Supplementary Appendix

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

Supplement to: Sly PD, Gangell CL, Chen L, et al. Risk factors for bronchiectasis in children with cystic fibrosis. N Engl J Med 2013;368:1963-70. DOI: 10.1056/NEJMoa1301725

Online Data Supplement

Risk Factors for the Onset of Bronchiectasis in Children with Cystic Fibrosis

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AREST CF Early Surveillance Program.

Study population

The AREST CF program covers a geographically-defined population. All children diagnosed with CF in Western Australia are managed at Princess Margaret Hospital, Perth and children diagnosed in Victoria (apart from those in the southern metropolitan area) are managed at the Royal Children's Hospital, Melbourne. The early surveillance program is well received by the clinic populations with >95% of eligible children participating. In Australia the diagnosis of CF is made in the majority of infants by 6 weeks of age, following detection by newborn screening[1]. At diagnosis the vast majority of infants (~80%) have no respiratory symptoms but may have covert pulmonary inflammation and infection[2].

Surveillance protocol

The AREST CF surveillance protocol includes assessment soon after diagnosis (approximately 3 months of age) and then annually, close to the child's birthday until 6 years of age. These assessments include:

- Chest CT Scanning at end inspiration (25 cmH_2O) and end-expiration (0 cmH_2O) under general anaesthesia.
- Bronchoscopy and bronchoalveolar lavage (BAL) follow the CT scan for assessment of pulmonary infection and inflammation.
- Lung function testing using infant lung function tests under chloral hydrate sedation at 3 months, 1 and 2 years; and using preschool techniques (unsedated) at 3 years and older.
- Collection of urine for assessment of biomarkers of inflammation, oxidative stress and lung damage.

Parents are informed that this is a clinically-directed surveillance program with some research aspects. Parents are given the opportunity to consent to each aspect (clinical or research) of the program separately.

The program has been approved by the ethics committees at both Princess Margaret Hospital, Perth and the Royal Children's Hospital, Melbourne.

CT Protocols

Chest CT and BAL were performed under general anesthesia. Children up to 6 years of age were initially intubated with a cuffed tracheal tube and a standardized recruitment manoeuvre, consisting of 10 consecutive slow breaths up to total lung capacity (Trans-respiratory pressure (P_{rs}) of 37-40 cmH_2O) over a positive end-expiratory pressure of 5 cmH_2O for 1-2 seconds after each inspiration, used to reduce procedure-related atelectasis. A volume-controlled limited slice CT scan (initial scan at 3 months of age) was performed with three slices taken at both end inspiration (P_{rs} of 25 cm H_2O) and end expiration (P_{rs}=0 cm H_2O) or a volume-controlled volumetric scan at end inspiration for older children (from 2007 in Perth and 2010 in Melbourne). Details of the scanners and setting used have been previously published[3,4].

Respiratory Symptoms at the time of CT and BAL

The clinical fellow or attending physician responsible for the patient took a standard clinical history asking about current respiratory symptoms, current medication use and changes in clinical status.

Microbiological protocols

Using a sterile disposable pipette a drop (10 – 50ul) of BAL fluid was inoculated onto agar plates and spread for single colonies using a sterile loop. The plates were inoculated in the following manner : Blood agar, Cytosine Lactose Electrolyte Deficient (CLED) agar, and blood agar + ticarcillin (for resistant *P. aeruginosa* identification) were incubated at 35ºC for 48hrs in a CO_2 incubator; PC plate (selective plate for *B.cepacia*) was incubated at 35ºC for 48hrs in a CO_2 incubator, then at room temperature for another 24hrs; MSA (Mannitol salt agar for *S. aureus*) was incubated at 35ºC for 48hrs in an aerobic atmosphere; Sabarouds agar (for fungi isolation) was incubated at 35ºC for 48hrs in a CO_2 incubator, then at 28ºC for 14 days; Fildes agar (for *Haemophilus* isolation) plates were incubated at 35ºC for 48hrs in an anaerobic environment (anaerobic incubation to prevent overgrowth by *P. aeruginosa*)

Gram stain was prepared and examined for bacteria, leucocytes and epithelial cells. Bacteria were identified by colony morphology, gram stain and biochemical tests including oxidase and C390 screening test specifically for *Pseudomonas*. *P. aeruginosa* were classified as smooth, rough or mucoid based on colony morphology on blood agar plates. *P. aeruginosa* colonies were unclassified if colony morphology was ambiguous. Growth was reported as isolated colonies, light, scanty, moderate or abundant growth based on the organism density on the plates which relate to colony counts of $<10^3$, 10^4, 10^5, 10^6, 10^7, respectively.

