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Original Article

The association between *Staphylococcus aureus* and subsequent bronchiectasis in children with cystic fibrosis

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Abstract

Background: Staphylococcus aureus (*S. aureus*) may be related to more rapid progression of cystic fibrosis (CF) lung disease.
Methods: In the AREST CF cohort study, children diagnosed with CF undergo annual bronchoscopies with bronchoalveolar lavage and ultra-low-dose, chest computed tomography (CT) up to 6-years-old. Spirometry was assessed 3-monthly from the age of 4 years. Associations between de novo *S. aureus* acquisition before school age and CT and lung function at ages 5–7 years were investigated. Models were adjusted for multiple markers of disease severity at baseline.
Results: De novo *S. aureus* acquisition at 3-years-old (n/N = 12/122) was associated with increased bronchiectasis score at age 5–6 years. This association decreased but remained significant after adjustment for confounders. *S. aureus* at 3 was associated with significantly reduced $FEF_{25–75}$ at age 5–7 years, but not with FEV_1-%-predicted.
Conclusion: De novo *S. aureus* acquisition at age 3 is associated with later bronchiectasis and $FEF_{25–75}$ in children with CF.

Keywords: CT scan; Structural lung damage; Infection and inflammation; Lung function

1. Introduction

Features of cystic fibrosis (CF) lung disease are inflammation and chronic pulmonary infection. *Staphylococcus aureus* (*S. aureus*) has been identified as one of the earliest and most common bacterial pathogens in airways of children with CF [1,2]. The deleterious effect of chronic *P. aeruginosa* infection on pulmonary structure and function has been repeatedly described, but knowledge of the impact of other pathogens, particularly *S. aureus*, in the development of early CF lung disease is lacking [2,3]. Consequently there is no international consensus on the need for *S. aureus* prophylaxis or eradication, even if the organism is detected in the lower airways.

There are in vitro and in vivo data showing an association between *S. aureus* and airway inflammation [4–6], reduced lung

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function [7,8] and reduced nutritional status [9]. Therefore, despite a lack of evidence for a causal role, pulmonary infections with *S. aureus* are generally believed to contribute to the cycle of infection, inflammation and destruction of lung tissue. Previous intervention trials using prophylactic anti-staphylococcal antibiotic treatment have not shown any positive effects on clinical outcomes in infancy or up to the age of 6 years [10–12]. Importantly, the spirometry outcomes used in these studies have repeatedly been demonstrated to be less sensitive for detecting early CF lung disease than chest CT scans [13].

The Australian Respiratory Early Surveillance Team for cystic fibrosis (AREST CF) surveillance program is a birth cohort of CF patients in Melbourne and Perth, with annual bronchoalveolar lavage (BAL) and CT scans up to the age of 6 years. Using these data we aimed to investigate the complex associations between lower airway *S. aureus* infections in early life and the later development of CF lung disease, taking into account the possible underlying pathophysiologic pathways. We hypothesised that newly acquired *S. aureus* infection in infants and young children with CF is not merely a marker for underlying CF disease severity, but an important independent factor for disease progression as reflected in later chest CT scan and/or spirometry. An important clinical implication of this would be that a more aggressive treatment of *S. aureus* may be indicated, possibly including eradication strategies as currently advised for *P. aeruginosa*.

2. Methods

2.1. Study population

The AREST CF program operates in the Royal Children's Hospital, Melbourne and Princess Margaret Hospital, Perth, Australia. Upon detection at new-born screening or presentation of symptoms suggestive of CF, a sweat test is conducted to confirm the diagnosis [14]. The surveillance program commences at 3 months of age and annually thereafter up to the age of 5 years (Melbourne) or 6 years (Perth). Since 2005, this program includes an annual bronchoscopy with BAL and ultra-low-dose chest computed tomography scan (CT) performed under general anesthesia. Assessment of clinical spirometry is attempted every 3 months in CF children starting at the age of 4 years. The current study used data collected in children born between 2005 and 2016. More detailed descriptions of the study protocol have been published previously [15,16] and the relevant routines have not changed since.

