

Concordant genotype of upper and lower airways *P. aeruginosa* and *S. aureus* isolates in cystic fibrosis

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Concordant isolates in CF-airways

ABSTRACT

Rationale:

Lower airway (LAW) infection with *Pseudomonas aeruginosa* (*P.a.*) and *Staphylococcus aureus* (*S.a.*) is the leading cause of morbidity in Cystic Fibrosis (CF). The upper airways (UAW) were shown to be a gateway for acquisition and reservoir of opportunistic bacteria. Therefore, tools for UAW-assessment within CF-routine care require evaluation.

Objectives:

Aims of the study were non-invasive assessment of UAW and LAW microbial colonization and genotyping of *P.a.* and *S.a.* strains from both segments.

Methods:

182 CF-patients were evaluated (age 0.4-68yrs, median 17yrs). LAW specimens were preferably sampled as expectorated sputum and UAW specimens by nasal lavage. *P.a.* and *S.a.*-isolates were typed in informative single nucleotide polymorphisms (SNPs) or by *spa* typing, respectively.

Results:

Of the typable *S.a.* and *P.a.* isolates from concomitant UAW- and LAW-positive specimens, 31 of 36 patients were carrying identical *S.a.* *spa* types and 23 of 24 patients identical *P.a.* SNP-genotypes in both compartments. Detection of *S.a.* or *P.a.* in LAW specimens was associated with a 15- or 88-fold higher likelihood to also identify *S.a.* or *P.a.* in a UAW specimen from the same patient.

Conclusions:

The presence of identical genotypes in UAW and LAW suggests that the UAW play a role as a reservoir of *S.a.* and *P.a.* in CF. Nasal lavage appears to be suitable for non-invasive UAW-sampling but further longitudinal analyses and comparison with invasive methods are required. While UAW bacterial colonization is typically not assessed in regular CF-care, our data challenge the need to discuss diagnostic and therapeutic standards for this airway compartment.

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INTRODUCTION

Cystic Fibrosis (CF) is the most frequent life threatening recessive genetic disorder in Caucasians. It is caused by mutations in the *Cystic Fibrosis Transmembrane conductance Regulator (CFTR)* gene. Chronic pulmonary infection with opportunistic bacteria is the major cause of morbidity and mortality in CF. *Staphylococcus aureus (S.a.)* is one of the first pathogens infecting CF airways for extended periods [1,2]. Later, up to 80% of CF-adults are chronically colonized with *Pseudomonas aeruginosa (P.a.)* [3] indicating progression of the pulmonary destruction [4] although aggressive antibiotic treatment can improve life expectancy [5]. The natural course of the disease can be ameliorated by preventing or postponing chronic *P.a.*-colonization. *P.a.*-eradication is possible in early colonization as long as colonies do not evolve to mucoid phenotypes. Therefore, further understanding of pathways leading to *P.a.*-acquisition is a key issue of contemporary CF research.

Similar to the lower airways (LAW), mucociliary clearance of the upper airways (UAW) is impaired by the causative CFTR defect. Therefore, mucus detention, chronic inflammation, and colonization of the UAW with opportunistic bacteria are typical in CF. Chronic rhinosinusitis and nasal polyps are pathognomonic signs of the disease [6,7,8]. Resulting symptoms are chronic nasal congestion, rhinorrhoea with anterior or postnasal drip, mouth-breathing, facial pain, anosmia, and sleep disturbances, which impair the overall health. Altogether, 30-67% of the patients are reported to suffer from chronic rhinosinusitis over all age groups [7,8]. Morphological abnormalities of the paranasal sinuses are detected in computed tomography from almost 100% of the patients [9], although in some cases this is due to hypoplastic frontal sinuses.

Sinonasal involvement in CF has been proposed as the major source for chronic broncho-pulmonary infection with opportunistic bacteria [7]. *S.a.* and *P.a.* are known to colonize CF-UAW [10] and therefore this site may function as a gateway and reservoir for subsequent pulmonary infection. If this were generally true in CF, the same bacterial clones should inhabit UAW and LAW and the detection of early airway colonization in UAW and subsequent therapeutic intervention could accordingly improve the success rate of effective eradication. However, despite its potential impact on the management of CF, the association between upper and lower airway colonization has never been systematically investigated. Hence, we set up a prospective clinical study to compare the microbial flora at the same time in upper and lower CF airways and to assess the genetic relatedness between *S.a.* and *P.a.* strains inhabiting the CF subject's UAW and LAW. Moreover, non-invasive and simple methods for sampling the UAW that are applicable to daily clinical practise were compared in their sensitivity.

MATERIALS AND METHODS

Patients

187 patients from 5 German CF outpatient clinics were enrolled for the study between December 2005 and April 2007. Microbiological sampling from both UAW and LAW was performed for 182 patients.

Inclusion criterion was an established diagnosis of CF confirmed by at least 3 positive sweat tests and/or two disease causing *CFTR* mutations. Exclusion criteria were sino-nasal surgery ≤ 6 months prior to recruitment and systemic antimicrobial *P.a.* therapy. Treatment with inhalative aminoglycosides and oral macrolides was reported. Patients were excluded from nasal lavage if they suffered from recurrent nasal bleeding, if the tympanum was perforated and/or if cooperation was not possible (age or other reasons).