Sensitivity testing of all pathogens, including *P. aeruginosa* and, *S. aureus* were performed by the agar dilution breakpoint method according to Clinical Laboratory Standards Institute (CLSI) guidelines. Sensitivity of isolates such as *Haemophilus* was performed by disc testing using CSLI guidelines. A wet preparation was made from the BAL fluid and examined for fungal elements.

Immunofluorescence and culture methods were used to identify viral infections. For immunofluorescence, specimens were prepared by washing the cell pellet in PBS until a 'tight' cell pellet was obtained. A glass pipette was used to make a smear of the cells within four wells of an 8-well Teflon coated slide for RSV; Parainfluenzae 1,2&3; influenzae A&B; and adenovirus. The slide was air dried at room temperature and fixed in acetone. Direct Fluroescent Antibody Testing was carried out using commercially available monoclonal antibodies. If no evidence of viral infection was detected then BAL fluid was processed for viral cell culture. Virus was cultured by inoculation of an aliquot of BAL on to cultured mammalian cell lines grown on round glass coverslips. Virus was identified by staining the cultured cells (on the coverslips) with monoclonal antibodies and examination of the cells under a fluorescence microscope.

Assessment of inflammation

BAL fluid was pooled and centrifuged for 5 minutes at 1500 rpm. Aliquots of supernatant were stored at -80ºC until needed for further analysis. The cell pellet was washed if required and resuspended in 1ml PBS. Total cell count using a haemocytometer was performed on 10ul of cell suspension and viability assessed using equal volume of tryphan blue stain. Cytospins were performed on 10^6 cells/ml and stained using Leishman stain. Differential cell counts were performed on 300 consecutive cells at 100x magnification. The following cells were counted: macrophages, neutrophils, lymphocytes and eosinophils.

B) Transport protocol (to Perth)

Samples frozen at -80ºC were transported to Perth from Melbourne overnight in an insulated container packed with dry ice to keep frozen. On arrival in Perth all samples were stored at -80ºC.

Analysis of inflammatory mediators IL-1β, IL-12, IL-6, IL-8, IL-10 and TNFα were conducted using a standard cytometric bead array human inflammation kit (BD Biosciences, San Diego, CA) with a working range between 20 and 5000 pg/ml. Analysis of IL-8 was completed using an ELISA (BD Opt EIA, BD Biosciences, San Diego, CA) with a working range between 0.01 and 6.40 ng/ml.

Free neutrophil elastase activity was assessed using an adapted ELISA. BAL fluid supernatant was serial diluted 1:2 with Tris buffer in duplicate. Tris buffer was the negative control and human neutrophil elastase diluted to 25 ug/ml was the standard. Substrate N-methoxysuccinyl-ala-ala-pro-val p-nitroanilide (dissolved in NMP) was added to each well. Activity was read immediately at 450nm. The plate was then incubated at 37ºC in a CO_2 incubator and read again at 20, 30 and 40 minutes. The results were calculated using AssayZap and the best time point taken. The lower limit of detection for this assay was 200ηg/ml.

Statistical Models

The results presented in Table 3 were generated using a generalized estimating equation (GEE) with binomial family, logit link, and AR(1) correlation matrix. GEEs are used to estimate the parameters of a generalized linear model with possibly unknown correlation between outcomes (in this case, between repeated measures on the same child).

Formulation of GEEs

Given a mean model, μ_{ij}, and variance structure, V_i, the estimating equation is formed via:

$$U(\beta) = \sum_{i=1}^{N} \frac{\partial \mu_{ij}}{\partial \beta_k} V_i^{-1} \{Y_i - \mu_i(\beta)\}$$

The parameter estimates solve $U(\beta)=0$. Note that the term "variance structure" refers to the algebraic form of the covariance matrix between outcomes, *Y*, in the sample. In this manuscript we have used an autoregressive lag-1 structure.