2.2. Data collection

In the program, baseline perinatal data are collected retrospectively at enrollment. At each 3-monthly clinic visit, questionnaire data are collected on respiratory symptoms and medication use. BAL is performed at 3 months, 1 year and yearly thereafter under general anesthesia. BAL fluid is sent for microbial cultures and analyses of inflammatory markers.

2.3. Outcome variables

The main outcome variable was defined as CF-CT bronchiectasis score at 6 years if available, or at 5 years otherwise. Volume-controlled, volumetric or limited slice chest CT images are obtained under anesthesia before BAL is performed, with images at end inspiration (trans-respiratory pressure = 25 cmH_2O) and end expiration (trans-respiratory pressure = 0 cmH_2O) as described previously [16]. If the CT was missing at both 5 and 6 years the outcome was considered missing. A simplified CF-CT scoring method was used [15,17]. Of the children with a CT scan available at age 5 or 6 years 85% had a full volumetric scan available, in the remaining 15% results from limited slice CT scan were used.

Spirometry is performed according to international guidelines on a 3-monthly basis when the child is able to achieve acceptable and repeatable measurements [18]. Results were expressed as %-predicted calculated using the Global Lung Initiative reference equations, the validity of which has been verified in the Australian population [19,20]. Measurement with the highest recorded FEV_1-%-predicted were selected from each year.

2.4. Exposure and confounder variables

The primary exposure variable was defined as de novo (i.e. first time) acquisition of *S. aureus* from BAL culture, regardless of culture density. Possible confounding and/or preceding factors included: CF-CT bronchiectasis, air trapping and bronchial wall thickening scores in previous year; homozygous Delta508 gene abnormality, pancreatic insufficiency & meconium ileus at baseline; BAL markers for inflammation in previous year (neutrophil elastase and interleukin 8); and positive BAL bacterial cultures for other bacteria (including *P. aeruginosa*) in previous years. Medication use was assessed by parental report, but those results were not available for the entire study period. During the study entire period it was standard practice at both centres to prescribe amoxicillin/clavulanate prophylaxis during the first two years of life. Prophylaxis could be continued beyond 2 years of age at the physician's discretion.

2.5. Statistical analysis

Statistical analyses were conducted using STATA version 14.0 (StataCorp). Associations between de novo *S. aureus* and bronchiectasis score were analysed using linear regression. In order to reduce the risk of reverse causation and confounding, previous *S. aureus* infections were excluded and associations were adjusted for markers of disease severity at time of de novo *S. aureus* acquisition. Multiple imputation was used for the multivariate analyses to avoid selection bias due to missing data on confounder variables (see Fig. S1 in online supplement for flowchart), using the multivariate normal regression procedure (mi impute mvn) in STATA 14.0 (creating 100 imputed datasets). Associations between *S. aureus* and repeated measures of spirometry were assessed using linear mixed models.

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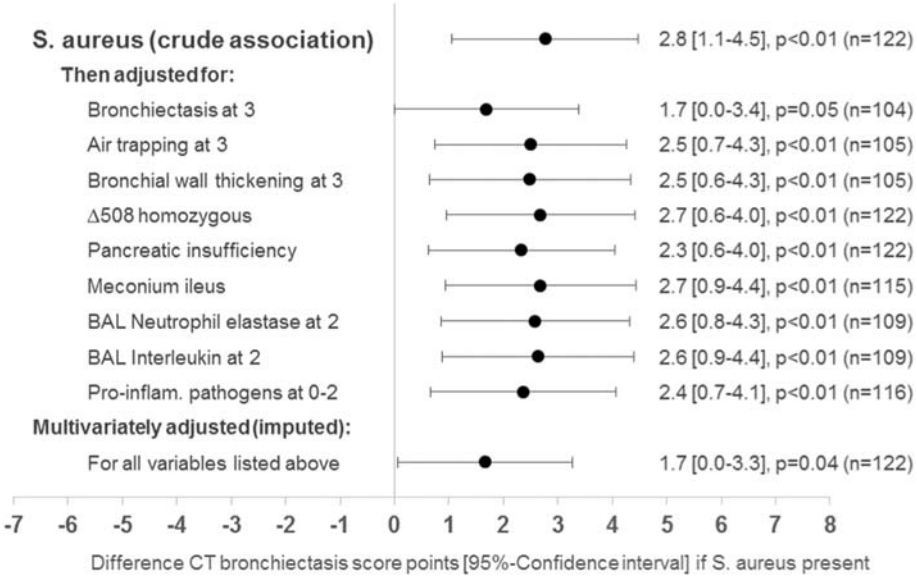

