Supplementary Online Content

Wainwright CE, Vidmar S, Armstrong DS, et al; ACFBAL Study Investigators. Effect of bronchoalveolar lavage-directed therapy on *Pseudomonas aeruginosa* infection and structural lung injury in children with cystic fibrosis: a randomized trial. *JAMA*. 2011;306(2):163-171.

eSupplement

This supplementary material has been provided by the authors to give readers additional information about their work.

eSUPPLEMENT

METHODS

Children were reviewed every 3-months for the study. All the clinics reviewed infants and pre-school children more frequently for clinical purposes although frequency of review varied from family to family. Exacerbation visits were also scheduled as needed and reviews of exacerbations scheduled to ensure exacerbations were resolved or the child admitted to hospital as per protocol. Use of pancreatic enzyme therapy, additional vitamins and added salt were managed according to local climatic conditions and available preparations. However, all sites had a uniform approach to starting fat soluble vitamin preparations and pancreatic enzyme supplements for children with pancreatic insufficiency. The approach to respiratory therapy was standardized across centers with regular coordination of respiratory therapists. Dornase alpha, inhaled hypertonic saline or azithromycin were either not available or used very infrequently in the pre-school age group during the time of the study.

RESULTS

BAL-cultures of *P. aeruginosa* at concentrations <10^3 CFU/mL during the study and/or at final BAL

Overall, 15 children on at least one occasion had <10^3 CFU/mL of *P. aeruginosa* detected in their BAL-cultures. Of these, 6 had *P. aeruginosa* present in their final BAL at age 5-years. Three of these 6 children had
P. aeruginosa at levels <10^3 CFU/mL at some point during the study and had positive cultures for *P. aeruginosa* at final BAL. One child received full eradication courses for *P. aeruginosa* at levels ≥10^3 CFU/mL on 5 separate occasions previously and was the only one to meet the criteria for chronic infection at 5-years of age. Another child with ≥10^3 CFU/mL of *P. aeruginosa* cleared the organism completely from their BAL after a full course of eradication therapy, but it was detected again in BAL-cultures in low concentrations (<10^3 CFU/mL) 3-months later during a further pulmonary exacerbation. In accordance with the study protocol this child received only 2-weeks of IV anti-pseudomonal therapy. *P. aeruginosa* was not identified in the next 3 BAL-cultures, but reappeared in the end of study BAL at a concentration of 10^6 CFU/mL. One child received a full eradication for *P. aeruginosa* at levels ≥10^3 CFU/mL and it was cleared completely from their BAL-cultures after a full course of eradication therapy, but it was detected again in BAL-cultures in low concentrations (<10^3 CFU/mL) on 3 occasions 12, 15 and 4-months after initial infection as well as at final BAL. This child also had BAL-cultures where *P. aeruginosa* was not detected on 5 separate occasions between these episodes. Finally, the other 3/6 children had *P. aeruginosa* detected in their BAL-cultures for the first time at their end of study BAL at levels <10^3 CFU/mL.

Sensitivity analysis

1. **Including patients who refused eradication treatment for *P. aeruginosa* infection and assuming they still had a *P. aeruginosa* infection at the time of their final BAL:**

   At age 5-years, 9/80 (11.3%) in the BAL group and 12/79 (15.2%) in the standard group had *P. aeruginosa* infection confirmed in their final BAL-cultures (Risk difference -3.9 % 95%CI -14.5%, 6.6%; P=0.46).

2. **Excluding patients with protocol violations:**

   Eight children in the standard group had BAL during the course of the study and another 2 children in the standard group had a pre-randomization BAL. Another 2 children randomized to standard therapy were withdrawn once it was realized that they did not have classical CF. Finally, Data from 1 other child could not be included as there were no end of study data available. This arose when a new pediatrician taking over the child's care decided to employ BAL-directed therapy as an integral part of clinical management. As a result the child was withdrawn from the study. At age 5-years, 8/79 (10.1%) in the BAL group and 7/67 (10.5%) in the standard group had *P. aeruginosa* in final BAL cultures (Risk difference -0.3 % 95%CI -10.2%, 9.6%; P=0.95).

eSUPPLEMENT (CONTINUED)

Analysis adjusting for effects of smoking during pregnancy and exposure to household smoking at enrolment

1.Prevalence of *P. aeruginosa* at final BAL:

Unadjusted Odds ratio for *P. aeruginosa* at final BAL (BAL group/standard group) is 0.84 (95%CI 0.31, 2.30; P=0.73). Adjusted for smoking during pregnancy or in the household at enrolment gives an Odds ratio of 0.90 (95%CI 0.32, 2.50; P=0.84).

