RESEARCH PAPER

Multicentre, population-based, case–control study of particulates, combustion products and amyotrophic lateral sclerosis risk

Anne E Visser,[1] Fabrizio D'Ovidio,[2] Susan Peters,[1,3] Roel CH Vermeulen,[3] Ettore Beghi,[4] Adriano Chiò,[2] Jan H Veldink,[1] Giancarlo Logroscino,[5,6] Orla Hardiman,[7] Leonard H van den Berg,[1] for the Euro-MOTOR consortium

For numbered affiliations see end of article.

Correspondence to
Leonard H van den Berg, Department of Neurology, Brain Centre Rudolf Magnus, University Medical Centre Utrecht, Utrecht 3584CX, The Netherlands; L.H.vandenBerg@umcutrecht.nl

AEV and FD contributed equally.

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ABSTRACT

Objective To investigate whether exposure to particulates and combustion products may explain the association between certain occupations and amyotrophic lateral sclerosis (ALS) risk in a large, multicentre, population-based, case–control study, based on full job histories, using job-exposure matrices, with detailed information on possible confounders.

Methods Population-based patients with ALS and controls were recruited from five registries in the Netherlands, Ireland and Italy. Demographics and data regarding educational level, smoking, alcohol habits and lifetime occupational history were obtained using a validated questionnaire. Using job-exposure matrices, we assessed occupational exposure to silica, asbestos, organic dust, contact with animals or fresh animal products, endotoxins, polycyclic aromatic hydrocarbons and diesel motor exhaust. Multivariate logistic regression models adjusting for confounding factors were used to determine the association between these exposures and ALS risk.

Results We included 1557 patients and 2922 controls. Associations were positive for all seven occupational exposures (ORs ranging from 1.13 to 1.73 for high vs never exposed), and significant on the continuous scale for silica, organic dust and diesel motor exhaust (p values for trend ≤0.03). Additional analyses, adding an exposure (one at a time) to the model in the single exposure analysis, revealed a stable OR for silica. We found similar results when patients with a *C9orf72* mutation were excluded.

Conclusion In a large, multicentre study, using harmonised methodology to objectively quantify occupational exposure to particulates and combustion products, we found an association between ALS risk and exposure to silica, independent of the other occupational exposures studied.

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INTRODUCTION

The aetiology of the fatal neurodegenerative disease, amyotrophic lateral sclerosis (ALS), is still largely unknown, but best described as the interplay of genetic and environmental factors.[1 2] Regarding environmental factors, occupational history has been studied extensively[3]; an association with ALS has been reported for farmers,[4] veterinarians,[3 5] firefighters,[6] flight attendants[7] and truck drivers.[8] Exposure to particulates (silica, asbestos, animal contact, endotoxin exposure) and combustion products (polycyclic aromatic hydrocarbons (PAH), diesel motor exhaust (DME)) could hypothetically link these occupations with ALS.

However, not all studies have reported positive associations for either the occupation or the proposed underlying exposure.[9–13] This may, in part, be due to methodological differences between these studies, such as study design or data collection; use of a single occupation to estimate exposure (eg, the longest or last held job) instead of full occupational histories; exploration of a single exposure instead of combined exposures; self-reported occupational exposures; or inferences about the underlying exposures when testing for association with occupational groups. In contrast, job-exposure matrices (JEM) provide an objective and agent-specific method for exposure assessment on lifetime occupational histories in case–control studies.

We aim to investigate previously suggested associations between ALS and occupational exposure to particulates and combustion products, based on full job histories and using JEMs in a large, multicentre, population-based, case–control study with detailed information on possible confounders.

METHODS

Study population

All participants were recruited as part of a case–control study undertaken by the Euro-MOTOR consortium between 2011 and 2014. Population-based cases representing patients with definite (laboratory supported), probable or possible ALS according to the revised El Escorial criteria[14] were matched to controls based on age, gender and residency. The study was carried out in three European countries, over five regions: the Netherlands, Ireland, Apulia, Lombardy, and Piedmont and Valle d'Aosta in Italy. The overall aim and a more extensive description of this project has been provided elsewhere.[15] All participants gave written informed consent.

