

Reduced effect of intravenous antibiotic treatment on sinonasal markers in pulmonary inflammation*

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Abstract

Background: Chronic bacterial rhinosinusitis is a common feature in Cystic fibrosis (CF) as mucociliary clearance in the sinonasal compartment is impaired. Aim of the present prospective study was to compare dynamics of inflammatory markers in the upper and lower airways (UAW/LAW) during systemic antibiotic therapy.

Methods: Nasal lavage and sputum of 16 CF-patients receiving an IV-antibiotic treatment against *Pseudomonas aeruginosa* and/or *Staphylococcus aureus* were collected before and during treatment (median after 7.5 days). Cytological changes, DNA concentration, and inflammatory markers interleukin (IL)-4, IL-8, IL-13 and Myeloperoxidase (MPO) were assessed in samples from both airway compartments.

Results: Total cell count declined significantly in LAW-samples but not in UAW. Although MPO and IL-8 decreased significantly in both airway compartments, this was considerably more pronounced for LAW (median decrease MPO: LAW = 9.8-fold vs UAW = 1.75-fold, respectively; IL-8: LAW = 3-fold vs UAW = 1.9-fold, respectively).

Discussion: This is the first publication demonstrating substantially lower effects of IV-antibiotic treatment on sinonasal than on pulmonary inflammatory markers. Consequently, our findings highlight limitations of systemic antibiotic treatment to control infection in the sinonasal compartment. Primarily, we attribute this to the paranasal sinus' structure: these hollow organs, which in bacterial sinusitis are frequently filled with pus, mucoceles and polyps, are not reached effectively by systemic antibiotic treatment.

Key words: Cystic fibrosis, upper airways, lower airways, nasal lavage, sputum, inflammation, cytokines, cytology

Introduction

Chronic rhinosinusitis (CRS), which is frequently associated with bacterial colonization of the upper airways (UAW) and paranasal sinuses is widespread, affecting 11% of the European and 13-17% of the US population⁽¹⁻³⁾. Direct health care costs are estimated to be about 8.8 billion USD per year, not including indirect costs generated by antibiotic resistances, antibiotic-related complications or missed workdays^(2,4). CRS

mostly affects immune competent patients and is associated to multiple factors such as air pollution, smoking or allergy^(1,5) but it is frequently associated to sinonasal pathogen colonization⁽³⁾. According to the assessment of different therapeutic strategies, incorporating the division in CRS with nasal polyposis (CRS_{NP}) and without nasal polyposis (CRS_{SNP}), there is a lack of evidence for systemic antibiotics⁽³⁾. The only exception is the long-term use of macrolides in a low dose for CRS_{SNP} based on macrolides

immune-modulatory impact rather than on their antibacterial effect ⁽³⁾.

Cystic fibrosis (CF) is interesting as a model for CRS associated to bacterial infection as CF patients' upper (UAW) and lower airways (LAW) are colonized almost regularly with pathogens. Rhinosinusitis is a hallmark of this inherited disease ⁽⁶⁻¹⁰⁾. The resulting prevalence of abnormal anatomy as seen in computer tomography reaches almost 100% while up to 50% of adult CF patients reveal nasal polyposis ⁽¹⁰⁻¹³⁾.

CF as the most frequent life threatening autosomal recessive disorder among Caucasians ⁽¹⁴⁾ is caused by a genetic defect of the cystic fibrosis transport regulator (CFTR) protein, a chloride channel across cell membranes. Altered ion composition leads to increased viscosity of secretions from exocrine glands of multiple organ systems. Regarding the respiratory system, which is most commonly affected, a consequential reduced airway surface liquid volume impairs mucociliary clearance ⁽¹⁵⁾. Mucus retention allows pathogens like *Staphylococcus (S.) aureus* and *Pseudomonas (P.) aeruginosa* to remain in the airways and cause chronic excessive airway inflammation that leads to a progressive damage of lung parenchyma as major cause of morbidity and premature mortality in about 90% of patients ^(9, 16, 17). Additionally, disorders like exocrine pancreatic insufficiency, diabetes, and hepatic involvement are frequent.