Results

Longitudinal data were available from 3 months of age for 127 patients with CF with 127 assessed at a mean (standard deviation) age of 0.35 (0.12) years, 109 assessed at 1.17 (0.20) years, 92 assessed at 2.17 (0.23) years and 81 assessed at 3.20 (0.22) years. The primary reason for the lower numbers of children with increasing age was that the children had not yet reached the assessment age by the end of the data collection period. 43 children had not received subsequent annual tests as at the time of analysis they were not old enough. Nine children did not have their 12 month test, 22 did not have their 24 month test, and 12 did not have their 36 month test. Of the other eight children with data missing at 3 years, four children missed their 36 month test and four had moved interstate/overseas.

Infecting organisms other than *S. aureus* and *P aeruginosa* that are included as “any infection” in Table 1 include: *A. flavus* (n=1), *A. fumigatus* (n=16), *A. niger* (n=1), Adenovirus (n=1), Aspergillus species (n=2), *C. albicans* (n=4), Candida species (n=9), *E. coli* (n=14), Enterobacter species (n=5), *H. influenza* (n=31), Haemophilus species (n=12), Klebsiella species (n=3), *M. catarrhalis* (n=2), mixed oral flora (n=175), Parainfluenza (n=2),

Penicillium species (n=4), Respiratory Syncytial Virus (n=2), *S. maltophilia* (n=2), *S. pneumonia* (n=8), *S. pyogenes* (n=1), *S. apiospermum* (n=1), Scedosporium species (n=2), Serratia species (n=2).

Table S1: Full data set used in analyses for bronchiectasis.

ID	Sex	3 months of age					1 year of age					2 years of age					3 years of age				
		Age	NE	Scan	Bx		Age	NE	Scan	Bx		Age	NE	Scan	Bx		Age	NE	Scan	Bx	
					A	S				A	S				A	S				A	S
1	M	0.26	1	L	0	0	1.20	0	L	0	0	2.08	1	L	1	1	3.31	1	L	1	1
2	M	0.23	0	L	0	0	1.29	0	L	1	1	1.96	1	L	0	1	-	-	-	-	1
3	F	0.34	0	L	0	0	1.05	1	L	0	0	1.98	0	L	0	0	2.98	0	L	0	0
4	M	0.48	1	L	0	0	1.00	1	L	1	1	2.03	1	L	1	1	3.10	0	L	1	1
5	M	0.29	0	L	0	0	1.03	0	L	0	0	2.11	0	L	1	1	3.09	1	L	1	1
6	F	0.30	0	L	0	0	1.06	0	L	0	0	2.23	0	L	0	0	3.07	0	L	0	0
7	M	0.24	1	L	0	0	0.99	0	L	0	0	1.98	0	L	0	0	2.98	1	L	1	1
8	F	0.29	0	L	0	0	1.04	0	L	0	0	-	-	-	-	0	3.42	0	V	1	1
9	F	0.31	0	L	0	0	1.04	0	L	1	1	2.00	1	L	0	1	3.01	0	L	1	1
10	M	0.23	0	L	0	0	0.99	0	L	1	1	1.97	1	L	1	1	3.10	0	L	1	1
11	M	0.28	0	L	0	0	1.17	1	L	1	1	2.20	0	L	0	1	2.95	1	L	1	1
12	M	0.49	0	L	0	0	1.43	1	L	1	1	-	-	-	-	1	3.02	0	L	0	1
13	F	0.30	1	L	0	0	1.00	0	L	0	0	1.98	0	L	1	1	3.07	0	V	1	1
14	M	0.55	0	L	0	0	-	-	-	-	0	2.05	0	L	1	1	3.49	0	V	0	1
15	M	0.42	1	L	1	1	1.00	1	L	1	1	2.01	1	L	1	1	3.05	1	V	1	1
16	M	0.43	0	L	0	0	0.94	1	L	1	1	1.98	1	L	0	1	2.98	0	V	0	1
17	M	0.39	0	L	0	0	1.23	0	L	1	1	1.94	0	L	1	1	3.11	0	V	0	1
18	M	0.40	0	L	0	0	0.96	1	L	0	0	1.95	0	L	0	0	2.97	0	V	1	1
19	M	0.36	0	L	0	0	1.05	1	L	1	1	2.09	0	L	1	1	3.09	1	V	0	1
20	M	0.39	1	L	0	0	1.10	0	L	0	0	1.96	0	L	0	0	3.34	0	V	1	1
21	F	0.71	1	L	0	0	1.39	0	L	1	1	2.09	0	L	1	1	3.03	0	V	1	1
22	F	0.54	1	L	1	1	1.22	1	L	1	1	1.96	1	L	1	1	3.57	1	V	1	1
23	F	0.43	1	L	0	0	1.19	0	L	0	0	2.00	0	L	0	0	3.11	1	V	1	1
24	F	0.30	1	L	0	0	1.46	0	L	0	0	2.38	1	-	9	0	3.15	0	V	1	1

