LUNG CANCER

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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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 ($p<0.0001$), 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.[1] 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.[2] 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.[3] 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.[4] The prognosis is generally poor with a reported median survival of 4–12 months in either untreated or treated (surgery, radiotherapy, or chemotherapy) patients.[5] Moreover, mesothelioma has proved resistant to classical chemotherapeutic and radiation regimes and the natural history has not been influenced by standard therapy.[6] Several clinical prognostic factors have been tentatively correlated to survival, histological type and performance status being the most valuable.[5 7–9]

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.[10–12] 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.[13–15] It has been shown that cultured human mesothelial cells contain cyclooxygenase activity[16] and that COX-2 expressing mesothelioma cell lines are associated with increased proliferative and invasive potential.[17] 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.[18] 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,[19] and several recent studies have investigated the potential prognostic value of p53, p21 and p27 in malignant mesotheliomas.[20–24]

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[23 24] and for the presence of SV40-like sequences.[25] 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.[25]

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℃. 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 experiments. The sections were then immunostained with the streptavidin-biotin system (Dako, Carpintera, CA, USA) using diaminobenzidine (DAB) as the final chromogen and haematoxylin as the nuclear counterstain. Negative controls for each tissue section were prepared by omitting the primary antibodies. A suitable positive control was run with each set of slides. All samples were processed under the same conditions.

The experimental conditions for the immunohistochemical analysis of p21 and p53, as well as for the detection of SV40 sequences, have been described elsewhere.[23–25]

Scoring and quantification of immunoreactivity

Two observers (AB and SS) estimated the staining pattern of p27 and COX-2 separately. For p27, each specimen was scored for the percentage of positive nuclei (<10% and >10% of cells expressing p27). For COX-2, staining intensity was scored as 0 (negative), 1 (weak), 2 (medium), and 3 (strong). 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]

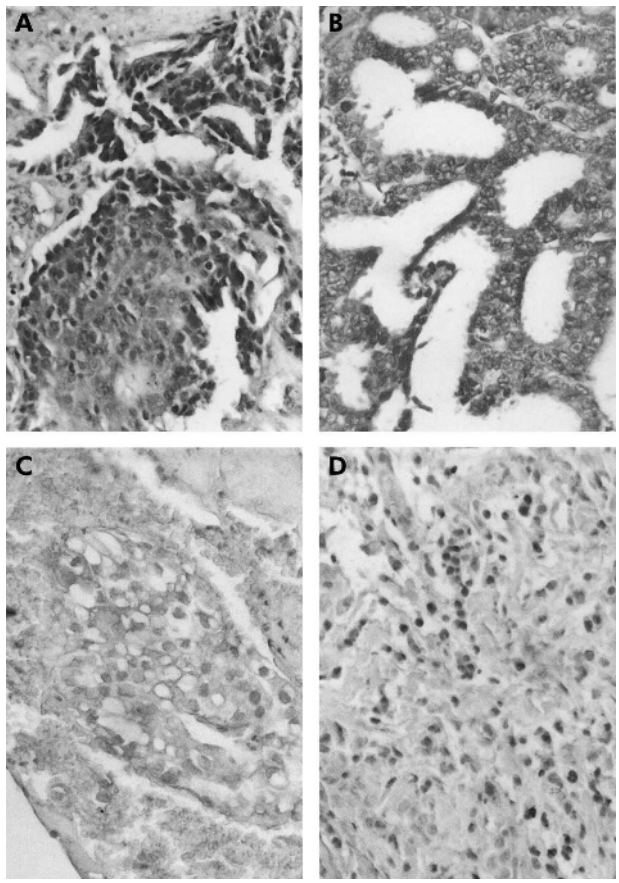

Figure 1 Immunhistochemical stainings of COX-2 (A and B) and p27 (C and D) in human pleural mesotheliomas (original magnification ×250).

Table 1 Characteristics of study patients

Patient no	Histology	COX-2 score*	p53 score†	p21 score†	p27 score†	Survival time (weeks)
1	Epithelial	1	2	2	2	18
2	Epithelial	1	1	2	2	15
3	Epithelial	2	1	1	2	6
4	Epithelial	2	2	1	1	1
5	Epithelial	1	2	2	2	12
6	Epithelial	2	1	2	2	10
7	Epithelial	1	2	2	2	18
8	Epithelial	2	1	1	1	9
9	Epithelial	2	2	1	1	7
10	Epithelial	1	1	1	2	14
11	Epithelial	1	1	2	2	13
12	Epithelial	2	1	1	1	4
13	Epithelial	2	1	1	1	3
14	Epithelial	2	1	1	1	5
15	Epithelial	1	2	2	1	10
16	Epithelial	1	1	2	2	14
17	Sarcomatoid	1	2	2	2	9
18	Sarcomatoid	2	1	1	1	3
19	Sarcomatoid	2	1	1	1	5
20	Sarcomatoid	2	2	1	1	4
21	Sarcomatoid	2	1	2	1	8
22	Sarcomatoid	1	2	2	2	16
23	Mixed	2	1	1	1	6
24	Mixed	2	1	1	1	4
25	Mixed	2	2	1	1	1
26	Mixed	2	2	2	2	11
27	Mixed	2	1	2	2	9
28	Mixed	2	1	2	2	15
29	Mixed	2	1	1	1	8

*1 = 0–4; 2 = >4.
†1 = 0–10%; 2 = >10%.

