Title: Ciprofloxacin during upper respiratory tract infections to reduce pseudomonas aeruginosa infection in paediatric cystic fibrosis: a pilot study

Running Title: Ciprofloxacin during viral infections in CF

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Abstract:

Introduction: Acute viral respiratory illnesses are associated with acquisition of *Pseudomonas aeruginosa* infection in Cystic Fibrosis (CF) patients.

Objectives: To pilot a protocol for a randomised controlled trial to determine whether oral anti-pseudomonal antibiotics used at the onset of such episodes might delay onset of infection with this organism.

Methods: Forty-one children with CF aged 2-14 years, without chronic *Pseudomonas* infection, were randomised to receive ciprofloxacin (n=28) or placebo (n=13) at the onset of acute viral respiratory infections on an intention to treat basis, during a study period of up to 32 months.

Results and Conclusions: There were no unexpected adverse events believed related to the use of the study medication. The rate of withdrawal from the study was low (approximately 7%) and did not differ between groups. Randomisation was effective and acceptable to participants. Primary and secondary outcome measures all favoured active treatment but there were no significant between group differences. The median rate of pseudomonas isolates was 0/patient/year, (interquartile range 0-0.38) in both the active and placebo groups. Kaplan Meier survival curves showed no significant difference in time to first *Pseudomonas* isolate between groups.

This study demonstrated the clinical feasibility of using oral ciprofloxacin in cystic fibrosis patients at times of viral infection. Within this sample size no significant association was found between active treatment and decreased growth of *Pseudomonas* on follow-up microbiological samples. A definitive study would require at least 320 children to demonstrate significant differences in the rate of pseudomonal isolates.

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Key words: Cystic Fibrosis, *Pseudomonas aeruginosa*, Ciprofloxacin, Randomised controlled trial

Ethical statement: UK NHS Ethics Approval was gained (08/H0504/110). The study was performed in accordance with the ethical standards laid down in the Declaration of Helsinki. Parents of all participating children gave informed written consent and children aged over 11 years were asked to provide written assent.

Introduction:

Cystic fibrosis (CF) disease-related morbidity and mortality are most commonly a result of progressive lung disease. This is characterised by a self-perpetuating cycle of bacterial infection and airway inflammation [1]. Studies have characterised the involvement of a range of bacterial, viral and fungal species in the pathogenesis of CF lung disease, [2] but *Pseudomonas aeruginosa* remains the most common organism causing chronic endo-bronchial infection associated with deteriorating respiratory health. Current concepts of care include the early use of anti-pseudomonal antibiotics after the initial detection of *P. aeruginosa* in respiratory samples[3,4]. This approach has been successful in temporarily eradicating *P. aeruginosa* and thus delaying the onset of chronic infection, but eradication attempts typically become less successful over time and most patients eventually become chronically infected [5]. Preventive strategies using regular inhaled anti-pseudomonal antibiotics [6] and routine bronchial lavage sampling to improve early identification and treatment of *P. aeruginosa* infection [7], have not been shown to be effective.

Laboratory studies have characterised the synergistic role of viruses as co-infecting respiratory pathogens predisposing to the onset of infection with *P. aeruginosa* [8,9]. In a prospective study of 38 children with CF, for 6 of the 7 children in whom *P. aeruginosa* was first isolated during the study period, first isolates of *P. aeruginosa* were identified during, or within 3 weeks of, a viral upper respiratory tract infection [10]. More recently, during one winter period, 6 of 20 prospectively studied children with CF (age range 0.1 to 7.4 years) had at least one *P. aeruginosa* positive culture from specimens collected at the time of acute viral respiratory infection [11]. Initial

onset of pseudomonal infections have been shown to have a seasonal pattern of occurrence temporally related to winter months when viral infections with pathogens such as RSV and influenza are more common [12-15].

These data suggest that individuals with CF might be at increased risk of infection with *P. aeruginosa* during episodes of viral respiratory tract infection but there are no randomised controlled trials of anti-pseudomonal prohylaxis during viral infections We investigated the feasibility of using a randomised controlled trial protocol to investigate the hypothesis that the oral anti-pseudomonal antibiotic ciprofloxacin, used at the onset of viral upper respiratory tract symptoms, can prevent the subsequent growth of *P. aeruginosa* from follow-up respiratory tract cultures.