Data collection

Using the same structured questionnaire, all five centres collected demographic characteristics of participants and data on their educational level, smoking and alcohol drinking habits and lifetime

occupational history. In order to allow comparability between cohorts, the highest educational degree obtained was categorised into International Standard Classification of Education (ISCED) 0–4 and ISCED 5–8, according to the ISCED 2011.[16] Data on age were collected at the start and cessation of smoking and alcohol drinking. Smoking and alcohol status was categorised as never, former or current. Detailed data were gathered on the lifetime job history of participants: they were asked to recall all their jobs and to describe all job-related activities performed, including the start and stop years and hours per week. Job titles were coded into the International Standard Classification of Occupations (ISCO), version 1968.[17]

In Ireland and Italy, using the questionnaire as a guide, face-to-face interviews were held to gather the data. In the Netherlands, participants filled in the questionnaire themselves. Participants were contacted by telephone to complete or correct the data where necessary. Clinical data were collected from patients' medical records.

Classification of occupational exposures

Occupational exposures were estimated to: silica (respirable crystalline silica) and asbestos; organic dusts in general and specifically animal contact (contact with animals or fresh animal products) and endotoxin; and PAH and DME. Estimations were performed using the general population DOM-JEM.[18–20] The DOM-JEM is based on the five-digit ISCO 1968 coding,[17] providing an exposure estimate (none, low, high) to each individual job code.

The exposure intensity scores of none, low and high were arbitrarily assigned values of 0, 1 and 4 to reflect the log-normal (multiplicative) nature of occupational exposure concentrations. The cumulative exposure scores per participant were calculated as follows: $\sum_{k=1}^{n} \frac{(\text{exposure intensity score}_k \text{ x duration in years}_k \text{ x hours per week}_k)}{40}$, where k represents a job from the lifetime occupational history and n the total number of jobs reported for the entire working career. Estimated cumulative exposure was consequently categorised into low and high exposures derived by the median of exposed controls. Military service or periods spent as a homemaker were excluded because of difficulties quantifying these activities. Because of considerable uncertainty about the exposure estimates before the Second World War, in the main analyses, we only included jobs starting after 1945 (>99% of all jobs).

Statistical analysis

We used a multivariate logistic regression model to investigate the association between ALS risk and the occupational exposures, adjusting for age at survey, gender, education, smoking, alcohol and cohort. All variables were calculated up to 3 years before survey date for both patients and controls to remove exposures that may have occurred after ALS onset.

We performed several additional analyses. First, when an occupational exposure showed a possible exposure–response association with ALS risk in the analysis of cumulative exposure, we analysed this exposure while mutually adjusting for the other exposures. Second, we performed a jack-knife analysis subsequently excluding one of the cohorts to investigate whether the results were not particularly driven by one cohort. Third, we tested whether the association between ever/never exposure to the seven exposures and ALS risk was different for men and women using an interaction term, and used the likelihood ratio test to compare the models with and without the interaction term. Fourth, since *C9orf72* repeat expansions are the most common genetic abnormality in ALS, and patients with this mutation may represent a subgroup with different lifestyle and environmental factors, we excluded cases with a *C9orf72* repeat expansion, determined by methods described previously.[21 22] Fifth, to explore the effect of educational level on the association between the occupational exposures and risk of ALS, we matched one patient to one control based on age, gender, centre

Table 1 Baseline characteristics

	Ireland		The Netherlands		Apulia		Lombardy		Piedmont and Valle d'Aosta	
	Patient	Control	Patient	Control	Patient	Control	Patient	Control	Patient	Control
n	177	349	791	1880	141	213	186	190	262	290
Male gender (%)	59.9	60.5	60.4	59.9	57.4	53.1	53.8	53.2	52.7	52.8
Bulbar site of onset (%)	23.7		35.3		26.5		29.6		37.0	
Age at survey, mean (SD), years	65.1 (11.5)	65.4 (11.0)	64.2 (10.3)	64.0 (9.6)	63.9 (10.8)	63.8 (11.7)	65.3 (10.6)	65.5 (11.1)	65.8 (10.8)	64.2 (11.7)
Education (%)										
ISCED 5-8	16.4	21.8	26.0	28.3	7.6	9.9	9.3	36.4	6.3	15.9
ISCED 0-4	83.6	78.2	74.0	71.7	92.4	90.1	90.7	63.6	93.7	84.1
C9orf72 repeat expansion* (%)	9.2		9.7		NA		8.8		8.8	
Smoking (%)										
Current	19.4	11.2	21.5	13.8	22.1	21.0	19.8	18.0	18.9	15.6
Former	36.6	40.2	45.9	52.6	33.6	26.7	36.3	34.4	28.7	34.6
Never	44.0	48.6	32.6	33.6	44.3	52.4	44.0	47.6	52.4	49.8
Alcohol (%)										
Current	72.7	75.4	78.1	87.7	54.3	40.5	59.6	62.7	56.4	61.3
Former	10.5	5.9	8.0	4.0	7.9	2.4	7.6	3.0	6.8	6.3
Never	16.9	18.6	13.9	8.3	37.8	57.1	32.7	34.3	36.8	32.4