25	M	0.51	1	L	0	0	-	-	-	-	0	1.95	0	L	0	0	2.93	0	V	1	1
26	F	0.38	1	L	0	0	1.11	0	L	0	0	2.08	0	-	9	0	3.14	0	V	1	1
27	F	0.38	0	L	0	0	0.91	0	L	0	0	1.95	0	-	9	0	3.00	0	V	0	0
28	M	0.40	1	L	1	1	1.09	1	L	1	1	1.88	0	-	9	1	3.26	1	V	1	1
29	M	0.27	0	L	0	0	1.04	0	L	1	1	2.15	1	-	9	1	3.13	9	V	1	1
30	F	0.34	0	L	0	0	1.06	0	L	0	0	2.00	0	-	9	0	3.01	0	V	0	0
31	F	0.41	0	L	0	0	1.17	0	L	1	1	1.98	1	-	9	1	3.03	1	V	1	1
32	M	0.52	1	L	1	1	1.21	0	L	1	1	2.03	0	-	9	1	3.05	0	V	1	1
33	F	0.33	0	L	1	1	1.14	0	L	0	1	2.14	0	-	9	1	3.02	1	V	1	1
34	M	0.48	1	L	0	0	1.31	1	L	1	1	2.02	0	-	9	1	3.26	9	-	9	1
35	F	0.32	0	L	1	1	0.91	0	L	0	1	2.05	0	-	9	1	2.97	0	V	1	1
36	M	0.34	0	L	0	0	1.07	0	V	0	0	2.01	1	-	9	0	3.41	0	V	1	1
37	M	0.25	0	L	1	1	1.00	0	V	0	1	2.00	0	-	9	1	2.88	9	-	9	1
38	M	0.38	0	L	1	1	1.15	0	V	0	1	2.11	0	-	9	1	2.99	0	V	0	1
39	M	0.50	0	L	0	0	1.00	0	V	1	1	2.09	0	-	9	1	3.36	9	-	9	1
40	M	0.34	1	L	0	0	1.20	1	V	0	0	2.03	1	-	9	0	2.91	1	V	1	1
41	M	0.25	0	L	0	0	0.99	1	V	1	1	2.03	1	-	9	1	-	-	-	-	1
42	F	0.32	0	L	1	1	1.15	0	V	1	1	2.01	0	-	9	1	-	-	-	-	1
43	M	0.25	0	L	0	0	1.04	0	V	1	1	-	-	-	-	-	-	-	-	-	1
44	M	0.34	0	L	0	0	1.36	0	V	0	0	-	-	-	-	-	-	-	-	-	-
45	M	0.29	9		9	0	1.16	0	V	0	0	-	-	-	-	-	-	-	-	-	-
46	F	0.27	0	L	0	0	0.87	9	V	0	0	-	-	-	-	-	-	-	-	-	-
47	M	0.29	0	L	0	0	1.00	0	V	0	0	-	-	-	-	-	-	-	-	-	-
48	F	0.25	0	L	0	0	1.02	0	V	0	0	-	-	-	-	-	-	-	-	-	-
49	F	0.27	0	L	1	1	1.47	0	V	0	1	-	-	-	-	1	-	-	-	-	1
50	F	0.35	0	L	1	1	1.06	0	V	0	1	-	-	-	-	1	-	-	-	-	1
51	F	0.25	0	V	0	0	1.00	0	V	0	0	-	-	-	-	-	-	-	-	-	-
52	M	0.35	0	L	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
53	M	0.53	0	L	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

