RESEARCH ARTICLE

Prospective multicenter German study on pulmonary colonization with *Scedosporium/Lomentospora* species in cystic fibrosis: Epidemiology and new association factors

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Abstract

Background

An increasing rate of respiratory colonization and infection in cystic fibrosis (CF) is caused by fungi of the *Scedosporium apiospermum* species complex or *Lomentospora prolificans* (*Sac-Lp*). These fungi rank second among the filamentous fungi colonizing the CF airways, after *Aspergillus fumigatus*. However, the epidemiology, clinical relevance and risk of pulmonary colonization with *Sac-Lp* are rarely understood in CF. The objective of the present prospective multicenter study was to study pathogen distribution and determine association factors of pulmonary *Sac-Lp* colonization in patients with CF.

Material and methods

Clinical, microbiological and laboratory data of 161 patients aged 6–59 years with CF in Germany were analyzed for *Sac-Lp* distribution and association factors. The free statistical software R was utilized to investigate adjusted logistic regression models for association factors.

Results

Of the 161 patients included in the study, 74 (56%) were male. The median age of the study cohort was 23 years (interquartile range 13–32 years). 58 patients of the total cohort (36%) were < 18 years old. Adjusted multivariate regression analysis revealed that *Sac-Lp* colonization was associated with younger age (OR 0.8684, 95%CI: 0.7955–0.9480, p<0.005) and less colonization with *H. influenzae* (OR 0.0118, 95%CI: 0.0009–0.1585, p<0.001). In
addition, *Sac-Lp*-colonized patients had more often allergic bronchopulmonary aspergillosis (ABPA) (OR 14.6663, 95%CI: 2.1873–98.3403, p<0.01) and have been colonized more often with the mucoid phenotype of *Pseudomonas aeruginosa* (OR 9.8941, 95%CI: 1.0518–93.0705, p<0.05).

**Conclusion**

Newly found association of ABPA and *Pseudomonas* revealed new probable risk factors for *Sac-Lp* colonization. Allergy might play a role in inducing immunologic host reactions which lead to a less effective response to species of *Sac-Lp*.

**Introduction**

Cystic fibrosis (CF) is the most frequent genetic disease in the European Caucasian population. In Germany, approximately 8000 patients are affected with CF; each year almost 200 patients are newly diagnosed with CF. The disease is caused by mutations in the CFTR (*Cystic Fibrosis Transmembrane Conductance Regulator*) gene located on chromosome seven, which results in a defective chloride channel. This directly influences the airway surface liquid (ASL) the reduction of which leads to a defective mucociliary clearance and the production of sticky bronchial mucus. This in turn makes the patient susceptible to chronic airway inflammation and infections. This vicious circle causes lung tissue damage. While the clinical relevance of bacteria in airway infection such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* has been well reviewed [1, 2], the role of filamentous fungi is poorly understood, except for *Aspergillus fumigatus*. During the last decade an increasing rate of bacterial and fungal colonization in the lungs of patients with CF and infection with some filamentous fungi belonging to the genus *Scedosporium* (family Microascaceae, division Ascomycota) was being recognized [3, 4]. Due to recent taxonomic changes, *Scedosporium apiospermum* is now considered to be a five-species complex, comprising *S. apiospermum* sensu stricto, *S. boydii* (formerly known as *Pseudallescheria boydii*), *S. aurantiacum*, *S. minutisspura* and *S. dehoogii* [3, 6]. As recently examined by Lackner et al. [6], *Scedosporium prolificans* is genetically distinct from the other *Scedosporium* species, which explains that it was reassigned to the genus *Lomentospora* and is now called *L. prolificans*. Molds of the *Scedosporium apiospermum* complex and *L. prolificans*, referred to in the following text as *Sac-Lp* [7], are recognized as opportunistic pathogens and can cause infections such as sinusitis, pneumonia and disseminated infections primarily in patients with immunodeficiency [8–11]. With an overall estimated prevalence of up to 14%, *Scedosporium/Lomentospora* species rank second among the filamentous fungi colonizing the airways of patients with CF, after *A. fumigatus* [4, 7, 12]. In patients with CF, especially after long-term treatment, we see an emerging rate of *Scedosporium/Lomentospora* species colonizing the respiratory tract over months or even years [12–14]. As colonization with *Sac-Lp* may negatively influence the outcome of lung transplantation, such bronchopulmonary colonization is discussed as a contraindication for lung transplantation [9, 15]. *Sac-Lp* can be detected in various different environmental sources including water, soil and dung [3, 16]. As yet there is a lack of knowledge about the clinical relevance of and predisposing factors for respiratory colonization by *Scedosporium* species in patients with CF. The present study aimed at closing this gap of knowledge by prospectively evaluating epidemiology and associated factors in 161 patients, in 26 patients of which *Sac-Lp* has repeatedly been isolated from respiratory specimens. The primary objective of this study was to evaluate epidemiologic patterns and clinical relevance of respiratory colonization with *Sac-Lp* in patients with CF and to determine clinical
association factors (possible predisposing factors) for respiratory colonization with these species in patients with CF. For this, samples from four distinct CF centers co-operating within the “German Network on Scedosporium and Lomentospora spp. in CF” founded in Germany in 2008 were examined. Currently (September 2016) 14 German centers for cystic fibrosis participate in this network.

