Comparison of exhaled and nasal nitric oxide and exhaled carbon monoxide levels in bronchiectatic patients with and without primary ciliary dyskinesia

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Primary ciliary dyskinesia (PCD) is a genetic disease characterised by defective motility of cilia resulting in impaired mucociliary clearance and infertility in most cases. Decreased ciliary motility with secondary microbial colonisation in the airways leads to chronic airway inflammation and bronchiectasis. Oxidants and inflammatory mediators released from activated inflammatory cells in airways may lead to the induction of inducible nitric oxide synthase (iNOS) producing nitric oxide (NO) and inductible heme oxygenase (HO-1) releasing carbon monoxide (CO). In pulmonary diseases associated with chronic airway inflammation, such as asthma, expression of iNOS and HO-1 is increased in the airways and levels of NO and CO are raised in exhaled breath. Increased levels of exhaled NO (eNO) and CO (eCO) correlate with other markers of airway inflammation in asthma. Concentrations of eNO and eCO are also increased in patients with bronchiectasis, which may reflect iNOS and HO-1 expression in macrophages and neutrophils in the airways of these patients. Patients with PCD may represent a unique group of subjects, however, as eNO and nasal NO (nNO) levels are abnormally low in patients with PCD and with documented bronchiectasis. Nasal NO is also lower in children with Kartagener’s syndrome (a condition in which PCD is associated with situs inversus) compared with healthy control children, suggesting a defect in the function and/or expression of NO synthases. The level of eNO and nNO in patients with PCD may not reflect the chronic inflammatory process in the airways and might not be used as a non-invasive inflammatory marker, but it may be useful in diagnosing PCD as a cause of bronchiectasis in these patients. Expression and/or function of HO-1, however, may not be defective in patients with PCD and the level of eCO may reflect the chronic inflammatory process in these subjects.

We have therefore measured eNO, eCO, and nNO levels simultaneously in bronchiectatic patients with PCD and compared these values with those obtained from age matched healthy subjects, and patients with non-PCD bronchiectasis, with and without cystic fibrosis (CF). We included bronchiectatic patients with CF because CF may present with symptoms of chronic inflammation of the upper and/or lower airways, similar to PCD. Furthermore, eNO and nNO levels are lower than normal not only in PCD, but also in CF.

Methods: The levels of exhaled nitric oxide (eNO), carbon monoxide (eCO) and nasal NO (nNO) from bronchiectatic patients with PCD (n=14) were compared with those from patients with non-PCD bronchiectasis without (n=31) and with cystic fibrosis (CF) (n=20) and from normal subjects (n=37) to assess the clinical usefulness of these measurements in discriminating between PCD and other causes of bronchiectasis.

Results: Exhaled NO levels were lower in patients with PCD than in patients with non-PCD non-CF bronchiectasis or healthy subjects (median (range) 2.1 (1.3–3.5) ppb vs 8.7 (4.5–26.0) ppb, p<0.001; 6.7 (2.6–11.9) ppb, p<0.001, respectively) but not lower than bronchiectatic patients with CF (3.0 (1.5–7.5) ppb, p>0.05). Nasal levels of nNO were significantly lower in PCD patients than in any other subjects (PCD: 54.5 (5.0–269) ppb, non-PCD bronchiectasis without CF: 680 (310–1000) ppb, non-PCD bronchiectasis with CF: 343 (30–997) ppb, control: 663 (322–1343) ppb). In contrast, eCO levels were higher in all patient groups than in control subjects (PCD: 4.3 (3.0–24.0) ppm, p<0.01, other bronchiectasis without CF: 5.0 (3.0–15.0) ppm, p<0.001; CF: 5.3 (2.0–23.0) ppm, p<0.001 vs 3.0 (0.5–5.0) ppm). Low values in both eNO and nNO readings (<2.4 ppb and <187 ppb, respectively) identified PCD patients from other bronchiectatic patients with a specificity of 98% and a positive predictive value of 92%.

Conclusion: The simultaneous measurement of eNO and nNO is a useful screening tool for PCD.
or primary ciliary disorientation (n=7, verified using a computerised improvisation image analysis system). Bronchiectasis was diagnosed clinically and confirmed by high resolution computed tomographic (CT) scanning of the thorax in all patients. CF was confirmed by genetic analysis and a positive sweat test (chloride values >60 mM). Patients with atopy, asthma, or reversible (>12%) airway obstruction were excluded from the study. Normal control subjects had no history of chronic disease, were not receiving any regular medication, and had been free of respiratory infections for at least 6 weeks before the study. None of the subjects reported being current cigarette smokers or being exposed to smoke for more than 0.5 hours/day; this was confirmed by testing their urinary cotinine level with NicCheck I (DynaGen Inc, Cambridge, MA, USA).

