Increased COPD exacerbations of likely viral etiology follow elevated ambient NOx

Running Title: Viral COPD exacerbations after higher ambient NOx

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At a Glance Commentary: (200 words)

Scientific Knowledge on the Subject: Diverse mechanisms by which air pollution could cause lung pathology have been reported but their importance *in vivo* remains uncertain. Previous time-series studies have shown inconsistent associations between COPD exacerbations and urban air pollution. COPD exacerbations are heterogeneous, being of infective and non-infective etiology, and this provides an investigative window to better examine associations with ambient pollution and underlying mechanisms. We therefore analyzed characterized exacerbations in the London COPD Cohort over the last 20 years, to study whether the effect of pollution on exacerbations differs by likely exacerbation etiology.

What This Study Adds to the Field: Our analysis of characterized COPD exacerbations revealed a consistently significant association between elevated ambient NOx, a tracer of traffic-related air pollution, and an increased incidence of COPD exacerbations 2-4 days later with symptoms of viral etiology. Daily diary card data from participants has allowed us for the first time to show these pollution-associated viral exacerbations are of significantly longer duration. Exacerbations, especially those of longer duration, have a major impact on quality of life of patients and this research supports the importance of studying mechanisms by which air pollution can increase susceptibility to, and severity of, respiratory tract infections.

Abstract

Rationale: Epidemiological research strongly supports an association between air pollution and COPD exacerbations. Numerous mechanisms may underlie any association as pollutants are toxic to pulmonary cells and may increase susceptibility to respiratory infections. The relationship between ambient pollution and exacerbation etiology has not been studied.

Objectives: To evaluate the characteristics of pollution-associated exacerbations and whether the association is specific to exacerbations of infective or non-infective etiology.

Methods: We analyzed the effect of preceding ambient PM_{10}, NOx and O_3 on characterized COPD exacerbations in a regression model adjusted for temperature, seasonality and long-term trend. We specifically examined associations with exacerbations of suspected viral and/or bacterial, or non-infective etiology. For the associations identified we further examined the characteristics of pollution-associated exacerbations.

Measurements and Main Results: 4173 exacerbations occurred over the 20 year study period. Higher ambient NOx was consistently associated with increased viral-type exacerbations at 2-4 days lag (p=0.010). Recovery for viral-type exacerbations following higher ambient NOx was significantly prolonged. These findings were consistent in the subset of 2841 exacerbations treated with oral corticosteroids or antibiotics, with recovery 1.29 (95% CI 1-17-1.42; p<0.001) times longer with 'viral-type' exacerbations of onset 3 days after above versus below median ambient NOx. A likely bimodal association of PM_{10} with infective exacerbations was also evident, and supported by a daily time-series analysis.

Conclusions: Higher levels of ambient NOx are associated with prolonged exacerbations of likely viral etiology, supporting toxicological effects of air pollution that increase susceptibility to, and severity of, infection.

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Introduction

The Great London Smog of 1952 provided unequivocal evidence that air pollution contributes to respiratory morbidity and mortality (1). Despite governmental initiatives, pollution still impacts on health particularly in people with chronic airflow limitation (2). Mechanistic studies have shown numerous direct and indirect routes by which oxides of nitrogen (NOx), ozone (O_3) and particulate matter (for example PM_{10}) can contribute to pulmonary inflammation and pathology. These include increasing susceptibility and severity of infection – of major importance given that infections trigger many exacerbations of airway diseases (3) (4) – but also other mechanisms unrelated to infection (5) (6). Epidemiological time-series studies of exacerbations of airways diseases (defined by healthcare presentations or mortality) compared to ambient concentrations of pollutants over the preceding days have reported heterogeneous findings (7) (8) (9) that could in part be explained by heterogeneity in the etiological origin of exacerbations but this has not been previously investigated.

Exacerbations of chronic obstructive pulmonary disease (COPD) are predominately triggered by respiratory viral infection, but many exacerbations are caused by new or flare-up of existing bacterial infection, or increase in airway inflammatory load, whilst some have no apparent inflammatory or infective origin (3) (10). Importantly, pollution may have specific effects on different types of exacerbation but this has not been previously investigated. This is a vital question if we are to better manage the continuing health impact of air pollution.

In this study, using our well phenotyped COPD cohort followed over 20 years, we have for the first time examined the hypothesis that air pollution triggers COPD exacerbations of particular etiology by analyzing whether exacerbations involving respiratory symptoms suggestive of infective etiology, or not, are more likely following elevated air pollution. A time-series model with distributed lags of increasing interval has been utilized to study onset of exacerbations at shorter and longer latent periods after pollution exposure, reflecting direct and more indirect mechanisms of toxicity. To further describe the nature of pollution-associated exacerbations we compared the clinical characteristics of exacerbations following high and low pollution episodes.