**Material and methods**

**Subject population and outcomes**

The prospective cohort study was conducted by four German CF centers belonging to the “German Network on Scedosporium and Lomentospora spp. in CF”: Cystic Fibrosis Centers (1) Berlin, (2) Bonn, (3) Munich and (4) Tübingen. These CF centers cared for 726 patients during the study period, equivalent to 14% (13.9%) of all patients from the German CF Registry (in 2012: 5242). All individuals with confirmed diagnosis of CF were prospectively screened for *Sac-Lp* in the four CF centers from 09/2011 to 03/2015.

Patients who fulfilled the following criteria were included in the study:

1. complete data set of the following parameters
   - *CFTR* mutation
   - age
   - gender
   - FEV1/% predicted
   - pancreatic status
   - CF-related diabetes status
   - ABPA status
   - exacerbation p.a.
   - total IgE
2. minimum of 4 airway samples in 12 months
3. fulfilled criteria for pulmonary *Sac-Lp* colonization (i) detection of *Sac-Lp* in sputum, throat swab or bronchoalveolar lavage twice or more in 6 months, (ii) molecular-based confirmation of *Sac-Lp* in sputum, throat swab or BAL during study period in the reference laboratory.

Exclusion criteria were lung transplantation in the past, *Sac-Lp* isolation as a single (arbitrary) finding during the study period or missing written consent to enrollment in the German cystic fibrosis patient registry Muko.web.

Furthermore, patients with CF without repeated evidence of *Sac-Lp* colonization who were not lung transplanted were enrolled as control cohort. Clinical data of study population was retrieved from patients’ health records and the certified server-based patient registry Muko.web. All *Sac-Lp* isolates or respiratory samples from patients with suspected *Sac-Lp* colonization were sent to the Robert Koch Institute for precise species identification.

**Ethical issues**

Written informed consent from patient or parent of patient has been obtained before enrollment in the study. Written consent was given as well to enrollment in the certified server-
based German cystic fibrosis patient registry Muko.web (version 1.4.7). The study was approved by the local Charité – Universitätsmedizin Berlin Institutional Review Board (approval ID: EA2/080/11). We confirm that the work was done in accordance with the appropriate institutional review board and carried out by observing the ethical standards set forth in the Declaration of Helsinki; Recommendations guiding physicians in biomedical research involving human subjects. Adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964, amended by the 29th World Medical Assembly, Tokyo, Japan, October 1975, the 35th World Medical Assembly, Venice, Italy, October 1983, and the 41st World Medical Assembly, Hong Kong, September 1989.

**Primary endpoint**

The primary outcome of the study was the determination of association factors of *Sac-Lp* colonization.

