Air pollution is associated to the multiple sclerosis inflammatory activity as measured by brain MRI

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This is the peer reviewed version of the following article: Air pollution is associated to the multiple sclerosis inflammatory activity as measured by brain MRI, which has been published in final form at 10.1177/1352458517726866. This article may be used for non-commercial purposes.

Keywords: Multiple sclerosis, air pollution, brain MRI, inflammation

ABSTRACT

BACKGROUND: Some environmental factors have been already associated to increased risk of multiple sclerosis (MS), but it is plausible that additional factors might play a role.

OBJECTIVE: To investigate in MS patients the relationship between inflammatory activity, detected by brain magnetic resonance imaging (MRI) with gadolinium (Gd), and air pollution, namely particulate matters with diameter less than 10 μm (PM_{10}).

METHODS: We analyzed from 52 remitting MS patients 226 brain MRIs, 34% with (Gd+MRI) and 66% without (Gd-MRI) T1-Gd-enhancing lesions. Daily recording of PM_{10} in the 30 days before MRI examination was obtained by monitors depending on the residence of subjects.

RESULTS: PM_{10} levels in the 5, 10, 15, 20 and 25 days before brain MRIs were higher (plus 16%, 21%, 24%, 25% and 21% respectively) with reference to Gd+MRI vs. Gd-MRI. There was a significant association between Gd+MRI and PM_{10} levels ($p=0.013$), independently of immune therapies, smoker status and season. In patients who had two

repeated MRIs with opposite outcomes (Gd-MRI and Gd+MRI), PM_{10} levels were strongly higher in concurrence with Gd+MRI ($p<0.0001$).

CONCLUSION: Our findings suggest that air pollution may be a risk factor for MS favouring inflammatory exacerbations.

INTRODUCTION

Multiple Sclerosis (MS) is an inflammatory demyelinating disorder of central nervous system which is characterized, mainly in its early phase, by periods of exacerbation and remission.

MS relapse amount varies during the year, with an increased relapse rate in autumn/winter [1].

Infections [2], stress [3], low vitamin D levels [4], have been associated to an increased risk to develop MS. However, none of these factors can explain the variability of MS course and relapse occurrence, thus it is plausible that additional environmental factors may play a role.

While smoking is related to the risk of MS [5] and to its unfavourable evolution [6], no association of tobacco use with clinical relapses or with magnetic resonance imaging (MRI) gadolinium (Gd) enhancing lesions has been found, indicating that inflammatory activity could be mediated through other mechanisms [7]. On the contrary, an increased number of clinical relapses has been found to be associated with ambient levels of air pollution as indicated by higher concentration of airborne particulate matter (PM) before relapses [8,9].

Aim of our study is to investigate in MS patients if particulate air pollution is related to MS inflammatory activity detected by post-Gd brain MRI.

PATIENTS AND METHODS

Patients

We included persons with the following characteristics:

- diagnosis of MS according to 2010 McDonald criteria [10]

- relapsing-remitting course (RR) [11]

- resident in the province of Pavia, northern Italy

- brain MRI performed in the Neuroradiology Department of C. Mondino National Neurological Institute in Pavia (Italy) in clinical practice, that is for a recent clinical relapse or for a standard monitoring outside clinical relapse

- free from corticosteroid therapy in the last month before MRI examination, in order to eliminate possible effect of acute treatment on MRI Gd-enhancement

- naïve to disease modifying therapies (DMT) or on first-line DMT (interferons, glatiramer acetate, teriflunomide, dymethylfumarate)

- informed consent form signed

At the day of MRI examination, we reported for each included patient: gender, age, disease duration, clinical course, DMT use (if applicable), lifetime smoking habits (current smoker or not).