*Missing in *C9orf72*: 4 (2.3%) in Ireland, 57 (7.2%) in the Netherlands, 139 (98.6%) in Apulia, 106 (57.0%) in Lombardy, 0 (0%) in Piedmont and Valle d'Aosta.
NA, not applicable.

Table 2 Cumulative occupational exposures and ALS risk

Exposure	Cumulative exposure*	Patients (n=1252), n (%)	Controls (n=2590), n (%)	OR†	95% CI	P value	P value for trend‡
Silica	Never	1147 (85.7)	2410 (91.2)	Ref			0.01
	≤21.9	96 (7.2)	116 (4.4)	1.67	1.23 to 2.25	0.0008	
	>21.9	96 (7.2)	116 (4.4)	1.73	1.28 to 2.33	0.0003	
Asbestos	Never	1085 (81.0)	2228 (84.3)	Ref.			0.68
	≤15.0	127 (9.5)	209 (7.9)	1.22	0.95 to 1.56	0.12	
	>15.0	127 (9.5)	205 (7.8)	1.16	0.90 to 1.51	0.25	
Organic dust	Never	800 (59.7)	1755 (66.4)	Ref			0.03
	≤17.0	241 (18.0)	444 (16.8)	1.14	0.94 to 1.38	0.18	
	>17.0	298 (22.3)	443 (16.8)	1.33	1.11 to 1.59	0.002	
Animal contact	Never	1228 (91.7)	2457 (93.0)	Ref.			0.72
	≤29.3	59 (4.4)	93 (3.5)	1.16	0.81 to 1.64	0.40	
	>29.3	52 (3.9)	92 (3.5)	1.13	0.78 to 1.61	0.52	
Endotoxin	Never	976 (72.9)	2085 (78.9)	Ref			0.13
	≤12.0	175 (13.1)	282 (10.7)	1.22	0.98 to 1.51	0.08	
	>12.0	188 (14.0)	275 (10.4)	1.36	1.10 to 1.68	0.005	
PAH	Never	1129 (84.3)	2340 (88.6)	Ref			0.24
	≤8.6	91 (6.8)	151 (5.7)	1.12	0.84 to 1.49	0.45	
	>8.6	119 (8.9)	151 (5.7)	1.47	1.12 to 1.92	0.005	
DME	Never	1019 (76.1)	2131 (80.7)	Ref			0.03
	≤22.0	170 (12.7)	256 (9.7)	1.40	1.12 to 1.75	0.003	
	>22.0	150 (11.2)	255 (9.7)	1.21	0.96 to 1.53	0.11	

*Low and high exposures based on control median: ≤median=low and >median=high; never exposed indicates background exposure, expressed in unit-years.
†Logistic regression, adjusted for age, gender, education, smoking, alcohol and cohort, background exposure as reference category.
‡P value for trend on a continuous scale.
ALS, amyotrophic lateral sclerosis; DME, diesel motor exhaust; PAH, polycyclic aromatic hydrocarbon.

and educational level. In these 'matched' analyses, all exposures were calculated up to onset of the disease of the patient for both the patient and the matching control, and we adjusted for smoking and alcohol. Sixth, we separately analysed the first and last jobs performed, and used an interaction term between the exposure intensity score and a newly created variable indicating the first/last performed job, to test whether late exposure (as expressed by the last performed job) or early exposure (as expressed by the first performed job) exerted greater influence on ALS risk. If a person had more than one first/last job, the job with the highest occupational exposure concentrations was used. Lastly, we investigated whether the occupational exposures were associated with the phenotype of ALS. Using bulbar or spinal site of onset as dependent variable in a multivariate model with the same confounders as previously described, we determined if the association between ever/never exposure to the seven exposures and ALS was different for bulbar or spinal-onset patients. Additionally, we formally tested whether the occupational exposures modified the age at onset in patients using an interaction term between age at survey (for patients this is highly correlated with age at onset) and ever/never exposure.