**Sample size determination**

The design of the study included independent cases and controls with the above-mentioned inclusion criteria for statistical analysis. Furthermore, it was assumed that about two explanatory variables were required to yield good results for predicting the binary outcomes. According to Harrell et al. [17], a minimum of ten cases per variable were needed for logistic regression modeling to get a stable model. Thus, 20 events in the case cohort were required, leading to a total sample size of at least 50 patients for adjusted multivariate regression analysis.

**Statistical analysis**

Multiple logistic regression analyses were performed to identify association factors of *Sac-Lp* colonization (yes, no). The following parameters have been adjusted in the multiple logistic regression analyses: age (in years), gender (male/female), predicted mean forced expiratory volume in one second (FEV1/predicted (%)), history of CF-related diabetes (CFRD) (yes, no), of allergic bronchopulmonary aspergillosis (ABPA) (yes, no), of exocrine pancreatic insufficiency (yes, no), number of annual exacerbations, deletion of phenylalanine at position 508 of the cystic fibrosis transmembrane conductance regulator gene (*CFTR* delF508: homozygous, non-homozygous), colonization with any bacterial and fungal species listed in Table 1 (each: yes, no). Patient with missing data were not included in the statistical analysis. Data distributions were checked by graphical inspections (box plots, QQ plots, histograms). Furthermore, distributions were arithmetically examined by skewness. All parameters were considered within the regression models and chosen for the final models by stepwise backward variable selection with AIC (Akaike information criterion). P values <0.05 were considered to be significant. All statistical analyses were performed using the free software R (Version 3.0.2, The R Project).

**Data sources, assessment and definitions**

Clinical data of the study population were retrieved from patients’ health records and Muko.web (updated version of Muko.doc). Colonization with bacteria or fungi was defined as at least one microorganism detected in respiratory secretions during the study period. But according to the definition of *Sac-Lp* colonization, two isolations in 6 months were needed. Annual pulmonary exacerbations according to Bilton et al. [18] were defined as average annual number of events within the study period. FEV1/predicted for *Sac-Lp*-colonized patients was defined
as FEV1/predicted at first detection time point of Sac-Lp colonization. ABPA, CFRD and exocrine pancreatic insufficiency were defined as occurring between the date of diagnosis of CF and the date of the last routinely performed follow-up before statistical analysis. For the control cohort (Non Sac-Lp colonization), FEV1/predicted was defined as best FEV1/predicted during the study period. Clinically proven ABPA has been evaluated according to the diagnostic criteria of ABPA in patients with CF proposed by the Cystic Fibrosis Foundation Consensus Conference and the United Kingdom CF Trust.

### Analysis of pathogen colonization

Identification and susceptibility testing of all examined pathogens and Sac-Lp were routinely performed by the certified hospital-associated microbiological diagnostic unit. Growing of the cultures was performed according to quality standard guidelines [19]. Expectorated sputa and/or bronchoalveolar lavage from four participating centers were cultured on standard mycological media. Fungal colonies were initially microscopically pre-identified including examination of hyphal and spore characteristics. Precise species identification of Sac-Lp isolates was performed or confirmed by the Reference Laboratory for Scedosporiosis at the Robert Koch Institute, Berlin, Germany, by molecular methods such as sequencing of the ITS region of ribosomal DNA or species-specific hybridization on LCD microarray [20].