Brain MRI

All imaging studies were performed on a 1.5 T Philips Gyroscan system (Koninklijke, The

Netherlands), using axial PD-T2 weighted (slice thickness = 3 mm, TR = 2500 ms, TE = 22/100 ms, number of slices = 44, FOV = 50 mm, matrix = 256 × 256, voxel size = 0,98 mm), axial FLAIR (slice thickness = 3 mm, TR = 8000 ms, TE = 125 ms, number of slices = 44, FOV = 250 mm, matrix = 256 × 512, voxel size = 0.98 mm), sagittal FLAIR (slice thickness = 5 mm, TR = 11000 ms, TE = 140 ms, number of slices = 22, FOV = 250 mm, matrix = 256 × 256, voxel size = 0.98 mm) sequences and also after gadolinium injection with axial T1-SE weighted (slice thickness = 3 mm, TR = 650 ms, TE = 12 ms, number of slices = 44, FOV = 250 mm, matrix = 256 × 256, voxel size = 0.98 mm) images.

Inflammatory activity of the disease was assessed on the basis of the presence (Gd+MRI) or absence (Gd-MRI) of at least one T1-weighted contrast enhancing lesion after Gd injection.

Air pollution measurement

Ambient concentrations of PM_{10}, which is physically defined by the mass median aerodynamic diameter <10 μm of the pollutant particles, were obtained from the regional air quality monitoring network (ARPA, Regional Environmental Protection Agency). Briefly, we geocoded the addresses of monitoring stations and study subjects in order to assign to each subject the daily PM_{10} concentration from the nearest monitor to home address. PM_{10} concentrations were obtained from the seven stations located in the Pavia province as all the study participants were resident in that area. The exposure was collected from the date of subject MRI to the 30 days before and 5-days averages were used in the models.

Statistical analyses

Descriptive indices were mean, standard deviation (SD), median and interquartile range (IQR) for continuous variables and proportions for categorical ones.

Normality distribution of PM_{10} was assessed graphically by the Shapiro-Wilk test and by the skewness and kurtosis values compared to their standard error. Due to the asymmetric

frequency distribution of PM_{10}, analyses were carried out on natural logarithm (ln)-transformed data.

In order to investigate the relationship between PM_{10} levels at time of detection prior MRI examination and presence of MRI Gd-enhancing lesions, a three ways analysis of variance mixed model was performed. The model took into account the effect of the time of detection prior MRI (repeated factor) and the effect of the presence of Gd-enhancing lesions (factor). The subject id variable was added as a random effect to model the correlation within brain MRIs. The model also considered the effect of interaction between time of detection prior and presence of MRI Gd-enhancing lesions. A linear model was used to estimate the p-values and the regression coefficients for the differences in percentage of PM_{10} level regarding the presence of Gd-enhancing lesions.

A logistic regression, adjusted for groups defined by subject id, was performed to quantify the risk of MRI Gd-enhancing lesions associated with PM_{10} levels in the previous 5-10-15-20-25-30 days, treatment with DMT, smoker status and season (winter, spring, summer, autumn).

For the subgroup of patients who had two repeated MRIs with opposite outcomes (Gd-MRI and Gd+MRI), a Mann-Whitney non-parametric test was used to assess the difference of PM_{10} medians in the times before MRIs.

Statistical significance was taken at the <0.05 level.

All analyses were conducted using STATA/SE for Windows, version 14 (StataCorp, College Station, TX, U.S.A.).

RESULTS

We analyzed 226 brain MRIs from 52 RRMS patients (average MRI/patients: 3.3±2.3) with the following characteristics: 15 males, 37 females; mean age 37.1±11 years; mean disease duration 10.7±5.5 years; mean EDSS 3.3±2; 24 smokers (46%); 38 on first line DMT treatment (73%).

Seventy-seven MRIs (34.1%) showed at least one T1-contrast enhancing lesions (Gd+MRI), indicating a current inflammatory activity, while 149 (65.9%) had no T1-contrast enhancing lesions (Gd-MRI). Twenty-nine Gd+MRIs were detected in concurrence with clinical relapse (38%), while 48 Gd+MRI (62%) were detected even in absence of clinical relapse.

Concerning exposure to air pollutants in the 30 days preceding MRI examination, PM_{10} levels differed along the year being, as expected, higher in winter and lower in summer (Fig.1).

