Isolation of Enterobacteriaceae in airway samples is associated with worse outcome in preschool children with cystic fibrosis.

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Abstract

Background: Increased abundance of *Enterobacteriaceae*(EB) in the respiratory microbiome of young CF patients was reported to precede *Pseudomonas aeruginosa*(PA) colonisation. We explored whether impending PA colonisation can be predicted by growth of EB in routine airway cultures and whether EB contribute to CF lung disease severity.

Methods: We retrospectively studied the records of 62 children with CF for growth of EB and PA during the first 5 years of life and subsequent best lung function at ages 5-7 and 9-11 years.

Results: At least one EB positive month occurred in 36/62 (58%) patients. Median (IQR) age at first EB isolation was 0.4 (0.2-0.8) years. PA isolation before age 5 was more frequent in the EB positive (23/36, 54%) than in EB negative children (10/26, 38%; $p=0.048$). EB isolation preceded PA isolation in 19/23 (83%) cases ($p=0.003$).

Median (IQR) FEV₁ at age 5 to 7 years was 105% (94-117) in the EB positive group and 108% (102-115) in the EB negative group ($p=0.154$). At age 9-11, FEV₁ was lower in EB positive children (99%(88-105) vs 105%(96-110); $p=0.035$).

Only PA isolation ($p=.002$) before age 5 years was a significant predictor of FEV₁ at age 5-7 years. Both EB isolation ($p=0.033$) and PA isolation ($p=0.023$) were predictors of the FEV₁ at age 9-11 years.

Conclusion: In preschool children with CF, EB were isolated in just over half of the children. In that subgroup PA isolation was more common. Both EB and PA isolation are associated with worse lung function at later age.

Keywords: cystic fibrosis, preschool, *Enterobacteriaceae*, *Pseudomonas aeruginosa*, outcome

Introduction

Cystic fibrosis (CF) lung disease starts in the first years of life. The etiology is multifactorial: low airway surface liquid height, defective mucociliary clearance, mucus retention, decreased airway defence and excessive inflammation all contribute to the chronic infection, inflammation, progressive airway obstruction and structural lung damage.[1]

Despite intensive treatment, lung function loss and structural lung damage are progressive from an early age on.[2] Chronic infection with *Pseudomonas aeruginosa* (PA), when it occurs, accelerates the decline in lung function.[3] Eradication of early PA infection to delay chronic infection has therefore become a major therapeutic goal.[4] This indeed increases the time between the first isolation and the onset of chronic colonisation.[5]

Recently, microbiome research has allowed defining bacterial taxa in greater detail. One report observed an increase in the relative abundance of *Salmonella* in the respiratory microbiome of young CF patients as well as a non-significant rise in *Klebsiella*, *Citrobacter* and *Enterobacter* prior to PA colonisation.[6] However, for routine follow-up, microbiome data are not available. Routine airway surveillance for identification of pathogens is done via standard culture techniques for CF pathogens.[4] Enterobacteriaceae (EB) are frequently isolated, but the significance of a positive culture for these pathogens is unclear.

We therefore explored whether impending PA colonisation can be predicted by the growth of EB in routine airway cultures and whether EB isolation is linked to worse outcome. We reviewed the electronic medical records of preschool children with CF followed at our centre.

Methods

Patient in and exclusion criteria

This retrospective medical record study received institutional review board approval. We thus explored the electronic medical records of all children with CF followed at the Leuven CF reference centre and born between January 1st 1998 (when early PA treatment became standard in our unit) and December 31st 2010 (insufficient follow-up time if born after this date) (n=85). Other inclusion criteria were: meeting CF diagnostic criteria: sweat chloride concentration >60 mmol/L or 2 disease causing *CFTR* mutations identified (n=83).[7] Exclusion criteria were: having had less than 3 airway samples/year in 3 different months since time of diagnosis (n=16); CF diagnosis past age 5 years (n=5). Sixty-two children thus met all in- and exclusion criteria (see also CONSORT statement in supplementary Fig. 1). Their baseline characteristics are shown in Table 1.