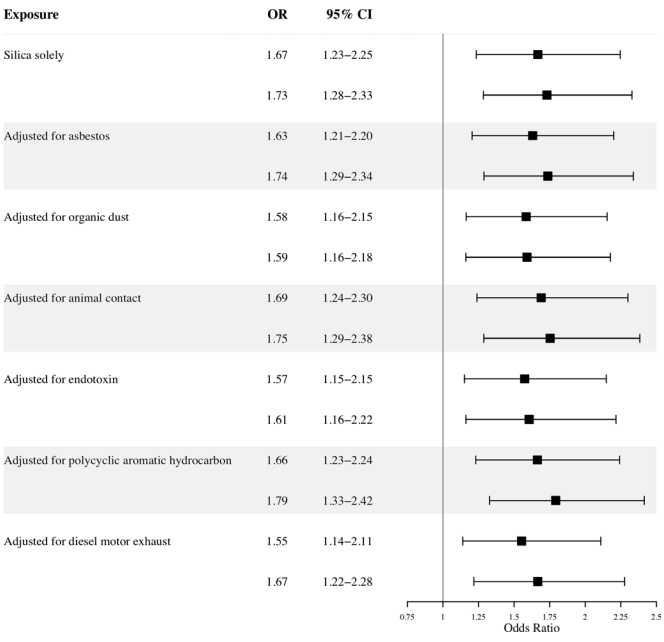

Figure 1 Pollutant models for silica with each of the other occupational exposures. The first row per exposure shows the OR with 95% CI of low versus background exposure, the second row shows this for high versus background exposure.

RESULTS

Study population and confounders

We included 1557 population-based cases and 2922 age, gender and geography-matched controls between 2011 and 2014 across three countries (table 1). Compared with cases, more controls completed higher levels of education. After exclusion of occupations starting before 1945, military occupations (28.7% patients and 29.7% controls performed at least one military job during their career), occupations with incomplete data (missing hours per week, start or stop year), or participants with missing data, 1252 patients and 2590 controls with available occupational information remained.

Single occupational exposure analyses

Most cases and controls (>50%) had only been exposed to background levels ('never'; table 2). Compared with controls, cases

Table 3 Cumulative occupational exposures and ALS risk with different categories across cohorts

