LUNG CANCER

Prognostic significance of cyclooxygenase-2 (COX-2) and expression of cell cycle inhibitors p21 and p27 in human pleural malignant mesothelioma

A Baldi, D Santini, F Vasaturo, M Santini, G Vicidomini, M Pia Di Marino, V Esposito, A M Groeger, G Liuzzi, B Vincenzi, G Tonini, M Piccoli, F Baldi, S Scarpa

Background: A study was undertaken to analyse the potential prognostic value of the immunohistochemical expression of cyclooxygenase-2 (COX-2) and p27 in 29 malignant mesotheliomas already screened for the expression of p21 and p53.

Methods: Immunohistochemistry was used to determine the expression of COX-2 and p27. The correlation with survival of these factors and of p21 and p53 expression was assessed by univariate and multivariate analyses.

Results: A positive statistically significant correlation was found between p27 and p21 expression (p<0.001), but there was a negative correlation between COX-2 expression and both p27 (p=0.001) and p21 (p<0.0001). No statistically significant correlation was recorded between p53 and all the other immunohistochemical parameters. Univariate analysis showed that overall survival was strongly influenced by p21, p27, and COX-2 expression, but multivariate Cox regression analysis showed that the only immunohistochemical parameter to influence overall survival of patients with mesothelioma was COX-2.

Conclusions: These findings suggest that COX-2 expression may be a useful prognostic parameter for mesothelioma.

Malignant mesothelioma is a rare, highly aggressive tumour which accounts for less than 1% of all cancer deaths in the world. This neoplasm arises from the surface serosal cells of the pleural (>90% of cases), peritoneal, and pericardial cavities and from the tunica vaginalis of the testis. Although the association between exposure to asbestos and the development of mesothelioma is commonly accepted, the exact mechanism whereby asbestos induces the mesothelioma is unknown. Several studies report the presence in some mesotheliomas of DNA encoding SV40 T antigen or SV40 T antigen protein expression, suggesting that the presence of this viral gene may also be associated with the pathogenesis of this neoplasm. The prognosis is generally poor with a reported median survival of 4–12 months in either untreated or treated (surgery, radiotherapy, or chemotherapy) patients. Moreover, mesothelioma has proved resistant to classical chemotherapeutic and radiation regimes and the natural history has not been influenced by standard therapy. Several clinical prognostic factors have been tentatively correlated to survival, histological type and performance status being the most valuable.

Cyclooxygenase-2 (COX-2) has been implicated in carcinogenesis of several neoplasms through the downregulation of cell mediated immunity, promotion of angiogenesis, inhibition of apoptosis, and the formation of carcinogenic metabolites such as malondialdehyde. Furthermore, COX-2 overexpression has been noted in many solid tumours and has been correlated with a worse prognosis in colorectal cancer, non-small cell lung cancer, and gastric cancer. It has been shown that cultured human mesothelial cells contain cyclooxygenase activity and that COX-2 expressing mesothelioma cell lines are associated with increased proliferative and invasive potential. A recent study showed that COX-2 expression is a strong prognostic factor in human mesothelioma, which contributes independently to the other clinical and histopathological factors in determining a short survival. It has been proposed that COX-2 exerts its influence on mesangial cell proliferation in vitro by a novel mechanism involving the tumour suppressor p53 and the cell cycle inhibitors p21 and p27, and several recent studies have investigated the potential prognostic value of p53, p21 and p27 in malignant mesotheliomas.

A study was undertaken to analyse the potential prognostic value of the immunohistochemical expression of COX-2 and p27 in 29 malignant mesotheliomas already screened for the expression of p21 and p53 and for the presence of SV40-like sequences. The correlation with survival of each factor in univariate and multivariate analysis was assessed.

METHODS

Clinical data and tumour specimen acquisition

All patients were treated at the Second University of Naples between 1980 and 1996. Clinical data were obtained by retrospective chart review. Survival was determined from the date of initial surgery. Follow up was available for all patients. No patients were excluded from survival analysis because of death unrelated to cancer, and no patients were lost during follow up. The two patients not included in the analysis of survival were excluded because they died in the postoperative period from surgery related complications.

