile, the relapse risk increased over fourfold (odds ratio: 4.143, $p < 0.001$), suggesting that inhalable airborne PM concentrations might be associated with MS activity. A recent work conducted in Iran evaluated the possible association between the distributions of MS prevalent cases with long-term air pollution levels. Prevalent cases showed a clustered pattern in Tehran City and a significant difference in PM10 exposure was observed in MS cases compared to controls.[7] More recently, a higher relative risk of hospitalization for MS relapse has been associated with PM10 exposure in the Lombardy region of Italy.[6] Hospital admission for MS relapse increased by 42% on the days following 1 week with PM10 levels in the highest quartile, with a highly significant (<0.001) p-value for the trend across quartiles. These findings have been confirmed in a study conducted in 536 relapsing MS patients in Strasbourg, France.[37] In the cold season, the PM10 average concentration registered from 1 to 3 days before relapse onset was significantly associated with the risk of relapse. All these observations strongly point out a possible role of air pollution as risk factor for brain inflammation.

PM2.5, which is mostly derived from combustion sources, has a carbon core coated with hydrocarbons, metals ions, and secondary particles derived from nitrogen oxides and sulfur oxides. The large surface area of PM2.5 contributes to the combination of toxic compounds, including polycyclic aromatic hydrocarbons, volatile organic compounds, and transition metals, but this composition may vary according to the pollutant that characterizes the territory analyzed.[38,39] PM2.5 is inhaled into the gas exchange area of human lungs[40,41] where the ultrafine component is released to the systemic circulatory system, thus potentially causing oxidative stress, inflammation, and genotoxicity in various organs, as observed in experimental models.[12] As a matter of fact, PM2.5 has the potential of inducing pro-inflammatory changes in the immune system.

The attempts to demonstrate an association between PM2.5 and MS incidence gave negative results. In a recently published Canadian study, no association was found between MS incidence and long-term exposures to PM2.5, NO_2, and O_3.[13] However, the mean concentrations of PM2.5 observed in Ontario was 9.6 μg/m³, a value below the quality limit indicated by WHO and about half of the values observed in the Province of Padua. Moreover, the follow-up

period of 13 years might not be long enough to observe an association between the increase in MS incidence and the increased PM2.5 exposure. In the US study on two large cohorts of nurses,[14] exposure to PM2.5 was not associated with MS risk. However, also this study, as correctly underlined by the authors, has several limitations, mainly related to the objective difficulties in designing studies aimed at identifying a pathogenic role of a given environmental factor in a complex-trait, multifaceted disease such as MS. Indeed, these studies need a quite long (decades) observational period, the definition of "period of exposure" and "disease duration," which may significantly vary among patients, and unchanged diagnostic criteria and procedures during the observational period (these have been changed at least three times in MS over the last two decades). Moreover, no possibility exists to identify the biological onset of the disease, thus making the preclinical disease phase extremely variable among patients. Hence, for all these reasons, we did not address the possible association between PM2.5 and MS incidence.

In our study, based on a large number of patients living in a highly polluted area, we found that the annual levels of PM2.5 in the period 1998–2015 were associated with the number of MS cases in urban areas. In addition, the worst class of air quality was associated with the highest prevalence rate. As mentioned above, the Province of Padua is exactly in the center of the Veneto Region, that is, one of the most industrialized areas of Italy, with high level of urban sprawl and land consumption.[42] Moreover, Padua City has been repeatedly recognized by the European Environment Agency as one of the most ozone-polluted city and air particulate-polluted city in Europe.[43] Combustion processes and traffic-related emissions constitute the main sources of PM2.5 for urban areas in the Veneto plain; in addition, also secondary aerosol and industrial emissions contribute to affect the PM2.5 air quality.[44] The occurrence of a bacterial community associated with PM is also a matter of concern. In addition, Padua's climate strongly contributes to a worsening of PM impact on health. Indeed, over the last decades, summers have become progressively hotter with temperatures often higher than 40°C, the number of rainy and windy days has progressively decreased, and fog is a common phenomenon in autumn and winter, even lasting all day long and sometimes even lasting several days (with a mean of about 50 foggy days/year). Therefore, the results of our study merit to be taken in consideration for several reasons. First, poor air quality may enhance the seasonal changes in MS relapse occurrence by an increased susceptibility to transmissible infections.[5] Second, exposure to air pollution could contribute to the development of autoimmunity by enhancing antigen presentation, thus up-regulating the effector arm of the immune response and, therefore, leading to autoimmunity.[2] Third, oxidative stress mechanisms have to also be considered since the main mechanism of PM toxicity, related to their physic-chemical characteristic, is related to their ability to induce oxidative stress.[45] Finally, PM may play an immunomodulatory role at the lung level, where autoreactive T cells may be licensed to enter the CNS.[46] However, we would like to point out that our findings suggest that the contribution of PM2.5 to MS risk is probably marginal and should be considered one of the pieces of the complex puzzle of MS susceptibility.