PM_{10} exposure levels in the 30 days preceding brain MRI examinations were higher in the presence of Gd-enhancing lesions (Fig.2).

The analysis of variance indicated that the mean levels of ln(PM_{10}) were statistically higher in the presence of Gd+MRI (3.91±0.38 *vs* 3.77±0.41; F=6.81, df=1,224; p=0.010). Indeed, the parameters of the linear model indicated statistically significant PM_{10} increases at 5, 10, 15, 20 e 25 days prior MRI examinations, in relation with Gd+MRI.

Furthermore, neither the effect of time nor the effect of the interaction between time and Gd+MRI were statistically significant (time: F=0.82, df= 2,478; p=0.45; interaction: F=1.93, df=2,478; p=0.143). No significant difference was found at 30 days before MRI (Table 1).

A logistic regression model, adjusted for the effects of variables possibly influencing the risk of inflammation, confirmed a significant association between Gd+MRI and PM_{10} levels in the 10 (OR=1.016, p=0.035) and 15 days (OR=1.021, p=0.013) before brain MRI examination, independently of DMT, smoker status and season (Table 2). This indicates that when PM_{10} increases of 10 µg/m^3 in the previous 15 days, the risk of inflammatory lesions increases of 23% ($OR_{10\mu g/m^3} = 1.021^{10}$). Thus, for an increment of 30 µg/m^3 (corresponding to the difference between the highest and lowest quartiles) the risk increases of 86% ($OR_{30\mu g/m^3} = 1.021^{30}$).

Finally, we analyzed data of 35 patients who had two repeated MRIs separated by at least 6 months, that showed opposite findings (Gd+MRI followed by Gd+MRI or vice versa): 22 who had a Gd+MRI followed by a Gd-MRI, and 13 who had a Gd-MRI followed by a Gd+MRI. PM_{10} levels were strongly higher when Gd+MRI (Table 3).

DISCUSSION

MS is recognized as a complex neurological disease, whose aetiology implicates genetic, epigenetic and environmental factors interacting with one another [12,13].

Among environmental factors, higher risk of MS has been recognized to be related with infective agents, mainly EBV [14], and non-infective factors, mainly levels of vitamin D and smoke habits [15]. Smoking can stimulate dysimmune/inflammatory responses through some mechanisms, such as by increasing pro-inflammatory cytokines and reducing Treg activities [16]. Anyway, the pro-inflammatory role of tobacco use does not seem to be due only to nicotine. Indeed, nicotine could have an anti-inflammatory effect, as suggested by the study of Hedström *et al.* [17] that reported that tobacco-snuffers have a lower risk of developing MS in comparison with general population and with tobacco smokers. Otherwise, the risk for MS is higher also in passive smokers [18] and in waterpipe smokers [19]. Those findings suggest that carbon monoxide and other combustion products, but not nicotine, are risk factors for MS. Combustion products are largely present in the air of industrialized areas, where prevalence and incidence of immune disorders, MS included, has steadily increased possibly due to the changes in environment [20].

One of the main outdoors pollutant is PM, that is a complex mixture of constituent chemicals. It is classified by particle size, although this physical classification oversimplifies the molecular makeup, which may vary from urban to rural areas,

including elemental and organic carbons, metals, sulfates, nitrates and microbial contaminants. Concerning PM_{10}, even if the vast majority of its sources are anthropologic (heating appliances, industry, fuel), there are also natural sources such as fires and volcano eruptions [21]. To this regard, the intriguing observation of a peculiar cluster of MS in Linguaglossa (a little town located in the side of the still active Etna volcano in Sicily, Italy) [22] suggests a relation between PM_{10} and MS. There are few large epidemiological studies that have considered the possible role of PM_{10} in MS risk and course. Ecological studies found a significant association between MS prevalence and PM_{10} levels in Georgia, USA [23] and in Tehran, Iran [24]. PM_{10} concentrations were also associated to the occurrence of MS relapses in Finland [8] and in France [9]. A large retrospective study in Lombardy region, the Italian area where air pollution has the higher impact, reported a 42% increase of hospital admission due to MS exacerbations when PM_{10} levels were in the highest quartile in the previous week [25]. All the above-mentioned studies referred to clinical manifestations of inflammatory activity, while we are aware that the presence of new/impaired neurological symptoms is just the 'tip of the iceberg' of the inflammatory manifestations. In clinical practice, brain blood barrier breakdown (BBB) as assessed by contrast-enhanced T1-weighted MR imaging is currently the gold standard marker of inflammation in MS [26], thus for the first time we focused on MRI examinations in concurrence with PM_{10} levels. We found a strong association between MS inflammatory activity assessed by MRI and

