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An association of particulate air pollution and traffic exposure with mortality after lung transplantation in Europe

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ABSTRACT

Background: Air pollution from road traffic is a serious health risk, especially for susceptible people. Single center studies showed an association with chronic lung allograft dysfunction (CLAD) and survival after lung-transplantation, but there are no large studies.

Methods: Thirteen lung-transplant centers in 10 European countries created a cohort of 5707 patients. For each patient, we quantified residential PM10 by land use regression models, and the traffic exposure by quantifying total road length within buffer zones around the residence, and distance to a major road or freeway.

Results: After correction for macrolide use, we found associations between air pollution variables and CLAD/mortality. Given the important interaction with macrolides, we stratified according to macrolide use. No associations were observed in 2151 patients taking macrolides. However, in 3556 patients not taking macrolides, mortality was associated with PM10 (HR 1.081 95%CI 1.000-1.167); similarly, CLAD and death were associated with road lengths in buffers of 200m to 1000m and 100m to 500m, respectively (HRs between 1.085 and 1.130). Sensitivity analyses for various possible confounders confirmed the robustness of these associations.

Conclusion: Long-term residential air pollution and traffic exposure were associated with CLAD and survival after lung-transplantation, but only in patients not taking macrolides.

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INTRODUCTION

There is a well-established relationship between long-term exposure to particulate matter (PM with aerodynamic diameter ≤ 2.5 and 10 µm, $PM_{2.5}$ and PM_{10}) and mortality in the general and susceptible population.

Lung transplant patients are of particular interest, as they could be extremely vulnerable due to their constant immune-compromised condition. Lung transplantation is the ultimate treatment option for patients with end-stage pulmonary disease, such as emphysema, pulmonary fibrosis or cystic fibrosis (1). With a median survival of 5 years, the long-term survival after lung transplantation remains low compared to other solid organ transplantations (2), mainly due to a higher incidence of chronic lung allograft dysfunction, CLAD (incidence of 50% five years post-transplant) (3). Currently, the only treatment that is able to influence pulmonary function after CLAD diagnosis is macrolide therapy, which reduces/postpones the incidence of CLAD in about 35% of patients (4). Besides small single-center studies, showing association with CLAD and survival, the role of long-term exposure in lung transplant recipients has not been established (5,6).

We assessed whether particulate air pollution and traffic exposure were associated with CLAD and mortality in lung transplant patients from 13 major lung transplant centers in 10 European countries. We hypothesized that road density within buffer zones and PM_{10} at the patient's home address would be associated with CLAD and mortality but that this would be influenced by the use of macrolides as we previously showed that azithromycin protected against air-pollution induced health effects.

MATERIAL AND METHODS

Study population

The target population consisted of all subjects having undergone a lung transplantation between 1987 and 2012 in 13 major lung transplant centers (Barcelona, Copenhagen, Essen, Groningen, Hannover, Leuven, Munich, Newcastle, Oslo, Pavia, Utrecht, Vienna, Zurich) from 10 different European countries and with follow-up data available until death or 31 December 2013. This study was approved by the central ethical committee in Leuven and by every participating centers' ethical committee (ML8653). All variables collected by the individual centers were anonymized and sent to the coordinating center (Leuven) for analysis. Study variables included age, gender, socio-economic status (occupation), smoking status pre-transplant, home address at the time of transplantation, underlying lung disease, date of transplantation, type of transplantation (single lung, sequential single lung, heart-lung), date of CLAD, date of all-cause mortality, macrolide usage (either azithromycin or clarithromycin). The general protocol for azithromycin usage is 250 mg three times a week and clarithromycin is given daily (500-1000 mg). All data was gathered by retrospective patients' chart analysis. CLAD was defined as every irreversible decline in pulmonary function with at least 20% in absence of an identifiable cause, as evidenced by individual chart analysis of every patient by the treating physician (7). Given the lack of uniform international criteria and the big time of patient inclusion, we did not sub-divide our patients in classical BOS and restrictive CLAD. Socio-economic status was coded using a scale from low to moderate to high according to the UK Office of Population Censuses and Surveys (13). Exclusion criteria were the lack of a home address, a post-transplant survival <90 days, distance to the transplant center > 700 km and lack of key covariate data (figure E1).