	Total study population			Ireland			The Netherlands			Apulia			Lombardy			Piedmont and Valle d'Aosta		
Exposure	CE*	P (n=1252)	C (n=2590)	CE	P (n=140)	C (n=305)	CE	P (n=605)	C (n=1663)	CE	P (n=107)	C (n=200)	CE	P (n=156)	C (n=144)	CE	P (n=244)	C (n=278)
Silica (%)	Never	85.7	91.2	Never	84.2	90.1	Never	88.6	91.7	Never	73.9	80.6	Never	85.1	98.8	Never	84.4	92.9
	Low	**6.6**	**4.5†**	≤8.00	3.4	5.1	≤17.50	5.6	4.2	≤57.38	12.6	9.7	≤31.95	**10.1**	**0.6†**	≤11.44	6.0	3.5
	High	**7.8**	**4.3‡**	>8.00	**12.3**	**4.8†**	>17.50	5.9	4.1	>57.38	13.5	9.7	>31.95	**4.8**	**0.6†**	>11.44	**9.6**	**3.5†**
Asbestos (%)	Never	81.0	84.3	Never	76.0	77.0	Never	82.7	84.5	Never	84.7	88.8	Never	82.7	93.3	Never	76.8	83.0
	Low	10.5	7.9	≤8.00	8.2	11.5	≤13.55	8.7	7.8	≤33.30	10.8	5.8	≤39.00	**14.9**	**3.7†**	≤17.38	13.2	8.5
	High	8.5	7.8	>8.00	15.8	11.5	>13.55	8.6	7.8	>33.30	4.5	5.3	>39.00	2.4	3.0	>17.38	10.0	8.5
Organic dust (%)	Never	59.7	66.4	Never	58.2	62.9	Never	63.3	66.0	Never	62.2	72.8	Never	67.9	76.2	Never	44.8	62.5
	Low	18.7	16.8	≤17.00	13.7	18.5	≤13.50	17.9	17.1	≤50.77	14.4	13.6	≤19.00	13.1	12.2	≤25.00	**29.6**	**18.7†**
	High	**21.5**	**16.7‡**	>17.00	28.1	18.5	>13.50	18.8	16.9	>50.77	**23.4**	**13.6†**	>19.00	19.0	11.6	>25.00	**25.6**	**18.7†**
Animal contact (%)	Never	91.7	93.0	Never	80.8	85.9	Never	94.4	93.4	Never	93.7	95.6	Never	89.3	98.2	Never	91.6	93.6
	Low	3.5	3.6	≤51.00	10.3	7.0	≤28.00	3.3	3.3	≤23.00	1.8	2.4	≤4.50	1.2	1.2	≤12.12	2.4	3.2
	High	4.8	3.4	>51.00	8.9	7.0	>28.00	2.3	3.3	>23.00	4.5	1.9	>4.50	**9.5**	**0.6†**	>12.12	6.0	3.2
Endotoxin (%)	Never	72.9	78.9	Never	73.3	78.6	Never	77.3	78.0	Never	66.7	82.5	Never	78.0	95.1	Never	60.4	72.4
	Low	**13.7**	**10.7†**	≤18.00	13.0	11.5	≤9.00	11.7	11.0	≤31.60	**20.7**	**8.7‡**	≤10.88	8.3	2.4	≤12.20	19.6	13.8
	High	**13.4**	**10.4†**	>18.00	13.7	9.9	>9.00	11.0	10.9	>31.60	12.6	8.7	>10.88	**13.7**	**2.4†**	>12.20	**20.0**	**13.8†**
PAH (%)	Never	84.3	88.6	Never	78.8	85.6	Never	88.1	88.9	Never	86.5	96.1	Never	85.7	96.3	Never	75.6	79.9
	Low	7.5	5.8	≤6.00	6.8	7.7	≤8.12	6.0	5.5	≤20.50	**7.2**	**1.9†**	≤52.12	**13.1**	**1.8†**	≤7.50	8.4	10.2
	High	**8.1**	**5.6†**	>6.00	**14.4**	**6.7†**	>8.12	5.9	5.5	>20.50	**6.3**	**1.9†**	>52.12	1.2	1.8	>7.50	**16.0**	**9.9†**
DME (%)	Never	76.1	80.7	Never	70.5	74.4	Never	77.9	81.0	Never	75.7	80.6	Never	81.0	91.5	Never	71.6	79.2
	Low	**13.1**	**9.7‡**	≤24.25	13.7	12.8	≤19.05	11.6	9.5	≤31.25	12.6	9.7	≤25.75	**11.3**	**4.3†**	≤22.75	**18.0**	**10.6†**
	High	10.8	9.7	>24.25	15.8	12.8	>19.05	10.5	9.5	>31.25	11.7	9.7	>25.75	**7.7**	**4.3†**	>22.75	10.4	10.2

Significant results are marked in bold.
*Low and high exposures in total study population based on control median: ≤median=low and >median=high; never exposed indicates background exposure.
†P<0.05.
‡P<0.005 in fixed effects meta-regression for the pooled analyses (total study population) and in logistic regression for the separate cohorts, adjusted for age, gender, education, smoking and alcohol, background exposure as reference category.
§
ALS, amyotrophic lateral sclerosis; C, controls; CE, cumulative exposure; DME, diesel motor exhaust; P, patients; PAH, polycyclic aromatic hydrocarbon.

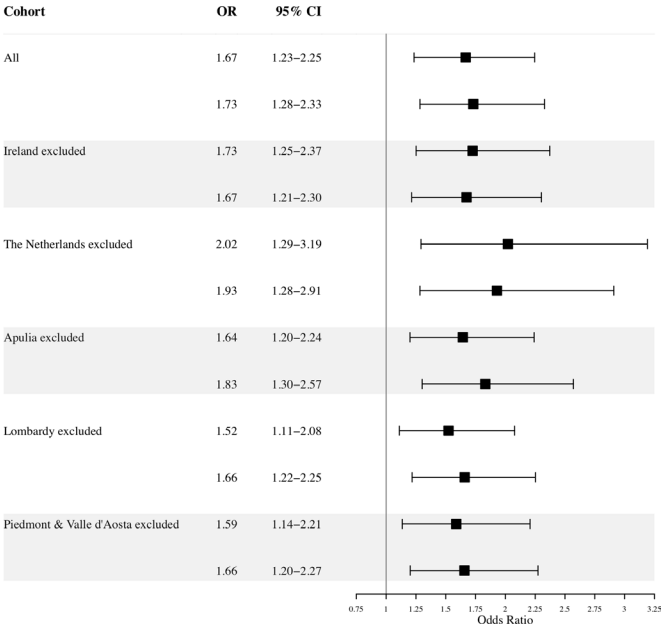

Figure 2 The main model for silica and subsequently excluding one of the cohorts. The first row per exposure shows the OR with 95% CI of low versus background exposure, the second row shows this for high versus background exposure.

were more frequently ever exposed, that is, to either low or high levels of all exposures. The risk of ALS was positively associated with all exposures, and statistically significant for high-level exposures to silica, organic dust, endotoxin and PAH. For silica and DME, statistically significant associations were observed for low levels of exposure. Treating the exposures as continuous predictors in the multivariate model resulted in significant linear associations for silica, organic dust and DME (table 2).