Fig. 1. Association of de novo *S. aureus* at age 3 with CT bronchiectasis score at 5–6 years. Pro-inflammatory pathogens include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Aspergillus* species. *: 'n' is number of children positive for de novo *S. aureus* acquisition at age 3, 'N' is total sample size.

3. Results

3.1. Study population

At time of data extraction 368 children were registered in the AREST CF cohort and 219 had reached the age of 5 years (baseline study population, Fig. S1). CT outcome data were available for 181 children of those. Of the 38 children with missing outcome, 17 were lost-to-follow-up (e.g. moved, withdrawn from study), and in the other 21 CT was missing for various reasons (e.g. no consent, technical issues CT, organisational, unknown reasons). Fig. 1 represents the flowchart for the analysis on *S. aureus* at age 3. For a summary table at other ages see the online supplement (Table S1). Meconium ileus, pancreatic insufficiency and any pro-inflammatory pathogens from BAL were significantly associated with bronchiectasis at 5–6 years (Table 1).

3.2. Association of Staphylococcus aureus with CT bronchiectasis

Prevalence of *S. aureus* ranged from 7% at age 1 to 17% at 3 years (Table 2A). The bronchiectasis score at age 5–6 years was higher in children with BAL culture positive for *S. aureus*, but this

Table 1
Characteristics of study population and CT bronchiectasis score (n = 181).

	n/N (%) or median (range), N	Difference in bronchiectasis CT score 5–6 years [a]	p-Value [c]
Descriptive variables	n/N (%)		
Gender (male)	91/181 (50%)	0.76 (−0.39–1.92)	0.195
Pancreatic insufficiency	150/177 (85%)	2.91 (1.35–4.48)	<0.001
Genetic abnormality			
Homozygous Δ 508	93/180 (52%)	0.54 (−0.63–1.70)	0.366
Heterozygous Δ 508	78/180 (43%)	Combined as reference	
No Δ 508	9/180 (5%)		
Meconium ileus (present)	34/170 (20%)	1.53 (0.04–3.01)	0.044
Any pro-inflammatory pathogens [b] in BAL at age 0–2 years.	77/156 (49%)	1.84 (0.67–3.01)	0.002
Outcomes	Median (range), N		
CT scores at 5–6 years			
CT Bronchiectasis score	3 (0−12), n = 181		
CT air trapping	3 (0–12), n = 179		
CT bronchial wall thickening	9 (0–12), n = 181		
Lung function at 6 years			
FEV_1-%-predicted at 6	102.6 (48.4–140.5), n = 176		
FVC-%-predicted at 6	109.4 (49.0–143.3), n = 176		
$FEV_{25–75}$-%-predicted at 6	95.7 (17.5–158.5), n = 175		

[a] Represented as difference in CT bronchiectasis score points (95%-CI) between the presence and absence of *S. aureus*.
[b] Includes *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Aspergillus* species.
[c] *t*-Test for difference in mean CT score. In order to be able to calculate median and range we report lung function assessed at age 6, in all other analyses we report repeated measurements between the aged of 5–7 years analysed using longitudinal mixed models.