Some of the results of these studies have been previously reported in the form of an abstract (11).

Methods

Patient recruitment

This study involved a total of 440 COPD patients enrolled in the London COPD cohort between 1st January 1996 and 31 December 2015. The cohort comprised 100-175 patients at any given time, with continuous recruitment from local clinics by consecutive invitation of patients (12), thereby replacing those who withdrew or died over the 20 years. The patients all lived in London and withdrew from the cohort if they moved to live elsewhere. At recruitment, a medical history was taken and spirometry performed with a Vitalograph Gold Standard spirometer (Vitalograph Ltd, Maids Moreton, UK) or FlowScreen II spirometer (Viasys Healthcare Ltd, Warwick, UK). Potential participants were screened with post-bronchodilator spirometry to ensure they met the criteria for COPD as defined at study

onset (13) (14), with Forced Expiratory Volume in 1 second (FEV_1) ≤80% of a normal value predicted from age, height, and sex, and a FEV_1/Forced Vital Capacity (FVC) ratio <0.7. As in previous studies, to ensure a reliable estimate of the annual exacerbation rate only patients who had completed at least 365 days of diary cards were included in this analysis.

The study was approved by the London-Hampstead research ethics committee and all patients gave written informed consent (REC 09/H0720/8).

Temperature and Pollution Data

Daily data for atmospheric pollutants in London, UK, were obtained for the North Kensington and Bloomsbury urban background monitoring sites from the Air Quality Information Archive databases (http://www.airquality.co.uk). North Kensington was chosen *a priori* as the site for the principal analysis as this has previously been shown as representative of exposure over London as a whole (15). The pollutants investigated were NOx, as an indicator of traffic-related air pollution (15) (16); PM_{10} as an indicator of total particulate matter; and O_3 to capture summer smog episodes (17) (18).

Daily temperature data was the average of hourly readings over 24 hours, from two sites (St. James and Hampstead, in London) obtained from the British Atmospheric Data Centre (www.badc.nerc.ac.uk). Missing daily temperature data was imputed from the other site prior to calculating the average of the two sites.

Exacerbations

Patients recorded any worsening in respiratory symptoms on daily diary cards. Total exacerbations were identified according to validated criteria of increases in any two major symptoms (dyspnea, sputum volume or sputum purulence) or one major and one minor symptom (a cold (nasal congestion/discharge), wheeze, cough, sore throat) over two consecutive days (19). Exacerbation recovery was defined as the number of days from exacerbation onset that increased respiratory symptoms were still being recorded, with the first of two consecutive symptom-free days indicating when the exacerbation finished. From October 1996 onwards, records were kept of treatment of exacerbations with antibiotics or oral corticosteroids, and these exacerbations are termed healthcare utilization (HCU) events. Occasionally, patients failed to record symptoms and exacerbations were defined by treatment and/or hospital admission and it was not possible to calculate an exacerbation recovery time.

The exacerbations were classified into four groups based upon the symptoms recorded by the patient between onset and recovery. The presence of cold symptoms (nasal congestion/discharge) was used a marker of a 'viral-type' exacerbation (3) (4), and symptoms of increased sputum volume or purulence as 'bacterial-type' (20). Exacerbations with symptoms suggestive of both viral and bacterial infection were categorized as 'with-both', and those that remained as 'with-neither'. When patients did not record symptoms, exacerbations were classified according to symptoms recorded by the physician in clinic who asked the patient.

Validation of symptom-defined exacerbations by PCR.

For a subset of exacerbations, sputum was collected within 7 days of onset prior to treatment and assayed by previous described quantitative polymerase chain reaction (PCR) for human rhinovirus and three common pathogenic bacteria (*H.Influenzae, S.Pneumonia, M.Catterhalis)* (4) (20). Samples were considered viral positive with viral load > 10 colony forming units (cfu) /ml and bacterial positive with > 10^4 cfu/ml. Whole blood was also collected prior to treatment into EDTA tubes, and sent for absolute and differential white cell counting, and after centrifugation, for measurement of plasma C-reactive protein (CRP).

Statistical methods

Patient characteristics are summarized as appropriate by a mean and standard deviation or standard error, or a median and inter-quartile range, or as a percentage, and compared by unpaired Student t-test, Mann-Whitney two-sample test or chi-squared test, as appropriate.