Informed written consent was obtained from patients and their parental guides. The study was approved by the local ethics committees.

Rhinoscopy

Nasal examination performed by flexible or rigid endoscopy included assessment of the mucosal status, sizing of nasal polyps if applicable and evaluation of secretions, crusts and other pathological alterations.

Nasal lavage

Nasal lavage was performed by applying 10ml of sterile isotonic saline in each nostril with a 10ml syringe in a slightly reclined position of the head during occlusion of the soft palate (fig. 1), as performed during the standard therapeutic nasal lavage [12]. Prior to the collection of nasal lavage, visual aids and verbal instructions were provided. Samples with contamination from the oral cavity were withdrawn and the procedure was repeated until non-contaminated samples were retrieved.

In general, patients elder than 6 yrs were able to accurately perform nasal lavage. Alternatively, deep-nasal swabs or swabs of nasal secretions sneezed into a handkerchief (blowing samples) were taken. Additionally, sputum samples or deep throat swabs were obtained. Specimens were processed by the local microbiology laboratory according to the German quality assurance guidelines for CF microbiology [13].

Molecular typing of *P.a.* and *S.a.*

P.a. isolates were typed in 16 SNPs with a custom-made microarray as described previously [14]. DNA amplified from the bacterial colony by cycles of multiplex primer extension was hybridized onto the microarray to yield an electronically portable binary multimarker genotype [14]. *S.a.* isolates were analyzed by *spa* typing. Briefly, the variable region of protein A was amplified with the following primers: *spa*-1113f (5'-TAAAGACGATCCTTCGGTGAGC-3') and *spa*-1514r (5'-CAGCAGTAGTGCCGTTTGCTT-3) [15] and sequenced. *Spa* types were determined with the Ridom StaphType software [16]. Numeric *spa* repeat and type codes were assigned.

Statistics

Data were evaluated with SPSS for Windows Version 11.5.1 (SPSS Inc., Chicago, Illinois 60606) and Microsoft Excel 2003 (Microsoft Corporation, Redmond, USA). We performed explorative analyses of data. The association between detection of bacteria and fungi in upper and in lower airways was estimated by phi-analysis. Agreement or concordance between both compartments (beyond what would be expected by chance) was measured by Cohen's Kappa. P-values of Kappa below 0.05 (global alpha) indicate a statistically significant difference from only chance agreement. Bonferroni adjustment was applied to control alpha for multiple comparisons. To describe the relative risk of finding bacteria or fungi in a UAW-sample we used the odds of finding them in LAW-positive and in LAW-negative subjects and calculated odds ratios and their 95% confidence intervals.

RESULTS

Demographic data

Baseline data from the altogether 187 CF-patients (81 f/106 m) recruited in five outpatient clinics are presented in table 1.

Table 1. Age, pulmonary function, body weight-related parameters and Shwachman-Kulczycki-Score of the included patients

		Median	Inner quartiles	Range	n
Age	[years]	17	8-24	0.4-68	187
FEV1	[%pred.]	83	60-102	17-150	161
FVC	[%pred.]	87	72-96	32-121	162
FEF25	[%pred.]	48	19-73	6-170	160
FEF75/25	[%pred.]	62	25-90	4-163	121
RV	[%pred.]	148	112-203	42-440	152
BMI (≥ 18 yrs)	[kg/m ²]	20	18-23	15-33	84
BMI (<18yrs)	[percentile] [32]	35	21-56	1-93	102
Shwachman-Score (without RX)	[pts] [33]	70	60-75	25-75	187

Abbreviation: FEV1, forced expiratory volume in one second; FVC, forced vital capacity; FEF25, forced expiratory flow at 25% of the forced vital capacity FVC; FEF75-25, forced expiratory flow at 75-25% of the FVC; RV, residual volume; BMI, body-mass-index; RX, chest roentgenogram; PC, percentile; n, number of patients.

The mutation F508del was detected in 163/187 patients (87.7%) with 78 (41.7%) being homozygous for this most frequent CFTR-defect in the German CF population. G551D was detected in 20 patients (5.3%), N1303K and G542X in 10 patients each (2.7%), R553X 9 (2.4%), R347P 8 (2.1%) and 3849+10kbC-->T 7 times (1.9%). Exogenous pancreatic insufficiency was present in 175/187 patients (93.6%) and 25/187 (13.4%) were diabetic. Altogether, allergic sensitization was found in 89/145 (61.4%) assessed patients by detection of specific IgE in serum. 36/187 (19.3%) CF patients presented with allergic rhinitis and 14/187 (7.5%) fulfilled the criteria for allergic bronchopulmonary aspergillosis (ABPA), according to the CF-consensus guidelines [17]. ENT-surgery had previously been performed in 79 of 187 recruited CF-patients, corresponding to a total percentage of 42.2%.

Upper and lower airway colonization

182 patients were assessed for microbial colonization in LAW and UAW (see table 2).