concentration of PM_{10} in the period preceding MRI examinations. This means that for an MS patient the probability to have an 'active' MRI is significantly higher if PM_{10} levels are elevated. In fact, for the increment of 30 $\mu g/m^3$ of PM_{10}, the risk of having an inflammatory lesion increases of 86%. The significant association between PM_{10} levels and MRI inflammatory activity was also confirmed when we adjusted for the effects of the variables that can influence the risk of inflammation, that is DMT, smoking, season. The relation between PM_{10} levels and Gd+MRI was even more impressive when we compared two different MRI examinations coming from the same patient, in which the main covariates (age, gender, clinical course, treatment) were obviously well controlled. The mechanism through PM_{10} stimulates dysimmune/inflammatory responses could be the same already described for smoking, mainly increasing pro-inflammatory cytokines expression [16], but other mechanisms are conceivable: free radicals' overproduction, BBB breakdown, vitamin D deficiency, mitochondrial dysfunction [27]. In addition, air pollutants might act as local stimulator of the lung, that is hypothesized as an immuno-reactive organ involved in MS ethiopathogenesis contributing to the activation of potentially auto-aggressive T cells [28].

We are aware of a possible confounding effect by UV radiation/Vitamin D levels. In fact, air pollution exposure, by limiting delivery of UVB to the ground, could indirectly reduce Vitamin D production [27] and consequently increase the risk of relapse. Thus, future studies on air pollution effects should include in the analyses Vitamin D levels.

In conclusion, our findings suggest that air pollution may be an additional environmental factor playing a role in inflammatory processes characterizing MS course.

REFERENCES

1. Spelman T, Gray O, Trojano M, et al. Seasonal variation of relapse rate in multiple sclerosis is latitude dependent. *Ann Neurol* 2014; 76:880–890.
2. Sotelo J, Ordoñez G, Pineda B, et al. The participation of varicella zoster virus in relapses of multiple sclerosis. *Clin Neurol Neurosurg* 2014;119:44-48.
3. Artemiadis AK1, Anagnostouli MC, Alexopoulos EC. Stress as a risk factor for multiple sclerosis onset or relapse: a systematic review. *Neuroepidemiology* 2011; 36:109-120.
4. Hartl C, Obermeier V, Gerdes LA, et al. Seasonal variations of 25-OH vitamin D serum levels are associated with clinical disease activity in multiple sclerosis patients. *J Neurol Sci* 2017; 375:160-164.

   ~~Olsson T, Barcellos LF, Alfredsson L. Interactions between genetic, lifestyle and environmental risk factors for multiple sclerosis. *Nat Rev Neurol* 2017; 13: 25-36.~~
5. Backhaus I, Mannocci A, Lemmens PH, et al. Smoking as a risk factor for developing Multiple Sclerosis: a meta-analysis of observational studies. *Clin Ter* 2016; 167: 82-92.
6. Manouchehrinia A, Tench CR, Maxted J, et al. Tobacco smoking and disability progression in multiple sclerosis: United Kingdom cohort study. *Brain* 2013;136: 2298-2304.
7. Kvistad S, Myhr KM, Holmøy T, et al. No association of tobacco use and disease activity in multiple sclerosis. *Neurol Neuroimmunol Neuroinflamm* 2016; 3: e260.