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Table 2
Infections in BAL at 0–4 years vs. CT and lung function at 5–7 years.

A. *Staphylococcus aureus* vs. CT bronchiectasis score at 5–6 years

Age BAL (years)	Overall prevalence *S. aureus*			De novo acquisition *S. aureus*		
	n/N[c]	Mean difference bronchiectasis[a]	p-Value	n/N[c]	Mean difference bronchiectasis[a]	p-Value
3 mths	12/114 (11%)	+0.46 (−1.91, 2.83)	0.701	12/114 (11%)	+0.46 (−1.91, 2.83)	0.701
1	9/133 (7%)	+1.55 (−1.06, 4.16)	0.241	7/121 (6%)	+1.83 (−1.10, 4.77)	0.218
2	18/147 (12%)	−0.05 (−1.94, 1.84)	0.960	15/128 (12%)	−0.38 (−2.44, 1.68)	0.717
3	26/155 (17%)	+2.48 (0.93, 4.03)	0.002	21/122 (17%)	+2.76 (1.05, 4.47)	0.002
4	21/151 (14%)	+1.76 (0.01, 3.51)	0.049	9/103 (9%)	+2.31 (−0.15, 4.77)	0.065
Any positive culture 0–4 years	64/181 (35%)	+1.59 (0.40, 2.79)	0.009	n.a.		

B. *Staphylococcus Aureus* vs. spirometry (FEV_1) at 5–7 years

Age BAL (years)	Overall prevalence *S. aureus*			De novo acquisition *S. aureus*		
	n/N[c]	Mean difference FEV_1-%-pred[b]	p-Value	n/N[c]	Mean difference FEV_1-%-pred[b]	p-Value
3 mths	15/130 (12%)	5.80 (−1.70, 13.3)	0.129	15/130 (12%)	5.80 (−1.70, 13.3)	0.129
1	12/150 (8%)	−1.68 (−9.92, 6.54)	0.688	10/135 (7%)	−6.02 (−14.7, 2.71)	0.176
2	21/163 (13%)	−3.37 (−10.3, 3.50)	0.336	15/138 (11%)	−2.38 (−10.4, 5.66)	0.561
3	30/170 (18%)	−6.16 (−11.8, −0.56)	0.031	22/131 (17%)	−6.37 (−13.0, 0.25)	0.059
4	26/163 (16%)	−9.45 (−15.5, −3.42)	0.002	9/108 (8%)	−4.19 (−13.9, 5.52)	0.398
Any positive culture 0–4 years	71/187 (38%)	−2.20 (−6.64, 2.24)	0.332	n.a.		

C. *Pseudomonas aeruginosa* vs. CT bronchiectasis score at 5–6 years

Age BAL (years)	Overall prevalence *P. aeruginosa*			De Novo acquisition *P. aeruginosa*		
	n/N[c] (%)	Mean difference bronchiectasis[a]	p-Value	n/N[c] (%)	Mean difference bronchiectasis[a]	p-Value
3 mths	4/114 (11%)	+1.64 (−2.30, 5.58)	0.412	4/114 (4%)	+1.64 (−2.30, 5.58)	0.412
1	15/133 (7%)	+2.31 (−0.26, 4.35)	0.027	14/131 (11%)	+1.99 (−0.12, 4.10)	0.064
2	13/47 (12%)	+1.20 (−0.97, 3.37)	0.278	12/130 (9%)	+1.30 (−0.91, 3.51)	0.247
3	17/155 (17%)	+1.72 (−0.16, 3.61)	0.073	14/127 (11%)	+1.91 (−0.08, 3.91)	0.060
4	14/151 (14%)	+2.74 (0.68, 4.81)	0.010	7/113 (6%)	+3.09 (−1.22, 7.40)	0.158
Any positive culture 0–4 years	51/169 (30%)	+1.92 (−0.67, 3.18)	0.003		n.a.	