All patients underwent cytoreductive surgery and 13 patients were further treated with radiotherapy or chemotherapy. Only in three cases did the clinical history show a clear exposure to asbestos, but this does not mean that the other patients were not also exposed to asbestos. In fact, all the patients were living in Campania, a region where contamination with asbestos is very high. Moreover, cases were selected who were still available for immunohistochemical studies from the original group of 35 already characterised for the presence of SV40 sequences.
Prognostic role of COX-2 and p27 in malignant mesothelioma

Tissue from 29 malignant mesothelioma specimens (16 epithelioid, six sarcomatoid, and seven mixed mesotheliomas) obtained from open biopsies or pleurectomies were collected and fixed in 10% formalin before being embedded in paraffin.

**Histological examination**

Five μm sections were cut from the formalin fixed, paraffin embedded samples and stained with haematoxylin and eosin. The histological diagnosis was re-examined by two pathologists (AB and FB) according to the World Health Organization histological typing of tumours. In addition, the most representative blocks were selected to be cut into new 5 μm thick sections for immunohistochemical studies.

**Immunohistochemistry**

All 29 cases had been assessed by immunohistochemistry for the presence of COX-2 and p27. Five μm sections were cut from each specimen, mounted on glass, and dried overnight at 37°C. All sections were then deparaffinised in xylene, rehydrated through a graded alcohol series, and washed in phosphate buffered saline (PBS). PBS was used for all subsequent washes and for antibody dilution. Endogenous peroxidase activity was blocked by 5% hydrogen peroxide. The primary antibodies for p27 (mouse monoclonal; sc-1641, Santa Cruz Biotechnology, CA, USA) and for COX-2 (goat polyclonal; sc-1745, Santa Cruz Biotechnology) were applied at room temperature for 1 hour at a dilution of 1:100. The optimal working dilution was defined on the basis of titration using diaminobenzidine (DAB) as the final chromogen and the streptavidin-biotin system (Dako, Carpintera, CA, USA) as the primary antibodies for p27. For COX-2 staining was always cytoplasmic, while p53, p21 and p27 staining was preferentially nuclear although cytoplasmic positivity was occasionally seen for p21 and p27. Both cytoplasmic and nuclear stainings were considered positive. Five cases were expressed as the detection of SV40 sequences, have been described elsewhere. 23–24

The level of concordance, expressed as the percentage of positive stained cells. The sum of the intensity and extent scores was used as the final staining score (0–7). The extent of staining was scored as 0 (0%), 1 (1–25%), 2 (26–50%), 3 (51–75%), and 4 (76–100%) according to the percentage of positive stained cells. The sum of the intensity and extent scores was used as the final staining score (0–7). The level of concordance, expressed as the percentage of agreement between the observers, was 93% (27 of 29 specimens). In the remaining specimens the score was obtained after collegial revision and agreement. The scoring and quantitation of immunoreactivity for p21 and p53 has been described elsewhere. 23–24

To select the cut off point for COX-2 expression the samples were divided into two groups (0–4 and >4) to give a low expression and high expression group. For all the other markers a cut off point of 10% was chosen because they are all nuclear molecules involved in cell cycle regulation whose loss of expression is strictly connected with tumour progression. A value of 10% therefore indicates whether or not the samples are expressing each of these markers.

**Statistical analysis**

Fisher’s exact test was used to assess the relationship between ordinal data (correlation matrix between immunostaining parameters). The correlation between COX-2, p27, and p21 was performed on the original ordinal units using the Spearman test. Univariate survival analysis for each prognostic variable on overall survival was estimated according to the Kaplan-Meier method. 26 The terminal event was death attributable to cancer. The statistical significance of the differences in survival distribution between the prognostic groups was evaluated by the log-rank test. 27 The Cox proportional hazards model was applied to the multivariate survival analysis. 28 The prognostic variables on overall survival included histological types, COX-2, p21, p27, and p53 expression. p values of <0.05 were regarded as statistically significant in two tailed tests. SPSS software version 10.00 (SPSS, Chicago, USA) was used for statistical analysis.

**RESULTS**

**Histology and immunohistochemistry**

The results of immunohistochemical analysis of 29 mesothelioma specimens are shown in table 1. Histologically, there were 16 epithelioid, six sarcomatoid, and seven mixed mesotheliomas. COX-2 staining was always cytoplasmic, while p53, p21 and p27 staining was preferentially nuclear although cytoplasmic positivity was occasionally seen for p21 and p27. Both cytoplasmic and nuclear stainings were considered positive. Figure 1 shows some typical immunohistochemical stainings for COX-2 (fig 1A and B) and p27 (fig 1C and D). p53 and p21 immunostainings on the same group of mesothelioma samples have been reported elsewhere. 23–24

![Figure 1](https://example.com/figure1.png) Immunochemical stainings of COX-2 (A and B) and p27 (C and D) in human pleural mesotheliomas (original magnification >250).