Data collection

Standard baseline data were assessed: sex, birth year, age at diagnosis, genotype, meconium ileus, pancreatic status.

Microbiological data obtained during the first 5 years of life were analysed. Airway samples are obtained at each routine 3-monthly outpatient visit. Extra samples are taken at supplementary visits or hospitalisations for exacerbations or complications. All respiratory samples taken were included in the analysis: cough swabs (most samples), sputum and/or broncho-alveolar lavage (BAL) fluid. Of the 62 children included, 23 had at least one BAL sample and 32 had at least one sputum sample. Microbiological results were pooled per month. Pathogens were considered *Enterobacteriaceae* if included in the NCBI taxonomy listing.[8] An EB positive month was defined as a month with one or more respiratory cultures positive for EB. Patients were then considered 'EB positive' or 'EB negative' depending on the presence or absence of at least one EB positive month before the age of 5 years. Similarly, patients were considered 'PA positive' or 'PA negative' depending on the presence or absence of at least one PA positive month before the age of 5 years.

For every patient, the total number of days with intravenous (IV) antibiotics between the time of diagnosis and the age of 5 years was calculated. The number of days on oral antibiotics was not taken into account because of insufficiently reliable data: oral antibiotics are at times prescribed by local doctors and this is not always recorded in the patient's chart. Use of omeprazole during the first 5 years of life was noted.

For every patient the best forced expiratory volume in once second (FEV₁) expressed as percent predicted between age 5 and 7 years and between 9 and 11 years were chosen, as well as the forced vital capacity (FVC) expressed as percent predicted at the same time points.[9] Only tests meeting ATS/ERS acceptability criteria were considered.[10] Weight, height and body mass index (BMI) expressed as z-scores[11] at the time of lung function were also noted.

Routine patient management during period of data collection

CF newborn screening had not yet been implemented during the study years. Routine anti-staphylococcal antibiotic prophylaxis is not used at our CF centre. When patients have increased pulmonary symptoms, they are treated with antibiotics, the choice being guided by airway sample results. Upon isolation of PA from the respiratory tract, patients receive PA eradication treatment according to the Danish protocol: nebulized colomycin (2 x 1 MU per day) plus oral ciprofloxacin (30 mg/kg/d) during 3 months unless when clearly symptomatic in which case a 14 day course with intravenous antipseudomonal antibiotics becomes the standard treatment.[4, 5] EB isolation is not treated routinely. When EB are isolated during exacerbations, antibiotic therapy is extended to include EB coverage.

Lung function tests are performed every 3 months from the age of 5 years on. FEV₁ and FVC are measured using a Master Screen spirometer (Jaeger, Hoechberg, Germany). Values are expressed as percentages of the predicted value.[9]

Airway sampling and sample processing during time of data collection

Airway samples (cough swabs, sputum, broncho-alveolar lavage fluid) were collected in sterile containers and stored at ambient temperature before being processed within 4 h from collection. The samples were inoculated and incubated onto media for the isolation of PA and EB using Columbia Agar Base (CMO331; Oxoid, Basingstoke, UK) with 5% defibrinated horse blood and MacConkey agar (CM0007; Oxoid). All media were incubated aerobically at 37°C for 48 hours. Gram-negative bacteria isolated were further identified using a combination of conventional methods, as well as the Vitek 2 system (BioMérieux, Marcy l'Etoile, France) and MaldiBiotyper.

Data analysis

As a primary analysis, we evaluated whether there was a significant association between being EB positive and PA positive before the age of 5 years (Chi square test).

Secondary analyses included Kaplan-Meier survival curves of remaining PA free in the EB positive and negative group and comparison of disease markers such as the total number of IV antibiotic days, use of proton pump inhibitors, best lung function between age 5 to 7 and 9 to 11 years, weight, height and BMI at time of best lung function.

A univariate linear regression was planned to relate the outcome of best lung function to being EB positive or PA positive before age 5 years.

Data were tested for normality with the Shapiro-Wilk test. Variables with a normal distribution were compared using an unpaired t-test; otherwise the Mann Whitney U test (MWU) was used. To compare proportions between groups, a chi square or Fisher exact test were used, as appropriate. A p-value <0.05 was considered statistically significant. Analyses were performed using IBM[®] SPSS[®] Statistics.