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### Table 1. Baseline characteristics of patients with CF without (control) and with (Sac-Lp) Sac-Lp colonization (total n = 161 patients).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients with CF</th>
<th>Control</th>
<th>Sac-Lp</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients, n (%)</td>
<td>161 (100)</td>
<td>135 (84)</td>
<td>26 (16)</td>
<td></td>
</tr>
<tr>
<td>Age at enrollment, year, median (IQR)</td>
<td>23 (13, 32)</td>
<td>24 (14.00, 33.00)</td>
<td>20 (12.50, 27.50)</td>
<td>0.1927</td>
</tr>
<tr>
<td>Gender, male</td>
<td>74 (45.96)</td>
<td>58 (43)</td>
<td>16 (61.5)</td>
<td>0.09012</td>
</tr>
<tr>
<td>CFTR dF508 homozygous, n (%)</td>
<td>84 (52.17)</td>
<td>72 (53.3)</td>
<td>12 (46.2)</td>
<td>0.5275</td>
</tr>
<tr>
<td>FEV1/predicted (%), median (IQR)</td>
<td>77 (57.50, 99.00)</td>
<td>74.5 (57.00, 96.00)</td>
<td>96 (62.50, 114.00)</td>
<td>0.3516</td>
</tr>
<tr>
<td>Exocrine pancreatic insufficiency, n (%)</td>
<td>142 (88.19)</td>
<td>123 (91.1)</td>
<td>19 (73.1)</td>
<td>0.01677*</td>
</tr>
<tr>
<td>ABPA, n (%)</td>
<td>47 (29.19)</td>
<td>40 (29.6)</td>
<td>7 (26.9)</td>
<td>0.97981</td>
</tr>
<tr>
<td>Exacerbations p.a., median (IQR)</td>
<td>2.03 (3.00, 5.00)</td>
<td>2.04 (1.00, 3.00)</td>
<td>1.37 (1.00, 2.75)</td>
<td>0.1879</td>
</tr>
<tr>
<td>Total IgE, median (IQR) (kU/L)</td>
<td>65 (33.00, 100.00)</td>
<td>52 (18.50, 175.25)</td>
<td>92 (29.45, 202.50)</td>
<td>0.1703</td>
</tr>
<tr>
<td>P. aeruginosa, n (%)</td>
<td>74 (45.96)</td>
<td>59 (43.7)</td>
<td>15 (57.7)</td>
<td>0.2046</td>
</tr>
<tr>
<td>S. aureus, n (%)</td>
<td>133 (82.61)</td>
<td>110 (82.2)</td>
<td>22 (64.6)</td>
<td>1</td>
</tr>
<tr>
<td>Burkholderia spp., n (%)</td>
<td>6 (3.72)</td>
<td>5 (3.7)</td>
<td>1 (3.8)</td>
<td>1</td>
</tr>
<tr>
<td>S. maltophilia, n (%)</td>
<td>39 (24.22)</td>
<td>36 (26.7)</td>
<td>3 (11.5)</td>
<td>0.134</td>
</tr>
<tr>
<td>A. xylosoxidans, n (%)</td>
<td>24 (14.91)</td>
<td>22 (16.3)</td>
<td>2 (7.7)</td>
<td>0.3722</td>
</tr>
<tr>
<td>H. influenzae, n (%)</td>
<td>62 (38.51)</td>
<td>61 (45.2)</td>
<td>1 (3.8)</td>
<td>3.369e-05****</td>
</tr>
<tr>
<td>A. fumigatus, n (%)</td>
<td>64 (39.75)</td>
<td>59 (60.8)</td>
<td>5 (19.23)</td>
<td>0.02733*</td>
</tr>
<tr>
<td>Other Aspergillus spp., n (%)</td>
<td>11 (6.83)</td>
<td>11 (6.8)</td>
<td>0</td>
<td>0.2142</td>
</tr>
<tr>
<td>C. albicans, n (%)</td>
<td>105 (65.22)</td>
<td>93 (68.9)</td>
<td>12 (46.2)</td>
<td>0.04125*</td>
</tr>
<tr>
<td>Other Candida spp., n (%)</td>
<td>50 (31.06)</td>
<td>48 (35.6)</td>
<td>2 (7.7)</td>
<td>0.004688***</td>
</tr>
</tbody>
</table>

IQR: interquartile range, n: number, p.a.: per annum; %: percent; SD: standard deviation; OR: odds ratio

*p<0.05

**p<0.01

***p<0.005

****p<0.001.

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Determination of ABPA

Proven ABPA has been evaluated according to diagnostic criteria of ABPA in patients with CF proposed by Cystic Fibrosis Foundation Consensus Conference and UK CF Trust [21, 22]. ImmunoCap assays for the analysis of total serum immunoglobulin E (IgE), specific A. fumigatus IgE and serological determination of Af antibodies were routinely performed by the certified hospital-associated microbiological diagnostic unit.