Times-series analyses:

Logistic regressions with random effects for subjects (xtlogit command in Stata 12.1) were used to model separately the effects of each pollutant on the incidence or not of each of the four types of exacerbations within individuals. In the distributed interval analyses, the independent variables were the pollutant and temperature averaged over days 0-1, 2-4, 5-8 and 9-13 preceding exacerbations, terms for seasonality (12 month sine and cosine) and a longitudinal trend term to adjust for any changes over time in disease severity or clinical care. Odds Ratios (ORs) for incidence of exacerbations per 10µg/m^3 increase in each ambient pollutant concentration were calculated. Further times-series analyses were conducted with similar models but with the daily concentrations on individual preceding days rather than averages for lag intervals.

Analysis of exacerbation characteristics dichotomized by preceding ambient pollution:

Duration of exacerbations and the numbers of days during recovery with particular symptoms were compared between exacerbations associated with high or low levels of pollution. Poisson or negative binomial regression models were used with adjustment for repeated measures on the same individual. Linear random effect models which adjusted for repeated measures were used to examine differences in CRP and % blood eosinophil count between types of exacerbations.

All statistical tests were two-sided, and $p<0.05$ considered significant.

Results

Patient Demographics and Exacerbations.

The 440 COPD patients studied had moderate to very severe COPD (Table 1). They were under observation for a total of 620,869 days. They experienced a total of 4173 exacerbations (2841 HCUs). 434 (271 HCUs) were of 'viral-type' with associated cold symptoms, 1645 (1089 HCUs) were of 'bacterial-type' with increased sputum purulence/volume, 1419 (1072 HCUs) had a combined character 'with-both' cold symptoms and increased sputum purulence/volume, and 675 (409 HCUs) 'with-neither' but which were characterized primarily by dyspnea, wheeze and/or cough.

Validation of symptoms and infective etiology

Between 2008-2014, 298 sputum samples were collected within 7 days of exacerbation onset and tested for rhinovirus and pathogenic bacteria. Sputum PCR detection for rhinovirus was significantly more likely in patients with 'cold' symptoms (Chi-squared test; p=0.006), and sputum PCR positivity for *H. influenzae*, *M. catarrhalis* or *S. pneumonia* significantly more likely in patients with increased sputum volume or purulence (p=0.025).

Blood eosinophil counts as a percentage were significantly lower (p=0.009 with adjustment for repeated measures) in exacerbations `with-both' bacterial and viral symptoms (mean of patient average = 2.47% (SD 1.6); n=137 patients), compared to exacerbations of 'with-neither' type i.e. with no infective symptoms (mean 3.46% (SD 2.85); n=46); 'viral-type' versus 'with-neither' type showed a tendency for a lower eosinophil count (p=0.08). The median of patients' plasma CRP levels at exacerbation onset was significantly higher in exacerbations `with-both' bacterial and viral symptoms (median=16.1 mg/dl (IQR 7-39)) and `bacterial-only' exacerbations (median=12.5 mg/dl (IQR 4.8-37.5)) compared to exacerbations of `with-neither' type (median=5.5 mg/dl (IQR 2-15); p=0.0001 and p=0.0041 respectively; Mann-Whitney).

Pollution

Over the 20 years between 1/1/1996 and 31/12/2015, PM_{10} data was available on 6946 days (95.1%); O_3 on 7070 days (96.8%); and NOx on 7068 days (96.8%) at the North Kensington site. Median daily levels recorded at North Kensington were 20.4 $\mu g/m^3$ (IQR

188 15.4-27.8), 35.4 μg/m^3 (20.7-49.0); and 48.5 μg/m^3 (31.6-78.1) respectively. As expected
189 there was clear seasonal variation in ambient pollutant concentrations (Figure 1) but also
190 evidence of long-term trends, with both NOx and PM_{10} falling by -2.4 and -0.7 μg/m^3/year
191 respectively (both, p<0.001) whilst O_3 rose by 0.63 μg/m^3/year (p<0.001).

192

193 *Associations between ambient pollution and onset of specific types of exacerbation*
194 The effects of the different atmospheric pollutants at distributed lag intervals on specific
195 types of COPD exacerbation were analyzed after accounting for lagged temperature,
196 seasonality and longitudinal trend (Table 2). `Viral-type' exacerbations were more likely
197 following higher ambient NOx at 2-4 days lag as measured at the North Kensington urban
198 background monitoring site, whilst no other significant associations were evident with
199 measures from this site. Notable increased odds ratios for exacerbations with both
200 bacterial and viral features were evident with higher ambient PM_{10} at 0-1 days lag and 9-13
201 days lag, but these did not reach significance.

202 To confirm the association between NOx and viral-type exacerbations we repeated the
203 analysis with the alternate Bloomsbury urban background monitoring site (Table E1 in the
204 online data supplement). Consistent with the North Kensington analysis an increased
205 incidence of `viral-type' exacerbations was evident following elevated NOx at 2-4 days lag
206 (OR=1.0239, p=0.033). The associations of increased exacerbations with both bacterial and
207 viral features after higher ambient PM_{10} at 0-1 days lag and 9-13 days lag were significant in
208 Bloomsbury analysis.