Patients and Methods:

Following UK NHS Ethics Approval (08/H0504/110), all children aged 2-14 years with a confirmed diagnosis of CF and attending either the regional CF service for full care or shared care with one of two referral hospitals were screened for study inclusion. Those with negative *Pseudomonas* ELISA serology and judged not to be chronically infected with *P. aeruginosa* [16] or any other gram negative organism, and who had not received regular anti-pseudomonal antibiotics in the previous 6 months, were invited to participate. Parents of all participating children gave informed written consent and children aged over 11 years were asked to provide written assent.

Children were randomised in blocks of 9 (6 active versus 3 placebo, see consort diagram figure 1). Restricted randomisation was used with stratification by clinic in a double blind manner. Participants received ciprofloxacin syrup or placebo treatment for viral infections for 32 months or until study end if this was sooner. Very low rates of infective episodes were reported during late spring and summer months and for this reason the study was stopped earlier than had originally been planned and before all study participants had been followed up for 32 months. The first patient was recruited into the study in December 2009 and the last in January 2011, the study ended in March 2013. Parents were instructed to contact the CF research nurses if their child experienced symptoms suggestive of acute viral respiratory illness including a runny nose, nasal congestion, sore throat and sneezing with or without fever. Parents also received fortnightly reminder texts from the research nurse to make contact in the event of these symptoms. When acute symptoms were reported, these were verified by the research team and arrangements made for the family to receive, according to randomisation group, a two week course of either ciprofloxacin syrup (Bayer plc; Dose according to the British National Formulary for Children 20mg/kg/day twice daily aged > 5years; 15mg/kg/day twice daily age 2-5 years) or a placebo of the syrup suspension alone prepared by the clinical trial pharmacy at University Hospital Southampton NHS Foundation Trust. If cough persisted for more than 48 hours, children were assessed and treated with additional oral antibiotics by the CF clinical team according to regional protocols. Microbiological specimens were obtained at out-patient clinic visits every two months and when clinically indicated. Separate to the study procedures, all positive isolates of *P. aeruginosa* were treated according to local clinical guidelines with 3 months inhaled colomycin plus oral ciprofloxacin (3 weeks for first isolates and 3 months for

subsequent isolates) or a combination of two intravenous antibiotics. Study medication was discontinued during these treatment periods. Children who became chronically infected with *P. aeruginosa* were no longer eligible for inclusion within the trial.

The pre-determined primary clinical outcome was the annual rate of positive isolates of *P. aeruginosa*. Secondary outcome measures included time to first detection of *P. aeruginosa*, change in pseudomonal serology and annual rates of nebulised and intravenous antibiotic prescription.

Statistical considerations:

All data were analysed on an intention to treat basis. We aimed to recruit 45 children into the study and used a 2:1 asymmetrical study design favoring active treatment to maximise safety and efficacy data for the study intervention. Based on published epidemiological studies [17], and review of PortCF data, we anticipated that *P. aeruginosa* would be detected in approximately one third of the control group. The number of children becoming chronically infected, according to the definition of >50% of samples positive for *Pseudomonas* over 12 months, was anticipated to be small[16]. However, the overall isolation rate was judged to be a clinically significant outcome of direct relevance to chronic colonisation. Given the absence of trial data relating to anti-pseudomonal prophylaxis during viral infaction, the likely size of any between group difference in overall isolation rate was unknown. This study was

designed to provide important safety and feasibility data for this treatment approach and to inform the design and sample size of further trials in this age group.

Many of the outcomes were positively skewed so non-parametric tests were used for between group comparisons. Bonferroni correction was considered over-conservative as the analyses were designed *a priori* to test a limited number of hypotheses and not all the tests were independent.[18] We focused our interest on results with P-values <0.05 and considered consistency of the findings in our interpretation. Stata® 11 (Stata Corp., College Station, TX) was used for all analyses.