Study design
Exhaled NO, eCO, and nNO were measured and spirometric tests were then performed. Forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC) were measured (range) 2.1 (1.3–3.5) ppb; p<0.001 and 3.0 (1.5–7.5) ppb; the end point of the measurement and values of eNO and eCO were read at this plateau. Participants repeated the manoeuvre until two acceptable tests were performed (difference between readings <5%).

Levels of nNO were measured using a Teflon tube which was inserted just inside one nostril while the contralateral nostril was left open. Air was sampled at 250 ml/min continuously from one nostril via the other nostril during a breath hold maintained as long as possible. NO concentration was recorded when the value reached the plateau. Nasal CO2 was also monitored to ensure exhalation was not taking place. This test was repeated twice and the mean value calculated.

The same model of analyser was used in both laboratories and measurements were performed by the same investigator to avoid any possible error arising from measurements taken at different sites. Both analysers were calibrated daily with certified NO mixtures (100 ppb) in nitrogen (BOC Special Gases, Guildford, UK). Two successive recordings were made and mean values were used in all calculations.

Statistical analysis
Age, FEV1, and FVC values are given as mean (SE). Mediator values are expressed as median (range). Differences between groups were analysed non-parametrically using the Kruskal-Wallis test (p values of <0.0001 were considered significant). Pairwise comparisons using the Dunn’s multiple comparison test were then carried out.

Spearman’s rank correlation was used to determine the relationship between variables. Mean – 2SD values were calculated for nNO to obtain a cut off value for determining the specificity and sensitivity of the measurements. A p value of <0.05 was considered significant.

RESULTS
Exhaled NO
The concentration of eNO in patients with PCD and CF was significantly lower than that in healthy subjects (median (range) 2.1 (1.3–3.5) ppb; p<0.001 and 3.0 (1.5–7.5) ppb;
Patients with primary ciliary dyskinesia (PCD) with documented bronchiectasis, and patients with non-PCD bronchiectasis with and without cystic fibrosis (CF). The Kruskal-Wallis test showed significant differences between the different groups of patients (fig 1). There were a few outliers in eCO data (24 ppm in the PCD group, 19 and 23 ppm in the CF group) so the results were also analysed without these values, giving the following results: PCD patients: 4 (3–8) ppm, CF patients: 5 (2–11) ppm; the values in both groups were significantly higher than those of the normal control group (p<0.01 and p<0.001, respectively). No significant difference was detected between patients with and without steroid treatment.

Sensitivity and specificity of the measurement of eNO and nNO

The sensitivity of eNO measurement in selecting PCD patients (number of PCD patients with low eNO reading/number of all patients with PCD in the population) was 79% when a cut off value of 2.4 ppb was chosen. The specificity of eNO measurement (number of non-PCD bronchiectatic patients with negative test/number of all non-PCD bronchiectatic patients) at this cut off value was 85%. The predictive value of a positive test (PPV; percentage of PCD patients with a positive result) was 69% and the predictive value of a negative test (NPV; percentage of patients with a negative test result from those patients who did not have PCD) was 94%.

The sensitivity of nNO measurement was 93% and the specificity was 95% when the mean – 2SD of the nNO level in healthy subjects (187 ppb) was used as a cut off value (only two patients with CF had a value below the cut off level). The PPV of nNO measurement was 87% and its NPV was 97% in discriminating between PCD and non-PCD bronchiectatic patients.

If a positive result was described as low values in both eNO and nNO readings together in the same patient (eNO <2.4 ppb + nNO <187 ppb), this identified PCD patients with a specificity of 98%, PPV of 92%, and NPV of 93%.

Correlations between variables

There was no correlation between lung function and eNO, nNO, or eCO values either when data were pooled or when data from steroid treated and non-treated subgroups were analysed separately or different subject groups were analysed separately (data not shown). Similarly, no relation was found between eNO and nNO readings or between eNO, nNO, eCO values and the age of subjects.

DISCUSSION

We have assessed the clinical usefulness of the simultaneous measurement of eNO and nNO in discriminating between PCD and other types of bronchiectasis. The reported specificity and sensitivity of the simultaneous measurements suggest that their combined use can be a valuable non-invasive screening tool for PCD. The measurement of nNO by itself has specific sensitivity was 93% and the specificity was 95% when the mean – 2SD of the nNO level in healthy subjects (187 ppb) was used as a cut off value (only two patients with CF had a value below the cut off level). The PPV of nNO measurement was 87% and its NPV was 97% in discriminating between PCD and non-PCD bronchiectatic patients.