Results

Cohort characteristics

During the 563.5 person-years of follow-up, 726 subjects with CF visited the four CF centers. 26 patients with CF and confirmed Sac-Lp colonization were enrolled, and 135 patients with CF but without Sac-Lp at these four CF centers served as controls. For this study cohort (in total 161 patients) demographic, clinical and microbiological colonization characteristics are summarized in Table 1. 74 were male and 87 were female. The median age of patients was 23 years (interquartile range 13–32 years). The median FEV1/% predicted was 77 (range 57.50–99). The majority of subjects had exocrine pancreatic insufficiency (n = 142, 88.2%) and 29.2% had CFRD (n = 47). Delta F508 was the most frequent CFTR mutation with 88.81% (homozygous mutation 52.17%, heterozygous 36.65%, other genotypes 11.18). The median annual exacerbation rate of the total cohort was 2.03.

The percentage of co-colonized patients was different between patients harboring Sac-Lp and the control group. Table 1 gives an overview of bacterial and fungal isolates in the total, control and Sac-Lp cohort. In the total cohort Methicillin-susceptible Staphylococcus aureus (n = 133, 82.6%), non-mucoid P. aeruginosa (n = 116, 72.1%), mucoid P. aeruginosa (n = 74, 46%) and Haemophilus influenzae (n = 62, 38.5%) have been most frequently isolated, followed by Stenotrophomonas maltophilia (n = 39, 24.2%), Achromobacter xylosoxidans (n = 24, 14.9%) and Burkholderia species (n = 6, 3.7%). The most common fungi reported were Candida albicans (n = 105, 65.2%) and A. fumigatus (n = 64, 39.8%), followed by other Candida species (n = 50, 31.1%) and other Aspergillus species (n = 11, 6.8%).

Prevalence of Sac-Lp colonization

In total, 29 patients (prevalence 4%) with CF from 4 German CF centers revealed confirmed Sac-Lp colonization during a time interval of 43 months (9/2011 to 3/2015), but three patients were excluded due to missing clinical data (exacerbation rate, total IgE).

Characteristics of Sac-Lp-colonized patients

Table 1 shows demographic and clinical characteristics of patients that were colonized with Sac-Lp species. The Sac-Lp cohort (n = 26) consisted of 16 males (61.5%) and 10 females (38.5%) (Table 1). The median age of these patients was 20 years (interquartile range 12.5–27.5 years). In the Sac-Lp group delta F508 was the most frequent mutation with 76.92% (homozygous 46.15%, heterozygous 30.77% and others 23.07%).

In 19 (73.1%) patients a pancreatic insufficiency could be evaluated. CFRD was described in 7 (26.9%) patients. In 11 (42.3%) patients the diagnosis of an ABPA could be confirmed. The median number of annual exacerbations was 1.00 (interquartile range 1.00–2.75). Total IgE was elevated above 100 kU/L in 4/26 patients (15.4%) and in 5/26 patients (19.2%) above 500 kU/L. The median total IgE in patients with CF colonized with Sac-Lp was 92 kU/L (interquartile range 29.5–202.5).
The most frequently detected Sac-Lp species were *S. boydii* (17/26 patients, 65.4%) and *S. apiospermum* (15/26, 61.5%), while significantly less *L. prolificans* (2/26 patients, 7.7%) and *S. aurantiacum* (2/26 patients, 7.7%) were cultured (Table 2). Eight of 26 (30.8%) patients with Sac-Lp colonization showed pulmonary infection (data not shown). Overall, in 18 of 26 Sac-Lp patients only one Sac-Lp isolate was recovered from respiratory culture.

Two different species within Sac-Lp were identified in 8/26 patients, while three species were found in only one of the 26 patients (Table 2).