Results

Baseline characteristics in the entire group and in the EB positive and EB negative group are presented in Table 1. From diagnosis until age 5 years, airway sample(s) had been taken at 3 monthly routine clinic visits and at times of exacerbation; in this cohort this resulted in a mean of 22.2 different months sampled per patient (e.g. out of a theoretical maximum of 60 months if a patient would have been diagnosed in the first month of life). Hence

microbiology information was available from about 1 month in 3 during the preschool years. A single sample was obtained in the vast majority of sampled months (86.4%), with more than 2 samples in only 3.2% of the months. The proportion of EB positive months was 9.6% in months with one sample taken and 18.2% both in months with 2 samples or with 3 or more samples.

At least one EB positive month had occurred in 36/62 (58%) patients. In 25 of these 36 patients, EB were isolated in two or more months. The median (IQR) number of EB positive months per patient was 3 (1-5.8). *Escherichia coli* (n=22) and *Enterobacter cloacae* (n=16) were isolated most commonly. The full list of EB species isolated and the proportion of subjects in whom they were isolated is shown in the online supplementary table S1.

The median (IQR) age at first isolation of EB was 0.4 (0.2-0.8) years. There was no difference in sex, distribution of *CFTR* genotype, proportion with pancreatic insufficiency or meconium ileus, and birth year between EB positive and EB negative subjects. However, in the EB positive group, age at diagnosis was significantly younger ($p=0.004$; MWU test), and the number of months with respiratory samples taken was significantly higher ($p=.001$; MWU test).

Primary and secondary outcomes

PA isolation before the age of 5 was more frequent in the EB positive group (23/36) than in the EB negative group (10/26) ($p=0.048$; Chi square test). In 19/23 EB positive patients, EB isolation preceded PA isolation ($p=0.003$ Fisher exact test). In 3 cases EB and PA were isolated simultaneously. In only 1 patient PA isolation preceded EB isolation. The median (IQR) interval to PA isolation in the EB positive group was 344 (122-843) days.

In the EB positive group, the mean number of days on IV antibiotics was significantly higher (22 (IQR 13-40) vs. 6 (IQR 0-14) ($p<0.001$; MWU test). Omeprazole use was possibly more frequent in EB positive than EB negative subjects ($p=0.057$). However, omeprazole treatment preceded EB isolation in only 1 subject.

The Kaplan-Meier plot of remaining PA free in either group is shown in the figure. The difference between both groups was not significant ($p=0.061$). The median age at first isolation of PA did also not differ between EB positive and EB negative subjects ($p=0.926$).

In the EB positive group, the median (IQR) FEV₁ at 5-7 years was 105% (94-117) versus 108% (102-115) ($p=0.154$; MWU test). At 9-11 years, FEV₁ was lower in the EB positive group: 99% (88-105) vs 105% (96-110) ($p=0.035$; MWU test). FVC, weight, height and BMI at 5-7 years and 9-11 years were not significantly different between the EB positive and EB negative group.

Similar results were obtained when patients with repeated EB isolation (≥ 2 positive months) were compared to patients with no or just one month with EB (supplementary Table S2).

Taking FEV₁ at age 5 to 7 years as outcome PA isolation ($p=.002$) but not EB isolation ($p=0.117$) was a significant predictor. Both EB isolation ($p=0.033$) and PA isolation ($p=0.023$) before the age of 5 years were predictors of the FEV₁ at age 9-11 years. Lung function data, weight, height and BMI in the PA positive and negative group are given in Table 2. At age 5-7 as well as at age 9-11, FEV₁, weight and BMI were significantly lower in the PA positive group.

Discussion

In this longitudinal cohort evaluation of preschool children with CF, EB were isolated in just over half of the children. In that subgroup PA isolation was more common and the PA isolation nearly always followed the EB isolation. Lung function outcome at age 9-11 years was worse in the EB positive group,

There is a scarcity of information about airway pathogens other than the classical CF pathogens like *S aureus*, *P aeruginosa*, *B cepacia* or mycobacteria. There are also no guidelines about how to decide whether an isolated pathogen is contributing to CF lung disease. One could consider association with worse outcome or repeated isolation as significant factors.