If a positive result was described as low values in both eNO and nNO readings together in the same patient (eNO <2.4 ppb + nNO <187 ppb), this identified PCD patients with a specificity of 98%, PPV of 92%, and NPV of 93%.

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There was no correlation between lung function and eNO, nNO, or eCO values either when data were pooled or when data from steroid treated and non-treated subgroups were analysed separately or different subject groups were analysed separately (data not shown). Similarly, no relation was found between eNO and nNO readings or between eNO, nNO, eCO values and the age of subjects.

DISCUSSION

We have assessed the clinical usefulness of the simultaneous measurement of eNO and nNO in discriminating between PCD and other types of bronchiectasis. The reported specificity and sensitivity of the simultaneous measurements suggest that their combined use can be a valuable non-invasive screening tool for PCD. The measurement of nNO by itself has good diagnostic value. However, if a low nNO value is observed, determination of eNO and the combined evaluation of the two test results improves the diagnostic value by increasing its specificity and PPV. The measurement of eNO, however, cannot be used by itself for this purpose because of its low sensitivity and PPV. This study shows for the first time...
that patients with PCD have lower nNO values than those with any other types of bronchiectasis. It also shows that eNO values are lower in patients with PCD than in those with non-PCD, non-CF bronchiectasis, although there is no difference in eNO values between patients with PCD and CF bronchiectasis. In addition, we have shown that the eCO concentration is increased in patients with PCD to a similar extent to that in other types of bronchiectasis.

There was a significant difference in the mean age of the patients with CF and the other groups. This difference, however, probably did not influence our results because eNO and eCO values have been found not to be age related in adults. Furthermore, there was no evidence of any relation between age and nNO values.

A modest increase in eNO levels in non-PCD, non-CF bronchiectatic patients compared with healthy subjects, with some overlap between the two groups. This increase was smaller than we had observed in patients with atopic asthma. This fits well with the notion that production of NO is linked to eosinophil inflammation and that eNO levels are not as high in patients with airway inflammation dominated by neutrophils as in those with allergic asthma.

Some of our patients were treated with inhaled and/or oral steroids, but we could not detect any difference in eNO and eCO levels between patients with or without this treatment. Corticosteroids have been shown to reduce eNO levels profoundly in asthmatic patients and to decrease nNO levels in patients with allergic rhinitis. However, in patients with chronic suppurrative upper and/or lower airway inflammation the results are somewhat contradictory. No difference was found in the eNO level between steroid treated and non-treated patients with CF, while in patients with bronchiectasis one study showed a difference in the eNO level but another did not. Differences between the results of these studies can be explained by differences in the techniques used for eNO measurement and by the fact that all of these studies (including the present one) were cross sectional. Prospective follow up studies are required to investigate the possible influence of steroid treatment on eNO values in bronchiectatic patients.

Because nasal symptoms (nasal polyps, sinusitis, persistent serous otitis media) are the main presenting features of PCD, it is important to consider the diagnosis of PCD in these cases. Could the measurement of nNO and eNO help to exclude PCD as an underlying cause of chronic upper airway symptoms? Nasal NO levels are extremely low in PCD. However, low NO levels are also found in patients with chronic rhinosinusitis and may therefore influence the diagnostic value of nNO measurement in discriminating between patients with PCD and those with rhinosinusitis from other causes. Although not specifically investigated in the present study, subjects with non-PCD, non-CF upper airway and sinus diseases had normal NO levels in the lower airways. The use of the combination of eNO and nNO measurements would therefore still have good specificity in identifying PCD.

In contrast to the results on NO levels, we found that eCO levels were raised in all patient groups studied compared with controls, with no difference between different conditions. In chronic airway infections increased HO-1 protein expression may result from the induction of the enzyme by inflammatory mediators such as interleukin-1β, tumour necrosis factor-α, interferon-γ and hydrogen peroxide (H₂O₂) present in the inflamed airways. HO-1 may be induced in various cells in the respiratory tract, including airway macrophages, epithelial cells, and infiltrating inflammatory cells such as neutrophils. In the present study there was no correlation between eCO levels and FEV₁. This is not surprising as the decrease in FEV₁ may be the result of structural changes due to repeated inflammation while increased eCO levels are more closely linked with the ongoing inflammatory process. Increased CO production may have multiple functions in the airways including antioxidant, pro-inflammatory, and anti-inflammatory effects.