Results:

Forty-one children were randomised (Figure 1)[19]. Twenty seven completed 32 months, 3 were withdrawn (7%) and 11 had completed less than 32 months (26-31 months) at study end. Two children withdrew from the intervention group, after 22 and 28 months, due to their families relocating out of region and one was withdrawn from the placebo group after 8 months because they were commenced on long-term nebulised antibiotics for clinical indications. There were no withdrawals attributable to no longer wishing to take the study medication or due to dissatisfaction with any other element of the study protocol. Withdrawal rate did not differ between groups. At recruitment there were no significant between group differences in demographic or

clinical features (Table 1). Participants did not demonstrate a better than chance ability to guess their group allocation.

The median number of occasions when a child was judged to require study medication was two in both groups. Twelve children in the placebo group received at least one course of oral ciprofloxacin during the study period. These individuals each received between one and eight courses. Despite this, the total number of courses of ciprofloxacin prescribed according to the study protocol combined with those prescribed at the discretion of the clinical care team was significantly higher in the treatment group (p=0.0002). Each child in the active treatment group received between one and eleven courses (Table 1).

946 samples were collected during the study period of which 22 (2.3%) yielded positive cultures for *P. aeruginosa*. Although there was an almost 50% reduction in the mean annual rate of *P. aeruginosa* isolates (0.17 versus 0.30), this difference was not significant (Table 2). Nine of the 28 children in the active treatment group (32%) had at least one positive isolate of *P. aeruginosa*. For seven children this occurred on one occasion. One child had two positive isolates and one child had positive isolates on three occasions. Six out of 13 children receiving placebo (46%) had at least one positive isolate of *P. aeruginosa*, one child isolated P. aeruginosa on five occasions and five children had one positive isolate each. A *post hoc* power calculation based on the annual rates of *Pseudomonas* isolates estimated that 80% power would require approximately 300 participants, therefore to account for a

withdrawal rate of approximately 7% at least 320 children would need to be recruited.

The number of episodes of intravenous or nebulised antibiotic usage did not differ between active treatment and placebo groups and there were no between group differences in ELISA serology (Table 2). Although the time to first *P. aeruginosa* isolate was shorter in the placebo group, this difference was not significant p=0.1, (Figure 2).

There were no between group differences in the rate of isolation of any other microbial or fungal species (Table 3). Median weight, height and FEV_1 SD scores all improved between the beginning and end of the study period and there were no significant between group differences. There were no cases of allergic bronchopulmonary aspergillosis and no reports of arthropathy or any other significant side effects in relation to the use of ciprofloxacin or placebo.

Discussion:

This study provides data about the safe use of two week courses of oral ciprofloxacin at times of acute respiratory infection in children 2 years of age or older who are not yet chronically infected with *P. aeruginosa.* Within this pilot study there was no significant between group difference in the annual rate of *P. aeruginosa* isolates.

The study was designed as a pilot and unlikely to have sufficient power to detect a significant difference between groups for any outcome. It is possible, however, that the strong seasonal association found in epidemiological studies [12] between *P.aeruginosa* infection and respiratory virus infections might not reflect direct facilitation of *P.aeruginosa* infection at the time of viral infection. Post-viral effects, such as mucus gland hypertrophy, might play a role in the seasonal associations reported. The study design might therefore have failed to find a significant between group difference because the period of antibiotic cover was insufficient. Nevertheless animal model data, demonstrate a facilitating effect upon *Pseudomonal* infection during early viral infection which supports investigation of this hypothesis in humans.[20]

IAn important finding of relevance to proceeding to a definitive study was that the overall number of *P. aeruginosa* isolates in the control group was higher than anticipated,probably reflecting the intensity of microbial surveillance during the study.Despite this, only one child receiving placebo became chronically infected with *P. aeruginosa* and ELISA serology remained <1.00 (not indicative of chronic infection) in all study participants. Up to 11 courses of ciprofloxacin were prescribed to participants in the treatment arm of the study. An important clinical consideration is to weigh reduction of this relatively rare event against possible adverse drug effects or effects upon the microbiome of frequent broad spectrum oral antiobiotic administration.