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Correlation between immunohistochemical and pathological parameters

A positive statistically significant correlation was recorded by rank correlation matrix between p27 and p21 expression, and a negative correlation was found between COX-2 expression and both p27 and p21 (table 2). Interestingly, there was no statistically significant correlation between p53 and all the other immunohistochemical parameters. No correlation was seen between immunohistochemical parameters and mesothelioma histological type.

Immunohistochemical and pathological parameters and patient survival

Univariate analysis showed that overall survival was strongly influenced by p21, p27, and COX-2 expression. The median survival in patients with low p21 or p27 expression was longer than in those with high p21 or p27 expression. The median survival in patients with low COX-2 expression was 14 months compared with 5 months in those with high COX-2 expression. On the other hand, p53 expression and histological type did not influence the overall survival in our patient population (table 3). Kaplan-Meier survival plots for all patients are shown in fig 2. There was a significant association between high levels of COX-2 or low levels of p27 and p21 and poor outcome (p<0.0001), while p53 levels did not significantly affect outcome.

Interestingly, chemotherapy and radiotherapy did not have any effect on overall survival in univariate analysis (data not shown).

Multivariate Cox regression analysis showed that the only immunohistochemical parameter to influence overall survival in patients with malignant mesothelioma was COX-2, the calculated relative risk of death in patients with low COX-2 expression being significantly lower (0.143; p = 0.01, table 4). The other two parameters significantly associated with prognosis by univariate analysis (p27 and p21) did not influence the overall survival when evaluated by multivariate analysis, although p27 achieved borderline significance in multivariate analysis (p = 0.066).

DISCUSSION

We have analysed the expression of COX-2, p27, p21 and p53 and correlated these data with several clinical parameters in a group of 29 mesothelioma specimens positive by polymerase

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**Table 1** Characteristics of study patients

<table>
<thead>
<tr>
<th>Patient no</th>
<th>Histology</th>
<th>COX-2 score</th>
<th>p53 score</th>
<th>p21 score</th>
<th>p27 score</th>
<th>Survival time (weeks)</th>
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<td>1</td>
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<td>2</td>
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<td>2</td>
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<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>8</td>
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</tbody>
</table>

*1 = 0–4; 2 = >4.

*†1 = 0–10%; 2 = >10%.

**Table 2** Rank correlation matrix (and statistical significance) between molecular markers in patients with malignant mesothelioma

<table>
<thead>
<tr>
<th>Histology</th>
<th>COX-2</th>
<th>p53</th>
<th>p21</th>
<th>p27</th>
</tr>
</thead>
<tbody>
<tr>
<td>COX-2</td>
<td>0.589</td>
<td>-2.557</td>
<td>-16.546</td>
<td>-16.163</td>
</tr>
<tr>
<td>p53</td>
<td>0.720</td>
<td>0.110</td>
<td>&lt;0.0001</td>
<td>0.001</td>
</tr>
<tr>
<td>p21</td>
<td>0.736</td>
<td>-2.557</td>
<td>1.691</td>
<td>0.279</td>
</tr>
<tr>
<td>p27</td>
<td>0.792</td>
<td>0.110</td>
<td>0.194</td>
<td>0.710</td>
</tr>
<tr>
<td>COX-2</td>
<td>0.262</td>
<td>-16.546</td>
<td>1.691</td>
<td>16.905</td>
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<tr>
<td>p53</td>
<td>0.000</td>
<td>-0.0001</td>
<td>0.194</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>p21</td>
<td>1.073</td>
<td>-16.563</td>
<td>0.279</td>
<td>17.905</td>
</tr>
<tr>
<td>p27</td>
<td>0.693</td>
<td>0.001</td>
<td>0.710</td>
<td>&lt;0.0001</td>
</tr>
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</table>
We did not find any relationship between the levels of expression of the parameters analysed and the histological types in these patients. These data agree with the lack of correlation reported by others between COX-2, p21, and p53 and type of mesothelioma, but other groups have found a significant correlation between p27 expression and epithelioid histotype.

We found that a high level of COX-2 expression and low p21 and p27 expression were associated with a statistically significant decrease in survival. In multivariate analysis, COX-2 expression predicted outcome independently of all other variables. These findings indicate that high COX-2 expression in tumour cells is associated with clinically more aggressive mesotheliomas and is a strong predictor of poor survival.