The finding that eCO is increased equally in different inflammatory airway diseases including asthma, bronchiectasis, and CF suggests that CO production has a profound link with inflammatory processes regardless of their atopic or non-atopic nature.

In conclusion, this study has shown that combined measurement of eNO and nNO levels discriminates between bronchiectatic patients with PCD and those with bronchiectasis from other causes, which suggests that this measurement can be used as a non-invasive screening tool for PCD. On the other hand, increased levels of eCO found in all patient groups indicate that the activation of CO production is generally involved in these airway conditions.

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REFERENCES


LUNG ALERT

Thrombolysis for submassive pulmonary embolism

This was a randomised placebo controlled trial of alteplase (100 mg over 2 hours) with heparin v placebo with heparin in 256 patients with submassive pulmonary embolism (PE) defined as echocardiographically detected PE; pulmonary artery hypertension with confirmation of PE by spiral computed tomography (CT); precapillary pulmonary hypertension with confirmation by CT; or new electrocardiographic signs of right ventricular strain followed by CT. Exclusion criteria included age >80 years, haemodynamic instability, and presentation more than 96 hours after onset of symptoms. In the alteplase group there was less escalation of treatment (p=0.004) and a greater chance of 30 day event free survival (p=0.005). There was no difference in mortality or adverse events between the two groups.

This is the largest trial of thrombolysis in PE ever conducted, and it looks at the controversial group of patients with submassive PE showing significant benefit of alteplase. The absence of haemorrhagic complications was surprising.

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LETTERS TO THE EDITOR

Variations in mortality in acute COPD may reflect nihilism as well as resources

I read with great interest the paper by Roberts et al. and the accompanying editorial by Rudolf. The study highlights important variations in the outcomes of patients with a common chronic disease, and once more illustrates that doctor:patient ratios may be an important contributor to this. It is also likely that some of the observed variation may arise as a result of variations in decision making by individual clinicians.

A recent study carried out in the eight hospitals in the Heart of England Critical Care network interviewed 98 clinicians who made the critical decisions for patients with chronic obstructive pulmonary disease (COPD). Each had made a median of 10 end of life decisions for COPD patients in the previous 12 months. There was considerable variability in the decision whether or not to admit identical patients to the critical care unit, with those choosing not to admit patients forming very pessimistic predictions of outcome compared with clinicians who would admit. It seems possible that poor outcomes for patients with COPD may not simply reflect a lack of resources, but also therapeutic nihilism that may have grown up over the years in response to the cognitive dissonance that arose when beds in critical care units could not be found for COPD patients in extremis. It seems likely that reversing variations in outcome will require both changes in resources and changes in clinicians’ expectations. In this respect, the GMC guidance on withholding and withdrawing life prolonging treatments may well be helpful, particularly section 20 which recommends that “where there is a reasonable degree of uncertainty about the appropriateness of providing a particular treatment, treatment which may be of some benefit to the patient should be started until a clear and final decision can be made”. The Heart of England Critical Care network study over one third of clinicians would not admit a 75 year old COPD patient with single organ respiratory failure, yet in a recent study of over 3700 admissions of COPD patients of median age 67 years to UK intensive care units, those with single organ respiratory failure had a hospital survival of over 70%.

It is important that chest physicians continue to advocate for COPD patients admitted as emergencies, and take every opportunity to point out to their colleagues in general medicine and intensive care how well patients with COPD can do with both invasive and non-invasive ventilation.

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References

Authors’ reply

We thank Dr Wildman for his letter suggesting a further possible reason for the variation in outcome that we reported for the acute care of COPD patients in different hospital centres. In a further unpublished multiple regression analysis of the RCP/BTS 2001 audit we found that 26% of the variation in the outcome of death at 90 days following admission could be accounted for by factors measured in the study that included patient characteristics such as performance status and resource and organisational issues, as described in our paper. Dr Wildman suggests that individual clinicians might vary in determining admission to the ITU for COPD patients in respiratory failure, and suggests that respiratory physicians need to be advocates for their patients in this arena.

Attitudes and beliefs in what might be achieved are important but are difficult to measure in clinical practice. They might account for some of the variation in outcome but, if so, the therapeutic nihilism would have to vary systematically between whole hospitals to explain the pattern seen in our study. Admission to the ITU depends on more than the individual attitude of the referring doctor. A lack of availability of beds may raise thresholds, and an institutional nihilism within the ITU may lead to rejection of suitable patients.