8. Oikonen M, Laaksonen M, Laippala P, et al. Ambient air quality and occurrence of multiple sclerosis relapse. *Neuroepidemiology* 2003; 22: 95–99.
9. Rouxa J, Barda D, Le Pabica E. Air pollution by particulate matter PM10 may trigger multiple sclerosis relapses. *Environmental Research* 2017; 156:404–410.
10. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol* 2011; 69: 292-302.
11. Lublin FD, Reingold SC, Cohen JA, et al. Defining the clinical course of multiple sclerosis: the 2013 revisions. *Neurology* 2014; 83: 278-286.
12. Ramagopalan SV and Ebers GC. Genes for multiple sclerosis. *Lancet* 2008; 371: 283–285.
13. 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.
14. Ascherio A and Munger KL. Environmental risk factors for multiple sclerosis: part I: the role of infection. *Ann Neurol* 2007; 61: 288–299.
15. Ascherio, A and Munger, KL. Environmental risk factors for multiple sclerosis. Part II: Noninfectious factors. *Ann Neurol* 2007; 61: 504–513.
16. Correale J and Farez MF. Smoking worsens multiple sclerosis prognosis: two different pathways are involved. *J Neuroimmunol* 2015; 281: 23-34.

17. Hedström AK1, Bäärnhielm M, Olsson T, Alfredsson L. Tobacco smoking, but not Swedish snuff use, increases the risk of multiple sclerosis. *Neurology* 2009; 73: 696-701.

18. Hedström AK, Bäärnhielm M, Olsson T, Alfredsson L. Exposure to environmental tobacco smoke is associated with increased risk for multiple sclerosis. *Mult Scler* 2011; 17: 788-793.

19. Abdollahpour I, Nedjat S, Sahraian MA, et al. Waterpipe smoking associated with multiple sclerosis: A population-based incident case-control study. *Mult Scler* 2016; 1352458516677867.

20. Rose NR. Prediction and Prevention of Autoimmune Disease in the 21st Century: A Review and Preview. *Am J Epidemiol* 2016; 183: 403-406.

21. Fano V, Cernigliaro A, Scondotto S, et al. Health effects of environmental contamination due to volcanic ash of Mount Etna in autumn 2002. *Epidemiol Prev* 2005; 29: 180-187.

22. Nicoletti A, Patti F, Lo Fermo S, et al. A possible spatial and temporal cluster of multiple sclerosis in the town of Linguaglossa, Sicily: an update. *Mult Scler* 2009; 15: 129-30.

23. Gregory AC, Shendell DG, Okosun IS, et al. Multiple Sclerosis disease distribution and potential impact of environmental air pollutants in Georgia. *Sci Total Environ* 2008; 396: 42–51.

24. Heydarpour P, Amini H, Khoshkish S, et al. Potential impact of air pollution on multiple sclerosis in Tehran, Iran. *Neuroepidemiology* 2014; 43: 233-238.
25. 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.
26. Rovaris M and Filippi M. Contrast enhancement and the acute lesion in multiple sclerosis. *Neuroimaging Clin N Am* 2000;10: 705-716.
27. Esmaeil Mousavi S, Heydarpour P, Reis J, et al. Multiple sclerosis and air pollution exposure: Mechanisms toward brain autoimmunity. *Med Hypotheses* 2017; 100: 23-30.
28. 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.

LEGENDS

Figure 1
PM10 average levels in the 30 days preceding MRI examination.