The current study provides arguments that isolation of EB from the CF airway of preschool children points towards worse disease: it was associated with more frequent subsequent PA isolation, higher antibiotic use, larger number of airway samples taken and worse lung function at age 9 to 11 years. In two thirds of the subjects, EB were isolated repeatedly. However, on average, the preschool children with CF in whom EB were isolated had been diagnosed earlier, possibly pointing towards more severe pre-existing disease. And although isolation of EB and PA were both associated with lung function outcomes at older age, the association was more consistent for PA, pointing towards the greater importance of PA as CF pathogen.

Whether infection with *Enterobacteriaceae* is the cause of worse disease evolution, or just a marker of more severe disease and thus a predictor of less favorable outcomes at later age cannot be differentiated with our data. There is a possibility that more frequent sampling triggered by respiratory exacerbations, as a marker of more severe disease, results in more frequent isolation of *Enterobacteriaceae*. Multivariate analyses required to distinguish individual contributions of specific risk factors to outcomes would require larger sample sizes. Studies in larger populations, as well as in different patients populations such as patients diagnosed after neonatal screening, would be welcome to further explore the importance of isolation of *Enterobacteriaceae*.

A few previous studies also hint towards *Enterobacteriaceae* and/or *Escherichia coli* contributing to CF lung disease. In a longitudinal German microbiology evaluation, *E coli* was isolated from the respiratory samples of a quarter of subjects with CF, mainly the very young subjects, and was isolated repeatedly, convincing the authors that it may be an 'unrecognized' CF pathogen.[12] Also in nasopharyngeal microbiota specimens from infants with CF, *Enterobacteriaceae* occur more commonly than in controls.[13] EB in airway samples were also associated with more inflammation.[14] On the contrary, longitudinal analysis of microbiome in 13 infants with CF pointed towards an increase in *Salmonella* preceding PA infection, but no significant association between the presence of *Enterobacteriaceae* and later PA isolation.[6]

It is unclear how the more frequent use of omeprazole in EB positive group should be interpreted. In CF, omeprazole is used to treat gastro-oesophageal (GE) reflux as well as to improve fat digestion in pancreatic insufficiency.[15] In the current historic cohort we could not confidently discern whether omeprazole was mainly used to treat GE reflux or to improve digestion. Does GE reflux or its treatment by itself contribute to CF lung disease[16, 17] or is

GE reflux and thus also its treatment just another marker of intrinsically worse lung disease severity? Previous studies have argued for either statement. In the current cohort, omeprazole treatment preceded the first EB isolation in only one subject. Hence, omeprazole use did not lead to colonization by EB.

The current study has limitations since it is a retrospective study. However, the standardized follow-up and data registration of children with CF mitigates this. In addition, the worse outcome seen in children with early PA colonization is in line with previous reports.[18] The current study does not provide information whether isolation of EB in preschool children without associated symptoms warrants any treatment. How then to use this information further? The current data demonstrate worse outcome in that group and hence support a closer follow up of preschool children in whom EB are isolated with specific attention to early colonization with PA.

In conclusion, this report adds to the information that EB isolation in preschool children with CF should be seen as a marker of worse disease course and that EB should be considered as a possible CF pathogen. These data highlight the importance of studies into pathogens other than *S aureus* and *P aeruginosa*, to further improve outcome in preschool children with CF. Indeed, isolation of EB occurred in over half of the preschool children with CF. In addition, studies linking classical microbiological airway results and microbiota findings would be helpful. At present routine airway surveillance is based solely on results from classical microbiology whereas most if not all current research efforts go towards the study of airway microbiota.

Conflict of Interest:

Prof. F. Vermeulen, Prof. M. Proesmans, Dr. M. Vermaelen have nothing to disclose.

Prof. K. De Boeck reports other from Boehringer, Protalix, Raptor, Teva, Novabiotics, Elox, Vertex, Galapagos, outside the submitted work.

