ORIGINAL ARTICLE

Effect of long term treatment with azithromycin on disease parameters in cystic fibrosis: a randomised trial

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Background: Relentless chronic pulmonary inflammation is the major contributor to morbidity and mortality in patients with cystic fibrosis (CF). While immunomodulating therapies such as prednisolone and ibuprofen may be beneficial, their use is limited by side effects. Macrolides have immunomodulatory properties and long term use dramatically improves prognosis in diffuse panbronchiolitis, a condition with features in common with the lung disease of CF.
Methods: To determine if azithromycin (AZM) improves clinical parameters and reduces inflammation in patients with CF, a 3 month prospective randomised double blind, placebo controlled study of AZM (250 mg/day) was undertaken in adults with CF. Monthly assessment included lung function, weight, and quality of life (QOL). Blood and sputum collection assessed systemic inflammation and changes in bacterial flora. Respiratory exacerbations were treated according to the policy of the CF Unit.
Results: Sixty patients were recruited (29 men) of mean (SD) age 27.9 (6.5) years and initial forced expiratory volume in 1 second (FEV_1) 56.6 (22.3)% predicted. FEV_1% and forced vital capacity (FVC)% predicted were maintained in the AZM group while in the placebo group there was a mean (SE) decline of –3.62 (1.78)% (p=0.047) and –5.73 (1.66)% (p=0.001), respectively. Fewer courses of intravenous antibiotics were used in patients on AZM (0.37 v 1.13, p=0.016). Median C reactive protein (CRP) levels declined in the AZM group from 10 to 5.4 mg/ml but remained constant in the placebo group (p<0.001). QOL improved over time in patients on AZM and remained unchanged in those on placebo (p=0.035).
Conclusion: AZM in adults with CF significantly improved QOL, reduced CRP levels and the number of respiratory exacerbations, and reduced the rate of decline in lung function. Long term AZM may have a significant impact on morbidity and mortality in patients with CF. Further studies are required to define frequency of dosing and duration of benefit.

C hronic bronchial sepsis and an exuberant host inflammatory response results in bronchiectasis and, ultimately, respiratory failure in cystic fibrosis (CF). Immunomodulatory agents such as prednisolone and ibuprofen have been shown to be beneficial, but prescribing is limited because of concern regarding long term efficacy and side effects.[1]

Macrolide antibiotics display immunomodulatory properties and are well tolerated. Long term, low dose erythromycin was efficacious in improving symptoms of chronic suppurative respiratory disease in patients with diffuse panbronchiolitis (DPB).[2–4] Cystic fibrosis has several features in common with DPB; both conditions are characterised by chronic bacterial colonisation and a marked neutrophil infiltrate within the airways.[2] The first suggestion that long term macrolide therapy may also be beneficial in CF was a small study demonstrating significant improvement in lung function in seven paediatric patients who took azithromycin (AZM) daily for several months.[5] We report the results of the first long term prospective randomised, double blind, placebo controlled trial of oral AZM in adult patients with CF on clinical, bacteriological, and inflammatory parameters.

METHODS

Sixty adult patients (29 men) of mean age 27.9 years (range 18–44) with CF and with clinically stable disease were recruited from a total population of 129 patients at two adult CF centres. Enrolment required a period of clinical stability defined as no change in symptoms or medication, no admission to hospital, and no intravenous antibiotic therapy for at least 2 weeks prior to enrolment. Patients were excluded if they had known allergies to macrolides, known or suspected intolerance to sputum induction, severe liver disease, or on personal request. During the study usual treatment was unchanged and monitored by the clinic physician and included physiotherapy, oral and/or inhaled antibiotics, bronchodilators, pancreatic and vitamin supplements.

Randomisation

Placebo and AZM were concealed in identical capsules. Medication was randomised prior to commencement of the study by the hospital pharmacy independently of trial staff. Randomisation was in blocks of 10 (5 placebo/5 AZM) and patients were automatically dispensed the next allocated treatment (containing either AZM 250 mg or placebo daily for 3 months). Unblinding occurred after statistical analysis.

Informed consent was obtained from all subjects and the trial was approved by the ethics committees of the Mater Adult Hospital and The Prince Charles Hospital.