Recent studies demonstrated evidence of cross colonization among both airway compartments. In patients colonized in both airway levels with *P. aeruginosa*, Mainz et al. revealed identical genotypes in the UAW and LAW by 96% ⁽⁹⁾. This was confirmed by Johansen et al. ⁽⁸⁾ and others. Further recent studies have shown that CF patients who undergo lung transplantation regularly become re-colonized in their lung grafts from sino-nasal reservoirs. *P. aeruginosa* clones in lung transplants were genotypically identical with those cultured before transplantation in both, the UAW and LAW ^(18, 19). These findings underline the thesis that CF upper airways are a gateway and a bacterial reservoir from where *P. aeruginosa* can spread into the LAW. Furthermore, paranasal sinuses may be a niche for adaptation, diversification and the development of antibiotic resistances of *P. aeruginosa* ⁽²⁰⁾. Hence, sinonasal involvement may play a key role in enhancing and maintaining chronic airway inflammation and the resulting aggravation of the clinical course. However, there appear to be differences in the pathophysiology of both airways levels. While the inflammation in lower airways is characterized by a neutrophil domination and high levels of IgG against *P. aeruginosa*, Johansen et al. showed that biofilms in the sinuses are surrounded by only a very few neutrophils, most likely as the result of an extensive IgA response in the upper airways ⁽⁸⁾. The authors propose that non-inflammatory properties of IgA may inhibit neutrophil recruitment in CF patients' sinuses

leading to a reduced and more differentiated mucosal immune response. In contrast, the systemic inflammatory response in the LAW is characterized by neutrophil-dominated inflammation and the release of proteases and oxygen radicals ^(8, 21, 22).

The use of intravenous antibiotics to treat and/or prevent pulmonary exacerbations in CF patients is well established and one of the major reasons for the increased life expectancy during the last four decades ⁽²³⁾. Elective IV-antibiotic therapy for eradication or reduction of the pathogen burden as well as the reduction of resulting inflammation is part of the standard care in many European CF Centers ^(24, 25).

The assessment of inflammatory markers in non-invasively collected nasal lavage (NL) and induced sputum (IS) of CF patients opens a field to monitor airway colonisation, host defence, inflammation, and the effects of therapeutic approaches. While IS has been considered repeatedly in the recent literature to be a useful method ⁽²⁶⁻²⁸⁾, assessment of NL has been investigated rarely so far. NL is a well-tolerated, fast and cheap method to obtain airway surface liquid from the upper airways for microbiological and immunological issues ⁽²⁹⁾. According to the 'unified-airway-concept' a recent study by Fischer et al. showed that UAW assessment can have a predictive value for chronic pulmonary colonisation with *P. aeruginosa* ⁽³⁰⁾. Further on, Pitrez et al. discussed nasal wash as an alternative to bronchoalveolar lavage in detecting early pulmonary inflammation in children with CF ⁽³¹⁾. Paats et al. stated that the determination of interleukin (IL)-6 and IL-10 in NL may provide an assessment tool for pulmonary exacerbations whereas Hentschel et al. were able to show a significant reduction of IL-6 in the UAW during IV-antibiotic therapy, even after a median therapeutic time of 6 days ⁽³²⁾.

The aim of the present study was to investigate the changes in upper and lower airway inflammation during systemic IV-antibiotic treatment. As non-invasive methods, practicable within routine care, NL was applied for UAW sampling and IS for assessment of LAW infection and inflammation. Before and during early intravenous antibiotic therapy concentration of DNA, cytology and the expressions of the inflammatory mediators myeloperoxidase (MPO), IL-8, IL-4, IL-13, IL-17A, regulated upon activation normal T-cell expressed, and presumably secreted protein (RANTES or CCL5) as well as human soluble intercellular adhesion molecule-1 (sICAM or sCD54) were assessed and compared for both airway compartments.