BAL is Broncho-alveolar lavage.

[a] Represented as difference in CT bronchiectasis score points (95%-Confidence Interval ($CI_{95\%}$)) between the presence and absence of *S. aureus* (panel A) or *P. aeruginosa* (panel C) at each age BAL was performed; results from linear regression.

[b] Represented as mean difference in %-predicted-FEV_1 ($CI_{95\%}$) between the presence and absence of *S. aureus* at each age BAL was performed; results from mixed linear models accounting for repeated measurements.

[c] 'n' is number of children positive for *S. aureus* (panel A and B) or *P. aeruginosa* (panel C), 'N' is total sample size with available data at that age.

increase was only significant at the ages of 3 and 4 years. Results for de novo acquired *S. aureus* infections, excluding those positive for *S. aureus* in any preceding year, are given in right panel of Table 2A. About three quarters of *S. aureus* infections at 1, 2 and 3 year were de novo acquired. Associations with bronchiectasis were similar for both the overall prevalence and de novo *S. aureus*, but significance was lost at age 4 years. A substantial decrease in numbers of de novo acquisitions at age 4 should be noted. Thus a significant association between de novo *S. aureus* acquisition and later bronchiectasis was demonstrated only at the age of 3 years. Hence, analyses to investigate if this association remained significant after adjustment for possible confounding or preceding factors focus on the association at 3 years. The prevalence of *Methicillin Resistant S. aureus* (MRSA) was low throughout the study with 2 cases at 2 and 6 years, and only 1 case at other ages.

Univariate adjustment for risk factors present before de novo *S. aureus* acquisition, led to a decrease in the association with bronchiectasis at 5–6 years, but it remained significant (Fig. 1). The multivariate analysis, including 9 possible confounding variables, was performed on 122 children (multiple imputed datasets). Results were similar to the univariate results and the association between *S. aureus* and CT bronchiectasis remained significant. Results of adjusted analyses at other ages are summarised in the online supplement (Figs. S2–S5).

3.3. Association of Staphylococcus aureus with spirometry

In line with the associations found with CT outcome, the overall *S. aureus* prevalences at age 3 and 4 years were significantly associated with lower FEV_1-%-predicted at 5–7 years (Table 2B). Children with de novo *S. aureus* acquisition at age 3 showed a 6.37% [$CI_{95\%}$ 0.25–13.0] decrease in FEV_1-%-predicted, a trend that did not reach statistical significance ($p = 0.06$; Fig. 2). Univariate and multivariate adjustments for confounder variables led to a further decrease in effect size and significance (p-value = 0.10). Overall prevalence and de novo *S. aureus* acquisition at age 3 were significantly associated with lower $FEF_{25–75}$, but not FVC. After multivariate adjustment for preceding markers of

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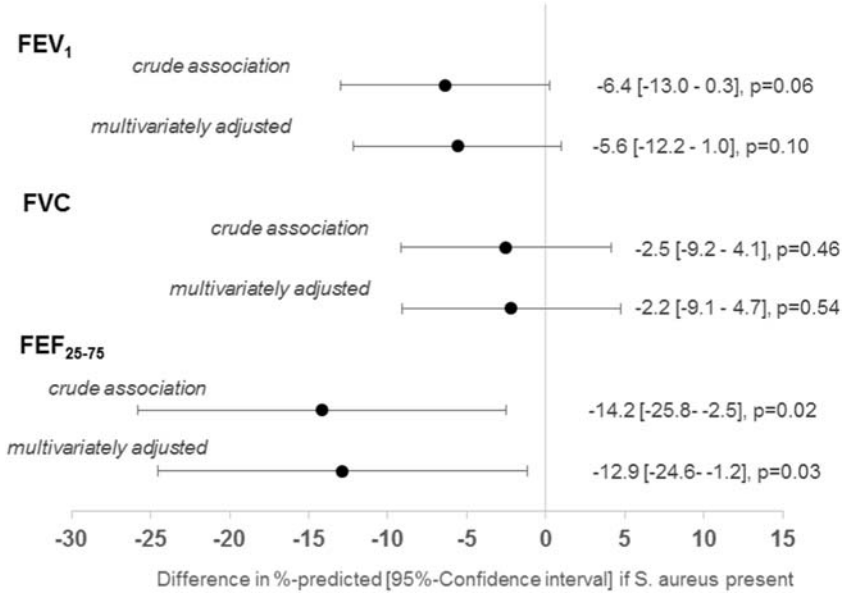