One concern about the increased use of ciprofloxacin in young children is the emergence of resistant opportunistic airway pathogens. Our results showed no between group difference in the annual rate of *S. aureus* isolates or any other known CF pathogens. One child in the active intervention group who grew MRSA had grown this organism intermittently prior to study entry as had one of two children who grew atypical mycobacterial species. Close surveillance for changes in the occurrence of other bacterial pathogens would be an important part of similar future studies.

The study was subject to several limitations. The underlying rate of reported acute upper respiratory tract episodes was much lower than anticipated, and varied widely between patients [21]. In particular, very low rates of infective episodes were reported during late spring and summer months and for this reason the study was stopped earlier than had originally been planned and before all study participants had been followed up for 32 months. It is possible that sub-clinical viral infections were not detected limiting the efficacy of the active treatment. Equally, efficacy might have been improved by rapid diagnostic testing during viral infections to identify infective agents since the risk of *Pseudomonal* infection may reflect the causative virus, respiratory syncytial virus and influenza being particularly implicated in increasing the risk.[9, 22] An unanticipated limitation was that the prescribing of ciprofloxacin as a treatment for respiratory symptoms during the study was higher than anticipated. Our current recommendations are for this to be used as a second line agent for two-week courses in children with ongoing cough, poorly responsive to first line therapies empirically treating *Staphlococcus aureus* and *Haemophilus influenzae*. We believe this additional ciprofloxacin prescribing resulted from the CF

clinical team and participating families being aware of the information that formed the basis of the study hypothesis, resulting in a lower threshold for ciprofloxacin use in routine clinical practice. This was highlighted and feedback to the clinical team by the trial's independent data safety monitor.

In general the clinical diagnosis of upper respiratory tract infection was felt to be accurate. For example, parents were questioned about seasonality of symptoms and contact with other symptomatic individuals in order to distinguish allergic rhinitis from symptoms more suggestive of infective episodes. Nevertheless viral testing would have both confirmed diagnosis and provided the added advantage of identifying the causative viral agent. Many participants reporting episodes had accompanying cough at the time of initial presentation and the study protocol did not permit the use of study medication for these episodes, which were treated according to recommended guidelines [23]. A better study design might be to treat such episodes with study medication *in addition* to first line antibiotics.

Cough swabs were the most commonly used microbial sample in this study with very few study participants able to expectorate sputum. The sensitivity of these specimens probably underestimates the true rate of infection but this was partly offset by very frequent sampling at least every two months and at times of acute coughing for more than 2-3 days. The negative ELISA assays provided further reassurances that the overall *Pseudomonas* bacterial load was low.

The optimal approach to treating initial isolates of *P. aeruginosa* in CF remains unclear and is the subject of ongoing clinical trials [24]. Whilst continuous prevention treatment has not been shown to be effective in preventing *Pseudomonas* infection, a more targeted use of anti-pseudomonal antibiotics at times of acute viral illness might be a more effective strategy. On the basis of our data, a larger multi-centre clinical trial recruiting at least 320 patients would be needed to further explore this hypothesis.

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Table 1: Comparison of baseline data between intervention and placebo groups.