Combined exposure analyses

Since silica, organic dust and DME were significantly associated with ALS risk in both the categorical and continuous exposure analyses, we analysed these further by subsequently adding one of the other exposures to the model. Only the OR for silica remained significantly associated with ALS risk (figure 1). In contrast, the ORs for organic dust and DME decreased significantly (>5% drop in OR) after adjustment for silica (data not shown).

Additional analyses

Except for low exposure in the Irish cohort, patients from all five cohorts were more frequently exposed to silica than controls (table 3). This difference in exposure was most obvious in the Lombardy region: 5%–10% of patients were exposed versus less than 1% of controls. Excluding the Lombardy cohort from the analysis, the association between silica and ALS risk remained (figure 2). Men and women had the same increased risk of ALS with the various types of exposures (all p values for interaction >0.11; data not shown). When we excluded 199 patients with a *C9orf72* repeat expansion, the effect of exposure to silica on the risk of ALS remained the same: low versus background exposure OR 1.73 (95% CI 1.27 to 2.34) and high versus background exposure OR 1.69 (95% CI 1.24 to 2.28; table 4). The effect was in the same direction, although somewhat stronger, when matching one patient to one control based on age, gender, cohort and education: low versus background exposure OR 2.24 (95% CI 1.50 to 3.35) and high versus background exposure OR 2.13 (95% CI 1.42 to 3.20; table 4). We found positive associations between all exposures and ALS risk for both the first and the last

Table 4 Overview of the additional analyses on cumulative occupational exposures and ALS risk

	Analysis excluding *C9orf72* patients				Post hoc matched analysis			
Exposure	Cumulative exposure*	OR	95% CI	P value	Cumulative exposure*	OR	95% CI	P value
Silica	Never	Ref			Never	Ref		
	≤21.9	1.73	1.27 to 2.34	0.0004	≤23.5	2.24	1.50 to 3.35	<0.0005
	>21.9	1.69	1.24 to 2.28	0.0008	>23.5	2.13	1.42 to 3.20	<0.0005
Asbestos	Never	Ref			Never	Ref		
	≤15.0	1.24	0.96 to 1.60	0.10	≤16.5	1.33	0.98 to 1.81	0.07
	>15.0	1.21	0.92 to 1.56	0.16	>16.5	1.14	0.83 to 1.57	0.43
Organic dust	Never	Ref			Never	Ref		
	≤17.0	1.12	0.92 to 1.36	0.27	≤20.0	1.31	1.03 to 1.66	0.03
	>17.0	1.33	1.10 to 1.60	0.003	>20.0	1.56	1.23 to 1.99	<0.0005
Animal contact	Never	Ref			Never	Ref		
	≤29.3	1.22	0.85 to 1.73	0.28	≤41.2	1.39	0.92 to 2.09	0.12
	>29.3	1.23	0.85 to 1.76	0.28	>41.2	0.94	0.58 to 1.51	0.79
Endotoxin	Never	Ref			Never	Ref		
	≤12.0	1.23	0.98 to 1.54	0.07	≤13.8	1.18	0.89 to 1.55	0.25
	>12.0	1.33	1.07 to 1.65	0.01	>13.8	1.70	1.28 to 2.27	<0.0005
PAH	Never	Ref			Never	Ref		
	≤8.6	1.10	0.82 to 1.48	0.51	≤10.0	1.12	0.80 to 1.57	0.51
	>8.6	1.57	1.20 to 2.07	0.001	>10.0	1.36	0.96 to 1.91	0.08
DME	Never	Ref			Never	Ref		
	≤22.0	1.46	1.16 to 1.83	0.001	≤26.2	1.35	1.03 to 1.75	0.03
	>22.0	1.24	0.97 to 1.57	0.08	>26.2	1.04	0.77 to 1.39	0.82

*Low and high exposures in total study population based on control median: ≤median=low and >median=high; never exposed indicates background exposure.
ALS, amyotrophic lateral sclerosis; DME, diesel motor exhaust; PAH, polycyclic aromatic hydrocarbon.

held jobs, with significant associations for silica and the highest category of organic dusts and endotoxin. This was confirmed by all seven insignificant interaction terms (all p values for interaction >0.41; data not shown).