Exposure assessment

The residential addresses of all lung transplant patients were geocoded (ArcGIS10) and linked with average levels of particulate matter with an aerodynamic diameter of less than 10 µm (PM_{10}, µg/m³). We used an existing EU-wide map of PM_{10} modeled on a 100x100 m resolution for the year 2007 based on land use regression, using predictor variables from EU-wide databases with satellite-derived PM estimates, North-South trend, land use, roads and altitude (9). PM data for Switzerland were not available within this study, although we could quantify all other variables. Residential proximity to major roads or freeway, and total length of roads within different buffers (50-100-200-500-1000m) around the residence were estimated, as explained in figure E2.

Statistical analysis

For the cohort-specific analysis, we performed Cox proportional hazards regression using SAS 9.3 (SAS institute Inc, Cary, NC, USA). Censoring was done at the time of death/re-transplantation, loss of follow-up or at end of follow-up, whichever came first. All hazard ratios shown are given per IQR increment. Patients with a re-transplantation were censored at the moment of re-transplantation (=loss of graft) and the re-transplantation was analyzed as a separate event (since donor organ is different). PM exposure was analyzed as a linear variable, traffic exposure was analyzed in each buffer zone, and potential confounders were identified a priori (age, gender, type of transplantation, underlying lung disease and transplant era) and included in the model. We only included cases where all these data was available in our final model. The effect of lung transplant center on the Cox model was taken into account as a random effect and separate sub-analyses were performed for every center. Sensitivity sub-analyses were performed with lung transplant center as a fixed effect, omission of each single center to determine center effect, re-transplantation status, socio-economic status, transplant

era, time of macrolide initiation and smoking history. The latter variables were not complete, therefore the exact number of patients used for analysis is explicitly stated. A p-value<0.05 was considered significant.

RESULTS

A total of 5707 patients were included in this study and contributed 33202 patient years of follow-up, with a median follow-up of 5.6 years. Of these 5707 patients, 2626 (46.0%) developed CLAD and 2577 patients (45.2%) died. Median time to CLAD was 6.4 years, while median survival was 8.9 years, however patients with early death were excluded from this analysis which will significantly impact survival. Age, gender, underlying disease and type of transplant were available for all subjects. Concentrations of air pollutants varied between and within study centers (figure 1). Specific characteristics per center and exposure characteristics are shown in table E1. We considered a priori that macrolide therapy could modify the relation between air pollution and outcomes, and we therefore divided the patients in two groups, according to whether they had ever (2151 patients; 37.7%) or never (3556 patients; 62.3%) been chronically treated with azithromycin or clarithromycin (table 1). This division was based on previous literature of the beneficial effect of azithromycin in lung transplant patients (11) and the protective effect of azithromycin on air pollution (6). We also performed analysis without stratification according to macrolide usage, implementing macrolide usage (yes/no) as a covariate (table E2), however these results should be interpreted with caution as we showed significant interaction between macrolides and air pollution variables (table E3). Macrolides were started at a median time of 26(9-63) months post-transplant and were started as a treatment for CLAD in 782 of 2107 (37.1%) patients where start date was available. A total of 640 (29.8%) patients died in the macrolide group and 1937 (54.5%) died in the macrolide-free group. Within the cohort of patients treated with macrolides no association between pollution

variables and mortality could be detected (table 2). However, in the macrolide-free group, PM_{10} (per IQR increment, HR 1.081; 95%CI 1.000-1.167) was significantly associated with mortality. A 10 µg/m³ PM_{10} increase was associated with a 13.8% increased risk of mortality. Analyzing PM_{10} in categories by quintile of exposure (reference =lowest quintile) demonstrated that PM_{10} values >20 µg/m³ implied a higher risk of dying (Q3+Q4+Q5 vs Q1 figure 3B). Also an association of all-cause mortality with road length in the 100 m, 200 m and 500 m buffer zones and a trend for the 50m buffer zone (Table 2) were observed. Figure 2 (A-B) demonstrates the HR for mortality and road length in a 200 meter buffer zone and PM_{10} for each center. Subdividing the road length in a 200 meter buffer zone in quintiles (lowest quintile as reference) showed that mainly the patients within the highest quintile of road length suffered from the highest risk of dying (figure 3A and table E4).