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Table 1. Patient characteristics and outcome according to isolation of Enterobacteriaceae in airway samples before age 5 years

	All patients n=62 (100)	EB < 5y n=36 (58)	No EB < 5y n=26 (42)	p-value
Male	33 (53)	19 (53)	14 (54)	0.934
Homozygous F508del	40 (65)	21 (58)	19 (73)	0.466
Heterozygous F508del	18 (29)	12 (33)	6 (23)	
Other/other	4 (6)	3 (8)	1 (4)	
Pancreatic insufficiency	59 (95)	35 (97)	24 (92)	0.567
Meconium ileus	10 (16)	7 (19)	3 (11)	0.499
Birth year	2005 (2002-2009)	2006 (2002-2009)	2005 (2001-2008)	0.434
<i>Age at diagnosis (yrs)</i>	<i>0.3 (0.1-0.9)</i>	<i>0.2 (0.0-0.4)</i>	<i>0.7 (0.1-2.8)</i>	<i>0.004</i>
<i>Number of months with airways sample(s)*</i>	<i>22.2 ± 1.1</i>	<i>25.3 ± 1.3</i>	<i>18.0 ± 1.7</i>	<i>0.001</i>
<i>Number of airway samples</i>	<i>1601</i>	<i>1089 (36)</i>	<i>512 (26)</i>	
<i>N Swab (n patients)</i>	<i>1415 (62)</i>	<i>939 (36)</i>	<i>476 (26)</i>	
<i>N Sputum (n patients)</i>	<i>154 (39)</i>	<i>123 (29)</i>	<i>31 (10)</i>	
<i>N BAL/Tracheal aspirate (n patients)</i>	<i>32 (21)</i>	<i>27 (18)</i>	<i>5 (3)</i>	
Age at 1st PA isolation (yrs)	2.2 (1.1-3.9) (n=33)	2.1 (1.1-3.9) (n=23)	2.5 (1.0-3.8) (n=10)	0.926
Age at 1st EB isolation (yrs)	-	0.4 (0.2-0.8)	-	-
Number of EB positive months	-	3.0 (1.0-5.8)	-	
EB positive months (%)	-	12.0 (6.2-23.4)	-	
<i>IV antibiotic therapy (days, ≤ age 5 yrs)</i>	<i>14 (4-30)</i>	<i>22 (13-40)</i>	<i>5.5 (0-14)</i>	<i><0.001</i>
<i>Omeprazole use (n)</i>	<i>21</i>	<i>17</i>	<i>4</i>	<i>0.057</i>
Outcome at age 5-7 yrs	All patients n=62	EB < 5y n=36	No EB < 5y n=26	p-value
FEV ₁ (%pred)	106 (97-117)	105(94-117)	108(102-115)	0.154
FVC (%pred)	110 (101-116)	109(97-119)	111(102-115)	0.721
Weight (z-score)	-0.4 ± 0.1	-0.5 ± 0.2	-0.3 ± 0.2	0.544
Height (z-score)	-0.6 ± 0.1	-0.7 ± 0.2	-0.5 ± 0.2	0.679
BMI (z-score)	-0.1 ± 0.1	-0.2 ± 0.2	0.0 ± 0.2	0.632
Outcome at age 9-11 yrs	All patients	EB < 5y	No EB < 5y	p-value

	n=55	n=32	n=23	
<i>FEV₁</i> (%pred)	101 (92-108)	99 (88-105)	105 (96-110)	0.035
FVC (%pred)	104 (97-111)	103 (98-111)	107(95-111)	0.682
Weight (z-score)	-0.5 ± 0.1	-0.6 ± 0.2	-0.4 ± 0.2	0.597
Height (z-score)	-0.6 ± 0.1	-0.7 ± 0.2	-0.4 ± 0.2	0.318
BMI (z-score)	-0.2 ± 0.1	-0.2 ± 0.2	-0.3± 0.2	0.831

Data are expressed as number (and percentage), mean (±standard error of the mean) or median (and interquartile range)