Recent studies have underlined the involvement of COX-2 in the natural history of mesothelioma and have also established its prognostic value. However, p21 and p27 have also been shown to have a significant role in the

<table>
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<th>Table 3</th>
<th>Univariate analysis of survival (in weeks) and pathological and immunohistochemical parameters in patients with malignant mesothelioma</th>
</tr>
</thead>
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<tr>
<td><strong>Histology</strong></td>
<td><strong>Median (95% CI) survival</strong></td>
</tr>
<tr>
<td>Epithelial</td>
<td>10.00 (8.06 to 11.94)</td>
</tr>
<tr>
<td>Sarcomatoid</td>
<td>5.00 (1.20 to 9.80)</td>
</tr>
<tr>
<td>Mixed</td>
<td>8.00 (3.87 to 13.13)</td>
</tr>
<tr>
<td>COX-2</td>
<td></td>
</tr>
<tr>
<td>0–4</td>
<td>14.00 (11.71 to 16.29)</td>
</tr>
<tr>
<td>&gt;4</td>
<td>5.00 (3.06 to 6.94)</td>
</tr>
<tr>
<td>p53</td>
<td></td>
</tr>
<tr>
<td>&lt;10%</td>
<td>8.00 (3.87 to 12.13)</td>
</tr>
<tr>
<td>&gt;10%</td>
<td>10.00 (5.68 to 14.32)</td>
</tr>
<tr>
<td>p21</td>
<td></td>
</tr>
<tr>
<td>&lt;10%</td>
<td>5.00 (3.47 to 6.54)</td>
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<tr>
<td>&gt;10%</td>
<td>12.00 (8.33 to 15.67)</td>
</tr>
<tr>
<td>p27</td>
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<tr>
<td>&lt;10%</td>
<td>5.00 (3.51 to 6.49)</td>
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<tr>
<td>&gt;10%</td>
<td>13.00 (10.56 to 15.44)</td>
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<table>
<thead>
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<th>Table 4</th>
<th>Multivariate Cox regression analysis of overall survival in patients with malignant mesothelioma</th>
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<tbody>
<tr>
<td><strong>Hazard ratio (95% CI)</strong></td>
<td><strong>p value</strong></td>
</tr>
<tr>
<td>COX-2</td>
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<tr>
<td>0–4</td>
<td>0.143 (0.032 to 0.646)</td>
</tr>
<tr>
<td>&gt;4</td>
<td>1</td>
</tr>
<tr>
<td>p21</td>
<td></td>
</tr>
<tr>
<td>&lt;10%</td>
<td>2.191 (0.685 to 7.005)</td>
</tr>
<tr>
<td>&gt;10%</td>
<td>1</td>
</tr>
<tr>
<td>p27</td>
<td></td>
</tr>
<tr>
<td>&lt;10%</td>
<td>4.016 (0.913 to 17.653)</td>
</tr>
<tr>
<td>&gt;10%</td>
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</table>
progression of human mesothelioma.\textsuperscript{20,21} Interestingly, while p53 overexpression is a common event in mesothelioma,\textsuperscript{24} it is rarely mutated or inactivated.\textsuperscript{29}

The data presented here showing COX-2 expression as the strongest predictor of a poor outcome not only confirm previous published results but also shed new light on the role of COX-2 in the pathogenesis and progression of mesothelioma. In vitro studies on mesangial cells have recently shown that COX-2 exerts its inhibition on cell proliferation by a mechanism that is independent of prostaglandin synthesis but involves p53, p21, and p27.\textsuperscript{27} Based on our immunohistochemical data, it is possible to hypothesise a model where the weaker the COX-2 mediated induction of p21 and p27, the more aggressive is the tumour and the poorer the prognosis.

Based on this model, we would expect to find that p53 behaves in a similar way to p21 and p27, but our data do not show any correlation between p53 and COX-2 expression. This can be explained by the fact that all the mesothelioma specimens analysed were positive for the presence of SV40-like sequences.\textsuperscript{23} Consistent with the possible role of SV40 oncoproteins in the pathogenesis of mesothelioma,\textsuperscript{4} it has been observed that mutations involving p53 are extremely rare in these neoplasms\textsuperscript{29} and that SV40-Tag isolated from mesotheliomas is able to bind to p53 and to the RB family proteins.\textsuperscript{21} Activation of p53 mediated by SV40 could at least partly account for this phenomenon, even if the relevance of SV40 in the pathogenesis of mesothelioma is not universally accepted.\textsuperscript{40} Another possible explanation could be derived from the observation that the activity of the COX-2 inhibitor NS398 is not affected by the antiproliferative effects of p53 in human mesothelioma cell lines,\textsuperscript{17} thus showing the lack of influence of p53 on the COX-2 pathway. However, the role of p53 in mesothelioma still remains unclear.