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

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

	Histology	COX-2	p53	p21	p27
COX-2	0.589		−2.557	−16.546	−16.163
	0.720		0.110	<0.0001	0.001
p53	0.736	−2.557		1.691	0.279
	0.792	0.110		0.194	0.710
p21	0.262	−16.546	1.691		16.905
	1.000	<0.0001	0.194		<0.0001
p27	1.073	−16.563	0.279	17.905	
	0.693	0.001	0.710	<0.0001	

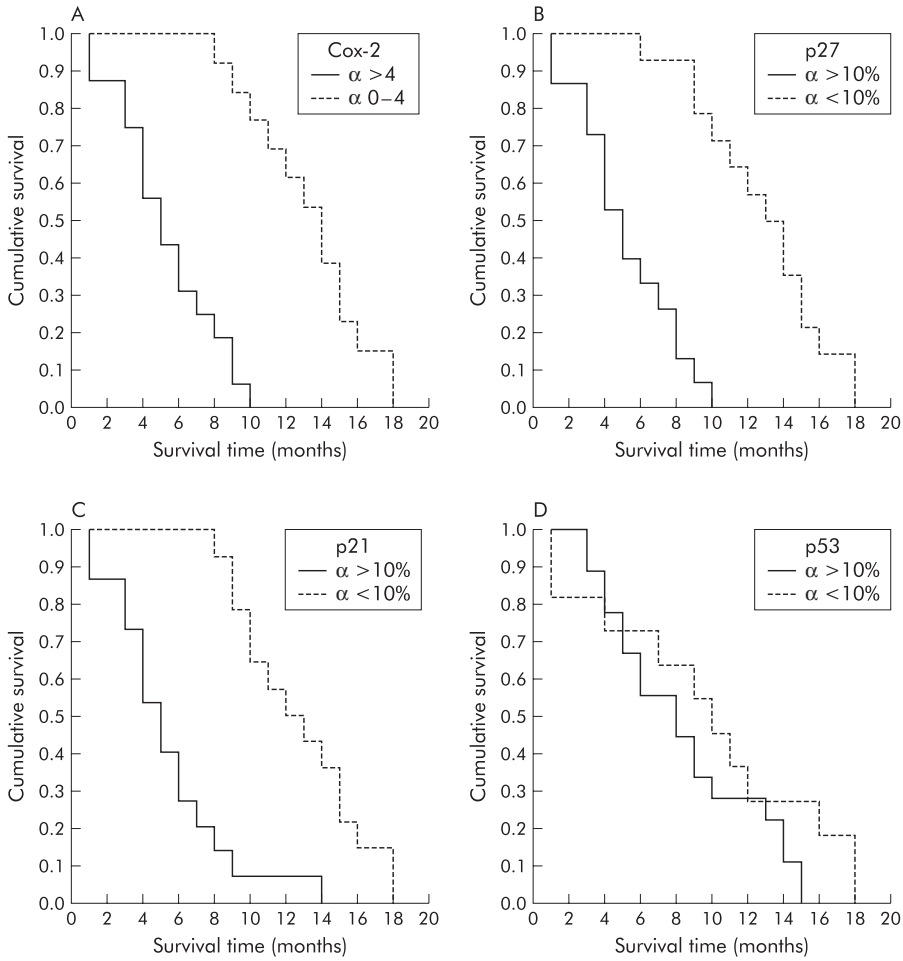

Figure 2 Kaplan-Meier survival plots for (A) COX-2, (B) p27, (C) p21, and (D) p53 levels.

chain reaction for SV40 sequences.[25] 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,[17 23 24] but other groups have found a significant correlation between p27 expression and epithelioid histotype.[20 21]

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.[17 18] However, p21 and p27 have also been shown to have a significant role in the

Table 3 Univariate analysis of survival (in weeks) and pathological and immunohistochemical parameters in patients with malignant mesothelioma

	Median (95% CI) survival	p value
Histology		
Epithelial	10.00 (8.06 to 11.94)	0.4927 (NS)
Sarcomatoid	5.00 (1.20 to 9.80)	
Mixed	8.00 (3.87 to 13.13)	
COX-2		
0–4	14.00 (11.71 to 16.29)	<0.0001
>4	5.00 (3.06 to 6.94)	
p53		
<10%	8.00 (3.87 to 12.13)	0.1977 (NS)
>10%	10.00 (5.68 to 14.32)	
p21		
<10%	5.00 (3.47 to 6.54)	<0.0001
>10%	12.00 (8.33 to 15.67)	
p27		
<10%	5.00 (3.51 to 6.49)	<0.0001
>10%	13.00 (10.56 to 15.44)	

Table 4 Multivariate Cox regression analysis of overall survival in patients with malignant mesothelioma