CLAD was present in 60.1% of the macrolide group and in 38.5% of the macrolide-free group. Analysis of the sub-group of patients taking macrolides showed no association of CLAD with any of the studied pollution variables, except for PM_{10} which showed an inverse association (table 2). However, in patients not taking macrolides, we observed an association between CLAD and road length in a 200, 500 and 1000 meter buffer zone around the patient's home address (table 2). Figure 2C demonstrates the HR's calculated for CLAD and road length in the 200 meter buffer zone per center. We could not detect an association between CLAD and PM_{10}, road length in a 50 and 100 meter buffer zone and distance to a major road or freeway, although PM_{10} tended to be significant (figure 2D). Bhinder et al. previously showed an association between patients living <100m from a highway and CLAD but not mortality (5), we observed a similar association with CLAD although not significant (HR 1.249; 0.994-1.570, p=0.056), but no effect on survival (HR 0.931; 0.760-1.140, p=0.49). Subdividing road length in a 200 meter buffer zone in quintiles showed that mainly patients within the highest quintile of road

length had the highest risk of CLAD (figure 3C); for PM_{10} this was mostly Q3 and Q5 (figure 3D). Distance to major road or highway was not associated with any of the outcomes.

Further sensitivity analyses were performed for road lengths in 200 meter buffer and for PM_{10}; analysis with road length in the 100 and 500 meter buffer zone did not change the results. Sub-analysis was performed for the possible confounding effect of smoking and socio-economic status (SES) as information was not available for the entire cohort. This analysis is shown in table E5 and demonstrates that both smoking and SES did not influence the association between mortality/CLAD and either road length or PM_{10}. The time of macrolide initiation could be an important confounder and consequently, we subdivided our cohort of macrolide users in macrolides at/after CLAD diagnosis (n=782) and contrasted these patients with macrolides users before CLAD diagnosis or never developing CLAD (n=1325) with 44 patients not having the exact date of macrolide initiation recorded. Stratification demonstrates that the deleterious effect of air pollution in the macrolide users is absent in patients taking macrolides before CLAD or not developing CLAD, while a trend towards an association is observed in patients who started with macrolide therapy at/after CLAD diagnosis (i.e. no protective effect on CLAD development possible as diagnosis is made at/after initiation and therefore macrolides are started 'to late'). A model without transplant centers, to prevent overcorrection for macrolide strategy demonstrated that air pollution increased the risk for CLAD and death in patient who started macrolides at/after CLAD diagnosis (table E6). Since macrolide therapy can be started any time after transplant, we analyzed macrolide usage as a time-dependent variable as well (table E7), confirming the increased risk of death and CLAD in patients not taking macrolides. Lastly, we performed an analysis where we censored CLAD and mortality for date of macrolide initiation, which confirmed that air pollution variables are associated with CLAD-free (HR 1.005, 1.000-1.011) and overall survival (HR 1.012; 1.003-1.021)

The distribution of road lengths varied between centers (figure E3). A subsequent sensitivity analysis for center as an indicator variable (fixed effect) instead of a random effect variable demonstrated that the association between road length in the 200 meter buffer zone and either mortality (HR 1.086; 95%CI 1.015-1.161) or CLAD (HR 1.110; 95%CI 1.023-1.204) remains unchanged, although there was a pronounced difference in mortality and CLAD rate across the centers ($p<0.0001$). Additional analysis to exclude an effect by a specific center was performed by removing one center at the time, and performing the analysis with the 12 remaining centers. These analyses confirmed the consistency of our results (figure E4).