209

210 *Increased HCU events with ambient pollution*

We performed a sensitivity analysis using only HCU events (Table 3). When we examined the effect of ambient pollutants measured at the North Kensington site on HCU events with specific characteristics, `viral-type' HCU events were as previously significantly more likely after higher ambient NOx at 2-4 days lag. Additionally 'bacterial-type' HCU events were more likely after higher ambient PM_{10} at the same lag.

In the confirmatory analysis using pollutant concentrations as measured at the Bloomsbury site (Table E2 in the online data supplement) the association between ambient NOx and 'viral-type' HCU events at 2-4 days lag was again present (OR=1.0290, p=0.044). However the association between 'bacterial-type' HCU events and PM_{10} was not significant in this analysis.

Association between ambient NOx and viral type-exacerbations in a daily time-series analysis

To better characterize this association between ambient NOx and viral-exacerbations at 2-4 days lag and to establish whether the increased risk for exacerbations strengthened and then declined over this interval in the manner expected for a biological association, we next examined the association in daily lag time-series model (Figures 2A, E1). The association was significant at a lag of 3 days with increasing odds ratios over the 2 preceding days and decreasing odd ratios over the following 2 days. We also examined the effect of preceding ambient NOx at the same daily lags on the odds ratios of a viral-type exacerbation being long (≥10 days) as opposed to short (<10 days). Consistent with the other data there was a significantly increased odds ratio of a viral-type exacerbation being of long duration for exacerbations of onset 3 days after higher ambient NOx (Figure 2B).

Analysis of effects of season and long-term time period on the association between viral-type exacerbations and ambient NOx

Given the differences in winter and summer pollution episodes we repeated the distributed interval analysis for NOx with viral-type exacerbations separately for the summer (April - September) and winter periods (October - March) (Table E3). The association between ambient NOx and viral-type exacerbations at 2-4 days lag was significant for winter (OR=1.0260, p=0.007) but not for summer periods (OR=1.0044, p=0.919). Similarly the association between PM_{10} and bacterial-type HCU exacerbations was significant for winter but not summer periods. Given the interaction between NOx and O_3, we also re-analysed the winter and summer periods in a two-pollutant model (Table E4), and the association between NOx and viral-type exacerbations remained significant in the winter (OR=1.0279, p=0.008) but not summer (OR=0.9614, p=0.437).

The types of road vehicles have changed over the 20 years studied with resulting changes in traffic related air pollution. In an exploratory analysis, we repeated the distributed interval analysis separately for the earlier years of the cohort study and later years (Table E5). The association between ambient NOx and viral-type exacerbations at 2-4 days lag was significant over the later 10 years (December 2005 – December 2015) (OR=1.0382, p=0.018, 285 patients and 356,608 data days) but not the initial 10 years (January 1996 – December 2005) (OR=1.0152, p=0.168, 234 patients with 239,487 data days). The association between PM_{10} and bacterial-type HCU exacerbations was also significant for the more recent but not the earlier period.

Characteristics of high pollution-associated exacerbations

To better appreciate the impact of ambient pollution, we further examined in exploratory analyses the characteristics of `viral-type' exacerbations and HCU events 3 days following above or below median ambient NOx.

Gender and smoking status have been suggested to affect susceptibility to pollution and we therefore studied whether disproportioniate numbers of viral-type exacerbations 3 days after high versus low ambient NOx occurred in men compared to women, or in smokers compared to non-smokers (21) (22). Whilst smoking status had no effect on distribution of NOx-associated exacerbations at 3 days lag, gender did have an effect with a greater proportion of viral-type exacerbations being associated with elevated ambient NOx in male than in female subjects (Chi-squared test; p=0.013).

Table 4, and Figure 3, show that the durations of both `viral-type' exacerbations and `viral-type' HCU events were significantly longer with higher preceding ambient NOx. In contrast the duration of exacerbations 'with-neither' viral or bacterial features was not significantly longer when preceded 3 days prior by above median NOx (Figure 3).

'Viral-type' HCU events 3 days after higher than median NOx were also significantly more likely to be characterised by more days of cough symptoms; there was a similar trend towards more days of cough symptoms in 'viral-type' exacerbations. Pollution did not have any significant effect on days of breathlessness or wheeze in these exacerbations.

Limited numbers of exacerbations or HCU events of each type of character had CRP or eosinophil count measured prior to treatment, and therefore equivalent analyses of the

effect of above/below median ambient pollution were not possible. However, for total exacerbations, CRP was higher (p=0.0165) for those exacerbations 3 days following above versus below median NOx.