	Hazard ratio (95% CI)	p value
COX-2		
0–4	0.143 (0.032 to 0.646)	0.011
>4	1	
p21		
<10%	2.191 (0.685 to 7.005)	0.186 (NS)
>10%	1	
p27		
<10%	4.016 (0.913 to 17.653)	0.066 (NS)
>10%	1	

progression of human mesothelioma.[20–22] Interestingly, while p53 overexpression is a common event in mesothelioma,[24] it is rarely mutated or inactivated.[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.[19] 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.[25] Consistent with the possible role of SV40 oncoproteins in the pathogenesis of mesothelioma,[4] it has been observed that mutations involving p53 are extremely rare in these neoplasms[29] and that SV40-Tag isolated from mesotheliomas is able to bind to p53 and to the RB family proteins.[25 30] Inactivation 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.[31] 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,[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.[32] 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

1 **Peto J**, DeCarli A, La Vecchia C, *et al.* The European mesothelioma epidemic. *Br J Cancer* 1999;**79**:666–72.
2 **Attanos RI**, Gibbs AR. Pathology of malignant mesothelioma. *Histopathology* 1997;**30**:403–18.
3 **Hughes JM**, Weill H. Asbestos exposure: quantitative assessment of risk. *Am Rev Respir Dis* 1986;**133**:5–13.
4 **Carbone M**, Fisher S, Powers A, *et al.* New molecular and epidemiological issues in mesothelioma: role of SV40. *J Cell Physiol* 1999;**180**:167–72.
5 **Sugarbaker DJ**, Flores RM, Jaklitsch MT, *et al.* Resection margins, extrapleural nodal status, and cell type determine postoperative longterm survival in trimodality therapy of malignant pleural mesothelioma: results in 183 patients. *J Thorac Cardiovasc Surg* 1999;**117**:54–63.
6 **Pass HI**, Robinson BW, Testa JR, *et al.* Emerging translational therapies for mesothelioma. *Chest* 1999;**116**:455–60S.
7 **Rusch VW**, Venkatraman ES. Important prognostic factors in patients with malignant pleural mesothelioma, managed surgically. *Ann Thorac Surg* 1999;**68**:1799–804.
8 **Johansson L**, Linden CJ. Aspects of histopathologic subtype as a prognostic factor in 85 pleural mesotheliomas. *Chest* 1996;**109**:109–14.
9 **Beer TW**, Buchanan R, Matthews AW, *et al.* Prognosis in malignant mesothelioma related to MIB 1 proliferation index and histological subtype. *Hum Pathol* 1998;**29**:249–51.
10 **Uefuji K**, Ichikura T, Mochizuki H. Cycloxygenase-2 expression is related to prostaglandin biosynthesis and angiogenesis in human gastric cancer. *Clin Cancer Res* 2000;**6**:135–8.
11 **Grosch S**, Tegeder I, Niederberger E, *et al.* COX-2 independent induction of cell cycle arrest and apoptosis in colon cancer cells by the selective COX-2 inhibitor celecoxib. *FASEB J* 2001;**15**:2742–4.
12 **Tsuji S**, Tsujii M, Kawano S, *et al.* Cyclooxygenase-2 upregulation as a perigenetic change in carcinogenesis. *J Exp Clin Cancer Res* 2001;**20**:117–29.
13 **Sheehan KM**, Sheahan K, O'Donoghue DP, *et al.* The relationship between cyclooxygenase-2 expression and colorectal cancer. *JAMA* 1999;**282**:1254–7.
14 **Hida T**, Yatabe Y, Achiwa H, *et al.* Increased expression of cyclooxygenase-2 occurs frequently in human lung cancers, specifically in adenocarcinomas. *Cancer Res* 1998;**58**:3761–4.
15 **Murata H**, Kawano S, Tsuji S, *et al.* Cyclooxygenase-2 overexpression enhances lymphatic and metastasis in human gastric carcinoma. *Am J Gastroenterol* 1999;**94**:451–5.
16 **Baer AN**, Green FA. Cyclooxygenase activity of cultured human mesothelial cells. *Prostaglandins* 1993;**46**:37–49.
17 **Marrogi A**, Pass HI, Khan M, *et al.* Human mesothelioma samples overexpress both cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (NOS2): in vitro antiproliferative effects of a COX-2 inhibitor. *Cancer Res* 2000;**60**:3696–700.
18 **Edwards JG**, Faux SP, Plummer SM, *et al.* Cyclooxygenase-2 expression is a novel prognostic factor in malignant mesothelioma. *Clin Cancer Res* 2002;**8**:1857–62.
19 **Zahner G**, Wolf G, Ayoub M, *et al.* Cyclooxygenase-2 overexpression inhibits platelet-derived growth factor-induced mesangial cell proliferation through induction of the tumour suppressor gene p53 and the cyclin-dependent kinase inhibitors $p21^{waf-1/cip-1}$ and $p27^{kip-1}$. *J Biol Chem* 2002;**277**:9763–71.
20 **Bongiovanni M**, Cassoni P, De Giuli P, *et al.* $p27^{kip1}$ immunoreactivity correlates with long-term survival in pleural malignant mesothelioma. *Cancer* 2001;**92**:1245–50.
21 **Beer TW**, Shepherd P, Pullinger NC. p27 immunostaining is related to prognosis in malignant mesothelioma. *Histopathology* 2001;**38**:535–41.
22 **Isik R**, Metintas M, Gibbs AR, *et al.* p53, p21 and metallothionein immunoreactivities in patients with malignant pleural mesothelioma: correlations with environmental asbestos exposure. *Respir Med* 2001;**95**:588–93.
23 **Baldi A**, Groeger AM, Esposito V, *et al.* Expression of p21 in SV40 large T antigen positive human pleural mesothelioma: relationship with survival. *Thorax* 2002;**57**:353–6.