Table 2. Bacteria and fungi in upper and lower airways from 182 individuals with CF

	Detection in LAW	Detection in UAW	Detection in both compartments		K [#]	p- value *
	n	n	% of UAW	% of LAW		
<i>P. aeruginosa (total)</i>	65	29	28	96.6	43.1	0.48 <0.001
<i>P. aeruginosa (mucoid)</i>	45	21	18	85.7	40.0	0.46 <0.001
<i>P. aeruginosa (non-mucoid)</i>	31	11	4	36.4	12.9	0.11 0.095
<i>P. alcaligenes / Comamonas testosteroni</i>	2	3	1	33.3	50.0	0.39 0.033
<i>Pseudomonadaceae ssp.</i>	3	4	1	25.0	33.3	0.27 0.065
<i>Staphylococcus aureus (S.a.) (without MRSA)</i>	60	52	38	73.1	63.3	0.54 <0.001
<i>methicillin-resistant S.a. (MRSA)</i>	4	3	3	100.0	75.0	0.85 <0.001
<i>Haemophilus influenzae</i>	18	11	6	54.5	33.3	0.37 <0.001
<i>coagulase-negative Staphylococci (CNS.)</i>	24	56	17	30.4	70.8	0.30 <0.001
<i>streptococci (α-hemolytic)</i>	64	27	19	70.4	29.7	0.26 <0.001
<i>S. pneumoniae</i>	6	7	4	57.1	66.7	0.60 <0.001
<i>S. pyogenes (β-hemolytic A)</i>	2	2	1	50.0	50.0	0.49 0.022
<i>γ-hemolytic streptococci</i>	20	9	5	55.6	25.0	0.30 0.001
<i>Enterococci</i>	12	8	1	12.5	8.3	0.05 1
<i>Micro-, Lacto-, Stomatococcus, Sarcina</i>	5	1	0	0.0	0.0	0.00 1
<i>Corynebacteria</i>	2	13	1	7.7	50.0	0.12 0.138
<i>Neisseria ssp.</i>	26	11	5	45.5	19.2	0.20 0.01
<i>Moraxella</i>	1	7	0	0	0	0.00 1
<i>Acinetobacter baumannii</i>	1	1	0	0	0	0.00 1
<i>Ralstonia pickettii</i>	4	2	2	100.0	50.0	0.66 <0.001
<i>Stenotrophomonas maltophilia</i>	18	2	2	100.0	11.1	0.18 0.009
<i>Ochrobactrum anthropi</i>	2	2	1	50.0	50.0	0.49 0.022
<i>Escherichia coli</i>	4	1	1	100.0	25.0	0.40 0.022
<i>Enterobacter cloacae</i>	4	1	0	0	0	0.00 1
<i>Klebsiella oxytoca, K. terrigena, K. planticola</i>	4	2	1	50.0	25.0	0.32 0.044
<i>Proteus mirabilis</i>	2	2	2	100.0	100.0	1.00 <0.001
<i>Serratia marcescens, S. proteamaculans</i>	2	2	0	0	0	0.00 1
<i>Morganella, Providencia, Edwardsiella, Hafnia</i>	1	2	0	0	0	0.00 1
<i>Haemophilus parainfluenzae</i>	12	3	1	33.3	8.3	0.11 0.186
<i>Candida spp (n.n.k.)</i>	31	4	1	25.0	3.2	0.02 1
<i>Candida albicans</i>	33	0	0	0	0	
<i>Aspergillus fumigatus</i>	26	1	1	100.0	3.8	0.06 0.143
<i>Penicillium</i>	2	1	0	0	0	0.00 1

A Kappa (K) value of 0 indicates only chance agreement, and a value of 1 indicates complete agreement.

* local p-values are given; after Bonferroni procedure only local p-values <0.0015 are statistically significant for the global alpha of 0.05.

Haemophilus influenzae (H.i.) was found in 9.9% (18) of LAW and 6.0% (11) of UAW-samples and in 6 of these, *H.i.* was detected in both compartments. *Aspergillus fumigatus* was recovered in 14.3% (26/182) LAW-cultures, whereas it grew in only one UAW-sample that had been retrieved from a patient who simultaneously had a positive LAW culture. *Candida albicans* was found in 18.1% (33) of the LAW-cultures but in none of the UAW-samples.

P. aeruginosa

History of chronic colonization of the airways with *P. aeruginosa* was defined according to Kerem et al. [18] as having at least 50% of *P.a.*-positive LAW-samples during a period of 12 months. 63 of 182 (34.6%) patients fulfilled the criteria of history of chronic colonization with *P.a.*

P.a. could be identified in upper airway-samples from 29 (46.0%) of the 63 patients with a history of chronic *P.a.*-colonization of the LAW. At the same time, the bacterium was found in 90.5% (57/63) of the LAW-cultures from these patients. The proportion of patients with detection of *P.a.* in both airway segments increased with age [13] (fig 2).

In one patient with history of chronic pulmonary colonization *P.a.* only could be detected in the nasal lavage but not in sputum. *P.a.* was 88-fold more likely detectable in a UAW-sample from a LAW-*P.a.* positive than from a LAW-*P.a.* negative subject (95% confidence-interval (CI): 11.5–667.5).