54	M	0.36	1	V	1	1	0.55	9	V	1	1	-	-	-	-	1	-	-	-	-	1
55	M	0.67	0	L	0	0	-	-	-	-	0	2.09	0	V	0	0	3.12	0	V	1	1
56	F	0.22	1	L	1	1	0.98	1	L	1	1	1.98	0	V	1	1	3.24	0	V	1	1
57	M	0.13	1	L	1	1	1.05	0	L	0	1	2.01	0	V	1	1	3.08	0	V	0	1
58	F	0.20	9	L	0	0	1.20	0	L	0	0	2.25	0	V	0	0	3.23	0	V	1	1
59	F	0.22	9	L	1	1	1.18	0	L	1	1	1.98	0	V	1	1	3.06	0	V	0	1
60	F	0.17	0	L	0	0	1.17	0	L	0	0	2.03	0	V	0	0	3.12	0	V	0	0
61	F	0.26	0	L	0	0	1.25	0	L	0	0	2.23	0	V	1	1	3.09	0	V	1	1
62	F	0.30	0	L	1	1	1.33	0	L	0	1	2.52	0	V	0	1	3.48	0	V	0	1
63	M	0.37	0	L	0	0	1.44	0	L	0	0	2.44	0	V	0	0	3.44	0	V	1	1
64	F	0.32	0	L	0	0	1.31	0	L	0	0	2.39	0	V	0	0	3.35	0	V	1	1
65	M	0.18	0	L	0	0	1.26	0	L	0	0	2.14	0	V	1	1	3.13	0	V	1	1
66	M	0.54	0	L	0	0	1.67	1	V	1	1	2.55	1	V	0	1	3.62	0	V	1	1
67	M	0.24	0	L	0	0	1.12	0	V	0	0	2.23	0	V	1	1	3.19	1	V	1	1
68	M	0.30	0	L	0	0	1.14	1	V	0	0	2.25	0	V	1	1	3.25	0	V	1	1
69	M	0.31	0	L	9	0	1.25	0	V	1	1	2.18	0	V	1	1	3.26	1	V	1	1
70	F	0.33	0	L	9	0	1.16	0	V	1	1	2.21	0	V	1	1	3.36	0	V	1	1
71	M	0.28	0	L	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
72	M	0.77	0	L	0	0	-	-	-	-	0	1.87	0	V	1	1	2.79	1	V	1	1
73	F	0.25	0	L	0	0	1.04	0	V	1	1	2.17	0	V	0	1	3.13	0	V	1	1
74	M	0.31	0	V	0	0	1.26	0	V	0	0	2.22	0	V	0	0	3.26	1	V	1	1
75	M	0.30	0	L	0	0	1.29	0	V	1	1	2.37	0	V	1	1	3.48	0	V	1	1
76	M	0.57	0	L	0	0	1.49	0	V	0	0	2.56	0	V	1	1	3.56	0	V	0	1
77	F	0.30	0	L	0	0	1.28	0	V	0	0	2.31	0	V	0	0	3.31	0	V	1	1
78	M	0.30	0	L	0	0	1.28	0	V	0	0	2.32	0	V	1	1	3.20	0	V	1	1
79	M	0.21	0	L	0	0	1.19	0	V	0	0	2.38	0	V	0	0	3.33	1	V	1	1
80	F	0.24	0	L	0	0	0.97	0	V	0	0	2.00	1	V	1	1	3.00	0	V	1	1
81	F	0.28	1	L	1	1	1.23	0	V	1	1	2.15	0	V	1	1	3.27	0	V	1	1
82	M	0.30	0	L	0	0	1.29	0	V	0	0	2.33	0	V	0	0	3.29	0	V	1	1