24 **Esposito V**, Baldi A, De Luca A, *et al*. p53 immunostaining in differential diagnosis of pleural mesothelial proliferations. *Anticancer Res* 1997;**17**:733–6.
25 **De Luca A**, Baldi A, Esposito V, *et al*. The retinoblastoma gene family pRb/p105, p107, pRb2/p130 and simian virus-40 large T-antigen in human mesotheliomas. *Nature Med* 1997;**3**:913–6.
26 **Kaplan EL**, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958;**53**:457–81.
27 **Peto R**, Pike MC, Armitage P, *et al*. Design and analysis of randomised clinical trials requiring prolonged observation of each patient. *Br J Cancer* 1977;**35**:1–39.
28 **Cox DR**. Regression models and life tables. *J R Stat Soc* 1972;**34**:187–220.
29 **Mor O**, Yaron P, Huszar M, *et al*. Absence of p53 mutations in malignant mesotheliomas. *Am J Respir Cell Mol Biol* 1997;**16**:9–13.
30 **Carbone M**, Rizzo P, Grimley PM, *et al*. Simian virus-40 large-T antigen binds p53 in human mesotheliomas. *Nature Med* 1997;**3**:908–12.
31 **Mulatero C**, Surentheran T, Breuer J, *et al*. Simian virus 40 and human pleural mesothelioma. *Thorax* 1999;**54**:60–1.
32 **Subbaramaiah K**, Altorki N, Chung WJ, *et al*. Inhibition of cyclooxygenase-2 gene expression by p53. *J Biol Chem* 1999;**274**:10911–5.

LUNG ALERT

Procalcitonin may guide antibiotic treatment for LRTI

▲ Christ-Crain M, Jaccard-Stoiz D, Bingisser R, *et al*. Effect of procalcitonin-guided treatment on antibiotic use and outcome in lower respiratory tract infections: cluster-randomised, single-blinded intervention trial. *Lancet* 2004;**363**:600–7

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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PostScript

LETTERS TO THE EDITOR

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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*[1] and the accompanying editorial by Rudolf[2]. 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).[3] 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[4] 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 clearer assessment can be made''. 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 67.8 years to UK intensive care units, those with single organ respiratory failure had a hospital survival of over 70%.[5]

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

1 **Roberts CM**, Barnes S, Lowe D, *et al*. Evidence for a link between mortality in acute COPD and hospital type and resources. *Thorax* 2003;**58**:947–9.
2 **Rudolf M**. Inpatient management of acute COPD: a cause for concern. *Thorax* 2003;**58**:914–5.
3 **Wildman MJ**, O'Dea J, Kostopoulou O, *et al*. Variation in intubation decisions for patients with chronic obstructive pulmonary disease in one critical care network. *Q J Med* 2003;**96**:583–91.
4 **General Medical Council**. Withholding and withdrawing life-prolonging treatments: good practice. In: *Decision-making*. London: General Medical Council, 2002.
5 **Wildman M**, Harrison D, Brady AR, *et al*. Unit and hospital outcomes for 3752 admissions to 128 UK adult critical care units between 1995 and 2001. *Thorax* 2003;**58**(Suppl III):iii23.

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.[1] 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 have been a factor 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*[2] reported a study in which questionnaires regarding initiation of mechanical ventilation in end stage COPD patients were completed by 350 doctors subdivided by speciality (intensivists, respiratory specialists and other physicians). As in the paper by Wildman *et al*,[3] 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 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 regarding 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

1 **Roberts CM**, Barnes S, Lowe D, *et al*. Evidence for a link between mortality in acute COPD and hospital type and resources. *Thorax* 2003;**58**:947–9.
2 **Perrin F**, Renshaw M, Turton C. Clinical decision making and mechanical ventilation in patients with respiratory failure due to an exacerbation of COPD. *Clin Med* 2003;**3**:556–9.
3 **Wildman MJ**, O'Dea J, Kostopoulo O, *et al*. Variation in intubation decisions for patients with chronic obstructive pulmonary disease in one critical care network. *Q J Med* 2003;**96**:583–91.

Do inhaled corticosteroids slow FEV_1 decline in COPD after all?

I question the findings of the meta-analysis by Sunderland *et al*[1] and the content of the associated editorial by Burge and Lewis.[2] 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.[3] The fact is that four long term, adequately powered RCTs have examined the effect of ICS. All of these studies showed no effect of ICS on the primary outcome measure of decline in forced expiratory volume in

1 second (FEV_1). 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 FEV_1 decline compared with placebo is of ''debatable clinical importance''.[1] 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.[5]

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.[4] 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.[6] 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.[2] 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

1 **Sutherland ER**, Allmers H, Ayas NT, *et al*. Inhaled corticosteroids reduce the progression of airflow limitation in chronic obstructive pulmonary disease: a meta-analysis. *Thorax* 2003;**58**:937–41.
2 **Burge PS**, Lewis SA. So inhaled steroids slow the rate of decline of FEV_1 in patients with COPD after all? *Thorax* 2003;**58**:911–3.
3 **Highland KB**, Strange C, Heffner JE. Long-term effects of inhaled corticosteroids on FEV_1 in patients with chronic obstructive pulmonary disease. A meta-analysis. *Ann Intern Med* 2003;**138**:969–73.
4 **The Lung Health Study Research Group**. Effect of inhaled triamcinolone on the decline in pulmonary function in chronic obstructive pulmonary disease. *N Engl J Med* 2000;**343**:1902–9.
5 News: NICE steroids advice for COPD thrown into doubt. *Pulse* 3 November 2003.
6 **CSM/MCA**. Reminder: Fluticasone propionate (Flixotide): use of high doses (>500 micrograms/twice daily). *Current Problems in Pharmacovigilance* August 2001.