**Association factors for Sac-Lp colonization**

Unadjusted analysis revealed a highly significant negative correlation between Sac-Lp colonization and exocrine pancreatic insufficiency (*p* = 0.017), negative co-colonization with *A. fumigatus* (*p* = 0.027), with *A. xylosoxidans* (*p* = 0.046), with *H. influenzae*, Hi (*p* = 3.369e-05), with *C. albicans* (*p* = 0.041) and with other *Candida* species (without further specification) (*p* = 0.005) (Table 3). Another significant relationship was seen between Sac-Lp colonization and a positive correlation for ABPA (*p* = 0.009) (Table 3). No differences in the isolation of co-colonizing pathogens were found with respect to age, gender or other variables and Sac-Lp colonization.

Demographic, clinical and microbiological association factors correlating with Sac-Lp colonization calculated in an adjusted multivariate regression model are shown in Table 3. The adjustment of variables is specified according to criteria in the methods section. The following variables identified in univariate analysis were confirmed by adjusted multivariate analysis as being statistically significantly associated with Sac-Lp colonization: ABPA (OR 14.6663, 95% CI: 2.1873–98.3403, *p* = 0.0057), co-colonization with Hi (OR 0.0118, 95%CI: 0.0009–0.1585, *p* = 0.0008) and with *Candida* species (OR 0.0151, 95%CI: 0.0013–0.1812, *p* = 0.0009). Furthermore, adjusted multivariate analysis revealed that Sac-Lp colonization was associated with negative odds for age (OR 0.8684, 95%CI: 0.7955–0.9480, *p* = 0.0016). Sac-Lp patients were younger than Non-Sac-Lp patients (median of 18 years vs. median of 24 years). In addition, colonization with Sac-Lp was associated with increased odds for co-colonization with the mucoid phenotype of *Pa* (OR 9.8941, 95%CI: 1.0518–93.0705, *p* = 0.0451). A higher proportion of Sac-Lp patients (57.7% vs. 43.7%) revealed co-colonization with this pathogen compared to controls. However, the adjusted model did not confirm results from unadjusted univariate analysis for the correlation of exocrine pancreatic insufficiency, co-colonization

**Table 2. Evidence of isolated Sac-Lp species per patient.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients, n (%)</td>
<td>26 (100)</td>
</tr>
<tr>
<td>1 species</td>
<td></td>
</tr>
<tr>
<td><em>S. boydii</em></td>
<td>17 (65.4)</td>
</tr>
<tr>
<td><em>S. apiospermum</em></td>
<td>9 (34.6)</td>
</tr>
<tr>
<td><em>S. aurantiacum</em></td>
<td>7 (26.9)</td>
</tr>
<tr>
<td>2 species</td>
<td></td>
</tr>
<tr>
<td><em>S. boydii</em> &amp; <em>S. apiospermum</em></td>
<td>1 (3.8)</td>
</tr>
<tr>
<td><em>S. boydii</em> &amp; <em>L. prolificans</em></td>
<td>1 (3.8)</td>
</tr>
<tr>
<td><em>S. apiospermum</em> &amp; <em>S. aurantiacum</em></td>
<td>1 (3.8)</td>
</tr>
<tr>
<td>3 species</td>
<td></td>
</tr>
<tr>
<td><em>S. boydii</em> &amp; <em>S. apiospermum</em> &amp; <em>L. prolificans</em></td>
<td>1 (3.8)</td>
</tr>
</tbody>
</table>

n: number, %: percent

doi:10.1371/journal.pone.0171485.t002
with *A. fumigatus* and co-colonization with *C. albicans* with respect to *Sac-Lp* colonization. There were no significant differences in other baseline characteristics, including FEV1/predicted and annual exacerbations comparing *Sac-Lp* patients and Non-*Sac-Lp* patients.

**Discussion**

Fungal complications such as lung deterioration and local inflammation in patients with CF are essentially caused by filamentous fungi. Colonization and infection with *Sac-Lp* in these patients is an emerging topic [8, 23]. The present multicenter study describes clinical association factors for *Sac-Lp* colonization in four German CF centers. Given the opportunity of inclusion of multiple German CF centers and selection on standardized highly selective SceSel + agar [24], we calculated an overall prevalence of *Sac-Lp* of 4% which is in the range of previously reported German prevalences of 3.1% [7] and 5.3% [25], of French prevalence of 8.6% [26] and of Australian prevalences of 17.4% and 25% [3, 23].