Assessment

Patients were assessed every month by lung function measured by spirometry, weight, and quality of life (QOL) survey. QOL was assessed using the Chronic Respiratory Disease Questionnaire (CRDQ)[6 7] at entry and at monthly intervals. This questionnaire has been used in previous research studies[7] and has also been investigated for its adaptation for use in CF.[8] Scores are obtained for the domains of fatigue, mastery, emotion, dyspnoea, and total quality of life. In addition, blood was collected each month for measurement of C reactive protein (CRP), erythrocyte sedimentation rate (ESR), and sputum for microbiological analysis.
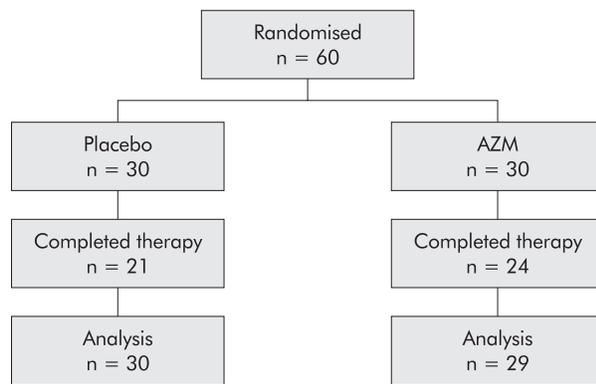
Figure 1 Trial profile.

Table 1 Baseline characteristics of azithromycin (AZM) and placebo arms

	Placebo (n=30)	AZM (n=30)
% male	66.7	30.0
Age (years)	28.1 (7.3)	27.7 (5.6)
Height (m)	1.71 (0.08)	1.66 (0.07)
Weight (kg)	65.1 (12.7)	56.6 (9.9)
BMI	22.5 (3.1)	20.6 (2.7)
FEV_1 (% pred)	62.3 (24.8)	50.9 (18.3)
FVC (% pred)	77.5 (19.9)	67.3 (19.0)

Values are mean (SD). BMI = body mass index; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity.

To determine whether clinical improvement was due to the treatment of organisms susceptible to azithromycin, serological evidence of infection (in the absence of available molecular techniques at that time) with *Legionella pneumophila*, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae* at baseline and study completion was sought.

Sputum was cultured for *Pseudomonas aeruginosa*, *Burkholderia cepacia*, other typical pathogens such as *Staphylococcus aureus* and *Haemophilus influenzae*, and atypical mycobacteria. Sputum was plated onto 5% horse blood, MacConkey and senal agar with colistin, nalidixic acid, (37°C, 5% CO_2), and also chocolate agar with bacitracin (anaerobic at 37°C) plus mannitol salt and Pseudocepacia agar with ticarcillin and polymixin B (37°C, 0% CO_2).

Quantitative bacterial counts of *P aeruginosa* were performed by serial dilutions of sputum with phosphate buffered saline. Colony counts were performed in duplicate on blood agar and were expressed logarithmically after incubation for 48 hours at 37°C, 5% CO_2.

An acute respiratory exacerbation was defined as increased cough, sputum, and/or dyspnoea, and/or reduced forced expiratory volume in 1 second (FEV_1) and a decision to treat with intravenous antibiotics (either inpatient or outpatient) by the usual treating CF physician. AZM was continued throughout these periods.

Statistical analysis and sample size

The primary study outcome was change in FEV_1 % predicted and was based on improvements in FEV_1 reported in the open label study by Jaffe.[5] A sample size of eight per group was required to detect a difference of 10% between drug and placebo groups with 90% power and 5% statistical significance. However, allowing for a potentially wide range of responses and anticipating loss to follow up, a sample size of 30 per group was considered optimal.

The analysis was blinded. An intention to treat analysis was performed. At the bivariate level of analysis, Fisher's exact tests were used to test the association between independent categorical variables. McNemar's test was used where categorical outcomes were compared over two time points (baseline and 3 months). Frequency distributions of all continuous variables were examined for outlying values and to determine whether or not they were approximately normal. The latter assumption was also formally tested with Kolmogorov-Smirnov tests for normality. Where the assumption could not be met, data were summarised in terms of medians rather than means and logarithmically transformed for analysis. Group means or log means were compared over time and by treatment group using repeated measures analysis of variance (ANOVA) models utilising a generalised estimating equations approach to accommodate the partially complete time series data (that is, all available data at each follow up point were utilised in the analyses). Trends over time were considered as categorical contrasts of each time point against baseline and, where these suggested appropriate, as linear contrasts. Models were adjusted for baseline FEV_1 % predicted as a covariate since this was determined to be substantially different between the two treatment groups. Statistical significance was quoted based on the conventional $p < 0.05$ level (two tailed). Analyses were performed using SUDAAN version 7.5.