83	M	0.49	0	L	0	0	1.64	0	V	0	0	2.75	0	V	1	1	3.78	0	V	1	1
84	F	0.60	0	V	1	1	1.75	0	V	1	1	-	-	-	-	1	3.66	0	V	1	1
85	M	0.23	1	L	9	0	1.26	0	V	0	0	2.22	0	V	0	0	3.30	0	V	1	1
86	F	0.41	1	L	1	1	1.45	1	V	1	1	2.52	1	V	1	1	3.48	1	V	1	1
87	F	0.37	1	L	1	1	1.37	0	V	1	1	2.60	0	V	1	1	-	-	-	-	1
88	F	0.21	0	L	0	0	1.28	0	V	0	0	2.28	0	V	0	0	3.39	0	V	0	0
89	F	0.51	1	L	1	1	1.05	1	V	1	1	2.28	0	V	1	1	3.39	1	V	1	1
90	F	0.41	0	L	0	0	1.41	0	V	1	1	2.37	0	V	1	1	3.29	9	V	1	1
91	F	0.64	0	L	0	0	1.79	0	V	0	0	2.78	0	V	0	0	3.93	0	V	1	1
92	F	0.43	0	L	1	1	1.46	0	V	1	1	2.46	0	V	1	1	3.49	0	V	1	1
93	F	0.25	0	L	0	0	1.02	0	V	0	0	2.13	0	V	0	0	3.28	0	V	1	1
94	F	0.27		L	1	1	1.30	0	V	0	1	2.34	0	V	1	1	3.29	0	V	0	1
95	F	0.25	0	L	0	0	1.17	0	V	1	1	2.21	0	V	1	1	3.13	0	V	0	1
96	M	0.75	0	L	0	0	-	-	-	-	0	1.74	0	V	1	1	2.82	0	V	0	1
97	F	0.41	0	L	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
98	F	0.28	0	L	0	0	1.24	0	V	0	0	2.24	0	V	1	1	3.23	0	V	1	1
99	M	0.41	0	L	0	0	1.49	0	V	1	1	2.75	0	V	0	1	-	-	-	-	1
100	M	0.28	0	L	1	1	1.20	0	V	1	1	2.16	1	V	1	1	3.24	0	V	1	1
101	F	0.17	0	L	0	0	1.16	0	V	0	0	2.51	0	V	1	1	-	-	-	-	1
102	F	0.21	0	L	0	0	0.98	0	V	1	1	3.09	1	V	1	1	-	-	-	-	1
103	F	0.36	0	L	1	1	-	-	-	-	1	-	-	-	-	1	-	-	-	-	1
104	F	0.30	0	V	0	0	1.30	0	V	1	1	2.26	0	V	0	1	-	-	-	-	1
105	M	0.16	1	V	0	0	1.15	0	V	1	1	2.19	0	V	0	1	-	-	-	-	1
106	M	0.37	1	L	1	1	1.36	0	V	1	1	2.28	0	V	0	1	-	-	-	-	1
107	F	0.35	0	L	0	0	1.00	0	V	1	1	-	-	-	-	1	-	-	-	-	1
108	F	0.28	1	L	0	0	1.01	0	V	1	1	2.05	0	V	0	1	-	-	-	-	1
109	F	0.65	0	L	1	1	-	-	-	-	1	2.10	0	V	1	1	-	-	-	-	1
110	M	0.24	9	L	1	1	1.04	0	V	0	1	2.00	1	V	1	1	-	-	-	-	1
111	M	0.46	0	L	0	0	1.76	9	V	0	0	-	-	-	-	-	-	-	-	-	-