Perrin et al. reported a study in which questionnaires regarding initiation of mechanical ventilation in end stage COPD patients were completed by 350 doctors subdivided by specialty (intensivists, respiratory specialists and other physicians). As in the paper by Wildman et al., there was considerable individual variation in decision making but no overall difference between the three types of specialist studied. However, no analysis by hospital or trust was made to identify local patterns. We believe all respiratory physicians will share Dr Wildman’s call that referring physicians should be advocates for their patients, and this has to be matched by a willingness of the ITU staff to accept such patients and the availability of beds within an ITU/HDU to accommodate them. Perrin’s paper provides hope of a generic match although individual disagreements may still occur. It is, however, not only admission to the ITU that matters, as in many hospitals non-invasive ventilatory support is provided on general wards by respiratory units without input from intensivists.

In the BTS/RCP 2003 national audit of the acute care of COPD patients, 95% of all acute admitting sites have now registered to participate and data collection is nearing completion. Within the clinical data gathering there is a question that attempts to document clinical decision making when a patient eligible for ventilation on blood gas criteria does not receive ventilatory support. In addition, data on available resources such as ITU beds, bed occupancy, and numbers of ITU candidates transferred off site will be recorded. We may be in a position to shed further light on the issues of individual versus institutional nihilism or rationing in due course.

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References

Do inhaled corticosteroids slow FEV1 decline in COPD after all?

I question the findings of the meta-analysis by Sunderland et al. and the content of the associated editorial by Burge and Lewis. The meta-analysis has drawn from available long term data from randomised controlled studies (RCTs) of inhaled corticosteroids (ICS) in chronic obstructive airways disease (COPD). The whole purpose of meta-analysis is to analyse such data systematically to answer a question. This study seems to use the data selectively to demonstrate an effect. Another recent meta-analysis—in my opinion properly conducted—drew on the same studies and reached the opposite conclusion. The fact is that four long term, adequately powered RCTs have examined the effect of ICS, all of these studies revealed no effect of ICS on the primary outcome measure of decline in forced expiratory volume in
Ethics of placebo-controlled studies of inhaled steroids for COPD

The meta-analysis by Sutherland et al.\(^1\) of the effect of inhaled corticosteroids on the progression of airflow limitation in patients with chronic obstructive pulmonary disease (COPD) found a small improvement in forced expiratory volume in 1 second (FEV\(_1\)) of 7.7–9.9 ml in FEV\(_1\) decline compared with placebo of “debatable clinical importance”.\(^2\) It is hardly something to shout about, as occurred following this publication (probably egged on by the editorial) which was quoted in the GP press as suggesting that current widespread ICS use (albeit “off-label”) was now clinically justified.\(^1\)

Another major problem with this study is that it does not analyse harm. For example, the largest RCT showed a significant reduction in bone mineral density of the lumbar spine and femur in patients receiving inhaled triamcinolone.\(^3\) People with COPD likely to receive ICS are frail and have poor mobility, so this finding raises particular concern as they are more likely to fall and falls could result in fracture. Even if inhaled triamcinolone is not used in the UK, fluticasone is. Fluticasone has been the subject of particular cautionary advice because of its ability to cause systemic effects at high doses.\(^4\) If there is indeed a marginal clinical benefit from using these drugs, I think these people deserve a better assessment of risk and benefit than was presented in this meta-analysis and the accompanying editorial.\(^5\) The editorial claimed that it is no longer ethical to do more long-term trials: surely the conclusion is the opposite? We need better data to justify the widespread use of ICS in COPD.

References


Authors’ reply

To make randomised controlled studies ethical, the investigator must believe that neither of these treatments is known to be superior to the other. If the trial is to be placebo controlled, the investigator must believe that no non-approved treatment is known to be of benefit. Both Drs Duerden and Hahn want more placebo controlled trials of inhaled corticosteroids (ICS) in COPD before they recommend their use. I was puzzled by this statement and would like to ask them why they reached this conclusion.

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unanswered questions are—at which stage to start and what dose to use? Randomised trials in these areas are badly needed. They will require large numbers, enthusiasm from respiratory clinicians, and are likely to need public rather than pharmaceutical industry funding.