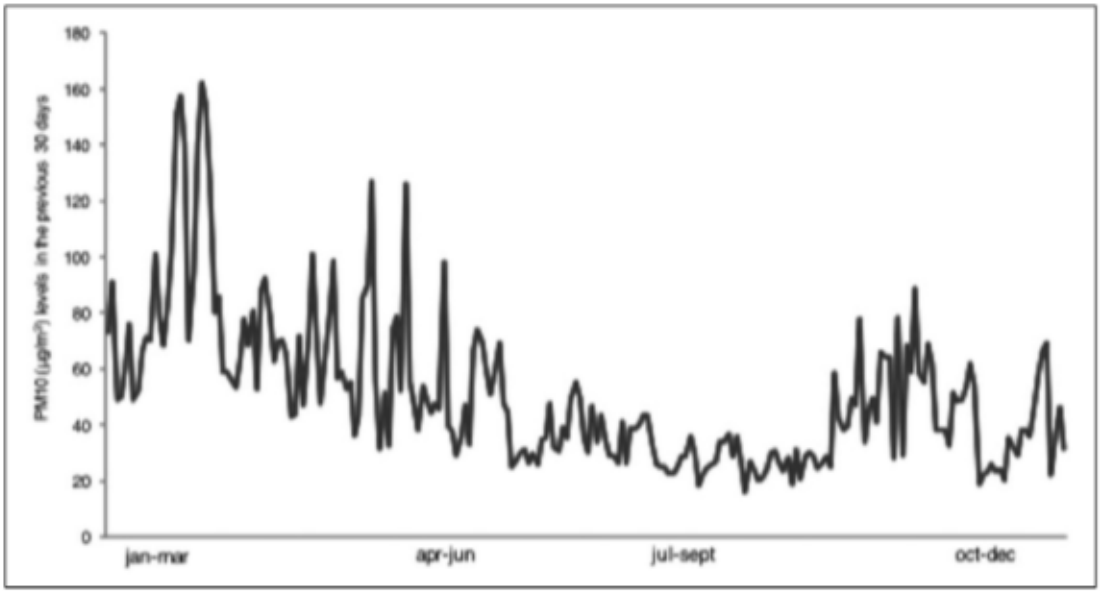
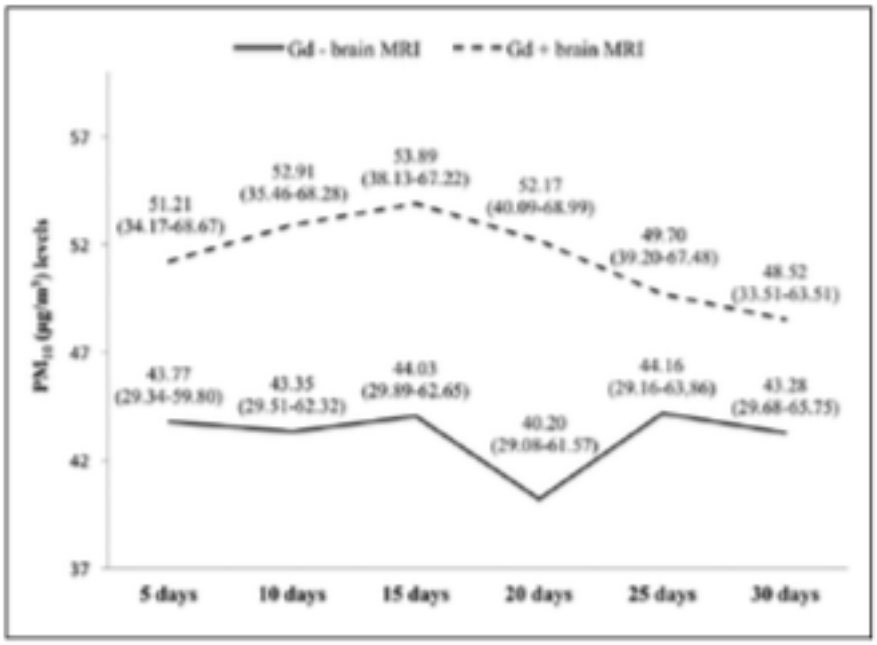


Figure 2.
Median PM10 exposure levels and IQR (in brackets) on the 30 days preceding brain MRI examinations, split on the basis of the presence (Gd+) or absence (Gd−) of Gd-enhancing lesions.