Patients, materials and methods

Patients

Sixteen adult CF patients were recruited prospectively between August and December 2011, either during their stay at the University Hospital of Jena or during regular visits in the CF

outpatient clinic. Inclusion criteria were an established diagnosis of CF based on two sweat tests and/or the recorded presence of two disease causing CFTR mutations as well as an intravenous antibiotic therapy planned to improve the clinical course of the patient. Written informed consent was obtained by patients before enrolment. The study was approved by the local ethics committee.

Nasal lavage (NL)

NL was performed as previously described⁽⁹⁾ using 10 mL of sterile saline (0.9 % NaCl, Braun, Melsungen, Germany) per nostril. Processing was performed as described by Fischer et al.⁽³⁰⁾. An aliquot was directly sent to microbiological analysis. After centrifugation the pellet was used for cytological analyses and the supernatant was stored in aliquots at -80°C with protease inhibitors (Protease Inhibitor Mix G, SERVA Electrophoresis GmbH, Heidelberg, Germany) for immunological approaches.

(Induced) Sputum (IS)

Sputum was collected by spontaneous coughing or after

induction by inhalation of 6% hypertonic saline (PARI GmbH, Starnberg, Germany). One aliquot was sent to microbiological analysis. Sputum was 1:5 weight-related diluted with sterile PBS, and homogenized by aspirating 20 times with an 18 Gauge canula followed by additional 1:4 dilution using 0.1% Sputolysin (Merck, Darmstadt, Germany) and vortexing. The sputum was filtered with a 40µm cell strainer to remove debris and mucus. 1ml of filtrated sputum mixed with 100µl FCS was sent to cytological analysis. The remaining volume was centrifuged (400rpm, 7min, room temperature) and aliquots of the supernatant were stored at -80°C with protease inhibitors.

Microbiological, cytological, DNA and mediator analysis

Before IV-treatment nasal lavage and sputum were examined microbiologically according to European standards⁽³²⁾. Physiological flora of the nasopharynx was considered as described recently⁽³⁰⁾. The definition of permanent and intermittent colonization (50% or more of cultures and less than 50% of cultures within the preceding 12 months positive for a pathogen) was applied according to the criteria published by Lee et al.⁽³³⁾.

Table 1. Clinical and serological characteristics of included patients (metric and ordinal variables).

Metric and ordinal variables	n	mean	SD	median	Range
Age (yr)	16	26.25	4.597	25.0	21-37
Weight (kg)	16	56.84	9.5	55.95	45.3-72.0
Height (cm)	16	160.56	8.97	167.5	156-186
BMI	16	19.9	2.26	19.45	16.6-23.9
ESR after 1/2 h (mm/h)	13/5	35.46/ 49.20	25.44/ 37.79	45/41	2-80/8-90
CRP (mg/l)	16	25.14	46.05	8.85	2.0-190.6
Leukocytes (Gpt/l)	16	12.25	4.16	10.8	6.6-21.7
Total IgG (g/l)	13	17.52	5.03	18.0	10.5-26.4
Total IgM (g/l)	12	1.58	0.36	1.59	1.09-2.22
Total IgA (g/l)	12	3.33	1.77	2.92	1.09-6.58
Total IgE (kU/l)	12	248.5	260.9	145.0	7.17-672
FEV1 (l)/ (% predicted)	15	1.77/ 50.45	0.83/ 24.73	1.74/42.6	0.77-3.22/ 24.0-109
IVC (l)/ IVC-P (% predicted)	15	2.84/ 67.01	0.99/ 20.16	2.44/ 60.0	1.57-4.66/ 41.0-112.0
MEF25 (l) / MEF25-P (% predicted)	15	0.47/ 20.89	0.35/ 16.7	0.32/13.1	0.11-1.4/5.0-70.0
MEF75/25 (l)/ MEF7525-P (% predicted)	15	1.03/ 23.97	0.76/ 18.91	0.69/16.0	0.24-2.85/ 6.0-74.0
RV (l)/ RV-P (% predicted)	15	3.19/ 210.67	1.17/ 71.28	3.02/219.0	1.29-4.96/ 99.0-324.0
Retrieved NL volume prior to therapy (ml)	16	9.15	2.08	9.25	
Retrieved NL volume during/after therapy (ml)	16	9.94	2.19	10.50	

Table 2. Clinical and serological characteristics of included patients (nominal variables).