In summary, our findings suggest an association between the risk of MS and PM2.5. Obviously, our study was not aimed at identifying a cause-effect relationship between air pollution and MS, but rather to take a picture of a worrying reality and provide elements of reflection. Since the epidemiological trends strongly indicate exogenous factors as responsible for the increased MS risk, our findings spur the planning of further studies aimed at elucidating the potential pathological role of air pollution in MS as well as in other autoimmune disorders.

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References

1. Thompson AJ, Baranzini SE, Geurts J, et al. Multiple sclerosis. *Lancet* 2018; 391: 1622–1636.

2. Ritz SA. Air pollution as a potential contributor to the "epidemic" of autoimmune disease. *Med Hypotheses* 2010; 74: 110–117.

3. Guo L, Zhu N, Guo Z, et al. Particulate matter (PM10) exposure induces endothelial dysfunction and inflammation in rat brain. *J Hazard Mater* 2012; 213–214: 28–37.

4. Calderón-Garcidueñas L, Solt AC, Henríquez-Roldán C, et al. Long-term air pollution exposure is associated with neuroinflammation, an altered innate immune response, disruption of the blood-brain barrier, ultrafine particulate deposition, and accumulation of amyloid beta-42 and alpha-synuclein in children and young adults. *Toxicol Pathol* 2008; 36: 289–310.

5. Oikonen M, Laaksonen M, Laippala P, et al. Ambient air quality and occurrence of multiple sclerosis relapse. *Neuroepidemiology* 2003; 22: 95–99.

6. Angelici L, Piola M, Cavalleri T, et al. Effects of particulate matter exposure on multiple sclerosis hospital admission in Lombardy region, Italy. *Environ Res* 2016; 145: 68–73.

7. Heydarpour P, Amini H, Khoshkish S, et al. Potential impact of air pollution on multiple sclerosis in Tehran, Iran. *Neuroepidemiology* 2014; 43: 233–238.

8. Bergamaschi R, Cortese A, Pichiecchio A, et al. Air pollution is associated to the multiple sclerosis inflammatory activity as measured by brain MRI. *Mult Scler*. Epub ahead of print 1 August 2017. DOI: 10.1177/1352458517726866.

9. Bernatsky S, Fournier M, Pineau CA, et al. Associations between ambient fine particulate levels and disease activity in patients with systemic lupus erythematosus (SLE). *Environ Health Perspect* 2011; 119: 45–49.

10. Hart JE, Laden F, Puett RC, et al. Exposure to traffic pollution and increased risk of rheumatoid arthritis. *Environ Health Perspect* 2009; 117: 1065–1069.

11. Van Eeden SF, Tan WC, Suwa T, et al. Cytokines involved in the systemic inflammatory response induced by exposure to particulate matter air pollutants (PM(10)). *Am J Respir Crit Care Med* 2001; 164: 826–830.

12. Cho CC, Hsieh WY, Tsai CH, et al. In vitro and in vivo experimental studies of $PM_{2.5}$ on disease progression. *Int J Environ Res Public Health* 2018; 15: 1380.

13. Bai L, Burnett RT, Kwong JC, et al. Long-term exposure to air pollution and the incidence of multiple sclerosis: A population-based cohort study. *Environ Res* 2018; 166: 437–443.

14. Palacios N, Munger KL, Fitzgerald KC, et al. Exposure to particulate matter air pollution and risk of multiple sclerosis in two large cohorts of US nurses. *Environ Int* 2017; 109: 64–72.

15. Carmona R, Linares C, Recio A, et al. Emergency multiple sclerosis hospital admissions attributable to chemical and acoustic pollution: Madrid (Spain), 2001–2009. *Sci Total Environ* 2018; 612: 111–118.

16. Grassivaro F, Puthenparampil M, Pengo M, et al. Multiple sclerosis incidence and prevalence trends in the Province of Padua, Northeast Italy, 1965–2018. *Neuroepidemiology*. Forthcoming 2018.

17. European Environment Agency. European Environment Agency's home page, https://www.eea.europa.eu/ (accessed 14 August 2018).

18. WHO. *Air pollution*. Geneva: WHO, 2018.

19. 15 ° Italian general censuses of population and housing 2011 (1% sample), https://www.istat.it/en/archive/196131 (accessed 14 August 2018).

20. Istat, https://www.istat.it/en/ (accessed 14 August 2018).

21. Regione del Veneto. Demografia ed Epidemiologia, http://www.regione.veneto.it/web/sanita/demografia-ed-epidemiologia (accessed 14 August 2018).