RESULTS

The design of the trial is shown in fig 1.

There were no significant differences between the two groups with respect to age, number of doses missed, or number of adverse events experienced during the trial. However, the placebo group contained more men and was also on average, taller, heavier, and had better lung function than those randomised to receive AZM (table 1). The chance differences observed between the two groups were not anticipated and there were no obvious reasons for these differences.

Table 2 Mean (SD) change in FEV_1 and FVC % predicted over time and by treatment group

	Change in FEV_1 % predicted*			Change in FVC % predicted*		
	Month 1	Month 2	Month 3	Month 1	Month 2	Month 3
Placebo group						
n	23	17	21	22	15	19
Mean (SD)	−1.32 (5.51)	−1.17 (5.85)	−0.91 (5.99)	−3.26 (6.98)	−1.82 (6.06)	−0.46 (7.83)
AZM group						
n	22	24	22	19	20	19
Mean (SD)	2.92 (7.72)	1.51 (8.84)	2.95 (9.22)	2.96 (8.19)	4.28 (6.46)	3.81 (6.76)
Mean (SE) excess effect of AZM over placebo		3.62 (1.78)%			5.73 (1.66)%	

*All changes are from baseline FEV_1 % predicted and adjusted for baseline FEV_1 % predicted levels in each case.

Table 3 Acute exacerbations of respiratory disease: antibiotics and admissions in placebo and AZM groups

	Placebo	AZM	p value
Number of courses of IV treatment	1.1 (0.5, 0–7)	0.4 (0, 0–2)	0.016
Total days IV treatment	7.1 (1, 0–44)	2.0 (0, 0–17)	0.009
Total days home IV treatment	2.1 (0, 0–14)	0.2 (0, 0–5)	0.037
Total hospital days	5.2 (0, 0–36)	2.1 (0, 0–15)	0.056

Values are mean (median, range).

Unless otherwise stated, these differences were accounted for in the analyses of treatment group differences over time by adjusting for sex, body mass index (BMI), and FEV_1 % predicted.

There was a significant overall difference in change in FEV_1 (p=0.047) and FVC (p=0.001) % predicted between the AZM and placebo groups. Over the 3 months of the study the mean FEV_1 % predicted was 3.62% (95% confidence interval (CI) –7.13 to –0.13%) less and the mean FVC % predicted was 5.73% (95% CI –8.98% to –2.47%) less in the placebo group than in the AZM group (table 2). The AZM group maintained lung function over time while the placebo group experienced deterioration in FEV_1 and FVC % predicted. The p value for the difference between AZM and placebo and the stated difference between AZM and placebo has been adjusted for baseline FEV_1 % predicted.

The AZM group had significantly fewer total days of intravenous antibiotic treatment for acute respiratory exacerbations (p=0.009), fewer days at home receiving intravenous antibiotics (p=0.037), and fewer courses of intravenous antibiotics during the study (p=0.016). The number of days spent in hospital for antibiotic treatment of acute respiratory exacerbations was lower in the AZM group (p=0.056, table 3).

Treatment had a significant effect on the time trend of CRP measurements (p<0.001). Median CRP values declined steadily over time on AZM but remained relatively constant in the placebo group (fig 2). At baseline, CRP was significantly negatively correlated with FEV_1 % predicted (p<0.001) and BMI (p=0.048). In patients who received AZM the reduction in CRP over time was strongly related to baseline CRP (p<0.001). Patients who received AZM with a CRP level of >10 μg/ml at baseline had significantly poorer lung function than patients receiving AZM with a normal CRP level at baseline (mean (SD) FEV_1 42 (16)% predicted v 60 (15)% predicted, p<0.01).

No statistically significant differences in ESR or BMI were detected over time, either overall or between treatment groups (p>0.10 in all cases).