S. aureus

S.a. was recovered in 55 UAW (30%) and 64 (35%) LAW specimens. *S.a.* isolates of 4/64 LAW specimens were *methicillin-resistant S.a. (MRSA)*. Samples from 41/64 (64%) patients were positive for *S.a.* in both the UAW and LAW specimens. Samples from 14 patients were only positive for *S.a.* in the UAW specimen, while samples from 23 patients were only positive in LAW specimens. *S.a.*-colonization of both airway sites was found in all age groups (fig 3). A statistically significant association for *S.a.*-carriage in both airway sites was found ($\Phi=0.568$, $p<0.001$). The Odds-ratio analysis revealed a LAW-*S.a.* carrier to be 15.4fold more likely *S.a.*-positive in the UAW than a LAW-*S.a.* negative subject (95% CI: 7.1–33.4).

Patients with a history of chronic *P.a.*-colonization less frequently carried *S.a.* in the upper and lower airways. *S.a.* was found in the upper airways of 14.3% (9/63) of the patients with chronic *P.a.*-colonization of the LAW, but in 38.7% (46/119) of the patients without chronic *P.a.*-colonization. Accordingly, the Odds ratio was 3.8 higher for nasal *S.a.*-detection in *P.a.*-negative patients (95% CI: 1.7–8.4).

Genetic relatedness of *P.a.* and *S.a.* strains detected in the UAW and LAW

P.a. strains of 29 patients were typed in 16 informative SNPs of the core genome [14]. Isolates from both UAW and LAW were available from 24 patients (Lower airways samples: 21 expectorated sputum and 3 deep throat-swabs. Upper airway samples: all 24 obtained by NL). Strain pairs with identical *P.a.* SNP-marker genotype had been retrieved from the UAW and LAW specimens of 23 patients. Distinct *P.a.* genotypes had been recovered from the UAW and LAW of only one patient (fig 4a). The isolates were classified into 21 different genotypes including eight common genotypes that each make up more than 1% of the *P. aeruginosa* population [14]. Seven genotypes of this study had not been observed before in our collection of more than 2,000 strains that represent 180 independent clones.

S.a. isolates from 36 patients were further analyzed. The isolates were cultured from nasal swabs (n=30) or nasal lavage (n=14) representative for the UAW or from deep throat swabs (n=24) or sputum (n=12) representative for the LAW. All *S. a.* isolates were subjected to *spa* typing [15] that does not only allow comparing the *S. a.* isolates within the same patient but also between different patients and between different centres. In most patients, the isolates from the UAW and LAW were genotypically identical (31/36; 86.1%) (fig 4 b). More than one *S.a.* genotype was retrieved from throat swabs of two patients. The isolates of 36 patients revealed 37 different *spa* types. Only 2 of 3 *MRSA*-couplets from both airway segments were available for typing. They were found to be genotypically identical in upper and lower airways for each patient.

Assessment of different methods to sample the UAW and LAW

Comparison of different methods to sample material from the UAW of 61 patients revealed remarkable discrepancies in the rate of *P.a.* positive cultures from patients with a history of chronic pulmonary *P.a.*-colonization. *P.a.* was detected in 56% of nasal lavages (25/45, $\Phi = 0.648$, $p < 0.001$), 29% of deep nasal swabs (11/38, $\Phi = 0.469$, $p < 0.001$) and 10% of blowing samples (1/10, $\Phi = 0.182$, $p = 0.667$). In case of the LAW specimens, *P.a.* was detected in 51 of 56 sputa and 6 of 7 deep throat-swabs (fig 5).

Of the 53 patients whose UAW were sampled simultaneously with nasal lavage and nasal swab, 7 patients were *P.a.*-positive with both methods, 4 patients *P.a.*-positive only with lavage and one patient only with nasal swab.

DISCUSSION

Chronic rhinosinusitis is a hallmark of CF [6-9]. The local symptoms frequently impair the quality of life, but – more importantly – chronic rhinosinusitis may remodel the UAW to become a niche for opportunistic pathogens that during periods of damage of the oropharyngeal barrier may descend into the lungs.

This study simultaneously sampled the UAW and LAW of CF patients with non-invasive methods. The cultured bacteria and fungi were classified into 30 groups (Table 2). In 50% of cases, the same microorganism was also recovered from the lungs if it had been cultured from the UAW. A few pathogens were predominantly recovered from only one habitat. *Coagulase-negative Staphylococci*, *Corynebacteria* and *Moraxella spp.* were frequently cultured from the UAW, but infrequently from the lungs in accordance with their preponderance in the nasopharyngeal tract as their natural niche in healthy carriers [19]. On the other hand, opportunistic pathogens [19] that typically are absent in the commensal flora of healthy individuals were more frequently cultured from CF LAW than from CF UAW. Examples are *Candida* and *Aspergillus*, *Haemophilus influenzae* and *Haemophilus parainfluenzae*, *S. maltophilia* and the leading pathogen in CF, *P. aeruginosa* (Table 2).

Mucus retention and insufficient aeration of the paranasal sinuses, and nasal polyps and congestion are obligatory symptoms in CF. Hence, one may wonder why the reasonable and fundamental question whether the UAW are reservoir and gateway for microbial colonization in CF has so far been addressed in only few studies [20-22]. The anatomical barriers to retrieve samples are one obvious reason. The direct assessment of the paranasal sinuses requires quite invasive methods such as endoscopy-guided sinus aspiration [23], sinus puncture or intraoperative sampling [20], which are unsuitable for routine clinical management. Thus, this study evaluated an alternative option of non-invasive methods to sample the UAW for bacteriological analysis. The detection of *P.a.* in UAW specimens retrieved from patients with chronic airway colonization with *P.a.* was taken as the standard for comparison. Recovery of *P.a.* from LAW specimens (sputum or deep throat swab) was about 90%. Nasal lavage turned out to be the method of choice for sampling the UAW with a recovery rate of close to 60% *P.a.*-positive cultures. If subjects were unable to perform the manoeuvre, blowing samples or deep nasal swabs were taken. However, these alternative procedures were not sensitive enough and therefore have to be classified as inappropriate.