22. Ranzato F, Perini P, Tzintzeva E, et al. Increasing frequency of multiple sclerosis in Padova, Italy: A 30 year epidemiological survey. *Mult Scler* 2003; 9: 387–392.

23. Puthenparampil M, Seppi D, Rinaldi F, et al. Increased incidence of multiple sclerosis in the Veneto region, Italy. *Mult Scler* 2013; 19: 601–604.

24. Welcome to the QGIS project, https://www.qgis.org/en/site/ (accessed 14 August 2018).

25. Van Donkelaar A, Martin RV, Brauer M, et al. Use of satellite observations for long-term exposure assessment of global concentrations of fine particulate matter. *Environ Health Perspect* 2015; 123: 135–143.

26. http://fizz.phys.dal.ca/~atmos/martin/?page_id=140

27. Handel AE, Giovannoni G, Ebers GC, et al. Environmental factors and their timing in adult-onset multiple sclerosis. *Nat Rev Neurol* 2010; 6: 156–166.

28. World Health Organization Regional Office for Europe. *Evolution of WHO air quality guidelines: Past, present and future*. Geneva: World Health Organization Regional Office for Europe, 2017.

29. Katsouyanni K, Schwartz J, Spix C, et al. Short term effects of air pollution on health: A European approach using epidemiologic time series data: The APHEA protocol. *J Epidemiol Community Health* 1996; 50(Suppl. 1): S12–S18.

30. Dockery DW, Pope CA, 3rd, Xu X, et al. An association between air pollution and mortality in six U.S. cities. *N Engl J Med* 1993; 329: 1753–1759.

31. Katsouyanni K, Touloumi G, Samoli E, et al. Confounding and effect modification in the short-term effects of ambient particles on total mortality: Results from 29 European cities within the APHEA2 project. *Epidemiology* 2001; 12: 521–531.

32. Calderón-Garcidueñas L, Reed W, Maronpot RR, et al. Brain inflammation and Alzheimer's-like pathology in individuals exposed to severe air pollution. *Toxicol Pathol* 2004; 32: 650–658.

33. Elder A, Gelein R, Silva V, et al. Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. *Environ Health Perspect* 2006; 114: 1172–1178.

34. Calderón-Garcidueñas L, Azzarelli B, Acuna H, et al. Air pollution and brain damage. *Toxicol Pathol* 2002; 30: 373–389.

35. Xu X, Ha SU and Basnet R. A review of epidemiological research on adverse neurological effects of exposure to ambient air pollution. *Front Public Health* 2016; 4: 157.

36. Chen H, Kwong JC, Copes R, et al. Living near major roads and the incidence of dementia, Parkinson's disease, and multiple sclerosis: A population-based cohort study. *Lancet* 2017; 389: 718–726.

37. Roux J, Bard D, Le Pabic E, et al. Air pollution by particulate matter PM_{10} may trigger multiple sclerosis relapses. *Environ Res* 2017; 156: 404–410.

38. Pandey P, Patel DK, Khan AH, et al. Temporal distribution of fine particulates ($PM_{2.5}$: PM_{10}), potentially toxic metals, PAHs and Metal-bound carcinogenic risk in the population of Lucknow City, India. *J Environ Sci Heal A* 2013; 48: 730–745.

39. Weinstein JP, Hedges SR and Kimbrough S. Characterization and aerosol mass balance of PM2.5 and PM10 collected in Conakry, Guinea during the 2004 Harmattan period. *Chemosphere* 2010; 78: 980–988.

40. Schulze F, Gao X, Virzonis D, et al. Air quality effects on human health and approaches for its assessment through microfluidic chips. *Genes* 2017; 8: 244.

41. Xing Y-F, Xu Y-H, Shi M-H, et al. The impact of PM2.5 on the human respiratory system. *J Thorac Dis* 2016; 8: E69–E74.

42. European Environment Agency. Towards an urban atlas: Assessment of spatial data on 25 European cities and urban areas, https://www.eea.europa.eu/highlights/20020807135903 (accessed 14 August 2018).

43. European Environment Agency. Air Quality e-Reporting (AQ e-Reporting), https://www.eea.europa.eu/data-and-maps/data/aqereporting-8 (accessed 14 August 2018).

44. Stefania S, Marta C, Elena I, et al. Urban air quality in a mid-size city — PM2.5 composition, sources and identification of impact areas: From local to long range contributions. *Atmospheric Research* 2017; 186: 51–62.

45. Crobeddu B, Aragao-Santiago L, Bui LC, et al. Oxidative potential of particulate matter 2.5 as predictive indicator of cellular stress. *Environ Pollut* 2017; 230: 125–133.

46. Odoardi F, Sie C, Streyl K, et al. T cells become licensed in the lung to enter the central nervous system. *Nature* 2012; 488: 675–679.