Discussion

In this study we have investigated for the first time associations between ambient pollution and COPD exacerbations categorized by symptom-defined etiology. We observed significant associations that were reproduced in subsequent analyses of exacerbations associated with health care utilization. An increased risk of exacerbations of probable viral etiology was consistently evident 2-4 days after elevated ambient NOx. This is in contrast to our previous study analyzing uncharacterized exacerbations (7) but is consistent with published literature in other patient groups. Previous studies have shown associations between higher NO_2 exposure at similar lag and increased visits to the doctor for respiratory symptoms in patients with chronic lung disease (23), more severe symptoms for respiratory tract viral infections in asthmatic children (24), and an association with combined hospital admissions for pneumonia and influenza (25).

We present the complete set of associations investigated as decided *a priori* to be necessary in the absence of clear evidence of a particular pollutant or lag to selectively study. Only 4173 exacerbations occurred over the more than 600,000 patient days studied limiting the power to conduct conservative multiple-comparisons tests without risk of a type 2 statistical error. Nevertheless, there are two strong lines of argument against the association

between ambient NOx and viral-type exacerbations being a chance finding. Firstly, sensitivity analyses consistently showed evidence of a significantly harmful effect of NOx at lag 2 to 4 days on viral-type exacerbations. Additionally the daily lag analysis showed a significant effect of ambient NOx on viral-type exacerbations at the day 3 midpoint of the interval, with the odds ratio increasing over the preceding days then decreasing consistent with this being a biologically real association.

Secondly, that traffic related air pollution leads to more and longer COPD exacerbations is strongly biologically plausible, being consistent with our understanding of the toxicity of NOx and engine exhaust particles (26) (27) (28), and their capacity to increase susceptibility to severe respiratory infections (29). Of particular relevance to viral exacerbations, NOx has been shown to increase epithelial expression of ICAM-1 (30) (31), the major entry receptor for human rhinoviruses and other pathogens (32) (33). Cell culture studies have shown a more-than-additive synergism between the pro-inflammatory effects of viral infection and NO_2 exposure (34).

That a similar association is not evident between NOx and exacerbations of combined viral-bacterial aetiology is interesting. Exacerbations with both viral and bacterial infection are not of a singular type – there are exacerbations where bacterial infection follows viral infection and exacerbations with both present from onset (4). Whilst the former would also be expected to be associated with ambient NOx, the pathogenesis of exacerbations with both bacterial and viral infection present from onset is different and therefore may not be similarly affected by NOx. Chronic airway colonization with pathogenic bacteria may be an independent risk factor for viral infection through mechanisms that overlap those of

pollutants (35). For example *Haemophilus influenzae* also increases epithelial ICAM-1 expression (36).

It is well known that exacerbations are more likely in the winter months, and in our analysis we made allowance for both outdoor temperature and for seasonality that might confound any relationship (37) (38). To further examine any effect of seasonal weather we separately examined winter and summer periods, finding the positive association between ambient NOx and viral-type exacerbations to be significant in the winter but not during summer periods. This seasonal difference might reflect an increased statistical power in the winter period when there are more viral respiratory tract infections and higher ambient NOx peaks. However the composition of air pollution does change between seasons beyond the NOx/O_3 interaction (39) with winter NOx potentially a tracer of other seasonal pollutant constituents. Alternatively other seasonal environmental factors may influence the effects of air pollution, for example patient vitamin D status (40).

Interestingly, the association between ambient NOx and viral-type exacerbations was significant only for the more recent 10 year period and not earlier 10 year period. Whilst this difference may result from less statistical power over the earlier years, NOx is a tracer of traffic related air pollution and the difference may be due to changes in road vehicles over the study period with increasing numbers of diesel engines (41) (42). Diesel emissions are thought particularly toxic to respiratory health, producing relatively greater harmful NO_2 as a proportion of NOx, and toxic diesel exhaust particles.

In addition to the association of viral-type exacerbations with ambient NOx, a likely bimodal association of PM_{10} with exacerbations characterized by both viral and bacterial symptoms was also evident and an association of bacterial-type HCU exacerbations with ambient PM_{10} at 2-4 days lag evident. The range of lags between pollution exposure and onset of exacerbations of different etiologies may reflect the range of possible underlying mechanisms for pollution toxicity identified in laboratory studies. Rapid effects include stimulating pro-inflammatory mediator release by epithelial cells and airway macrophages (43), and stimulation of neuronal reflex responses in the lung (5). Lagged mechanisms include stimulation of pathological adaptive immune responses (6), and actions of air pollution to increase susceptibility to more severe infection including impaired epithelial barrier integrity (44), impaired macrophage phagocytosis (45) (46), and perturbation of IFNγ-mediated immune responses (47) (48). Oxidative stress is a common pathological pathway for many mechanisms and gender-related differences in oxidative stress responses may underlie sex differences observed (21) (22).