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 ml/year (95% CI −14.2 to −1.3) which is similar to the results of the meta-analysis performed by Highland *et al*[2] (−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 FEV_1. The selected primary studies suffered from potential drop-out bias and significant selection bias, in that almost all of the studies subjected to these meta-analyses excluded patients with a bronchodilator response.[2] Studies of asthma and COPD as separate entities are limited because asthma and COPD (observed in cross section) represent a continuum,[3] 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.[4] Given all the uncertainties, questions and limitations, Highland *et al*[2] 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 Lewis[5] 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 acknowledged, I was puzzled by this statement and would like to ask them why they reached this conclusion.

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References

1 **Sutherland ER**, Allmers H, Ayas NT, *et al*. Inhaled corticosteroids reduce the progression of airflow limitation in chronic obstructive pulmonary disease: a meta-analysis. *Thorax* 2003;**58**:937–41.
2 **Highland KB**, Strange C, Heffner JE. Long-term effects of inhaled corticosteroids on FEV_1 in patients with chronic obstructive pulmonary disease. *Ann Intern Med* 2003;**138**:969–73.
3 **Brand PL**, Kerstjens HA, Postma DS, *et al*. Long-term multicentre trial in chronic nonspecific lung disease: methodology and baseline assessment in adult patients. Dutch CNSLD Study Group. *Eur Respir J* 1992;**5**:21–31.
4 **Hahn DL**. Chlamydia/mycoplasma: do they cause new-onset asthma in adults? In: Papadopoulos NG, ed. *Respiratory infections in allergy and asthma*. New York, Basel: Marcel Dekker, 2003;**178**:645–62.
5 **Burge PS**, Lewis SA. So inhaled steroids slow the rate of decline of FEV_1 in patients with COPD after all? *Thorax* 2003;**58**:911–3.

Authors' reply

To make randomised controlled studies ethical, the investigator must believe that neither 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. Our editorial pointed out that ICS were of established benefit in reducing exacerbations of COPD,[1] 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 FEV_1 <50% predicted have exacerbations over a 3 year period,[2] 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 selected with a reduced FEV_1/FVC ratio, the majority of whom had an FEV_1 above 80% predicted.[3] This leaves the group with an FEV_1 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.[4 5] 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.[6 7] This leaves the presymptomatic population in whom a decline in FEV_1 is the only practical outcome measure. No randomised study using an intention to treat analysis has shown a reduction in FEV_1 decline with any treatment in any disease, including ICS in asthma, although several studies have shown an improvement in FEV_1 with ICS in COPD.[2 4 6] Our editorial tried to explain why changes in FEV_1 decline were difficult 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 Duerdin 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.[5] 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 0.988 to 0.985 g/cm^2 and the femoral neck from 0.762 to 0.747 g/cm^2 in the triamcinolone group. EUROSCOP studied bone density measurements in 194 subjects[4] and showed very small changes which were significantly less at the femoral trochanter 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

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 FEV_1 in patients with COPD after all? *Thorax* 2003;**58**:911–3.
2 **Burge PS**, Calverley PMA, Jones PW, *et al*. Randomised, double blind, placebo controlled study of fluticasone propionate in patients with moderate to severe chronic obstructive pulmonary disease: the ISOLDE trial. *BMJ* 2000;**320**:1297–303.
3 **Vestbo J**, Sorensen T, Lange P, *et al*. Long-term effects of inhaled budesonide in mild and moderate chronic obstructive pulmonary disease: a randomised controlled trial. *Lancet* 1999;**353**:1819–23.
4 **Pauwels RA**, Lofdahl C, Laitinen LA, *et al*, for the European Respiratory Society Study on Chronic Obstructive Pulmonary Disease. Long-term treatment with inhaled budesonide in persons with mild chronic obstructive pulmonary disease who continue smoking. *N Engl J Med* 1999;**340**:1948–53.
5 **The Lung Health Study Research Group**. Effect of inhaled triamcinolone on the decline in pulmonary function in chronic obstructive pulmonary disease. *N Engl J Med* 2000;**343**:1902–9.
6 **Calverley PMA**, Pauwels R, Vestbo J, *et al*, for the TRISTAN Study Group. Combined salmeterol and fluticasone in the treatment of chronic obstructive pulmonary disease: a randomised controlled trial. *Lancet* 2003;**361**:449–56.
7 **Szafranski W**, Cukier A, Ramirez A, *et al*. Efficacy and safety of budesonide/formoterol in the management of chronic obstructive pulmonary disease. *Eur Respir J* 2003;**21**:74–81.