Phenotype

We did not find any suggestion that the occupational exposures were associated with a different site of onset, that is, bulbar and spinal patients were equally exposed (data not shown). Regarding the age at onset, we found a younger age at survey (for patients this was highly correlated with age at onset) for patients exposed (mean of 63.5 years) to asbestos when compared with patients who were non-exposed (mean of 64.9 years). This was not found for any of the other six occupational exposures.

DISCUSSION

In this large, multicentre, population-based, case–control study, we found an association between occupational exposure to particulates and combustion products and the risk of ALS. However, this association appeared only noteworthy for exposure to silica, independent of important confounders, such as age, gender, education, smoking and alcohol, gathered using a validated and methodologically harmonised questionnaire, and after adjustment for the other occupational exposures. The exposure-response trend seen for silica, that is, increasing ORs with higher level of exposure, together with the significant linear association, strengthens the robustness of our results.

To the best of our knowledge, this is the first study describing the association between silica and risk of ALS. There are studies, however, that describe associations with occupations in which silica could play a role: a recent nested case–control study with prospectively collected data on 1826 cases and 1 826 000 controls showed higher odds of ALS for male construction workers, adjusted for socioeconomic status, residential location and marital status.[23] A finding consistent with results from two other case-control studies.[11 24] In a prospective study using ALS mortality data, on the other hand, the authors did not find an association with occupations in which silica exposure could play a role, possibly due to limited power.[13]

Rather than studying occupational groups without information on potential underlying risk factors, we were able to study a large group of population-based patients and controls with respect to the specific elements to which they were exposed to. Moreover, we had information on several occupational exposures, which made it possible to correct for another exposure in a bipollutant model. In this study, participants were essentially blinded for the hypothesis being tested; the questionnaire contained questions on many other exogenous factors. However, recall bias cannot be completely ruled out as it is a commonly held belief in the general public that occupational exposures can lead to neurodegenerative disease and therefore patients may be more likely to have thought through and recalled details that may not have seemed important to controls.

The observed effect of silica would seem to be driven by the Lombardy region (table 3), but this region accounts for only 10% of the total study population and when subsequently excluding one cohort as a sensitivity analysis, the ORs remain stable (figure 2). This regional variation cannot be explained by differential recall or systemic measurement bias (face-to-face interviews in Ireland and Italy and self-administered questionnaires in the Netherlands), as occupational exposures were objectively assessed by the application of JEMs. The difference in educational level between cases and controls was most prominent in the Lombardy region (table 1). The regional variation, however, was not explained by selection bias on educational level since the effect sizes become even stronger after one-to-one matching of patients and controls for education (table 4). The median levels of exposure differed between cohorts (table 3), while in the main analyses we pooled all controls in order to create low and high exposure groups. Therefore, in order to rule out possible aggregation bias, we additionally performed a meta-regression analysis confirming the association with silica exposure (table 3). We included 86% of patients and 90% of controls after exclusion due to incomplete data, occupations starting before 1945 or military occupations. It would have been interesting to include military activities, as some military personnel are probably highly exposed to some of the studied constituents during training and operations. We had to exclude these occupations because the exposures could easily be misclassified due to the diversity of job activities that can be performed within the military service. We excluded the same percentage of activities in patients and controls (~29%). Moreover, a well-recognised limitation of using JEMs is that every person performing the same job is assigned an equal exposure (intensity), but not every person performs the same tasks in the same way.[25] However, this probably does not lead to differential misclassification. With regard to silica exposure, compared with case-by-case expert assessment, the DOM-JEM has shown good performance in a multicentre study on lung cancer.[18]