We excluded patients living further away than 700 km from their transplant center, as it may be a confounder, given that worse survival has been demonstrated for patients living further from their transplant center after kidney and stem cell transplantation (10,11). Analyses of different thresholds are shown in table E8. In order to include as many patients as possible, we decided to use a threshold of 700 km from the transplant center.

Sensitivity analysis was also performed using only patients transplanted after 1997 as we used PM10 maps of 2007. In patients not taking macrolides (n=2959), the association between the road length and overall (HR 1.013; 1.003-1.024, p=0.011) and CLAD-free survival (HR 1.014; 1.001-1.027, p=0.035) persisted, while there was no association in macrolide-users.

Regarding re-transplantation, we omitted all data regarding the second transplantation from those patients (n=58) and considered the moment of re-transplantation as death (since graft is lost) and demonstrated that the road length in the 200 meter buffer zone remained associated with both survival (HR 1.093; 95%CI 1.023-1.169) and CLAD (HR 1.119; 95%CI 1.030-1.213).

The World Health Organization (WHO) recommended to limit the annual mean exposure to PM_{10} to a maximum of 20 $\mu g/m^3$; so, we analyzed the effect of road length in buffer zones around patients´ residences separately for patients not taking macrolides experiencing levels <

or ≥20 µg/m³ of PM_{10}. The road length in the 200 meter buffer zone was only associated with mortality in patients with PM_{10}≥ 20 µg/m³ (2605 patients, HR 1.119; 95%CI 1.038- 1.204) and not in patients with PM_{10}< 20 µg/m³ (951 patients, HR 0.941; 95%CI 0.808-1.094). Similarly, for CLAD only an association was present for the patients with a PM_{10} exposure ≥ 20 µg/m³ (2598 patients, HR 1.119; 95%CI 1.015-1.231) and no association was observed for the patients with a PM_{10}<20 µg/m³ (945 patients, HR 0.993 95%CI 0.832-1.187).

DISCUSSION

This is the first study investigating the effect of air pollution and traffic exposure in a large multi-center cohort of lung transplant patients geographically dispersed over almost the entire European continent. Interestingly, the effect of air pollution was not limited to mortality but we also found an association with CLAD. However, this was only observed in patients not taking macrolides, suggesting a protective role of macrolides against air pollution.

This seemingly protective effect of macrolides on air pollution is not unexpected as these drugs are known to reduce innate (neutrophilic) immune activation in many chronic respiratory disorders like COPD (12), cystic fibrosis (13), non-cystic fibrosis bronchiectasis (14), severe asthma (15) and CLAD (4). More indirect evidence to support a protective effect of macrolides originates from a randomized placebo-controlled trial that demonstrated that maintenance azithromycin therapy is able to prevent COPD exacerbations (12), which are also linked with short-term exposure to air pollution (17). Moreover, in lung transplantation patients, PM_{10} was associated with airway inflammation on transbronchial biopsies and lavage samples, but again only in those patients not taking azithromycin (18). It might be counter-intuitive that there was a higher CLAD rate in the macrolide group, however nowadays at CLAD diagnosis, guidelines propose to start azithromycin as soon as possible (12). This explains why the majority of the patients in the macrolide group suffers from CLAD. Stratification of our macrolide cohort

according to the time of macrolide initiation, provided further support for the protective effect of macrolides. We acknowledge that macrolide usage is an important confounder in this analysis, however adding macrolide usage to our model binary, as a time-dependent variable, censoring for macrolide start data and stratified analysis according to timing of macrolide start relative to CLAD data, all proved that the observed associations are robust.