		Intervention (n= 28)	Placebo (n=13)	P-value
Demographics				
Age at recruitment, years (median (IQR))		6.61 (3.49, 11.38)	7.19 (2.74, 11.73)	0.90
Gender (n (%))				
Male		15 (53.6)	6 (46.1)	0.74
Female		13 (46.4)	7 (53.8)	
Centre (n (%))				
Southampton		18 (64.3)	7 (53.8)	0.73
Poole/Winchester		10 (35.7)	6 (46.1)	
Clinical data				
Genotype DeltaF508:DeltaF508 (n (%))				
Yes		17 (60.7)	9 (69.2)	0.73
No		11 (39.3)	4 (30.8)	
Newborn screened (n (%))				
Yes		4 (14.3)	4 (30.8)	0.24
No		24 (85.7)	9 (69.2)	
Number of previous pseudomonas isolates (n (%))	0	12 (42.9)	7 (53.9)	0.72
	1	7 (25.0)	2 (15.4)	
	2	4 (14.3)	2 (15.4)	
	3	1 (3.6)	0 (0.0)	
	4	1 (3.7)	1 (7.7)	
	5	2 (7.1)	1 (7.7)	
	6	1 (3.6)	0 (0.0)	
Weight SDS at recruitment (median (IQR))		-0.08 (-0.85, 0.36)	-0.77 (-1.08, -0.09)	0.19
Height SDS at recruitment (median (IQR))		-0.43 (-0.87, 0.21)	-0.89 (-1.04, -0.58)	0.13
ELISA at recruitment (median (IQR))		0.49 (0.36, 0.61)	0.45 (0.31, 0.64)	0.64
FEV_1 at recruitment, percentage predicted (median (IQR))		97.8 (91.0, 109.0)	95.0 (84.0, 102.1)	0.14
Study data				
Length in study, months (median (IQR))		32 (32, 32)	30.5 (27.5, 32)	0.08
Number of viral infections treated under the study protocol per year in study (median (IQR))		0.75 (0, 1.5)	0.75 (0.38, 1.5)	0.58
Number of courses of ciprofloxacin per year in study, total of those according to protocol and at clinical discretion (median (IQR))		1.88 (1.02, 2.26)	0.75 (0.38, 0.87)	<0.001

Binary outcomes were compared by Fishers exact test, categorical outcomes by a χ^2 test for trend, and continuous variables using ranksum test.

Table 2. Outcome data (1) – Between group comparisons of rate of pseudomonas isolates, treatment, ELISA serology, growth and lung function

Outcome	Active intervention					Placebo					Rank sum test
	mean	SD	median	IQR	n	mean	SD	median	IQR	n	P-value
Episodes of pseudomonas/patient/year	0.17	0.29	0	0, 0.38	28	0.30	0.54	0	0, 0.38	13	0.41
Episodes of intravenous antibiotics/patient/year	0.18	0.22	0	0, 0.38	28	0.25	0.42	0	0, 0.39	13	0.75
Episodes of nebulised antibiotics/patient/year	0.82	0.94	1	0, 1	28	0.85	0.90	1	0, 1	13	0.84
Change in ELISA serology	0.16	0.27	0.10	0.00, 0.30	16	0.10	0.37	0.1	-0.21, 0.42	4	0.90
Change in weight SDS*	0.18	0.53	0.02	-0.28, 0.61	27	0.47	0.66	0.31	-0.12, 1.12	13	0.18
Change in height SDS*	0.34	0.69	0.13	-0.10, 0.88	27	0.26	0.55	0.24	-0.02, 0.57	13	0.97
Change in absolute FEV1 SDS*	0.51	0.49	0.43	0.12, 0.71	14	0.78	0.55	0.83	0.26, 0.86	7	0.25

* All changes from study entry were in the direction of improvement for both study groups

Table 3: Outcome data (2) – Between group comparisons of rate of isolation of other bacterial isolates

Outcome	Active intervention					Placebo					P-value
	mean	SD	median	IQR	n	mean	SD	median	IQR	n	
Episodes of *Staph aureus*/patient/year	0.82	0.93	0.59	0-1.03	28	0.59	0.92	0.38	0-0.44	13	0.496
Episodes of *H. influenzae*/patient/year	0.14	0.28	0	0-0.19	28	0.21	0.34	0	0-0.44	13	0.518
Episodes of MRSA/patient/year	0.12	0.45	0	0-0	28	0	0	0	0-0	13	0.329
Episodes of other bacterial isolates/patient/year	0.14	0.28	0	0-0.19	28	0.27	0.53	0	0-0	13	0.838
Episodes of fungal isolates/patient/year*	0.66	2.0	0	0-0.38	28	0.58	1.86	0	0-0	13	0.625

* Candida and aspergillus species.

Figure 1 Study participants flow diagram (Consort 2010)

Figure 2 Kaplan-Meier survival estimate: Time to pseudomonas isolation