* All cultures taken in the same month were combined into a single data point

Abbreviations: EB *Enterobacteriaceae*; PA *Pseudomonas aeruginosa*; yrs years; n number; FEV₁ forced expired volume in one second; %pred percent predicted; FVC forced vital capacity; BMI body mass index

Table 2 Outcome according to Pseudomonas aeruginosa isolation before age 5yrs

Outcome at age 5-7 yrs	All patients n=62	PA < 5y n=33	No PA < 5y n=29	p-value
<i>FEV₁ (%pred)</i>	<i>106 (97-117)</i>	<i>100(92-111)</i>	<i>111(104-122)</i>	<i>0.004</i>
<i>FVC (%pred)</i>	<i>110 (101-116)</i>	<i>104(97-112)</i>	<i>115(106-121)</i>	<i>0.008</i>
<i>Weight (z-score)</i>	<i>-0.4 ± 0.1</i>	<i>-0.8 ± 0.2</i>	<i>-0.1 ± 0.1</i>	<i>0.003</i>
<i>Height (z-score)</i>	<i>-0.6 ± 0.1</i>	<i>-0.8 ± 0.2</i>	<i>-0.5 ± 0.2</i>	<i>0.485</i>
<i>BMI (z-score)</i>	<i>-0.1 ± 0.1</i>	<i>-0.4 ± 0.1</i>	<i>0.3 ± 0.2</i>	<i>0.001</i>
Outcome at age 9-11 yrs	All patients n=55	PA < 5y n=25	No PA < 5y n=30	p-value
<i>FEV₁ (%pred)</i>	<i>106 (97-117)</i>	<i>99(87-107)</i>	<i>105(96-110)</i>	<i>0.040</i>
<i>FVC (%pred)</i>	<i>110 (101-116)</i>	<i>103(95-108)</i>	<i>107(100-113)</i>	<i>0.094</i>
<i>Weight (z-score)</i>	<i>-0.4 ± 0.1</i>	<i>-0.7 ± 0.1</i>	<i>-0.2 ± 0.2</i>	<i>0.046</i>
<i>Height (z-score)</i>	<i>-0.6 ± 0.1</i>	<i>-0.7 ± 0.2</i>	<i>-0.4 ± 0.2</i>	<i>0.654</i>
<i>BMI (z-score)</i>	<i>-0.1 ± 0.1</i>	<i>-0.5 ± 0.1</i>	<i>0.0 ± 0.1</i>	<i>0.010</i>

Data are mean and standard error of the mean, or median and interquartile range
Abbreviations: PA pseudomonas aeruginosa, n number; FEV₁ forced expired volume in
one second; FVC forced vital capacity; BMI body mass index

Figure: Kaplan-Meier plot of remaining free of *Pseudomonas aeruginosa* colonisation in preschool children with CF in whom *Enterobacteriaceae* were isolated or not (p=.06).

Online supplementary Table S1.

All different species of *Enterobacteriae* isolated from airway samples of preschool children with CF. The absolute number and the proportion of children in whom the pathogens were isolated are reported in patients with and without isolation of *Pseudomonas aeruginosa* before the age of 5 years.

	N (%) in whom isolated		
	PA negative patients (n=29)	PA positive patients (n=33)	All patients (n=62)
<i>Escherichia coli</i>	7 (24)	15 (45)	22 (35)
<i>Enterobacter cloacae</i>	4 (14)	12 (36)	16 (26)
<i>Klebsiella oxytoca</i>	2 (7)	5 (15)	7 (11)
<i>Klebsiella pneumoniae</i>	1 (3)	6 (18)	7 (11)
<i>Morganella morganii</i>		4 (12)	4 (6)
<i>Serratia marcescens</i>	2 (7)	1 (3)	3 (5)
<i>Proteus mirabilis</i>	1 (3)	2 (6)	3 (5)
<i>Serratia liquefaciens</i>	1 (3)	1 (3)	2 (3)
<i>Enterobacter aerogenes</i>		1 (3)	1 (2)
<i>Citrobacter braakii</i>		1 (3)	1 (2)
<i>Hafnia alvei</i>	1 (3)		1 (2)
<i>Enterobacter species</i>		1 (3)	1 (2)