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References
1 Burge PS, Lewis SA. So inhaled steroids slow the rate of decline of FEV1 in patients with COPD after 8 years (range 0.5–62) and 133 patients (84.3%) reported that their bed partner had previously complained of the sleep related breathing disorders among the general population and probably among health professionals. The delay in diagnosis is likely to have significant effects on morbidity, and in recent preliminary work it has been shown that those with OSASH have structural changes in brain morphology compared with healthy controls.2 In addition to the health and quality of life benefits to the individual to be gained by prompt diagnosis, there are also economic aspects in favour of prompt diagnosis and treatment2 and early benefits in terms of driving performance.3

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Delays in diagnosis of OSAHS
We very much enjoyed the first paper in the review series on sleep and admired Stradling and developments of antibiotic resistance.4 Recently published research has shown benefits of long term azithromycin treatment in patients with bronchiectasis.5 These results led us to consider using azithromycin as prophylaxis in patients with non-cystic fibrosis bronchiectasis with frequent infective exacerbations.

Exclusions included allergy to macrolides and abnormal liver function tests. The study schedule was 500 mg once daily for 6 days, 250 mg once daily for 6 days, then 250 mg on Monday/Wednesday/Friday of each week. A safety blood examination was organised 1 month after starting treatment. The patients were fully reviewed at least 4 months after commencement of azithromycin prophylaxis and lung function tests repeated. Sputum culture results before and after starting prophylaxis were noted. Statistical analysis was performed using a paired t test and non-parametric Wilcoxon test.

Thirty nine patients were studied. Fifteen had idiopathic bronchiectasis and the remainder consisted of 13 with post childhood infections, five with primary ciliary dyskinesia, five with common variable immunodeficiency, and one with Young’s syndrome. Their mean (±SD) age was 51.9 (±16.1) years (range 18–77) with a 2:1 female predominance. All patients had had more than four documented exacerbations during the previous 12 months. Six patients stopped taking the azithromycin prophylaxis because of side effects: abnormal liver function tests (n = 2), diarrhoea (n = 2), rash (n = 1), and tinitus (n = 1). All occurred during the first month of treatment. Other side effects experienced were mild and mainly gastrointestinal. Five patients were on long term oral corticosteroids with no change in dosage, in two new inhaled corticosteroids were introduced, and one patient was given a short day reducing course of oral corticosteroids. The mean (SD) length of time taking azithromycin, excluding those who stopped because of side effects, was 20 (±10.1) months (range 4–38). Twenty six patients are continuing with the prophylaxis at the present time; in the other seven treatment was discontinued because of improvement in their condition.

Spam culture results (bacteria isolated) before commencement showed no growth (n = 13), Pseudomonas aeruginosa (n = 8), Staphylococcus aureus (n = 6), Haemophilus influenzae (n = 6), Streptococcus pneumoniae (n = 3), Stenotrophomonas maltophilia (n = 2), Moraxella catarrhalis (n = 1), not done (n = 4). After 4 months the results were no growth (n = 18), P aeruginosa (n = 5),

Prophylactic antibiotic treatment of bronchiectasis with azithromycin
Once a treatable cause of bronchiectasis such as hypergammaglobulinaemia has been excluded, management largely involves physiotherapy and treatment of infective exacerbations with appropriate antibiotics.6 In a proportion of patients this is not adequate to prevent frequent infective exacerbations. Prophylactic antibiotic treatment can be used to try to prolong the exacerbation free period. This may be administered orally, via a nebuliser, or a long regimen of intravenous antibiotics. Prophylactic treatment may be problematic due to side effects and development of antibiotic resistance.7 Macrolide antibiotics exhibit immunomodulating properties. Long term, low dose erythromycin has been shown in diffuse panbronchiolitis, a disease with some similarities to idiopathic bronchiectasis, to be effective in controlling chronic suppurative airways disease.7 Recently published research has shown benefits of long term azithromycin treatment in patients with bronchiectasis.5 These results led us to consider using azithromycin as prophylaxis in patients with non-cystic fibrosis bronchiectasis with frequent infective exacerbations.

Patients attending the outpatients department between February 1999 and April 2002 who fulfilled the following criteria were considered for azithromycin prophylaxis:1

• bronchiectasis defined by CT scan;
• any causal condition had been treated if possible;
• general management optimised;
• >4 documented infective exacerbations requiring oral or intravenous antibiotic treatment during the last 12 months;
• Pseudomonas aeruginosa respiratory infection, if present, had not responded to nebulised antibiotic prophylaxis or this had not been tolerated;
• failure to control chronic symptoms.

These results suggest a lack of awareness of sleep related breathing disorders among the general population and probably among health professionals. The delay in diagnosis is likely to have significant effects on morbidity, and in recent preliminary work it has been shown that those with OSASH have structural changes in brain morphology compared with healthy controls.2 In addition to the health and quality of life benefits to the individual to be gained by prompt diagnosis, there are also economic aspects in favour of prompt diagnosis and treatment2 and early benefits in terms of driving performance.3

1 Stradling JR, Davies RJO. Sleep - 1: Obstructive sleep apnoea/hypopnoea syndrome: definitions, epidemiology and natural history.Thorax 2004;59:73–8


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S. aureus (n = 1), S. pneumoniae (n = 1), not done (n = 10). In three patients who had cultured P. aeruginosa before starting azithromycin prophylaxis the organism was not recultured at follow up.