In addition, the present multicenter work describes new association factors of pulmonary *Sac-Lp* colonization in CF. Adjusted multivariate analysis revealed that patients with *Sac-Lp* colonization showed significantly reduced co-colonization with *H. influenzae* and with *Candida* species and increased co-colonization with the mucoid phenotype of *P. aeruginosa*. Although the *in vitro* results by Kaur and colleagues showed that *P. aeruginosa* inhibits the growth of *S. aurantiacum* [27], our *in vivo* data could not confirm these findings. One reason could be the low prevalence of *S. aurantiacum* in Germany compared to other countries such as France [7, 28]. Furthermore, the adjusted model showed that patients harboring *Sac-Lp*
were on average younger than non-colonized patients and had significantly more often ABPA. Correlation with acquisition of Sac-Lp at a young age may be explained by the fact that children tend to spend a lot of time outdoors. These outdoor activities may include a high risk of playing with Sac-Lp-contaminated water, soil and dung where these fungal pathogens can ubiquitously be found. A prospective 5-year-study of 128 French patients with CF aged 7.8 to 21 years confirmed acquisition of Sac-Lp at a young age, i.e. mean of 14.5 years although not recovered in children [26]. The median age of Sac-Lp-colonized patients of the present German work correlates well with the mean age of 73 Sac-Lp-colonized patients of another German study in 2011 (mean 22 years, range 6–51 years [7]), some of them overlapping with those of the current study.

Our results show an increased co-colonization with mucoid phenotype of P. aeruginosa in Sac-Lp-colonized patients compared to controls. This result is in contrast to earlier observations made by Blyth and colleagues [23] who found that mucoid P. aeruginosa colonization “protects” against Sac-Lp colonization in 12 Sac-Lp-colonized patients compared to 57 non-Sac-Lp-colonized patients with CF in Australia. However, the results of the Australian colleagues are based on unadjusted univariate analysis only which could not be confirmed by adjusted multivariate analysis. Furthermore, differences in results may possibly stem from limited size and older age (> 20 years) of the Australian Sac-Lp cohort. In addition, unlike Blyth and colleagues we did not just include a history of P. aeruginosa colonization but prospectively analyzed pathogen co-colonization during the study period.

We could show significantly decreased co-colonization with H. influenzae (Hi) in patients with Sac-Lp colonization. The "protective effect" of Sac-Lp colonization for Hi colonization was not dependent on age or gender since we adjusted for these among other factors in the present work. By looking for prediction or association factors of Hi colonization in CF, we found a significant positive correlation between exacerbation and Hi colonization (Table 4). Hi has the ability to form a biofilm structure in CF. Persistence of Hi strongly correlates with