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Response	ln(PM_{10})	Independent variable	b	SE	p-value	Δ(PM_{10})%
PM_{10} level	5 days	Gd+ brain *vs* Gd - brain MRI	0.150	0.067	0.027	16
(logarithmic scale)	10 days	Gd+ brain *vs* Gd - brain MRI	0.174	0.064	0.008	19
	15 days	Gd+ brain *vs* Gd - brain MRI	0.183	0.060	0.003	20
	20 days	Gd+ brain *vs* Gd - brain MRI	0.175	0.006	0.007	19
	25 days	Gd+ brain *vs* Gd - brain MRI	0.143	0.058	0.015	15
	30 days	Gd+ brain *vs* Gd - brain MRI	0.065	0.068	0.339	7

Table 1 - Parameter estimation of the linear model to quantify the variation of PM_{10} in presence of Gd enhancing lesions on brain MRI (Gd+).

b=regression coefficients; SE= standard error (b); Δ(PM_{10}) %= percentage increase of PM_{10} in presence of Gd enhancing lesions on brain MRI.

	5 days			10 days			15 days		
Variables	**OR**	**CI(95%)**	**p**	**OR**	**CI(95%)**	**p**	**OR**	**CI(95%)**	**p**
PM_{10} ($\mu g/m^3$)	1.010	0.10-1.02	0.146	1.016	1.00-1.03	**0.035**	1.021	1.00-1.04	**0.013**
DMT									
Absence	1			1			1		
Presence	0.813	0.46-1.43	0.475	0.821	0.46-1.45	0.497	0.843	0.47-1.50	0.559
Smoke									
Absence	1			1			1		
Presence	0.631	0.34-1.16	0.137	0.617	0.33-1.14	0.121	0.570	0.31-1.06	0.076
Season									
Winter	1			1			1		
Spring	1.203	0.49-2.94	0.686	1.424	0.56-3.57	0.452	1.739	0.66-4.57	0.262
Summer	0.515	0.19-1.34	0.175	0.655	0.24-1.78	0.409	0.834	0.29-2.40	0.738
Autumn	1.039	0.48-2.23	0.921	1.109	0.51-2.40	0.793	1.290	0.57-2.87	0.534
	20 days			**25 days**			**30 days**		
Variables	**OR**	**CI(95%)**	**p**	**OR**	**CI(95%)**	**p**	**OR**	**CI(95%)**	**p**
PM_{10} ($\mu g/m^3$)	1.010	0.10-1.02	0.150	1.012	0.10-1.03	0.162	1.001	0.10-1.01	0.811
DMT									
Absence	1			1			1		
Presence	0.830	0.47-1.46	0.520	0.835	0.47-1.47	0.533	0.823	0.47-1.45	0.500
Smoke									
Absence	1			1			1		
Presence	0.586	0.32-1.08	0.089	0.599	0.32-1.10	0.100	0.629	0.34-1.15	0.132
Season									

Winter	1			1			1		
Spring	1.211	0.48-3.00	0.678	1.263	0.47-3.35	0.639	0.912	0.37-2.24	0.842
Summer	0.524	0.19-1.40	0.200	0.558	0.18-1.70	0.306	0.343	0.12-0.94	0.039
Autumn	1.094	0.49-2.42	0.825	1.124	0.49-2.57	0.782	0.876	0.37-2.05	0.763

Table 2. Association of risk factors with presence or absence of Gd enhancing lesions on brain MRI after adjustment for groups defined by subject id, in the 5, 10, 15, 20, 25 and 30 days before MRI examination.

		day 5	day 10	day 15	day 20	day 30
	percentile					
Gd-MRI	25	23.40	22.06	22.83	23.33	23.19
	50	29.54	29.88	29.24	30.09	29.67
	75	47.75	48.74	46.04	46.49	48.35
	percentile					
Gd+MRI	25	36.46	39.48	41.92	41.85	41.19
	50	64.65	63.51	62.37	62.22	61.85
	75	76.63	72.30	78.38	75.25	78.26
Mann-Whitney test	U	304	262	233	234	230
	p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Table 3. PM_{10} levels prior brain MRIs in 35 patients who had two examinations with opposite outcomes: MRI with enhancing lesions (Gd+MRI) followed by MRI without enhancing lesions (Gd-MRI) or vice versa.