A major strength of this study is the detailed characterization of the patients. The daily diary cards recorded by patients, together with examination in clinic by a specialist physician at exacerbation, provided a wealth of clinical data that allowed investigation of characterized exacerbations and atmospheric pollutants. This has not been possible before as detailed symptomology from onset to recovery are not routinely collected at primary care consultations or hospital attendances. The diary card data allowed us to clinically characterize these pollution-associated exacerbations and show them to be associated with increased days of coughing as well as of increased total duration. The increased duration was not only statistically significant but also clinically significant with 'viral-type' HCU events

1.29 times longer after above median ambient NOx. Duration of exacerbation is of major importance in determining the effect of COPD exacerbations on patient's health-related quality of life and economic costs (12) (49).

In this study pollution exposures were analyzed using ambient concentrations at an urban background monitoring site, raising the possibility of exposure misclassification. This type of error can lead to an underestimate of the effects of pollution (50). The North Kensington monitoring site was chosen *a priori* as it has previously been shown to be representative of London background ambient pollution (15). Importantly our patients lived over a small geographic region and inner London is known to have relatively homogenous background air pollution. In an additional analysis using data from the monitoring station closest to patient's home address the association between NOx and viral-type exacerbations remained significant (Table E6), consistent with previous research showing this may not improve accuracy in determination of urban personal exposure (51). Although an individual's exposure to pollution could be estimated based on home address, other unrecorded variables need to be considered for such models to be accurate such as ventilation of the home (52). Patients in our COPD cohort also go outdoors most days (53) with exposure to traffic-related air pollution that would not be captured by models based on patient's home address. The ideal would be to capture patient location and activity across the day, including physical activity (54). Studies using personalized monitoring are being conducted but are generally of too small a sample size to investigate the relation between pollution exposure and different types of exacerbations. Other ambient pollutants are also likely important in the health impact of air pollution, such as finer particles within PM_{10} (e.g.

395 $PM_{2.5}$), but we were restricted to pollutants measured continuously over the entire 20 year
396 period.

397 We used symptoms to define viral or bacterial-induced exacerbations and thus we will have
398 misclassified some exacerbations, however upper respiratory tract ('cold') symptoms are an
399 established marker of exacerbations of viral etiology (3) (4), and increased sputum
400 purulence or volume a marker of exacerbations with bacterial etiology (20) (55).
401 Significantly lower blood eosinophil counts in exacerbations `with-both' bacterial and viral
402 infection features is consistent with previous findings that eosinophil counts decrease
403 during COPD exacerbations with confirmed bacterial infection (56).

404 The definition of COPD has evolved since the cohort was established. Importantly if only
405 patients with FEV1/FVC less than the Lower Limit of Normality (LLN), as per current
406 definitions, are included (415 patients) the association between viral-type exacerbations
407 and NOx remains significant (Table E7).

408

409 Our findings are important as they provide a link between mechanistic studies showing
410 changes in inflammation and susceptibility to infection in response to air pollution, and
411 epidemiological studies of patient events. Additionally our study shows the value of
412 considering exacerbation phenotype in epidemiological studies. An effect of ambient NOx
413 on a subset of exacerbations characterized by viral-type symptoms was consistently evident
414 across analyses, but possible effects on other types of exacerbation were also identified that
415 need further study. The association with viral-type exacerbations also raises the question of
416 whether the economic impact of pollution may include effects on both incidence and
417 duration of recovery for common viral respiratory infections in healthy individuals. In

conclusion, our study adds further evidence of the danger of traffic-related air pollution in COPD, and supports an association between air pollution and infection-mediated exacerbations of COPD that warrants further mechanistic research.

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600

FIGURE LEGENDS

Figure 1: Seasonal and long-term trends in indices of ambient pollution and air temperature, London, 01/01/1996 – 21/12/2015.

Daily ambient PM_{10}, NOx and O_3 measured at North Kensington background monitoring site and averaged daily air temperatures, London 01/01/1996 – 21/12/2015.

Figure 2: Daily time-series analysis of effect of ambient NOx on viral-type exacerbations

Effect of daily ambient concentrations of NOx 1-5 days prior to onset of viral-type exacerbations, after adjustment for long-term trend, seasonality and preceding temperature, on odds ratios of (A) incident COPD exacerbations occurring and (B) exacerbations being of long (≥ 10 days) duration. Error bars show Odds Ratios with 95% Confidence Intervals. *, $p < 0.05$. Figure 2A shows a subset of the daily lag data presented in Figure E1 in the online data supplement.

Figure 3: Duration of exacerbations of onset 3 days after below-median and above-median ambient NOx.