Our findings corroborate the proposed WHO limit for air pollution, since the effect of long-term air pollution exposure was only found among those patients with $PM_{10} \geq 20$ μg/m³. Two thirds of our lung transplant patients were exposed to PM_{10} values above the WHO (annual) standard of 20 μg/m³. Based on our data, a reduction of the PM_{10} concentrations below the WHO recommendation could potentially prevent 9.9% of the observed mortality in lung transplant recipients not taking macrolides. Our observed associations regarding mortality (13.8% for increase in 10 μg/m³ in PM_{10}; 6.7% for 5 μg/m³ in PM_{10}) are somewhat more pronounced than in other similar studies. The ESCAPE study found a 4% increased risk in mortality for every 5 μg/m³ in PM_{10} (19). Similarly, a recent study in the Netherlands showed an 8% increased risk of non-accidental mortality for every 10 μg/m³ increase in PM_{10} (20).

The strength of our study is the unique large cohort of lung transplant patients spread over a large geographical area and the close follow-up of these patients in combination with uniform measures of air pollution exposure. A limitation of the study is the incomplete data for some variables (e.g. SES and smoking status); however, when sensitivity analyses were restricted to cohorts for which this information was available, the estimates were robust Although we used a single European-wide exposure model, exposure misclassification might be cohort-specific due to differences in model performance for different areas across Europe (21). We used modeled data on PM_{10} from 2007 and applied these to addresses of patients at baseline (for some patients even 20 years earlier), based on evidence showing that the spatial distribution of particulate air pollution is stable over a decade (22). Indeed, four separate studies have

demonstrated that the distribution of particulate air pollution is stable over a decade and that existing land use regression models are good predictors of historic spatial contrasts (22–24). We conducted a sensitivity analysis using a more recent cohort patients transplanted after the year 1997 and saw similar associations. Nevertheless, there have been changes in clinical practice and outcome that could confound our analysis. It would be of interest to associate $PM_{2.5}$ with incidence of CLAD and mortality given its important effects in respiratory diseases but this data was unfortunately not available. Lastly, diagnosis of CLAD remains subjective and in a multi-center study this might cause bias (25). We opted not to sub-phenotype our patients as there are no uniform diagnostic criteria and even so using these on a big retrospective cohort is very difficult.

In conclusion, our findings suggest that a significant association exists between air pollution and mortality/CLAD in lung transplant patients. Additionally, the possible protective effect of macrolide therapy against the detrimental effects of air pollution warrant further investigation.

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FIGURE LEGENDS

Figure 1: Geographical distribution of the lung transplant patients across Europe of the 13 different lung transplant centers from 10 different European countries (left) and the average PM_{10} concentration in Western Europe (right). For Zurich, no PM_{10} values were available. Every dot represents a single patient.

Figure 2: A) HR and 95% CI for mortality and road length within 200m buffer zone around patient's home residence. B) HR and 95% CI for all-cause mortality and PM_{10}. C) HR and CI for CLAD and road length in 200m buffer region. D) HR and 95% CI for CLAD and PM_{10}. Each HR(95%CI) is per IQR increase. The HR shown are from the non-macrolide users and are corrected for LTx era, type of transplantation, underlying disease, gender and age. In Zurich, no PM_{10} measurements were available for analysis. The size of the signal reflects the number of subjects and the lines represent the 95% confidence interval.

Figure 3: Distribution of the HR and 95% CI of all-cause mortality (A) or CLAD (C) associated with quintiles of road length in a 200m buffer zone around patient's home residence. HRs and 95% CIs for the association of mortality (B) and CLAD (D) and quintiles of PM_{10}. Q1 is used as reference in all these analyses. PM_{10} quintiles are Q1:<19µg/m³; Q2: ≥19 µg/m³; and <21 µg/m³;; Q3: ≥21 µg/m³; and <24 µg/m³;; Q4 ≥24 µg/m³; and <26 µg/m³;; Q5≥26 µg/m³; road length quintiles in 200m buffer region are Q1 <763m; Q2 ≥763m and <1075m Q3: ≥1075m and <132m3; Q4 ≥1323m and <1667m; Q5≥1667m. The ~~exact~~ HR and 95% CI are shown in supplemental table 2.