One patient was excluded from the end point analysis after commencing long term prednisone on the same day as the study. Fifteen patients (25%, nine placebo) did not complete the 3 months of treatment, three because of adverse events and the remainder because of non-compliance or personal request. Of the 15 patients who did not complete treatment, four stopped their medication before completing 1 month of treatment, a further five stopped before 2 months, and the remaining six stopped their medication before completing 3 months. Nineteen patients (31.7%, 12 placebo) reported missing doses of medication throughout the treatment period. Thirty one patients (16 placebo) used maintenance antibiotics (oral and/or nebulised) either continuously or intermittently during the study.

Quality of life
There were significant improvements in all dyspnoea scores over time overall in both groups (p=0.042, table 4). Improvements in dyspnoea, emotional, mastery and total scores were significantly higher for those receiving AZM compared with placebo. Improved fatigue scores were seen only in the AZM group.

Adverse events
There were 16 adverse events in 15 patients (seven on placebo). Of the three patients who discontinued treatment due to adverse events, urticarial reaction in a patient receiving AZM was considered "likely" to be related to the study drug, while neutropenia in a patient in the AZM group and "swelling" in a patient in the placebo group were considered "possibly" related to the study drug. A further two events were considered "possibly" related to the study drug (rash in each of
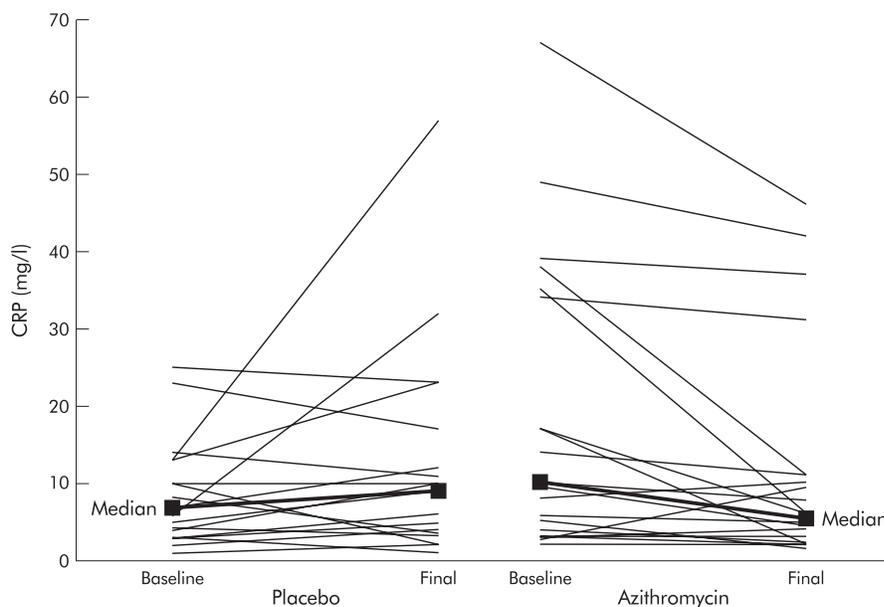
Figure 2 Effect of treatment group on C reactive protein (CRP) levels over time.

Table 4 Mean (SD) QOL scores over time: baseline and final assessment (month 3)

		Placebo		Azithromycin		
		n	QOL score	n	QOL score	p value*
Dyspnoea	Baseline	28	22.75 (4.86)	28	21.96 (4.68)	0.042
(range 5–35)**	Final	19	25.82 (5.27)	22	28.16 (4.76)	
Fatigue	Baseline	30	18.87 (5.37)	30	17.17 (5.50)	0.003
(range 4–28)	Final	20	18.85 (4.70)	24	21.04 (4.12)	
Emotion	Baseline	30	37.97 (6.84)	30	34.87 (7.63)	0.012
(range 7–49)	Final	20	39.70 (5.66)	24	41.33 (7.13)	
Master	Baseline	30	24.37 (3.69)	30	22.70 (5.47)	0.035
(range 4–28)	Final	20	25.25 (3.64)	24	25.54 (3.45)	
Total	Baseline	28	103.14 (14.00)	29	95.72 (18.68)	0.035
(range 20–140)	Final	19	109.30 (14.78)	23	116.11 (14.67)	

*Interaction of treatment effect and time. **Minimum and maximum score possible for each domain.

the AZM and placebo groups). All events resolved without complication.