2.Total CF-CT score:

Unadjusted mean difference (BAL group – standard group) 0.19 (95%CI -0.94, 1.33; P=0.74). Adjusted for smoking during pregnancy or in the household at enrolment gives a mean difference of 0.27 (95%CI -0.88, 1.41; P=0.64).

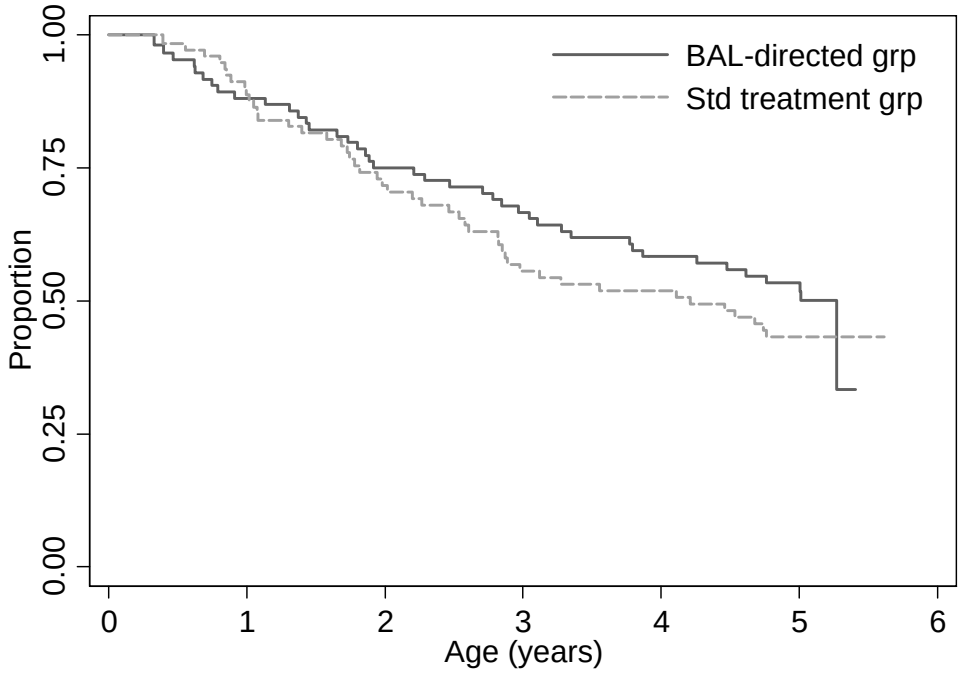
Adverse events associated with courses of *P. aeruginosa* eradication therapy

TOBI® administration was associated with a hoarse voice in 6 children, conjunctivitis in 2 and epistaxis in another. Development of a rash in 2 children and wheezing in another resulted in the drug's discontinuation with full resolution of these symptoms. The first dose of TOBI® was always administered in hospital prior to being discharged home. One child was inadvertently administered 300mg of tobramycin intravenously, this resulted in elevated serum tobramycin levels afterwards and mild bilateral sensorineural hearing impairment at age 5-years.

In addition, 3 children had generalised erythematous rash while receiving parenteral antibiotics and another had transient arthropathy resulting in cessation of treatment while taking ciprofloxacin.

eFigure. Time to the First Diagnosis of *Pseudomonas aeruginosa* Infection, by Treatment Group

Hazard ratio, 0.81 (95% CI, 0.53-1.23; P=0.33).