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>LCL</th>
<th>HCL</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at enrollment, year, median (IQR)</td>
<td>0.8563</td>
<td>0.7959</td>
<td>0.9212</td>
<td>0.0000</td>
</tr>
<tr>
<td>Gender, male</td>
<td>1.3453</td>
<td>0.4211</td>
<td>4.2978</td>
<td>0.6167</td>
</tr>
<tr>
<td>CFTR dF508 homozygous, n (%)</td>
<td>0.4049</td>
<td>0.1243</td>
<td>1.3192</td>
<td>0.1335</td>
</tr>
<tr>
<td>FEV1/% predicted (median [IQR])</td>
<td>0.9913</td>
<td>0.9742</td>
<td>1.0086</td>
<td>0.3202</td>
</tr>
<tr>
<td>History of exocrine pancreatic insufficiency, n (%)</td>
<td>9.5030</td>
<td>1.2902</td>
<td>69.9955</td>
<td>0.0271</td>
</tr>
<tr>
<td>CF-related diabetes, n (%)</td>
<td>0.6083</td>
<td>0.1641</td>
<td>2.2553</td>
<td>0.4572</td>
</tr>
<tr>
<td>History of ABPA, n (%)</td>
<td>0.2525</td>
<td>0.0563</td>
<td>1.1317</td>
<td>0.0721</td>
</tr>
<tr>
<td>Exacerbations p.a. (median [IQR])</td>
<td>1.5328</td>
<td>1.0917</td>
<td>2.1521</td>
<td>0.0136</td>
</tr>
<tr>
<td>P. aeruginosa, n (%)</td>
<td>0.6857</td>
<td>0.1471</td>
<td>3.1967</td>
<td>0.6310</td>
</tr>
<tr>
<td>P. aeruginosa (mucoid), n (%)</td>
<td>1.0013</td>
<td>0.1989</td>
<td>5.0407</td>
<td>0.9987</td>
</tr>
<tr>
<td>S. aureus, n (%)</td>
<td>13.5759</td>
<td>0.9586</td>
<td>192.2710</td>
<td>0.0538</td>
</tr>
<tr>
<td>Burkholderia spp., n (%)</td>
<td>0.8128</td>
<td>0.0364</td>
<td>18.1495</td>
<td>0.8959</td>
</tr>
<tr>
<td>S. maltophilia, n (%)</td>
<td>1.2035</td>
<td>0.3400</td>
<td>4.2598</td>
<td>0.7740</td>
</tr>
<tr>
<td>A. xylosidans, n (%)</td>
<td>0.1863</td>
<td>0.0318</td>
<td>1.0911</td>
<td>0.0624</td>
</tr>
<tr>
<td>C. albicans, n (%)</td>
<td>0.4447</td>
<td>0.1246</td>
<td>1.5871</td>
<td>0.2119</td>
</tr>
<tr>
<td>Other Candida spp., n (%)</td>
<td>0.3602</td>
<td>0.0798</td>
<td>1.6265</td>
<td>0.1843</td>
</tr>
</tbody>
</table>

IQR: interquartile range, n: number, p.a.: per annum; %: percent; SD: standard deviation; vs: versus, OR: odds ratio, LCL: lower confidence limit, HCL: highest confidence limit, adjustment was performed with all indicated variables in the table.

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biofilm production [29]. Whether the reduced growth of Hi in Sac-Lp patients with CF is the result of additional antibiotic treatment (also against the increasingly colonized Pa) has to be examined in further prospective trials, including therapeutic assessment of Sac-Lp-colonized patients.

Colonization with Hi was associated with a decreased risk for filamentous A. fumigatus colonization [30]. Such a dichotomous colonization pattern for Hi and filamentous fungi (i.e. Sac-Lp) colonization has been observed as well in the present study. Besides the significance of Sac-Lp for post-transplant fungemia and infections [31], emerging significance of these organisms in relation to allergic bronchopulmonary mycosis has to be considered [26, 32–34]. In addition, Paugam and colleagues described a correlation of Sac-Lp colonization with ABPA [35]. These observations are now completed by confirming multivariate results of the present prospective multicenter study. We do know that A. fumigatus can sometimes cause APBA which is the most common fungal disease in this context and has well-reviewed clinical markers such as IgE, specific IgE for A. fumigatus and antibodies rAsp 4 and rAsp 6 for this disease [36, 37]. Also there is a well-established therapy for ABPA [22]. The pathomechanism for ABPA causing Sac-Lp colonization is not known, but we know Aspergillus itself is associated with a broad reaction of the innate and adaptive immune system that might influence the defense of Sac-Lp as described by Chortirmall and colleagues [38]. The known risk for the development of bronchiectasis after ABPA underlines the hypothesis of a damaged local host defense due to ABPA [39].

Despite our intriguing new findings, the cross-sectional design of the present work limits us in the causal interpretation of the relation between pulmonary Sac-Lp colonization and association factors found. In summary, analysis revealed that Sac-Lp-colonized patients had significantly more often ABPA and have been colonized more often with the mucoid phenotype of P. aeruginosa. In addition, Sac-Lp colonization was associated with younger age.

Based on our observations, we would suggest to regularly screen for ABPA in patients with Sac-Lp colonization. For further research it remains an interesting proposition to examine the chronological correlation of Sac-Lp colonization, the pathogen–pathogen interaction and the immunological host response to allergy and to infection or colonization with Sac-Lp.

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References