Microbiology

Fifty seven patients had sputum analysis at baseline. A mucoid strain of *P aeruginosa* was isolated from 83.3% of subjects and a non-mucoid strain from 66.6%. *S aureus* was isolated from 41.3% of patients tested at baseline, *B cepacia* from four patients, and *H influenzae* from one patient. No mycobacteria were isolated during the course of the study. 77% of the *S aureus* isolates from the AZM group and 69.2% from the placebo group were sensitive to erythromycin.

There was no significant difference in organisms isolated or bacterial counts between the two groups at baseline, nor in the type or quantity of microorganisms at the final assessment (p>0.095 in all cases). The presence of *B cepacia, S aureus*, mucoid and non-mucoid forms of *P aeruginosa* was not influenced by sex, but the presence of *S aureus* was significantly correlated with reduced dyspnoea scores (p=0.021).

There was no evidence to suggest that clinical improvement was due to treatment of bacteria susceptible to macrolide therapy. Twenty nine patients underwent serological tests before and after treatment and a further 22 patients had incomplete testing (only initial titres performed). Only one patient (in the placebo group) had a fourfold rise in chlamydia and mycoplasma titres.

DISCUSSION

This is the first prospective randomised, double blind, placebo controlled trial of oral AZM in CF. Treatment with AZM significantly reduced the rate of decline in FEV_1 and FVC % predicted over time compared with placebo. AZM treatment was associated with significantly fewer acute respiratory exacerbations requiring intravenous antibiotics and significantly improved QOL scores compared with placebo. There was evidence of reduced host systemic inflammation with a decline in CRP levels over time in subjects receiving AZM.

Randomisation was moderately successful with the exception of sex, lung function, and weight, biasing the results in favour of a reduced effect of AZM. The imbalance was considered to be of clinical significance and adjustments were made in the modelling. Stratification for disease severity within the groups was not performed. While it may be argued that those with a more severe phenotype may have had a greater scope for improvement, it is important to note that all parameters were adjusted for baseline values in the statistical analysis, reducing the chance of any observed difference being the result of reversion to the mean.

The natural history of CF is one of persistent pulmonary inflammation as a consequence of chronic bacterial infection, resulting in intermittent exacerbations of pulmonary symptoms and with temporary clinical improvement after treatment. More severe disease is generally associated with more frequent exacerbations requiring intravenous treatment. Although randomisation produced two clinically dissimilar groups, all patients were stable before enrolment. Despite evidence that the group randomised to receive AZM had more severe disease than the placebo group, they required significantly fewer courses of intravenous antibiotics and fewer days of intravenous treatment during the study period. Furthermore, patients in the AZM group had higher baseline CRP levels and treatment was associated with significant reductions in CRP despite fewer courses of systemic antibiotics. This finding provides strong evidence that the small but significant differences in lung function between the two groups and the significant reduction in systemic inflammation over time in the AZM group represent real benefit to patients receiving AZM and supports a systemic anti-inflammatory effect.

The small changes in lung function seen in this study contrast with those reported by Jaffe *et al*[5] who reported an increase of 11% in FEV_1 % predicted after treatment with AZM. The mean age of patients in their study was 12.1 years and the mean duration of AZM treatment was 6 months compared with a mean age of 27.9 years and maximum treatment duration of 3 months in our study. Although changes in lung function were small, the differences from placebo were statistically significant. Treatment of an older group with potentially more advanced and permanent lung disease may limit any benefit achievable with anti-inflammatory therapy. Patients in this study generally had more severe disease than in the study of Jaffe *et al*[5] which is likely to be responsible for the smaller FEV_1 response to treatment. We have demonstrated maintenance rather than improvements in lung function in a group with severe disease. Over the 3 months of the study the placebo group experienced a change in FEV_1 % predicted of –0.91%, which equates to a drop of approximately 3–4% over 12 months. These changes are similar to changes in FEV_1 reported by Ramsey *et al*[9] in a study to determine the effect of inhaled tobramycin in CF. Over a 20 week treatment period they reported a fall in FEV_1 % predicted of 2.64% in their adult placebo group which equates to a change of approximately 6–7% over a 12 month period. Maximum benefits of such treatment may be achieved in subjects without established disease. Trials in children or with populations large enough to allow stratification of results based on disease severity are necessary to assess maximum achievable benefits from early onset of treatment.