It is not possible to exclude the possibility that molecular factors other than p21 and p27 might contribute to the complex COX-2 mediated inhibition of proliferation. In fact, the combined action of a number of factors is necessary to induce COX-2 dependent cell cycle arrest. Nevertheless, the tumorigenic effects of COX-2 could be divided into two distinct types: a direct effect on tumour cells and an effect on non-tumour cells such as tumour-nurturing blood vessels and immune competent cells.\textsuperscript{20} Our finding that only COX-2 expression was significantly associated with survival in multivariate analysis further confirms this hypothesis. One important limitation of this study is the absence of data on recognised clinical variables associated with prognosis such as performance status and the fact that it did not detect an association between histological type and prognosis. Moreover, the data are retrospective and essentially observational in nature and cannot therefore explain the functional mechanisms by which COX-2 promotes tumour growth. Further studies on larger independent groups of patients are needed to elucidate the interactions between oncogenes, tumour suppressor genes, and COX-2 expression in mesotheliomas.

In conclusion, this is the first study of which we are aware of the relationship between COX-2, p21, p27 and survival in patients with mesothelioma. The data suggest that COX-2 expression may be a useful prognostic parameter and thus support further investigations into the clinical usefulness of COX-2 inhibitors in the treatment of malignant mesotheliomas.

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**REFERENCES**


Procalcitonin may guide antibiotic treatment for LRTI

It is often difficult to decide which patients with suspected lower respiratory tract infection (LRTI) should be prescribed antibiotics. Circulating calcitonin precursors are higher in severe bacterial infections than in other illnesses. In this prospective single blind study 243 patients with suspected LRTI were randomised to a standard care group (n = 119) treated with antibiotics on the basis of clinical findings and routine investigations, or a procalcitonin group (n = 124) guided by a new procalcitonin assay with a sensitivity of 0.06 μg/l. In this group, antibiotics were strongly discouraged if procalcitonin levels were <0.10 μg/l, discouraged if levels were ≥0.10–0.25 μg/l, advised if levels were ≥0.25–0.50 μg/l, and strongly recommended if levels were >0.50 μg/l, with re-evaluation after 6–24 hours in both groups.

The primary end point was the use of antibiotics: 99 (83%) subjects in the standard care group and 55 (44%) in the procalcitonin group received antibiotic treatment. The adjusted relative risk of antibiotic exposure in the procalcitonin group was 0.49 (95% CI 0.44 to 0.55; p<0.0001) compared with the standard group. The reduction in antibiotic use was 47% in LRTI and 56% in acute exacerbations of COPD. There was no difference in laboratory and clinical outcomes including mortality, frequency and length of admission, need for ITU care, and rates of re-exacerbation of COPD and readmission after a mean (SD) of 13.0 (5.4) days. Re-evaluation after a mean (SD) of 5.3 (1.1) months showed no difference.

The fact that the study was single blind was a source of possible bias. There is no equivalent study using CRP as an alternative test. However, this study may have important clinical and economic implications.

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Prognostic significance of cyclooxygenase-2 (COX-2) and expression of cell cycle inhibitors p21 and p27 in human pleural malignant mesothelioma

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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 end of life decisions for patients with chronic obstructive pulmonary disease (COPD).1 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 treatments2 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 firm decision can be made”.3

In 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%.4

It is important that chest physicians continue to be strident advocates 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.5 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 fit 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 150 doctors subdivided by speciality (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/HUD facility 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.1 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 synthesise 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.1 The fact is that four long term, adequately powered RCTs have examined the effect of ICS in COPD, of which these studies were a part. The effects of ICS on the primary outcome measure of decline in forced expiratory volume in...
1 second (FEV₁). There may be a subset of people in whom the exacerbation rate is reduced, which was a secondary outcome in some of these studies.

In any case, as the authors point out, an annual difference of 7.7–9.9 ml in FEV₁ decline compared with placebo is of “debatable clinical importance”. 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.

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. 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. 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. 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.