Delays in diagnosis of OSAHS

We very much enjoyed the first paper in the review series 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 use of hospital resources and use of cardiovascular medication is high in those with undiagnosed obstructive sleep apnoea.[2 3] 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 could recall having symptoms at the time of diagnosis. In 155 cases (93.4%) 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 had been 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 OSASHS have structural changes in brain morphology compared with healthy controls.[4] 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 treatment[5 6] and early benefits in terms of driving performance.[7]

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References

1 **Stradling JR**, Davies RJO. Sleep · 1: Obstructive sleep apnoea/hypopnoea syndrome: definitions, epidemiology and natural history. *Thorax* 2004;**59**:73–8.
2 **Kryger MH**, Roos L, Delaive K, *et al*. Utilisation of health care services in patients with severe obstructive apnoea. *Sleep* 1996;**19**:S111–6.
3 **Otake K**, Delaive K, Walld R, *et al*. Cardiovascular medication use in patients with undiagnosed obstructive sleep apnoea. *Thorax* 2002;**57**:417–22.
4 **Morrell M**, McRobbie D, Quest R, *et al*. Changes in brain morphology associated with obstructive sleep apnoea. *Sleep Med* 2003;**4**:451–4.
5 **Pelletier-Fleury N**, Meslier N, Gagnadoux F, *et al*. Economic arguments for the immediate management of moderate to severe obstructive sleep apnoea syndrome. *Eur Respir J* 2004;**23**:53–60.
6 **Douglas NJ**, George CFP. Treating sleep apnoea is cost effective. *Thorax* 2002;**57**:93.
7 **Turkington PM**, Sircar M, Saralaya D, *et al*. Time course of changes in driving simulator performance with and without treatment in patients with sleep apnoea/hypopnoea syndrome. *Thorax* 2004;**59**:56–9.

Prophylactic antibiotic treatment of bronchiectasis with azithromycin

Once a treatable cause of bronchiectasis such as hypogammaglobulinaemia has been excluded, management largely involves physiotherapy and treatment of infective exacerbations with appropriate antibiotics.[1] 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 intravenous antibiotics. Prophylactic treatment may be problematic due to side effects and development of antibiotic resistance.[2] 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.[3] Recently published research has shown benefits of long term azithromycin treatment in patients with cystic fibrosis.[4] 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:

- 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 abnormal liver function tests. The dosing 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 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 7 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.

Sputum culture results (all 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), *Morexella catarrhalis* (n = 1), not done (n = 4). After 4 months the results were no growth (n = 18), *P aeruginosa* (n = 5),

Table 1 Change in symptoms while taking azithromycin prophylaxis

	Mean	SD	SE	p value
Sputum volume	1.6	0.8	0.14	<0.001
Sputum colour	2.1	0.7	0.13	<0.001
Sputum consistency	2.5	0.6	0.11	0.006
Cough	2.4	0.7	0.12	0.001
Fatigue	2.1	1.0	0.18	0.001
Exercise tolerance	3.8	0.9	0.16	0.002
Wheeze	2.6	0.8	0.14	0.011
Breathlessness	2.3	0.7	0.13	0.002

Symptoms scored on a 5-point scale: 1 = large decrease, 2 = decrease, 3 = no change, 4 = increase, 5 = large increase in symptoms.

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 signficance ($p = 0.01$).

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 secretion.[1 5]

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 (to 20% of pretreatment volume) than in the placebo group, but did not demonstrate any reduction in infective exacerbations.[6] 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

1 **Wilson R**. Bronchiectasis. In: Gibson J, Geddes D, Costabel U, eds. *Respiratory medicine*, 3rd ed. Edinburgh: WB Saunders, 2002:1145–464.
2 **Rayner CF**, Tillotson G, Cole PJ, *et al*. Efficacy and safety of long-term ciprofloxacin in the management of severe bronchiectasis. *J Antimicrob Chemother* 1994;**34**:149–56.
3 **Kudoh S**. Erythromycin treatment in diffuse panbronchiolitis. *Curr Opin Pulm Med* 1998;**4**:116–21.
4 **Wolter J**, Seeney S, Bell S, *et al*. Effect of long term treatment with azithromycin on disease parameters in cystic fibrosis: a randomised trial. *Thorax* 2002;**57**:212–6.
5 **Tsang KW**, Ho PI, Chan KN, *et al*. A pilot study of low-dose erythromycin in bronchiectasis. *Eur Respir J* 1999;**13**:361–4.
6 **Currie DC**, Garbett ND, Chan KL, *et al*. Double-blind randomized study of prolonged higher-dose oral amoxycillin in purulent bronchiectasis. *Q J Med* 1990;**76**:799–816.

Early life antibiotics and asthma

Cullinan *et al*[1] 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 casual link are strengthened by demonstration of an association when antibiotics were used for symptoms not associated with asthma. The earlier study by Farooqui and Hopkins[2] 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.[3] 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 3 antibiotic prescriptions over 5 years, while we found in a recent case-control study[4] 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 wheezers and 6.3 and 5 for non-wheezers. There is also 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

1 **Cullinan P**, Harris J, Mills P, *et al*. Early prescription of antibiotics and the risk of allergic disease in adults: a cohort study. *Thorax* 2004;**59**:11–5.
2 **Farooqui IS**, Hopkin JM. Early childhood infection and atopic disorder. *Thorax* 1998;**53**:927–32.
3 **Holgate ST**. The epidemic of allergy and asthma. *Nature* 1999;**402**:B2–4.
4 **Thomas M**, Murray CS, Simpson B, *et al*. Early life antibiotic exposure and subsequent risk of asthma: a case control study. *Thorax* 2003;**58**:iii67.