At the time of this trial there was no validated disease-specific questionnaire available for the measurement of QOL in CF. The QOL questionnaire used for this study was the CRDQ, developed for use in patients with chronic obstructive pulmonary disease (COPD) who share many clinical features in common with CF patients on a day to day basis. This questionnaire has been used in a previous clinical trial with adult CF patients[7] and has also been validated for its adaptation for use in CF.[8]

There was no evidence that AZM influenced the microbiological profile of sputum after 3 months of treatment. Improvement was not related to a bacterial effect on organisms sensitive to AZM. However, in the absence of accessible molecular techniques, serological testing was performed to determine whether clinical improvement was due to the treatment of susceptible organisms. Serological testing may have been inadequate to rule out an acute infection. In HIV/AIDS patients weekly treatment with AZM has been used for long periods and has not been associated with development of resistance.[10]

AZM was chosen over other macrolides such as erythromycin because of a superior gastrointestinal side effect profile and ease of administration. It was well tolerated but, despite a once daily regimen, adherence with treatment was moderate with almost one third of patients reporting missed doses at some time during the trial. These adherence levels were within the range of previously published studies of adherence in chronic diseases, including CF, which quote ranges of non-adherence from 15% to 50% for most areas of treatment.[11 12]

Although relatively costly, long term treatment with AZM may result in direct cost reductions associated with reduced number of acute respiratory exacerbations requiring intravenous antibiotic therapy as well as reduced indirect costs associated with improved QOL. Although the outcomes of this study are promising, the choice of macrolide, dose, dose interval, duration of effect, and impact of long term treatment on disease progression and the microbiological environment of the lung are unknown. Macrolides including AZM accumulate intracellularly,[13] but concentration/effect ratios are poorly defined both for the treatment of infection and inflammation. Reports of reduced adhesion of bacteria to epithelial cells after twice weekly AZM suggest that less frequent dosing than was used in this trial may be possible.[14]

The mechanism by which macrolides downregulate the host inflammatory response is unclear but is almost certainly multifactorial. Suppression of interleukin (IL)-8 secretion may be important[4 15 16] via inhibition of IL-8 gene transcription.[17 18] Other effects include suppression of tumour necrosis factor (TNF)-α synthesis and modulation of IL-1 and GM-CSF production by lipopolysaccharide stimulated monocytes.[15 19] Macrolides also interfere with neutrophil function.[20 21] In addition to effects on the host immune response, macrolides inhibit alginate production, protease activity, flagellin expression, and bacterial motility of *P aeruginosa*.[22–24] AZM reduces adherence of *P aeruginosa* to epithelial cells,[14] opening the possibility of a preventative role for these agents.

In summary, we have shown that long term oral AZM treatment is associated with improvements in several key disease indicators in CF. Treatment with AZM was associated with a significantly reduced rate of decline in lung function over a 3 month period. It significantly improved CRP levels as a marker of systemic inflammation, QOL, and reduced the number of acute respiratory exacerbations compared with placebo. We conclude that macrolides may have a role in the long term management of patients with CF.

ACKNOWLEDGEMENTS

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REFERENCES

1 **Oermann CM**, Sockrider MM, Konstan, MW. The use of anti-inflammatory medications in cystic fibrosis. *Chest* 1999;**115**:1053.

2 **Ichikawa Y**, Ninomiya H, Koga H, *et al*. Erythromycin reduces neutrophils and neutrophil-derived elastolytic-like activity in the lower respiratory tract of bronchiolitis patients. *Am Rev Respir Dis* 1992;**146**:196–203.

3 **Kobayashi H.**, Takeda H., Sakayori S, *et al*. Study on azithromycin in treatment of diffuse panbronchiolitis (abstract). *Kansenshogaku Zasshi* 1995;**69**:711–2.

4 **Mukae H.**, Kadota J, Ashitani J, *et al*. Elevated levels of soluble adhesion molecules in serum of patients with diffuse panbronchiolitis. *Chest* 1997;**112**:1615–21.

5 **Jaffe A**, Francis J, Rosenthal M, *et al*. Long-term azithromycin may improve lung function in children with cystic fibrosis. *Lancet* 1998;**351**:420.