In the 33 patients completing at least 4 months treatment there was a statistically significant reduction in infective exacerbations requiring oral antibiotics from a mean of 0.71 per month to 0.13 per month (p < 0.001). There was also a reduction in the requirement for intravenous antibiotics from a mean of 0.08 courses per month to 0.003 courses per month (p < 0.001).

Subgroup analysis of patients with P. aeruginosa isolated before starting azithromycin prophylaxis showed no difference compared with all patients included (p = 0.22). Twenty five patients had lung function tests before and after at least 4 months of treatment (range 4–20 months). There was an improvement in all lung function parameters but the improvement in carbon monoxide transfer factor (TlCO) was the only one to reach statistical significance (p = 0.01).

Symptom data were collected from 32 patients and scored on a 5-point scale (table 1). Statistical analysis using a non-parametric Wilcoxon test showed that there was a significant improvement in all symptoms.

The mechanism by which azithromycin reduces the number of infective exacerbations and chronic symptoms is unknown, but it is likely to be multifactorial. It may be due to downregulation of the host immune response by azithromycin, so decreasing host mediated tissue damage as postulated in the vicious circle hypothesis. It might also benefit patients by reducing bacterial load and therefore the stimulation for neutrophilic inflammation, or by influencing the pathogenic mechanisms of bacteria. Macrolide antibiotics have also been shown to reduce mucus formation, or by influencing the pathogenic mechanisms of bacteria.

Currie et al compared high dosage amoxicillin with placebo over an 8 month period and found a greater reduction in the volume of purulent sputum between exacerbations in the amoxicillin group (20% of pretreatment volume) than in the placebo group, but did not demonstrate any reduction in infective exacerbations. The superior findings of our study suggest that the anti-inflammatory effects of azithromycin were important in achieving the results obtained. This study was performed with patients who were sufficiently unwell to preclude consideration of a placebo group. The patients therefore acted as their own controls. The results are sufficiently impressive to encourage the design of a randomised study, either enrolling less sick patients and having a placebo comparator or using a comparator antibiotic without immunomodulating properties.

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References

Early life antibiotics and asthma
Cullinan et al present interesting data on the association between exposure to antibiotics in early life and the subsequent expression of atopy and asthma. In keeping with other studies, they report a positive association between antibiotic receipt over the first 5 years of life and asthma. The association was, however, largely accounted for by a history of asthma in the first year, while in the Ashford study only 396 prescriptions were issued to 746 subjects in the first year, so a maximum of 53% children received any antibiotics.

It seems likely from the data presented that antibiotic exposure did not play a major causal role in promoting the asthma phenotype, but rather that those who used antibiotics had asthma already or developed it subsequently. The data from our study are consistent with this and suggest that antibiotic exposure was more important for young children significantly less than 2 years of age, but the question of whether it may now be a significant and potentially modifiable factor remains unanswered.

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Recurrence of acute respiratory failure following use of waterproofing sprays
Between January and March 2003 six patients were admitted to hospital in the Lausanne area of Switzerland with acute respiratory failure following use of a waterproofing spray for clothes and leather. Within hours of exposure all patients developed a dry cough and rapidly progressive respiratory failure.

The clinical picture included severe hypoxaemia, increased white blood cell count, raised C-reactive protein, and reduced carbon monoxide...
In the past, several outbreaks of acute respiratory symptoms have been recorded in different countries including 550 in Oregon in 1992,\(^1\) in Pennsylvania and Virginia in 1993,\(^3\) in Quebec in 1993,\(^4\) and in Japan between 1992 and 1993.\(^5\) Most of these epidemics followed a modification of the composition of the spray. One untreated patient developed a pulmonary fibrosis during a German outbreak in the 1980s\(^6\) and one death was reported in Japan in the 1990s.\(^7\)

Following these outbreaks, various suggestions were proposed to explain these intoxications.\(^8\) In our opinion, the most likely explanation for the present outbreak is that the heptane solvent, which is more volatile than the previous one (isopropanol), allows the mist containing the new fluorinated resin to spread further in the tracheobronchial tree and to reach the alveoli where it might produce reactive metabolites inducing an alveolitis. However, the exact chemical reaction remains unknown. Because of the potentially lethal aspect of these intoxications and the possibility of new outbreaks, we consider that more research is needed on the effect of mist particle size and large analytical and epidemiological studies are required to investigate this phenomenon further.