Fig. 2. Associations of de novo *S. aureus* at age 3 with spirometry at 5–7 years *(n/N† = 22/131). Results for FEV_1, FVC and $FEF_{25–75}$ expressed as mean difference in %-predicted if *S. aureus* present. Multivariate adjustment performed for all confounders as listed in the figure. *: Results from mixed model with repeated measurements of FEV_1 at age 5, 6 and 7 years. All children with at least 1 successful spirometry measurement between ages 5–7 included, on average 2.5 measurements per child obtained. †: 'n' is number of children positive for de novo *S. aureus* acquisition at 3 years, 'N' is number of children in total sample size. Multiple imputations used for missing variables for confounders.

disease severity, the association with $FEF_{25–75}$ remained significant (Fig. 2). See Tables S2 & S3 (online supplement) for associations between *S. aureus* and spirometry at other ages. It was noted that children with *S. aureus* cultured in BAL at age 3 months had significantly higher $FEF_{25–75}$ later in life, which was not in line with any results at other ages.

3.4. Pseudomonas aeruginosa, Staphylococcus aureus and later outcomes

The prevalence of *P. aeruginosa* in BAL was lower than *S. aureus* at all ages except at 1 year (Table 2C). Previous reports found prophylactic treatment for *S. aureus* to be associated with higher rates of *P. aeruginosa*, suggesting the presence of *S. aureus* may protect against *P. aeruginosa* colonisation. Table 3 reports the odds ratios for *P. aeruginosa* positive BAL at each year, given the child had an *S. aureus* positive BAL in the preceding year. These results do not support an inverse association between *S. aureus* and later *P. aeruginosa* infection. Overall prevalences of *P. aeruginosa* at 1 and 4 years were associated with more bronchiectasis at 5–6 years, but none of the associations with de novo *P. aeruginosa* acquisition were significant. *P. aeruginosa* was not associated with spirometry outcomes at any age (see Table S3 in the online supplement for FEV_1).

4. Discussion

In the AREST CF longitudinal birth cohort we found associations between de novo *S. aureus* infections at 3 years and the development of more bronchiectasis and lower $FEF_{25–75}$-%-predicted at 5–7 years. These associations remained significant after adjustments for markers of pre-existing disease. The adverse association between *S. aureus* and structural lung damage at early school age was similar to associations found for *P. aeruginosa*. Our findings suggest that *S. aureus* infection in early life is independently associated with developing structural CF lung disease.

The isolation rate of *S. aureus* in our study was approximately 10–20%, despite the standard use of anti-staphylococcal prophylaxis in the first two years of life. Previous studies and international CF data registries have reported prevalences of up to 55%, but those include sputum samples, and nasal and throat swabs, likely reflecting in part upper airway colonisation [1,21]. Prevalence of *S. aureus* in lower airways as assessed by BAL in preschool children was previously reported at 24% [6], similar to our findings, and this is generally thought to reflect a pathologic situation [3].