6 **Guyatt GH**, Berman LB, Townsend M, *et al*. A measure of quality of life for clinical trials in chronic lung disease. *Thorax* 1987;**42**:773–8.

7 **Wolter JM**, Bowler SD, McCormack JG. Home intravenous therapy in cystic fibrosis: a prospective randomised trial examining clinical, quality of life and cost aspects. *Eur Respir J* 1997;**10**:896–900.

8 **Bradley J**, Dempster M, Wallace E, *et al*. The adaptations of a quality of life questionnaire for routine use in clinical practice: the chronic respiratory disease questionnaire in cystic fibrosis. *Qual Life Res* 1999;**8**:65–71.

9 **Ramsey BW**, Pepe MS, Quan JM, *et al*. Intermittent administration of inhaled tobramycin in patients with cystic fibrosis. *N Engl J Med* 1999;**340**:23–30.

10 **Oldfield EC**, Fessel WJ, Dunne MW, *et al*. Once weekly azithromycin therapy for prevention of Mycobacterium avium complex infection in patients with AIDS: a randomised, double-blind, placebo-controlled multicenter trial. *Clin Infect Dis* 1998;**26**:611–9.

11 **Conway SP**, Pond MN, Hamnett T, *et al*. Compliance with treatment in adult patients with cystic fibrosis. *Thorax* 1996;**51**:29–33.

12 **Abbott J**, Dodd M, Bilton D, *et al*. Treatment compliance in adults with cystic fibrosis. *Thorax* 1994;**49**:115–20.

13 **Carbon C**. Clinical relevance of intracellular and extracellular concentrations of macrolides. *Infection* 1995;**23**(Suppl 1):10–14.

14 **Fisher JJ**, Baumon U, Gudowius P, *et al*. Azithromycin reduces epithelial adherence of P aeruginosa in patients with cystic fibrosis (abstract). *Pediatr Pulmonol* 1999;Suppl 19:265.

15 **Takizawa H**, Desaki M, Ohtoshi T, *et al*. Erythromycin modulates IL-8 expression in normal and inflamed human bronchial epithelial cells. *Am J Respir Crit Care Med* 1997;**156**:266–71.

16 **Khan AA**, Slifer TR, Araujo FG, *et al*. Effect of clarithromycin and azithromycin on production of cytokines by human monocytes. *Int J Antimicrob Agents* 1999;**11**:121–32.

17 **Desaki M**, Takizawa H, Ohtosh T, *et al*. Erythromycin suppresses nuclear factor-kappa B and activator protein-1 activation in human bronchial epithelial cells. *Biochem Biophys Res Commun* 2000;**267**:124–8.

18 **Abe S**, Nakamura H, Inove S, *et al*. Interleukin-8 gene repression by clarithromycin is mediated by the activator protein-1 binding site in human bronchial epithelial cells. *Am J Respir Cell Mol Biol* 2000;**22**:51–60.

19 **Morikawa K**, Watabe H, Araake M, *et al*. Modulatory effect of antibiotics on cytokine production by human monocytes in vitro. *J Antimicrob Agents Chemother* 1996;**40**:1366–70.

20 **Sugihara E**. Effect of macrolide antibiotics on neutrophil function in human peripheral blood (abstract). *Kansenshogaku Zasshi* 1997;**71**:329–36.

21 **Villagrasa V**, Berto L, Cortijo J, *et al*. Effects of erythromycin on chemoattractant-activated human polymorphonuclear leukocytes. *Gen Pharmac* 1997;**29**:605–9.

22 **Molinari G**, Guzman CA, Pesce A, *et al*. Inhibition of Pseudomonas aeruginosa virulence factors by subinhibitory concentrations of azithromycin and other macrolide antibiotics. *J Antimicrob Chemother* 1993;**31**:681–8.

23 **Howe RA**, Spencer RC. Macrolides for the treatment of Pseudomonas aeruginosa infections? *J Antimicrob Chemother* 1997;**40**:153–5.

24 **Kawamura SK**, Iinuma Y, Hasegawa T, *et al*. Effect of subinhibitory concentrations of macrolides on expression of flagellin in Pseudomonas aeruginosa and Proteus mirabilis. *Antimicrob Agents Chemother* 2000;**44**:2869–72.