The sensitivity of nasal lavage can probably be improved with the subject's increasing experience in sampling. Although we provided meticulous visual and oral instructions to each study subject, some patients did not adhere to the protocol. Instead of rinsing the full volume of 10 mL of saline, they just applied portions of 2-3 mL having their head in the forward position. Supposedly these individuals predominantly rinsed the nasal vestibulum, but not the nasal conchae with the orifices of the paranasal sinuses. In conclusion, nasal lavage seems to be a promising technique for sampling the UAW in clinical practice. The subject needs to be trained as it is necessary for lung function testing. Since both methods require the subject's cooperation, they are not suitable for young children. However, it is just this group of young minors who would benefit most from an early detection of microbial pathogens in the UAW prior to their descent into the LAW. In case of the major pathogen *P.a.* numerous studies have convincingly demonstrated a 80% success rate of the eradication of *P.a.* from the airways [5, 24] during the stage of early colonization prior to bacterial adaptation to and diversification in the niche [25, 26]. Considering the impact for the prevention and therapy of infection, the protocols of UAW sampling for bacteriological analysis, particularly for the risk group of young children, should be further optimized in prospective trials and then introduced into the management of CF.

S.a. and *P.a.* are the dominant pathogens in CF [1, 3, 26]. Correspondingly, the major goal of our multicentre study was the analysis of the genetic relatedness of *S.a.* and *P.a.* strains that had been retrieved at the same time from the UAW and LAW of a CF subject. To address this issue, the currently most informative molecular typing methods for *S.a.* [15] and *P.a.* [14] were applied. The outcome was unequivocal. 86% of the *S.a.* strain pairs and 95% of the *P.a.* strain pairs were genotypically identical. In other words, the majority of *S.a.* carriers and almost all *P.a.* carriers are harboring the same clones in the upper airways and the lungs. Therefore, the CF patient's UAW and LAW are colonized with the same populations of *S.a.* and *P.a.*

This major finding based on the prospective analysis of a CF cohort of all age groups and grades of disease severity is supported by the few published data on preselected patient subgroups [20-22]. Walter *et al.* [22] typed LAW *P.a.* isolates from patients prior to and after lung transplantation. Within a few months, the *P.a.*-free donor lungs became colonized with the *P.a.* genotype of the explanted lungs. The authors concluded that the patients' UAW were the reservoir for the colonization of the lung transplant. During end-stage CF pulmonary disease UAW and LAW were colonized with the same *P.a.* clones. This conclusion was found to be also true for less advanced stages of lung disease. Taylor *et al.* [21] identified the same *P.a.* macrorestriction fragment genotypes in sputa and UAW samples from adults with CF. Recently, Muhlebach *et al.* [20] compared the bacteriology of the sinus with that of oropharyngeal swabs and bronchoalveolar lavage fluid (BALF) in children with CF. Samples were taken during operation of subjects requiring sino-nasal surgery. If *S.a.* or *P.a.* were isolated from both the sinus and the LAW specimen, the concordance of genotypes was 83%. This figure corresponds reasonably well to our data on LAW and UAW strain pairs retrieved by non-invasive procedures.

Considering the possible role of the UAW as a reservoir for lung infections with the major CF pathogens that has been demonstrated by Muhlebach's and our studies, future comparative investigations on the recovery of culturable microbes by invasive and non-invasive sampling of the UAW will be of high interest. If the current protocol of nasal lavage or a modified version would achieve similar detection rates to the direct analysis of sino-nasal tissue, the nasal lavage could become a routine measure for the bacteriological surveillance in CF and a valid surrogate parameter for antimicrobial intervention trials. Lastly, our results disprove earlier claims [27, 28] that the sino-nasal compartment is no reservoir for the descending infection to the lung in CF. On the contrary, as several authors had postulated previously [21, 29-31] the UAW appear to play a role in the acquisition and persistence of opportunistic bacteria in CF which requires further investigation [34].

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FOOTNOTES:

Contributors:

JM was principal investigator. LN and MK conducted and supervised the study in participating CF-centres. SM and GS were co investigators in participating ENT units. JM contributed to the concept and design of the study together with BT, JFB, IS and MS. BT, BCK, LW, NC and WP performed the microbiological analyzes including genotyping of *P.a.* and *S.a.*-strains. BT, BCK, IS, MS and BW contributed to the analysis and interpretation of data.

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Competing interests:

All authors confirm that they are not involved in any organisation or entity with a financial interest in or financial conflict with the subject matter or materials discussed in this manuscript.

Ethics approval:

The trial was approved by the Jena University Ethics Committee.