Table 3
Association between *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Age (years)	Bacteria	Overall prevalence	OR for *P. aeruginosa* if *S. aureus* positive in previous year
		n/N (%)	OR ($CI_{95\%}$), p-value
3 months	*S. aureus*	15/138 (10.9%)	n.a.
	P. aeruginosa	8/138 (5.8%)	
1	*S. aureus*	8/126 (6.4%)	1.1 (0.2–5.2), p = 0.94
	P. aeruginosa	16/126 (12.7%)	
2	*S. aureus*	20/151 (13.3%)	2.6 (0.5–13.4), p = 0.26
	P. aeruginosa	12/151 (8.0%)	
3	*S. aureus*	28/166 (16.9%)	1.4 (0.4–5.2), p = 0.63
	P. aeruginosa	17/166 (10.2%)	
4	*S. aureus*	22/162 (13.6%)	0.7 (0.2–3.3), p = 0.66
	P. aeruginosa	17/162 (10.5%)	

Numbers at each row reflect children with BAL data available at that age and the previous year, with either a CT or a lung function outcome available at end of follow-up. OR is odds ratio.

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Multiple studies, including AREST CF, have previously demonstrated associations between *S. aureus* and poorer markers of health in CF. *S. aureus* infections were associated with poorer nutritional status [9], more ventilation inhomogeneity [22] in infancy, and decreased lung function at early school age [8]. In line with previous reports we found significant associations between overall *S. aureus* prevalence and later lung function. After selection of newly acquired infections and adjustments for disease severity, the association with FEV_1 lost significance. $FEF_{25–75}$, likely measuring small airways disease, remained independently associated with de novo *S. aureus*. $FEF_{25–75}$ may be a more sensitive measure in young children with CF [23]. Furthermore, with our main outcome CT scans, the gold standard to diagnose bronchiectasis, we found a significant independent relationship with de novo *S. aureus* at age 3. Bronchiectasis reflects structural and likely irreversible lung damage in CF and has been validated with clinically relevant outcomes such as clinical exacerbations [24]. If associations between *S. aureus* and later outcomes are causal, than effective treatment of *S. aureus* would be expected to lead to improved outcomes. In contrast, a previous randomised trial by Stutman et al., investigating anti-staphylococcal prophylaxis in infants with CF, showed effective *S. aureus* suppression but reported no significant benefits in spirometry at the age of 6 years [11]. At that age the majority of children will have developed some bronchiectasis, despite FEV_1 appearing to be well-preserved [23]. Hence, contradictory findings might partially be explained by a higher sensitivity of structural outcomes compared to spirometry in detecting harmful effects of de novo *S. aureus* infections in this age group.

A potential risk of anti-staphylococcal treatment that was previously highlighted is the fact that it may result in lower *S. aureus* rates, but simultaneously in higher rates of *P. aeruginosa* colonisation [11,12]. Importantly this association was confirmed in a recent retrospective analysis of the UK CF Trust registry data [25]. Our observational data cannot be used to assess the direct effects of prophylaxis, but they offer no support for an inverse association between *S. aureus* and later *P. aeruginosa* acquisition. *P. aeruginosa* is assumed to be an important pathogen with a causal role in the development of CF lung disease. Our repeated BAL data in preschool children suggest that both *P. aeruginosa* and *S. aureus* were associated with a similar risk of developing later bronchiectasis. In fact, at the age of 3 years only de novo acquisition of *S. aureus*, and not *P. aeruginosa*, was associated with later bronchiectasis. The difference is likely related to the lower prevalence of de novo *P. aeruginosa* at that age. Also stringent eradication regimes for *P. aeruginosa* could have decreased associations with later sequelae. Previous analyses of BAL fluid showed that the inflammatory responses to *S. aureus* and *P. aeruginosa* were similar [4]. Our current findings indicate that like *P. aeruginosa*, de novo *S. aureus* acquisition may be a factor of clinical importance in this age group.