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 dyspnoea. The clinical picture included severe hypoxaemia, increased white blood cell count, raised C-reactive protein, and reduced carbon monoxide

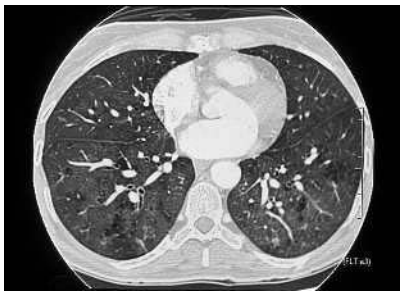

Figure 1 CT scan of thorax of a patient showing diffuse ground glass opacities.

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 TLCO (<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 solvent had to be replaced with a heptane solvent. Consumers started complaining of respiratory symptoms in October 2002 and the first severe case requiring admission was reported in January 2003. The three products were withdrawn from the market at the beginning of March. During this 6 month period 153 cases of respiratory symptoms 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.[1] These sprays were also withdrawn from the Dutch market. Surprisingly, no case has yet been recorded in the UK.

However, only sprays for public use were withdrawn, not the industrial liquids. In Switzerland two additional patients developed a chemical pneumonitis with similar symptoms and diffuse bilateral ground glass opacities after using industrial waterproofing liquid with a nebuliser. Workers in the above mentioned countries should therefore be warned not to use the liquid form with nebulisers.

In the past, several outbreaks of acute respiratory symptoms have been recorded in different countries including 550 in Oregon in 1992,[2 3] in Pennsylvania and Virginia in 1993,[4] in Quebec in 1993,[5] and in Japan between 1992 and 1993.[6] 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[7] and one death was reported in Japan in the 1990s.[8]

Following these outbreaks, various suggestions were proposed to explain these intoxications.[9] 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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References

1 **Bonte F**, Rudolphus A, Tan KY, *et al*. Severe respiratory symptoms following the use of waterproofing sprays. *Ned Tijdschr Geneeskd* 2003;**147**:1185–8.
2 **From the Centers for Disease Control and Prevention**. Acute respiratory illness linked to use of aerosol leather conditioner—Oregon, 1992. *JAMA* 1993;**269**:568–9.
3 **Smilkstein MJ**, Burton BT, Keene W, *et al*. Acute respiratory illness linked to use of aerosol leather conditioner—Oregon, December 1992. *MMWR Morb Mortal Wkly Rep* 1993;**41**:965–7.
4 **Burkhart KK**, Britt A, Petrini G, *et al*. Pulmonary toxicity following exposure to an aerosolised leather protector. *J Toxicol Clin Toxicol* 1996;**34**:21–4.
5 **Laliberté M**, Sanfacon G, Blais R. Acute toxicity linked to use of a leather protector. *Ann Emerg Med* 1995;**25**:841–4.
6 **Shintani S**, Ishizawa J, Endo Y, *et al*. A progress report of toxicovigilance activity for acute inhalation poisonings by waterproofing spray in Japan (abstract). *Clin Toxicol* 1996;**34**:589.
7 **Schicht R**, Hartjen A, Still V. Alveolitis after inhalation of leather impregnation spray. *Dsch Med Wochenschr* 1982;**107**:688.
8 **Ota H**, Koge K, Tanaka H, *et al*. Acute respiratory failure due to inhalation of aerosol water proof agent (Japanese). *Nihon Kokyuki Gakkai Zasshi* 2000 Jun;**38**:485–9.
9 **Hubbs AF**, Castranova V, Ma JY, *et al*. Acute lung injury induced by a commercial leather conditioner. *Toxicol Appl Pharmacol* 1997;**143**:37–46.

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 3] 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,[4] 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* 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 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 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% 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℃ in 5% CO_2 using the Miles and Misra technique.[5] 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 1:64 were performed. Control wells contained only broth and wells for sterility checks contained PM_{10} alone, broth alone and inoculum alone. Organism suspension, 50 μl *S pneumoniae* ATCC 49619, was added into each test and control column of the wells and incubated at 37℃ in 5% CO_2 for 5 hours. The Miles and Misra technique[5] 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.

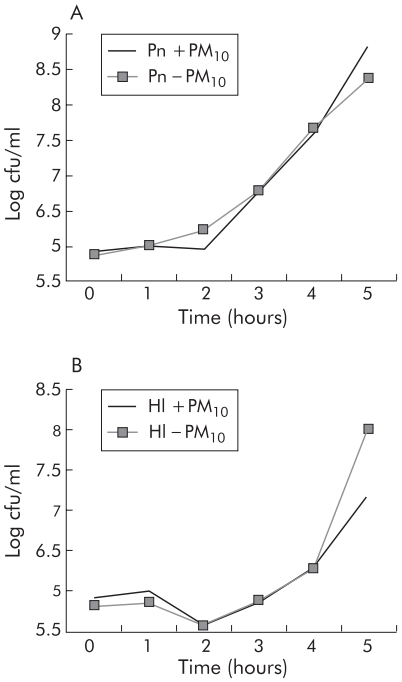

Figure 1 Growth curve against time with and without PM_{10} solution for (A) *S pneumoniae* ATCC control strain and (B) *H influenzae* NCTC control strain.