Days for recovery, as a percentage of exacerbations, for exacerbations of 'viral-type' and of 'with-neither' type, at 3 days lag after ambient NOx of <54.7 μg/m^3 as compared to those after ambient NOx >54.7 μg/m^3. Days for recovery was defined as the number of days from exacerbation onset that increased respiratory symptoms were still being recorded, with the first of two consecutive symptom-free days indicating when the exacerbation finished.

Online Figure E1: Daily time-series analyses of effects of air pollutants on Odds Ratios for onset of characterised COPD exacerbations.

Odds Ratios for COPD exacerbations categorised by presence/absence of symptoms suggestive of viral etiology, bacterial etiology, both or neither. Effect of daily ambient concentrations of PM_{10}, NOx and O_3 0-13 days prior to onset of the exacerbations after adjustment for long-term trend, seasonality and preceding temperature. Error bars show the standard errors (SEs) associated with the Odds Ratios.

633 **TABLES**

634 **Table 1: Demographics of London COPD cohort patients in the study.**

	440 COPD patients	
	Mean	**SD**
Age (years)	68.6	8.4
FEV_1 (l)	1.19	0.48
FEV_1 (% predicted)	46.9	16.6
FVC (l)	2.6	0.86
FEV_1/ FVC (%)	46.3	12.1
Smoking history (pack-years)	52.4	37.8
BMI (kg/m^2)	26.0	5.5
	Median	**IQR**
Distance of residential address to North Kensington monitoring site (km)	10.3	(5.8-12.9)
Exacerbations per year	2.13	(0.98-3.41)
HCU events per year	1.32	(0.60-2.32)
Observation (days)	1081	(667-1839)
	N	**%**
Male	283	64.3
Smoker at recruitment	151	34.6

635

636 HCU (healthcare utilization) events; exacerbations with associated antibiotic or oral

637 corticosteroid prescription.

638 **Table 2: Odds Ratios for characterized exacerbations following higher ambient pollutant concentrations at lag intervals.** Effects of ambient

639 pollutants measured at North Kensington monitoring site at 0-1, 2-4, 5-8 and 9-13 days lag intervals on Odds Ratios (ORs) for characterized

640 COPD exacerbations after accounting for lagged temperature, seasonality and longitudinal trend in logistic regressions per 10μg/m^3 increase.

	Lag Interval Days 0-1			**Days 2-4**			**Days 5-8**			**Days 9-13**		
	OR	95% CI	p	OR	95% CI	p	OR	95% CI	P	OR	95% CI	p
With-Both viral and bacterial-type symptoms												
NOx	1.0041	0.9971-1.0142	0.429	0.9991	0.9874-1.0110	0.884	1.0037	0.9914-1.0161	0.556	1.0089	0.9964-1.0214	0.158
PM_{10}	1.0547	0.9971-1.1156	0.063	0.9797	0.9161-1.0478	0.549	1.0180	0.9520-1.0886	0.602	1.0559	0.9905-1.1257	0.096
O3	0.9945	0.9515-1.0394	0.806	0.9926	0.9435-1.0443	0.775	0.9759	0.9247-1.0299	0.374	1.0249	0.9708-1.0821	0.374
Viral-type symptoms only												
NOx	0.9983	0.9807-1.0162	0.853	**1.0231**	**1.0054-1.0411**	**0.010**	1.0074	0.9870-1.0281	0.480	0.9952	0.9719-1.0141	0.692
PM_{10}	0.9795	0.8830-1.0865	0.695	1.0675	0.9504-1.1989	0.270	0.9942	0.8801-1.1231	0.926	1.0035	0.8908-1.1304	0.954
O3	1.0748	0.9919-1.1645	0.078	0.9222	0.8397-1.0127	0.090	0.9987	0.9039-1.1034	0.979	0.9950	0.8996-1.1004	0.922

641

Bacterial-type symptoms only												
NOx	0.9927	0.9821-1.0035	0.183	1.0046	0.9936-1.0157	0.418	1.0001	0.9882-1.012	0.983	1.0003	0.9876-1.0132	0.961
PM_{10}	0.9726	0.9216-1.0264	0.312	1.0595	0.9975-1.1254	0.060	1.0191	0.9569-1.0855	0.556	0.9859	0.9267-1.0489	0.654
O3	1.0192	0.9779-1.0622	0.367	0.9645	0.9190-1.0123	0.143	1.0193	0.9684-1.0723	0.468	0.9782	0.9294-1.0296	0.399
With-Neither viral or bacterial symptoms												
NOx	1.0057	0.9906-1.0211	0.460	1.0017	0.9841-1.0196	0.853	0.9980	0.9788-1.0176	0.842	1.0005	0.9808-1.0205	0.961
PM_{10}	1.0716	0.9869-1.1636	0.099	0.9190	0.8291-1.0187	0.108	0.9923	0.8962-1.0988	0.883	0.9840	0.8922-1.0853	0.747
O3	1.0159	0.9533-1.0827	0.627	0.9924	0.9222-1.0681	0.840	1.0443	0.9661-1.1289	0.275	1.0005	0.9249-1.0823	0.990