A major strength of this study is the relatively large sample size of CF children with prospective follow-up. The detailed annual assessments of disease characteristics including invasive and non-invasive tests allowed us to select de novo infections and define a large range of markers of disease severity. Despite the large overall sample size, the power of the study is determined by the number of children with positive cultures for *S. aureus* at each given year. Secondly, the fact that microbiologic samples from BAL were available allowed us to differentiate actual lower respiratory tract infections with *S. aureus* from possibly benign colonisation of the upper airways [3]. We cannot be certain how our BAL results relate to commonly used throat swab results, because these are not standardly performed for *S. aureus* in AREST CF. Thirdly, >95% of patients' parents are participants of the AREST CF surveillance program, making our study population a good representation of the overall CF population. The use of multiple imputation techniques for the multivariable analyses further reduces the risk of selection bias. Finally, the availability of CT outcomes in addition to spirometry has likely increased the power of this study to detect any effects of *S. aureus* infections in this cohort of young children. Our group recently developed a new CF scoring system (PRAGMA), but this score was not available for all children at age 3, 5 and 6 years. In order to maximise sample size and power, results on the basis of the CF-CT score are reported here.

Some limitations need to be considered. Because the association with de novo *S. aureus* was only significant at the age of 3 years, it should be acknowledged that the association may reflect a chance finding. The absence of a significant association with *S. aureus* at the ages 0–2 years may partly be explained by the clinical policy of amoxicillin/clavulanate prophylaxis between the ages of 0–2 years. We do not rigorously assess adherence to prophylaxis, but available data from parental reports suggests adherence is >50% in the first two years. Firstly, this likely reduced the overall *S. aureus* prevalence and thereby the power to detect any significant associations. Secondly, the continuous use of *S. aureus* suppressing antibiotics may have decreased the association with later structural lung damage, even if *S. aureus* was not eradicated completely. We attempted to adjust for all confounders and predisposing factors, and reported an independent association between *S. aureus* and later bronchiectasis. However, this is an observational study with risk of residual confounding. Hence, causality cannot be proven. Finally, although our study cohort is a good reflection of children with CF in Australia, differences in genetics, local practices and microbiological environment may complicate generalisation to other countries.

What are the implications of this study? Conflicting evidence from observational studies linking *S. aureus* with adverse health outcomes and the negative results from a randomised trial on *S. aureus* prophylaxis with 6 year follow-up have resulted in large differences in practice guidelines on anti-staphylococcal treatment around the world [26]. Our study cannot give the definitive answer to the important question whether *S. aureus* prophylaxis or eradication regimens are beneficial. However, our data support the notion that lower airway infections with *S. aureus* in this age group are not harmless and may lead to CF lung disease, similar as is assumed for *P. aeruginosa*. Our findings provide a possible explanation as to why a previous randomised trial did not find any health benefits of *S. aureus* prophylaxis, by using a relatively less sensitive measure of lung damage (i.e., FEV_1) in young children as main outcome. The previously suggested increased risk for *P. aeruginosa* infections associated with *S. aureus* prophylaxis was not supported by our data, but it remains a valid concern. As

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has been advocated previously [2,27] our study underlines the need for well-designed clinical trials investigating *S. aureus* prophylaxis or eradication regimens with long term follow-up, using CT outcomes to detect early structural lung damage.

Authors' contributions

DC, LT, JN, NHdK, TR, GLH, SCR, SMS all had substantial contributions to the conception of the study work, the broader AREST CF group contributed to the data acquisition. All authors contributed in the analysis and interpretation of data. All authors contributed in drafting and revising the manuscript critically and approve of the version to be published. All authors are accountable for all aspects of the work and ensure that any possible questions related to the accuracy or integrity of any part of the work will be appropriately investigated and resolved.

Conflict of interest

DC, LT, JN, NdK, GH, SR, PS and SS have nothing to disclose. TR has a patent WO 2016183609 A1 pending. GH reports grants from National Health and Medical Research Council of Australia and the USA CF Foundation, during the conduct of the study.

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Ethical approval

The study protocol was approved by the medical ethics committees of the participating university hospitals.

Take home messages

Staphylococcus aureus infection first acquired at age 3 years was associated with higher bronchiectasis score at age 5–6 years, despite adjustment for markers of disease severity.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcf.2017.12.002.

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