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Figure legends:

- 1 Method of diagnostic nasal lavage
- 2 Fraction of patients with detection of *P. aeruginosa* in the upper and lower airways in correlation to their age (n=182).
- 3 Patients with *S. aureus* in the upper and lower airways in correlation to age (n= 182).
- 4a and 4b Genetic relatedness of *P. aeruginosa* and *S. aureus* isolates from the upper and lower airways of individuals with CF.
- 5 Recovery of *P.a.* in specimens taken from the upper or lower airways of patients with a history of chronic *P.a.*-colonization of the lower airways (UAW n = 183; LAW n = 184).

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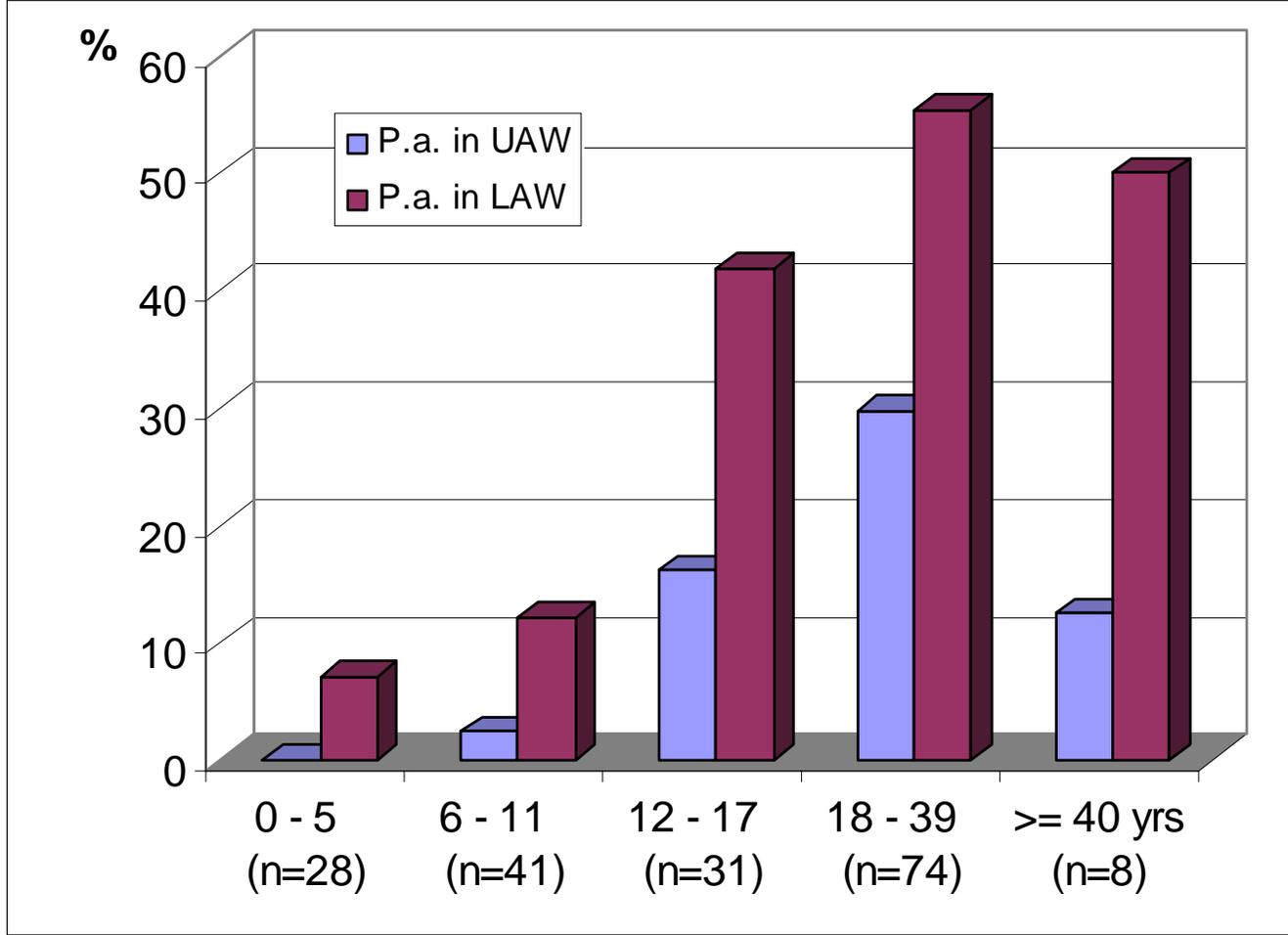