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References

Ethics of placebo controlled studies of inhaled steroids for COPD
The meta-analysis by Sutherland et al1 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 (FEV₁) of −7.7 ml/year (95% CI −14.2 to −1.3) which is similar to the results of the meta-analysis performed by Highland et al2 (−5 ml/year (95% CI −11.2 to 1.2)) using a very similar data set. The meta-analyses employed slightly different study selection criteria and analytical techniques, and questioned the clinical significance of such small differences in FEV₁. The selected primary studies suffered from potential drop-out bias and significant selection bias and most of the studies subjected to these meta-analyses excluded patients with a bronchodilator response. Studies of asthma and COPD as separate entities are limited because asthma and COPD (observed in cross section) represent a continuum, and the small number of available prospective observations indicates that asthma and COPD are sometimes different clinical manifestations of the same underlying aetiology evolving over time.3

Given all the uncertainties, questions and limitations, Highland et al2 concluded (correctly in my opinion) that “additional studies are needed to evaluate the effects on quality of life, risk for systemic side effects, dose–response relationships in corticosteroid-responsive patients, and the economic effect of inhaled corticosteroids”. On the other hand, in an editorial accompanying the paper by Sutherland et al, Burge and Lewis4 state: “It is no longer ethical to do more long term placebo controlled studies [of inhaled corticosteroids in COPD]”. Given the uncertainties, questions and limitations which Burge and Lewis acknowledge, I was puzzled by this statement and would like to ask them why they reached this conclusion.

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Authors’ reply
To make randomised controlled studies ethical, the investigator must believe that neither no treatment is known to be superior to the other. If the trial is to be placebo controlled, the investigator must believe that no non-allowed 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. They point out that ICS were of established benefit in reducing exacerbations of COPD, so any future placebo controlled study would need to be in patients without a history of exacerbations. As exacerbations are associated with disease severity, and as about 80% of patients with an FEV₁ <50% predicted had at least one exacerbation over a 3 year period, any trial would need to be in those with early disease. The Copenhagen City Lung Study found that inhaled budesonide 800 μg/day had no benefit in a population of smokers with a reduced FEV₁/FVC ratio, the majority of whom had an FEV₁ above 80% predicted. This leaves the group with an FEV₁ between 50% and 80% predicted, many of whom have not been identified by their medical practitioners. This was the group included in the EUROSCOP and Lung Health 2 studies, where the results included in the meta-analysis were the most divergent, probably because of the relatively low dose of ICS used in the Lung Health study.5,6 In the symptomatic patient with more severe disease, the combination of a long acting β agonist and an ICS has been shown to be superior to either alone and is now the treatment of choice.7 This leaves the presymptomatic population in whom a decline in FEV₁ is the only practical outcome measure. No randomised study using an intention to treat analysis has shown a reduction in FEV₁ decline with ICS in any disease, including ICS in asthma, although several studies have shown an improvement in FEV₁ with ICS in COPD.8

Our editorial tried to explain why changes in FEV₁ decline are less likely to show in patients with COPD. Patients with progressive disease are likely to be given ICS by their clinicians outside any trial, reducing the power of any study.

Any treatment should weigh the potential risks against any benefit. Dr Duerden wants a better analysis of the risks of ICS in patients with COPD, particularly related to bone loss, and points out the reduced bone mineral density in the triamcinolone group in the Lung Health 2 study.7 The reported results are in 359/412 of a convenience sample who had three measurements of bone density. After 3 years the lumbar spine density reduced from 0.985 to 0.983 g/cm² and the femoral neck from 0.762 to 0.747 g/cm² in the triamcinolone group. EUROSCOP studied bone density measurements in 194 subjects’ and showed very small changes which were significantly less at the level of 95% confidence in the budesonide group (0.04%/year v 0.36%/year in the placebo group). Randomised controlled studies are probably not the best method for assessing the extent of long term adverse effects, but the evidence from the randomised studies to date shows that the risks are relatively minor compared with the risks of death from the natural progression of the disease. Introducing ICS at an earlier stage may alter the risk/benefit ratios. The adverse effects on the bones are probably best studied in asthmatics of whom many are already taking long term ICS in equivalent doses.