Number at risk							
BAL-directed grp	0	74	63	56	48	32	0
Std treatment grp	0	74	58	45	42	22	0

eTable 1. Frequency of Chest High-Resolution Computed Tomography Scan Scores by Individual Component Score Category at Age 5 Years According to Treatment Group

	BAL group (n=78)	**Standard group** (n=77)	**P value***
Bronchiectasis score	n=77†	n=76†	
Zero	32 (42%)	34 (45%)	0.84
>0, ≤5%	35 (45%)	31 (41%)	
>5%	10 (13%)	11 (14%)	
Air trapping score	n=77†	n=77†	
Zero	42 (55%)	42 (55%)	0.97
>0%, ≤20%	20 (26%)	19 (25%)	
>20%	15 (19%)	16 (21%)	
Mucous plugging score	n=77†	n=76†	
Zero	41 (53%)	48 (63%)	0.22
>0, ≤5%	9 (12%)	11 (14%)	
>5%	27 (35%)	17 (22%)	
Airway wall thickening score	n=77†	n=76†	
Zero	49 (64%)	58 (76%)	0.18
>0, ≤5%	19 (25%)	14 (18%)	
>5%	9 (12%)	4 (5%)	
Parenchymal disease score	n=77†	n=76†	
Zero	35 (45%)	28 (37%)	0.44
>0, ≤5%	30 (39%)	31 (41%)	
>5%	12 (16%)	17 (22%)	

* Chi-square test (2 degrees of freedom); † Number of scans of sufficient quality that were able to receive a score.

eTable 2. Inflammatory Parameters in Bronchoalveolar Lavage (BAL) Fluid at Age 5 Years, by Treatment Group

	BAL group (n=79)	**Standard group (n=77)**	**Mean difference or ratio**	**95% CI**	**P-value**
Total cell count (10^4 cells/mL) Median (IQR)	n=70 46 (19, 120)	n=70 54 (22, 147)	0.92*	(0.56,1.49)	0.73
Neutrophil count (10^4 cells/mL) Median (IQR)	n=66 15.4 (4.3, 57)	n=67 11.2 (5.0, 78)	1.14*	(0.50,2.62)	0.75
Neutrophil % Mean (SD)	n=71 41 % (27%)	n=69 40 % (30%)	0.4%	(-9.1%, 9.9%)	0.93
IL 8 (pg/mL) Median (IQR)	n=72 2126 (677, 9578)	n=74 2604 (428, 10915)	1.16*	(0.56,2.40)	0.68

IQR, interquartile range; SD, standard deviation.
* ratio of geometric means

eTable 3. Distribution of the Total Number of *Pseudomonas aeruginosa* Infections Diagnosed per Child During the Conduct of the Study, by Treatment Group and Including Final Outcome Data

P-value (Fisher exact) for difference between groups = 0.62.

Number of *P. aeruginosa* infection episodes diagnosed	**BAL group** (n=79)	**Standard group** (n=76)
0	40 (51%)	31 (41%)
1	22 (28%)	20 (26%)
2	12 (15%)	18 (24%)
3	3 (4%)	4 (5%)
4	2 (3%)	2 (3%)
5	0 (0%)	1 (1%)

eTable 4. History of Previously Diagnosed *Pseudomonas aeruginosa* Infections Among 17 Participants With *P. aeruginosa* Infection at Final Bronchoalveolar Lavage (BAL)

	BAL group n=8	**Standard group** n=9
No previous *P. aeruginosa* infections	2	2
Previous *P. aeruginosa* infection diagnosed by oropharyngeal culture only	0	6
Previous *P. aeruginosa* infection where both oropharyngeal and BAL cultures were positive	5	1*
Previous *P. aeruginosa* infection where only the BAL culture was positive	1	0
Met criteria for chronic *P. aeruginosa* infection at time of final BAL	1	0

* This child was a protocol violation.

eTable 5. Use of Concomitant Medications at Least Once Prior to Final Outcomes

	BAL group n=80	**Standard group** n=77
Inhaled hypertonic saline	19 (23.8%)	13 (16.9%)
Dornase alpha	3 (3.8%)	0
Azithromycin	1 (1.3%)	2 (2.6%)
Inhaled corticosteroids	37 (46.3%)	26 (33.8%)