Figure 2

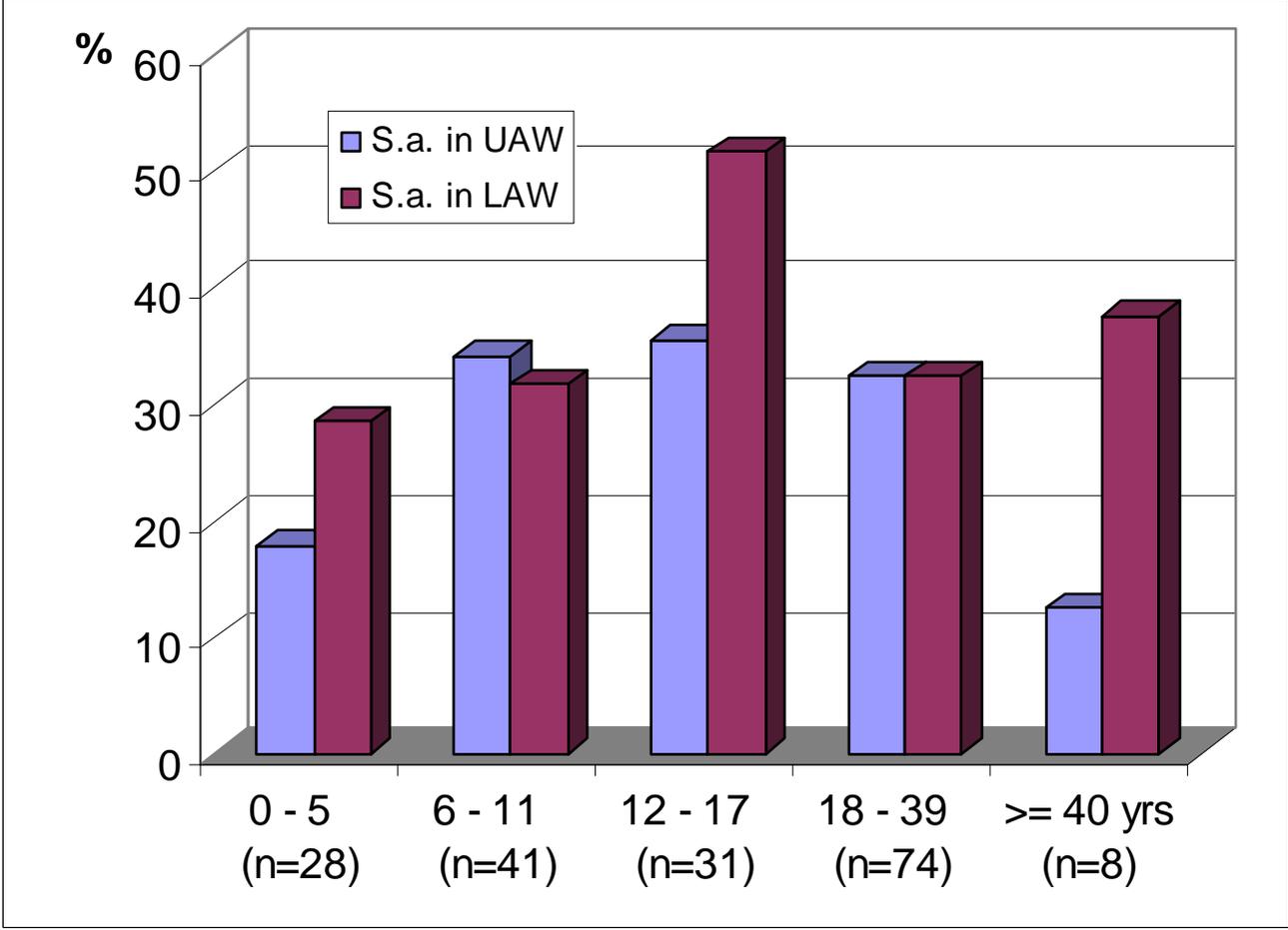
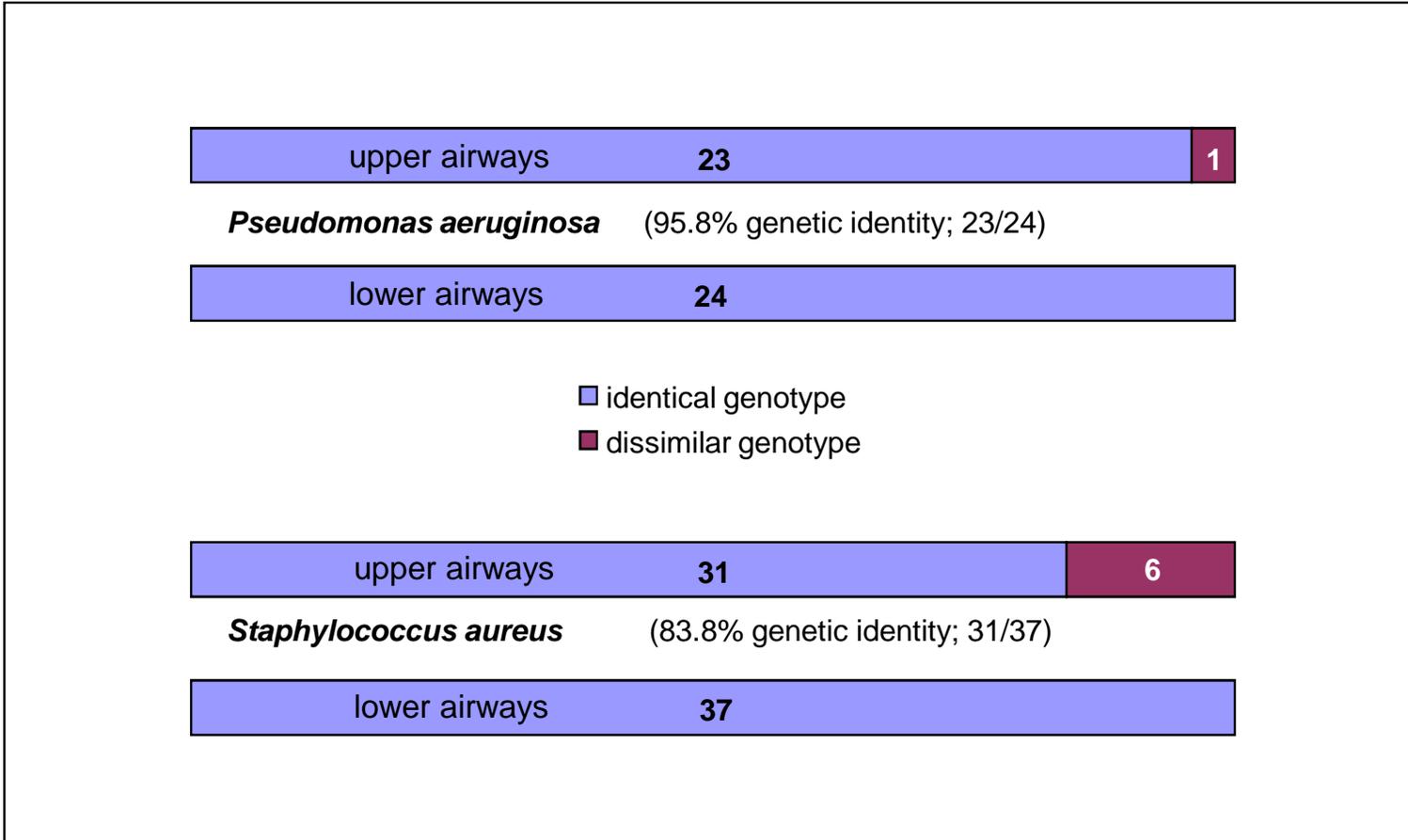