There is a striking difference between the way that cardiac and respiratory physicians greet new treatments whose individual effects are present but relatively minor. There has been a meaningful reduction in cardiac deaths attributed to the combination of several treatments with modest individual effects. This has resulted in more smokers living to develop significant COPD. It is likely that improvements in the quality and quantity of life in patients with COPD will come from a combination of treatments, among which ICS have a place. The main
Delays in diagnosis of OSAHS

We very much enjoyed the first paper in the review section on sleep and admired Stradling and Davies’s honest appraisal of the current difficulties in defining disease and the lack of a relationship between symptoms and the results of investigations.1 One of the problems of truly determining the size of the health burden associated with the obstructive sleep apnoea/hypopnoea syndrome (OSAHS) is that much of the burden may occur before the diagnosis is made. Others have shown how obstructive sleep apnoea/hypopnoea is associated with cardiovascular events.2

We administered a questionnaire to 166 consecutive patients with diagnosed OSAHS on continuous positive airway pressure treatment and asked them to identify how long they were able to recall having symptoms at the time of diagnosis. In 155 cases (93.8%) someone had previously complained of the patient’s loud snoring and first mention of this had been made a median of 12 years (range 2–52) before diagnosis of OSAHS. In 84.3% of respondents excessive daytime sleepiness was present for a median of 8 years (range 0.5–62) and 133 patients (80.1%) reported that their bed partner had witnessed apnoeas a median of 8 years (range 1–49) before diagnosis. We also found that, of the 119 (71.7%) who were drivers, 26 (21.8%) reported at least one or more automobile crashes in the previous 5 years, with seven respondents having had two and one having had four.

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 OASHS have structural changes in brain morphology compared with healthy controls.3 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 treatment4 and early benefits in terms of driving performance.5

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Prophylactic antibiotic treatment of bronchiectasis with azithromycin

Once a treatable cause of bronchiectasis such as hypergamaglobulinaemia 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 using a cyclical regimen of oral antibiotics. This may be administered orally, via a nebuliser, or using a cyclical regimen of oral antibiotics. Effective in controlling chronic suppurative airways disease.7 Recently published research has shown benefits of long term azithromycin treatment in patients with bronchiectasis.8 These results led us to consider using azithromycin as prophylaxis in patients with non-cystic fibrosis bronchiectasis with frequent infective exacerbations.

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

- 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.

Exclusions included allergy to macrolides and normal liver function tests. The median weight was 100 kg. The median 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 azithromycin because of side effects such as abdominal liver function tests (n = 2), diarrhoea (n = 2), rash (n = 1), and tinnitus (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 course of a systemic corticosteroid. 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 were continuing with the prophylaxis at the present time; in the other seven treatment was discontinued because of improvement in their condition.

Randomised controlled trials (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), Moorella xanthalis (n = 1), not done (n = 4). After 4 months the results were no growth (n = 18), P aeruginosa (n = 5),

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tics have also been shown to reduce mucus mechanisms of bacteria. Macrolide antimicrobial, or by influencing the pathogenic patients by reducing bacterial load and therefore vicious circle hypothesis. It might also benefit to downregulation of the host immune reactions and chronic symptoms is unknown, but there were no patients who were subsequently studied using a non-parametric Wilcoxon test showed that there was a statistical significance (p = 0.01). 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 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 neutrophil infiltration, or by influencing the pathogenic mechanisms of bacteria. Macrolide antibiotics have also been shown to reduce mucus secretion.

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 well 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 prescriptions issued for respiratory illnesses, and the authors conclude that reverse causation was the likely explanation for this association.

The inappropriate use of antibiotics for respiratory symptoms caused by unrecognised asthma is the main potential confounding factor in observational studies attempting to demonstrate a causal link between antibiotic receipt and atopic illnesses. It is certainly plausible that GPs may prescribe antibiotics in children with symptoms such as cough and wheeze in early life. Suggestions of a causal link are strengthened by demonstration of an association when antibiotics were used for symptoms not associated with asthma. The earlier study by Faroqui and Hopkins did, indeed, observe an association with non-respiratory use of antibiotics and asthma; in the study by Cullinan et al the association between non-respiratory indicated antibiotics and atopic asthma narrowly failed to reach statistical significance. The authors acknowledge that the study was only powered to show a doubling of the odds ratio for the association between early life antibiotic use and asthma, so an association remains possible in this cohort.

The most important limitation of the study, however, is the timing of the observed early life events in relation to secular changes in asthma prevalence and antibiotic prescribing, and hence the applicability of the results to modern day settings. This study observed events occurring 30 or more years ago in the parents of the Ashford birth cohort. As is well described, the prevalence of asthma has increased greatly over the last 30 years. There may also have been significant increases in antibiotic prescribing over this time.