Silica exists in numerous forms in nature; of which inhaled crystalline silica is studied most frequently regarding its toxic effects. The toxicity most likely depends on particle size, with the respirable fraction that reaches the alveoli of the lungs being the fraction of interest with respect to health effects.[26] As an element of particulate matter, silica could reach the brain by translocation through the systemic circulation following deposition in the pulmonary region after inhalation. Particulate matter has been hypothesised to be neurotoxic, mainly through potentially increased oxidative stress and increased activation of brain microglia, the primary regulators of neuroinflammation.[27] One should note, however, that this is based on indirect inferences since silica has never been specifically studied as an ALS risk factor. If future studies confirm our findings, it would be interesting to perform gene/silica interaction studies. Assuming oxidative stress and neuroinflammation are the pathogenic mechanisms, interaction with mutations in the gene encoding Cu/Zn superoxide dismutase (*SOD1*) could be of added value. Especially since this mutation is common in Italy, but rare in Ireland and the Netherlands.[28–30]

This is the first study to explore the association between particulates and combustion products and ALS risk using JEMs. In this large, multicentre, population-based, case-control study using full job histories, we found a positive association between occupational silica exposure and the risk of ALS.

Author affiliations
[1]Department of Neurology, Brain Centre Rudolf Magnus, University Medical Centre Utrecht, Utrecht, The Netherlands
[2]Rita Levi Montalcini Department of Neuroscience, University of Torino, Torino, Italy
[3]Division of Environmental Epidemiology, Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, The Netherlands
[4]Department of Neuroscience, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy
[5]Unit of Neurodegenerative Diseases, Department of Clinical Research in Neurology, University of Bari 'Aldo Moro', Pia Fondazione Cardinale G Panico, Lecce, Italy
[6]Department of Basic Medical Sciences, Neurosciences and Sense Organs, University of Bari 'Aldo Moro', Bari, Italy
[7]Academic Unit of Neurology, Trinity Biomedical Sciences Institute, Trinity College, Dublin, Ireland

Collaborators Rosanna Tortelli, MD, PhD (Unit of Neurodegenerative Diseases, Department of Clinical Research in Neurology, University of Bari Aldo Moro, at Pia Fondazione 'Card G Panico', Tricase, Lecce, Italy; Department of Basic Medical Sciences, Neurosciences and Sense Organs, University of Bari Aldo Moro, Bari, Italy); Chiara Zecca, MSc (Department of Clinical Research in Neurology, University of Bari, 'Pia Fondazione Cardinale G Panico', Lecce, Italy); Elisabetta Pupillo (Department of Neurosciences, IRCSS, Mario Negri Institute of Pharmacological Research, Milan); Giancarlo Comi, MD; Nilo Riva, MD (IRCCS San Raffaele Hospital, Milano, Italy); Christian Lunetta, MD; Francesca Gerardi, DrBT (NEMO Clinical Centre, Serena Onlus Foundation, Niguarda Ca' Granda Hospital, Milano, Italy); Massimiliano Filosto, MD; Maria Sofia Cotelli, MD; Fabrizio Rinaldi, MD (Civil Hospital of Brescia, Brescia, Italy); Luca Chiveri, MD (Ospedale Valduce, Como, Italy); Maria Cristina Guaita, MD; Patrizia Perrone, MD (AO Civil Hospital, Legnano, Italy); Ceroni Mauro, MD; Luca Diamanti, MD (IRCCS National Neurorological Institute 'C Mondino', Pavia); Carlo Ferrarese, MD; Lucio Tremolizzo, MD (University of Milano-Bicocca, 'San Gerardo' Hospital, Monza); Maria Luisa Delodovici, MD; Giorgio Bono, MD ('Ospedale di Circolo, Fondazione Macchi', Varese); Mark Heverin, MSc; Alice Vajda, PhD (Academic Unit of Neurology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland); Anneke J van der Kooi, MD, PhD (Department of Neurology, Amsterdam Medical Centre, University of Amsterdam, Amsterdam, The Netherlands); Joost Raaphorst, MD, PhD (Department of Neurology, Donders Institute for Brain, Cognition and Behaviour, Centre for Neuroscience, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands); Andrea Calvo, MD, PhD; Cristina Moglia, MD, PhD; Federico Casale, PhD; Giuseppe Fuda, MSc; Antonio Canosa, MD, PhD; Umberto Manera, MD; Alessandro Bombaci, MD; Maurizio Grassano, MD; Rosario Vasta, MD; Paolina Salamone, MSc, PhD; Giuseppe Marrali, MSc, PhD; Barabara Lazzolino, PsyD ('Rita Levi Montalcini' Department of Neuroscience, University of Torino, Torino, Italy); Letizia Mazzini, MD (ALS Centre, Department of Neurology, 'Maggiore della Carità', University Hospital Novara, Italy).

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