642

643 **Table 3: Associations of healthcare use (HCU) exacerbations with ambient pollution.** Effects of ambient pollutants measured at North

644 Kensington monitoring site at 0-1, 2-4 5-8 and 9-13 days lag intervals on Odds Ratios (ORs) for characterized HCU events after accounting for

645 lagged temperature, seasonality and longitudinal trend in logistic regressions per 10μg/m^3 increase.

	Lag Interval Days 0-1			**Days 2-4**			**Days 5-8**			**Days 9-13**		
	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
With-Both viral and bacterial-type symptoms												
NOx	1.0032	0.9913-1.0152	0.601	0.9952	0.9813-1.0094	0.509	1.0091	0.9951-1.0233	0.201	1.0113	0.9970-1.0260	0.122
PM_{10}	1.0468	0.9797-1.1184	0.176	0.9773	0.9034-1.0572	0.567	1.0425	0.9547-1.1265	0.293	1.0759	0.9988-1.1589	0.054
O3	1.0019	0.9523-1.0541	0.942	0.9886	0.9325-1.0481	0.700	0.9687	0.9107-1.0304	0.313	1.0146	0.9534-1.0797	0.648
Viral-type symptoms only												
NOx	0.9918	0.9680-1.0163	0.509	**1.0255**	**1.0024-1.0490**	**0.030**	1.0149	0.9896-1.0408	0.250	0.9893	0.9582-1.0214	0.509
PM_{10}	0.9145	0.7964-1.0502	0.206	1.0865	0.9389-1.2573	0.265	1.0120	0.8698-1.1774	0.289	1.0120	0.8698-1.177	0.877
O3	1.0634	0.9613-1.176	0.233	0.9416	0.8375-1.0586	0.314	0.9737	0.8586-1.041	0.677	1.0448	0.9209-1.185	0.496

646

Bacterial-type symptoms only												
NOx	0.9965	0.9836-1.0096	0.060	1.0088	0.9953-1.0225	0.203	0.9953	0.9801-1.0107	0.546	1.0039	0.9882-1.0199	0.630
PM_{10}	0.9577	0.8941-1.0257	0.217	**1.0921**	**1.0122-1.1783**	**0.023**	0.9683	0.8919-1.0512	0.442	0.9764	0.9019-1.0571	0.556
O3	0.9996	0.9501-1.0517	0.988	0.9577	0.9026-1.0162	0.153	1.0305	0.9679-1.0971	0.347	0.9627	0.9043-1.0248	0.233
With-Neither viral or bacterial symptoms												
NOx	1.0107	0.9915-1.0302	0.278	1.0040	0.9805-1.0281	0.742	0.9237	0.9552-1.0110	0.229	0.9956	0.9687-1.0232	0.751
PM_{10}	1.0467	0.9372-1.1688	0.418	0.9442	0.8247-1.0811	0.406	0.9724	0.8475-1.1158	0.690	0.930	0.8338-1.0892	0.480
O3	0.9951	0.9164-1.0806	0.907	0.9615	0.8746-1.0572	0.417	1.0773	0.9741-1.1914	0.147	0.9837	0.8890-1.0886	0.751

647

Table 4: Characteristics of exacerbations and their Incidence Rate Ratios (IRRs) when preceded by above-median compared to below-median ambient pollutant exposure, with adjustment for temperature, season (with sine and cosine terms) and longitudinal trend.

	NOx lagged 3 days, < or > 54.7 ug/m3			**NOx lagged 3 days, < or > 54.7 ug/m3**		
	Viral-type exacerbations; (384 exacs, 177 subjects) (missing data on 49 exac)			**Viral-type HCU; (233 HCU, 131 subjects) (missing data on 38 HCU)**		
	IRR	**95% CI**	**P-value**	**IRR**	**95% CI**	**P-value**
Recovery time (days)	1.15	1.07-1.24	**0.001**	1.29	1.17-1.42	**0.001**
Breathlessness (yes versus no)	1.14	0.95-1.36	0.172	1.19	0.93-1.52	0.161
Wheeze (yes versus no)	1.14	0.85-1.55	0.362	1.14	0.80-1.62	0.469
Cough (yes versus no)	1.33	1.00 -1.77	0.052	1.63	1.16 -1.23	**0.005**

Footnote: Recovery time analysed assuming a Poisson distribution with allowance for repeated measures.