The subjects in this study received an average of 3.1 and a median of 5 antibiotic prescriptions over 5 years, while in a recent case-control study of 37 children with atopy and wheezing and 37 without either that the average and median number of antibiotic courses received during the first 5 years of life was 9.9 and 7 for children aged 6, 7 and 3 for non-wheezers. There is evidence of earlier prescribing of antibiotics in recent times; in our study group 89% of wheezers and 68% of non-wheezers received one or more courses of antibiotics 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 30 years ago when both the prevalence of asthma and antibiotic prescribing to young children were significantly less than they are now, but the question of whether it may now be a significant and potentially modifiable factor remains unanswered.

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References

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 hypoxia. Empirical antibiotic therapy was commenced. The clinical picture included severe hypoxaemia, increased white blood cell count, raised C-reactive protein, and reduced carbon monoxide
transfer factor (TLCO). All patients had diffuse bilateral ground glass opacities on a high resolution CT scan, most often sparing the subpleural areas (fig 1). Every patient improved following treatment with oral prednisone (0.5–0.9 mg/kg) but residual dyspnoea and reduced TLC (<80% of predicted value) could be seen for more than 2 weeks.

Acute respiratory failure was attributed to inhalation of the waterproofing spray in view of the sudden occurrence of symptoms following exposure, the diffuse ground glass opacities without other abnormalities on the CT scan, and the absence of any other detected cause. In particular, BAL fluid was sterile for bacteria, mycobacteria, viruses and fungi. Serological tests for chlamydia and mycoplasma were performed on two patients and were negative. A nasal swab for influenza was performed on one patient and was negative.

We were, however, surprised that the patients used three different spray brands. Waterproofing sprays contain three types of components—a propellant gas (propane butane), a waterproofing agent (fluorocarbon resin), and a solvent. It appeared that the manufacturer of the fluorinated resin changed during the summer of 2002 (the same for the three brands) and that the isopropanol had to be replaced with a heptane solvent. Consumers started complaining of respiratory symptoms in October 2002 (the same for the three brands) and that the isopropanol related to waterproofing sprays were reported to the Swiss Toxicological Information Centre, whereas less than 10 cases per year had been reported in the previous 7 years.

The same fluorinated resin was also distributed in Germany, the Netherlands, and the UK. In Germany the waterproofing sprays were withdrawn before they reached the consumers. During the same period five patients were admitted to hospital in the Netherlands with the same complaints. Patients were admitted to hospital in the UK. In Germany the waterproofing sprays had been reported in the previous 7 years.

In the past, several outbreaks of acute respiratory symptoms have been recorded in different countries including 550 in Oregon in 1992, 1994 in Pennsylvania and Virginia in 1993, in Quebec in 1993, and in Japan between 1992 and 1993. 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 and one death was reported in Japan in the 1990s.

Following these outbreaks various suggestions were proposed to explain these intoxications. 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 epidemiological studies are required to investigate this phenomenon further.

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Effect of PM10 on H influenzae and S pneumoniae

That air pollution, and specifically particles, are harmful to health is well accepted, causing direct effects such as lung inflammation resulting in exacerbations of lung and cardiac conditions 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, 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 PM10 (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 PM10 on the growth of H influenzae and Streptococcus pneumoniae grown in liquid broth and the effect of PM10 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 11391 and S pneumoniae ATCC 49619 were used. Particles were collected on a tapered element oscillating microbalance situated in central Birmingham, representative of an 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 studies of inflammatory responses to which have shown pro-inflammatory effects.

In the first experiment a 1:20 dilution of PM10 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% CO2 using the Miles and Misra technique. In the growth kinetic experiment equal volumes of PM10 solution and ISA 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 only broth and wells for sterility checks contained PM10 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% CO2 for 5 hours. The Miles and Misra technique 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 PM10. This test was repeated five times using the same strain to check for reproducibility.

References

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 H. influenzae and 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 H. influenzae and 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 of 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 particles. This interactive mechanism is supported by the association of ambient nitrogen dioxide levels with admissions for croup, and is analogous to the potential of the airway response to inhaled allergen by both nitrogen dioxide and ozone. Finally, it is possible that the particles have an effect on bacterial virulence and toxin production rather than growth. This possibility has not been tested here but warrants further study.

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Figure 1 Growth curve against time with and without PM10 solution for (A) S pneumoniae ATCC control strain and (B) H influenzae NCTC control strain.