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**Effect of PM\(_{10}\) on H influenzae and S pneumoniae**

That air pollution, and specifically particles, are harmful to health is well accepted,\(^1\) causing direct effects such as lung inflammation resulting in exacerbations of lung and cardiac conditions\(^2\) and being associated with admissions for pneumonia. In the 1960s Lawther et al showed that ambient particles stimulated the growth of *Haemophilus influenzae* in vitro,\(^3\) suggesting a direct effect of particles on bacteria themselves. However, it is not known whether this remains so for modern ambient particles where the sources are different.

To address this we have assessed the effect of PM\(_{10}\) (particles essentially less than 10 μm in diameter) on the respiratory pathogens commonly associated with acute exacerbations of chronic obstructive pulmonary disease (COPD) and pneumonia. The effect of dilutions of extracts of PM\(_{10}\) on the growth of *H influenzae* and *Streptococcus pneumoniae* grown in liquid broth and the effect of PM\(_{10}\) on microbial growth kinetics of *S pneumoniae* was assessed.

Fresh isolates of *H influenzae* and *S pneumoniae* were obtained from clinical specimens and the control strains *H influenzae* NCTC 11931 and *S pneumoniae* ATCC 49619 were used. Particles were collected on a tapered element oscillating microbalance situated in central Birmingham, representative of the urban background site. To obtain a usable sample the surface of the filter was wetted and rinsed with two sequential aliquots of 0.5 ml saline using a Gilson pipette until visual inspection showed no more particles coming off the filter. The two aliquots were combined and sonicated for 2 minutes to disperse the particles and aggregates. This procedure usually gives a yield of 50–300 μg/ml particles (Donaldson, personal communication). It is not known for certain how these concentrations relate to likely concentrations in the epithelial lining fluid, but this approach has been used in previous in vitro studies of inflammatory responses which have shown pro-inflammatory effects.

In the first experiment a 1:20 dilution of PM\(_{10}\) was made by adding 0.5 ml to 9.5 ml Iso sensitest broth (ISTA; Oxoid Ltd, Basingstoke, UK) supplemented with 5% lysed horse blood and 20 μg/ml NAD. The same volume of normal saline was added to controls. Test and control bottles were inoculated with 0.5 ml of organism suspension at a density of 0.5 McFarland. A viable count was performed hourly for 5 hours while incubating at 37°C in 5% CO\(_2\) using the Miles and Misra technique.\(^4\) In the growth kinetic experiment equal volumes of PM\(_{10}\) solution and ISTA broth (supplemented with 5% lysed horse blood and 20 μg/ml NAD) were added to the first column of a sterile microtitre tray. Serial broth dilutions to a final dilution of 10\(^{-6}\) were performed. Control wells contained broth only and wells for sterility checks contained PM\(_{10}\) alone, broth alone and inoculum alone. Organism suspension, 50 μl *S pneumoniae* ATCC 49619, was added to each test and control column of the wells and incubated at 37°C in 5% CO\(_2\) for 5 hours. The Miles and Misra technique\(^4\) was used to estimate the viable count of organism in each well and the differences in log cfu/ml between test and control were plotted against serial dilutions of PM\(_{10}\). This test was repeated five times using the same strain to check for reproducibility.
In the first experiment the number of viable cells increased progressively and in the expected pattern over time (Fig 1A and B), whether in the presence or absence of PM10, for both \textit{H} influenzae and \textit{S} pneumoniae. In the growth kinetics experiment the only consistent finding was an inhibition of growth at a PM10:broth medium dilution of 1:1 compared with the PM10 free control.

Growth of \textit{H} influenzae and \textit{S} pneumoniae is therefore neither inhibited nor promoted by incubation with PM10, at concentrations of diluted particles which are known to be able to exert pro-inflammatory effects in vitro. There was a constant inhibitory effect at a PM10 dilution of 1:1, possibly due to the particles themselves or to dilution of the broth by the added saline. These findings suggest that the association of air pollution with hospital admissions for exacerbations of COPD and for pneumonia is probably not mediated through direct promotion of bacterial growth. If particles alone are responsible for these effects, they are likely to be mediated by particles causing lung inflammation, thus encouraging penetration and growth of bacteria in the respiratory tract. Alternatively, gaseous pollutants may be responsible for the epidemiological findings, either directly or in conjunction with...