112	F	0.36	0	L	1	1	0.97	0	V	1	1	1.97	0	V	0	1	-	-	-	-	1
113	M	0.28	0	V	1	1	1.08	0	V	1	1	-	-	-	-	1	-	-	-	-	1
114	M	0.29	0	L	1	1	0.98	0	V	0	1	-	-	-	-	1	-	-	-	-	1
115	M	0.28	0	L	1	1	1.05	0	V	0	1	-	-	-	-	1	-	-	-	-	1
116	M	0.26	0	L	1	1	0.95	0	V	0	1	-	-	-	-	1	-	-	-	-	1
117	F	0.29	0	V	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
118	F	0.30	9	V	1	1	-	-	-	-	1	-	-	-	-	1	-	-	-	-	1
119	F	0.27	9	V	0	0	0.96	0	V	0	0	-	-	-	-	-	-	-	-	-	-
120	F	0.34	1	L	0	0	0.92	0	V	0	0	-	-	-	-	-	-	-	-	-	-
121	F	0.29	0	L	1	1	0.98	1	V	1	1	-	-	-	-	1	-	-	-	-	1
122	F	0.31	0	L	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
123	M	0.27	0	L	1	1	-	-	-	-	1	-	-	-	-	1	-	-	-	-	1
124	M	0.25	0	V	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
125	F	0.26	0	V	1	1	-	-	-	-	1	-	-	-	-	1	-	-	-	-	1
126	F	0.27	0	V	1	1	-	-	-	-	1	-	-	-	-	1	-	-	-	-	1
127	F	0.60	0	V	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

NE = neutrophil elastase activity in BAL; Scan type: L= limited slice scan, V=volumetric scan; Bx = bronchiectasis, A = actual data, S = data used in sensitivity analysis - if bronchiectasis has been present on a previous scan all subsequent scans are considered positive, or if a CT data are missing between two scans the scan is considered negative for bronchiectasis unless positive on a previous scan; Data structure: 0 = negative, 1= positive, 9 = missing, - = no annual review primarily due to the child not reaching the assessment age during the study period.

Table S2: Longitudinal analysis of risk factors for bronchiectasis from 3 month to 3 years of age: sensitivity analysis.

Variable	Odds Ratio (95% CI)	p
Univariate analyses		
BMI Z-score	0.98 (0.84-1.15)	0.83
Male Sex	1.06 (0.57-1.99)	0.84
Meconium Ileus	4.99 (2.04-12.17)	<0.001
Pancreatic insufficiency	2.13 (0.96-4.74)	0.064
Severe genotype	2.25 (1.02-4.94)	0.044
Respiratory symptoms[1]	1.62 (1.09-2.42)	0.018
NE present in BAL	2.16 (1.47-3.17)	<0.001
Infection in BAL[2] • Any infection • *S. aureus* • *P. aeruginosa*	 1.31 (0.94-1.82) 1.42 (0.85-2.39) 1.42 (0.98-2.04)	 0.11 0.18 0.059
Gas trapping on Chest CT	1.35 (0.94-1.94)	0.10
Multivariate analysis		
Meconium Ileus	5.16 (2.15-12.38)	<0.001
NE present in BAL	2.20 (1.41-3.45)	<0.001

Analysis conducted using a generalised estimating equation with binomial family, logit link and AR(1) correlation matrix. For the sensitivity analysis once bronchiectasis was present it was assumed to remain present on subsequent scans. [1]Respiratory symptoms present at the time of the CT/BAL. [2]Any infection defined as $\geq 10^5$ colony forming units per ml of fluid returned except for *P. aeruginosa* where any colony count was considered as infected.

Associations between the presence of free NE activity in the BAL at 3 months of age and bronchiectasis from 3 months to 3 years of age are shown in the form of a Latin Square (Table S3).

Table S3: Latin Square representation of the associations between free neutrophil elastase activity (NE) in the bronchoalveolar lavage at 3 months of age, the presence of bronchiectasis (Bx) on chest CT scan at 3 months of age and the presence of bronchiectasis on Ct scans a 12, 24 and 36 months of age.

Status at 3m	3m (n)	12m (n)	24m (n)	36m (n)
NE- / Bx-	68	55	29	18
NE- / Bx+	22	32	31	39
NE+ / Bx-	15	4	4	1
NE+ / Bx+	12	15	10	18
NEunknown / Bx-	2	2	0	0
NEunknown / Bx+	4	1	0	2
NE- / Bxunknown	2	0	12*	0
NE+ / Bxunknown	1	0	6*	0
NEunknown / Bxunknown**	1	18	35	49

* the major reason for children appearing in this group is that they were assessed at the Melbourne site which stopped doing scans at 24m during the study period but continued with BALs. ** the major reason for children appearing in this group is that they had not reached the assessment age during the study period

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