	Macrolide-free group	Macrolide group
Patients, n (%)	3556	2151
Recipient age (year)	46.5 (±14)	45.5 (±13.8)
Gender (male), n (%)	1836 (52%)	1146 (53%)
Underlying disease, n (%)		
Emphysema	1535 (43%)	798 (37%)
Cystic fibrosis	707 (20%)	474 (22%)
Interstitial lung fibrosis	737 (21%)	502 (23%)
Pulmonary arterial hypertension	274 (8%)	164 (8%)
Other	303 (9%)	213 (10%)
Type of transplantation, n (%)		
Double or heart lung	2559 (72%)	1652 (77%)
Single	997 (28%)	499 (23%)
Period of lung transplantation, n (%)		
1987-1995	479 (14%)	94 (4%)
1996-2000	710 (20%)	254 (12%)
2001-2005	936 (26%)	644 (30%)
2006-2011	1431 (40%)	1159 (54%)
CLAD, n (%)		
No	2185 (62%)	891 (40%)
Yes	1367 (39%)	1259 (60%)
Unknown	5 (0%)	1 (0%)
Death or graft loss, n (%)	1937 (55%)	640 (30%)

Table 1: Patient characteristics of the lung transplant cohort divided in patients taking macrolides or not. Data are shown as mean (±standard deviation) of absolute number (%). Some of the data will not mount to 100% due to rounding of numbers.

Death		Macrolide-free group n=3556			Macrolide group		n=2151
	IQR	HR	95% CI	**P**	HR	95% CI	P
Road length in buffer zone							
50 m	108m	1.055	0.955-1.112	0.076	0.989	0.824-1.047	0.23
100 m	279m	**1.111**	**1.025-1.202**	**0.0094**	1.003	0.875-1.150	0.95
200 m	752m	**1.094**	**1.030-1.779**	**0.0054**	0.978	0.872-1.094	0.66
500 m	4092m	**1.085**	**1.000-1.130**	**0.0356**	1.085	0.960-1.226	0.15
1000 m	15403m	1.047	0.985-1.131	0.12	1.080	0.970-1.202	0.17
PM_{10}	6 µg/m³	**1.081**	**1.000-1.167**	**0.049**	0.982	0.859-1.120	0.77
Distance to freeway	1233m	0.987	0.964-1.012	0.16	1.000	0.883-1.025	0.31
Distance to major road	241m	1.000	0.976-1.024	0.68	0.976	0.907-1.000	0.092

CLAD	IQR	Macrolide-free group n=3551			Macrolide group		n=2150
Road length in buffer zone							
50 m	108m	1.025	0.956-1.099	0.49	0.997	0.927-1.073	0.93
100 m	279m	1.076	0.975-1.190	0.14	0.943	0.848-1.048	0.28
200 m	752m	**1.110**	**1.023-1.204**	**0.0114**	0.949	0.872-1.030	0.21
500 m	4092m	**1.130**	**1.042-1.226**	**0.0010**	0.960	0.884-1.085	0.62
1000 m	15403m	**1.113**	**1.031-1.202**	**0.0115**	0.970	0.897-1.063	0.48
PM_{10}	6 µg/m³	1.093	0.988-1.208	0.076	**0.886**	**0.803-0.976**	**0.013**
Distance to freeway	1233m	0.988	0.964-1.012	0.26	1.012	1.000-1.038	0.13
Distance to major road	241m	1.000	0.976-1.024	0.73	1.024	0.976-1.049	0.44

Table 2: Overview of main results investigating the association of particulate air pollution and traffic exposure with mortality and CLAD in lung transplant patients. All parameters are analyzed for every IQR increase. In the Cox analysis we corrected for patient age, patient gender, native disease (COPD vs ILD vs cystic fibrosis and bronchiectasis vs pulmonary hypertension vs others), type of transplantation (single versus sequential single), era of transplantation (1987-1995 vs 1996-2000 vs 2001-2005 vs 2006-2011).