Figure 3



Figures 4a and 4b

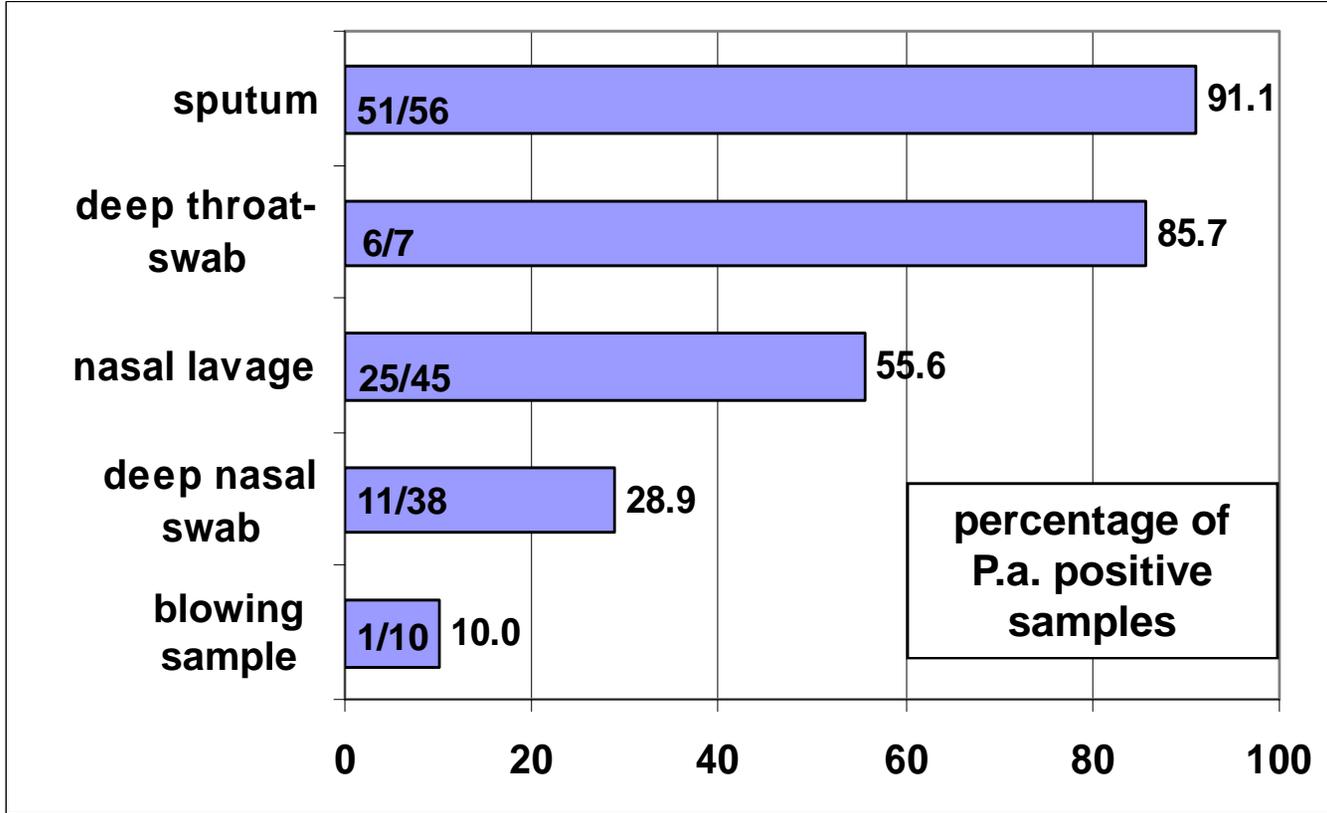


Figure 5