Online supplementary Table S2

Table S2. Patient characteristics and outcome according to isolation of Enterobacteriaceae in airway samples before age 5 years

	All patients n=62 (100)	0 or 1 month with EB < 5y n=41 (34)	2+ months with EB < 5y n=21 (66)	p-value
Male	33 (53)	24 (59)	9 (43)	0.242
Homozygous F508del	40 (65)	27 (66)	13 (73)	0.828
Heterozygous F508del	18 (29)	11 (27)	7 (23)	
Other/other	4 (6)	3 (7)	1 (4)	
Pancreatic insufficiency	59 (95)	38 (93)	21 (92)	0.545
Meconium ileus	10 (16)	9 (22)	1 (11)	0.143
Birth year	2005 (2002-2009)	2005 (2001-2008)	2006 (2003-2009)	0.114
Age at diagnosis (yrs)	0.3 (0.1-0.9)	0.6 (0.1-1.6)	0.1 (0.0-0.3)	0.002
Number of months with airways sample(s)*	22.2 ± 1.1	19.61 ± 1.31	27.24 ± 1.66	0.001
PA before 5 years	33(53)	21(51)	12(57)	0.658
Age at 1st PA isolation (yrs)	2.2 (1.1-3.9) (n=33)	2.6 (1.4-4.2) (n=21)	1.2 (0.8-3.1) (n=12)	0.926
Age at 1st EB isolation (yrs)	-	0.6 (0.4-2.8) (n=15)	0.2 (0.2-0.4) (n=21)	0.003
Number of EB positive months	1.0 (0.0-3.0)	0.0 (0.0-1.0)	4.0 (3.0-5.0)	<0.001
EB positive months (%)	4.1 (0.0-11.5)	0.0 (0.0-3.8)	13.0 (11.1-20.0)	<0.001
IV antibiotic therapy (days, ≤ age 5 yrs)	14 (4-30)	14 (0-27)	22 (13-37)	0.029
Omeprazole use (n)	21	11 (27)	10(48)	0.102
Outcome at age 5-7 yrs	All patients n=62	0 or 1 month with EB < 5y n=41 (34)	2+ months with EB < 5y n=21 (66)	p-value
FEV ₁ (%pred)	106 (97-117)	107.1 (97.7-117)	104 (93.3-111)	0.151
FVC (%pred)	110 (101-116)	110.3 (101.4-116.5)	109.2 (96.8-115.5)	0.489
Weight (z-score)	-0.4 ± 0.1	-0.24 ± 0.11	-0.83 ± 0.23	0.028
Height (z-score)	-0.6 ± 0.1	-0.49 ± 0.14	-0.88 ± 0.22	0.125
BMI (z-score)	-0.1 ± 0.1	-0.5 ± 0.11	0.42 ± 0.22	0.036
Outcome at age 9-11 yrs	All patients n=55	0 or 1 month with EB < 5y n=38	2+ months with EB < 5y n=17	p-value
FEV ₁ (%pred)	101 (92-108)	104.7 (96.1-110.3)	96.5 (87.7-103.1)	0.009

FVC (%pred)	104 (97-111)	107.1 (98.3-111.4)	100.5 (97.5-104.9)	0.215
Weight (z-score)	-0.5 ± 0.1	-0.46 ± 0.13	-0.63 ± 0.2	0.464
Height (z-score)	-0.6 ± 0.1	-0.49 ± 0.16	-0.83 ± 0.26	0.249
BMI (z-score)	-0.2 ± 0.1	-0.24 ± 0.11	-0.23 ± 0.21	0.965

Data are expressed as number (and percentage), mean ($\pm$ standard error of the mean) or median (and interquartile range)

* All cultures taken in the same month were combined into a single data point

Abbreviations: EB *Enterobacteriaceae*; PA *Pseudomonas aeruginosa*; yrs years; n number; FEV₁ forced expired volume in one second; %pred percent predicted; FVC forced vital capacity; BMI body mass index

Online Supplementary figure 1: CONSORT diagram

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