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 PM_{10}, for both *H influenzae* and *S pneumoniae*. In the growth kinetics experiment the only consistent finding was an inhibition of growth at a PM_{10}:broth medium dilution of 1:1 compared with the PM_{10} free control.

Growth of *H influenzae* and *S pneumoniae* is therefore neither inhibited nor promoted by incubation with PM_{10} 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 PM_{10} 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 particles. This interactive mechanism is supported by the association of ambient nitrogen dioxide levels with admissions for croup, and is analogous to the potentiation of the airway response to inhaled allergen by both nitrogen dioxide[6] and ozone.[7] 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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References

1 **Pope CA**, Bates DV, Reizenne ME. Health Effects of particulate air pollution: time for reassessment? *Environ Health Perspect* 1995;**103**:472–80.
2 **Donaldson K**, MacNee N. The mechanism of lung injury caused by PM_{10}. In: Hester RE, Harrison RM, eds. *Air pollution and health issues in environmental science and technology* No 10. 1998:21–32.
3 **Li XY**, Gilmour PS, Donaldson K, *et al*. Free radical activity and pro-inflammatory effects of particulate air pollution (PM_{10}) in vitro and in vivo. *Thorax* 1996;**51**:1216–22.
4 **Lawther PJ**, Emerson TR, O'Grady FW. Haemophilus influenzae growth stimulation by atmospheric pollutants. *Br J Dis Chest* 1969;**63**:45–7.
5 **Miles AA**, Misra SSK, Irwin JO. The estimation of the bactericidal power of the blood. *J Hygiene* 1938;**38**:732–49.
6 **Tunnicliffe WS**, Burge PS, Ayres JG. Effect of domestic concentrations of nitrogen dioxide on airway responses to inhaled allergen in asthmatic patients. *Lancet* 1994;**344**:1733–6.
7 **Jorres R**, Nowak D, Magnussen H. The effect of ozone exposure on allergen responsiveness in subjects with asthma or rhinitis. *Am J Respir Crit Care Med* 1996;**153**:56–64.

CORRECTIONS

doi: 10.1136/thx.2004.020307corr1

PAPER BY HORVATH *ET AL* (*THORAX* 2003;58:68–72)

In the paper entitled ''Comparison of exhaled and nasal nitric oxide and exhaled carbon monoxide levels in bronchiectatic patients with and without primary ciliary dyskinesia'' by I Horvath, S Loukides, T Wodehouse, *et al* published in the January 2003 issue of *Thorax* (2003;**58**:68–72), there was an error in the labelling of fig 1. The correct version of the figure is printed here. The publishers apologise for this error.

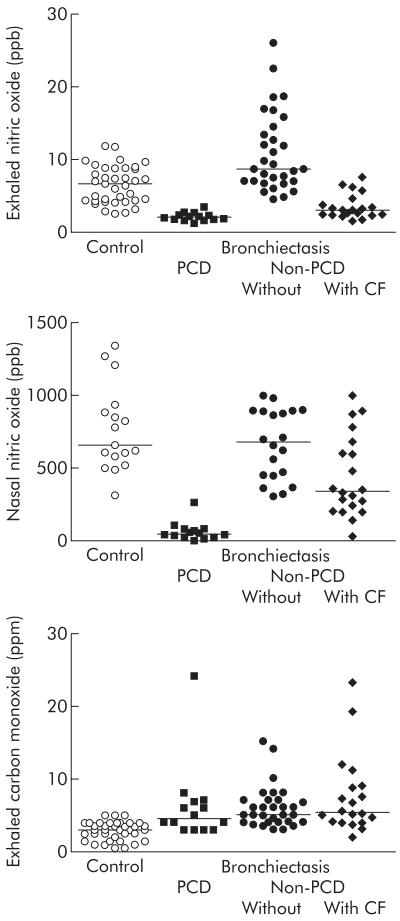

Figure 1 Exhaled NO, nasal NO and exhaled CO concentrations in normal subjects, patients with primary cell dyskinesia (PCD) with documented bronchiectasis, and patients with non-PCD bronchiectasis with and without CF. The Kruskal-Wallis test showed siginificant differences between mediator levels ($p<0.0001$).

doi: 10.1136/thx.2003.008912corr1

PAPER BY BALDI *ET AL* (*THORAX* 2004;59:428–32)

In the paper entitled ''Prognostic significance of cyclooxygenase-2 (COX-2) and expression of cell cycle inhibitors p21 and p27 in human pleural malignant mesothelioma'' by A Baldi, D Santini, F Vasaturo, *et al* published in the May 2004 issue of *Thorax* (2004;**59**:428–32) there was an error in the sentence beginning on line 14 of the left hand column on page 430. The sentence should have read ''The median survival in patients with low p21 or p27 expression was **shorter** than in those with high p21 or p27 expression.'' The publishers apologise for this error.