Nominal variables	n	Absolute frequency
gender (f/m)	16	9 (56.3 %) / 7 (43.7 %)
nasal polyps	16	4/16 (25%)
history of sinonasal surgery:		
once	16	7/16 (43.8%)
3 times		2/16 (12.5%)
adenotomy		1/16 (6.3%)
CRS	16	7/16 (43.8%)
exocrine pancreatic insufficiency	16	13/16 (81.3%)
diabetes mellitus	16	9/16 (56.3%)
hepatopathy	16	3/16 (18.8%)
allergic sensitisation to:		
<i>Aspergillus fumigatus</i>	16	11/16 (68.8%)
house dust mite		4/16 (25%)
grass pollen		2/16 (12.5%)
pet hair		2/16 (12.5%)
IgG increased		8/13 (61.5%)
IgM increased	12	0/12 (0%)
IgA increased	12	3/12 (25%)
IgE increased	12	7/12 (58.3%)
Therapy:		
nasal/topical:		
current use of decongestant nose drops		1/16 (6.3%)
current nasal topical steroids		4/16 (25%)
bronchial:		
inhalative anticholinergics	16	1/16 (6.3%)
inhalative NaCl (0.9% or 6%)		11/16 (68.8%)
current inhalative antibiotics		11/16 (68.8%)
inhalative DNase		8/16 (50%)
systemic:		
current oral antibiotics		7/16 (43.8%)
current azithromycin		14/16 (87.5%)
current antimycotics		7/16 (43.8%)
current mucolytics		13/16 (81.3%)
current systemic steroids		2/16 (12.5%)

By using fluorescence flow cytometry (Sysmex XE-5000, Sysmex Deutschland GmbH, Norderstedt, Germany) total cell count (TCC) and percentages of polymorphonuclear- (PMN) and mononuclear (non-PMN) cells were determined. For differential cell count based on morphology (100 cells) cell pellets were prepared using cytospin (100xg, 3min) and May-Gruenwald staining technique.

Levels of extracellular DNA were determined in native nasal

lavage fluid (NLF) and sputum supernatant at 260 nm using NanoDrop (NanoDrop ND 1000, PEQLAB, Erlangen, Germany.) For immunological analysis IS supernatant was diluted 1:10 with sterile PBS. As recently described ⁽⁶⁾ concentrations of IL-4, IL-8, IL-17A, IL-13, RANTES, MPO and sICAM were measured by using a Cytometric Bead Array (FlowCytomix Human Simplex Kits, eBioscience, San Diego, USA) and a flow cytometer (FACSCalibur, BD Biosciences, Franklin Lakes, CA, USA). Results of the analysis were appraised with the FlowCytomix Pro 2.4 software.

Statistics

Biometrical analysis was performed with MS Excel 2010, IBM SPSS Statistics 20 and GraphPad Prism 5. Dilutions made during procession and analysis of IS were subtracted to obtain values for the native material as obtained from the patient. Concentrations below the detection limit assessed by Cytokine Bead Array were defined as 0.1 ng/ml below detection limit (0.1 µg/ml, respectively). Longitudinal changes of DNA-content, cell counts and inflammatory mediators were assessed with the Wilcoxon-test for matched pairs while correlations were tested with Spearman's-rho-test for both dates (before and during therapeutic intervention). A value of $p < 0.05$ was considered statistically significant. Mann-Whitney-U-Test was performed to compare different groups and odds ratios (OR) were calculated for quantifying how strongly permanent colonization with *S. aureus* in both airway compartments are associated to each other. Using descriptive statistics clinical characteristics of the study population and test results were pictured.

Results

Demographic data

The mean age of the 16 CF-patients (9 female/7 male) recruited for this prospective study was 26.25 years (range 21-37, SD 4.6). Eight patients were homozygous for genotype F508del, 7 heterozygous for F508del and one patient homozygous for genotype CFTRdele2,3. In the heterozygous patients class 1-3 mutations (R553X, N1303K) and class 4-5 mutations (R347P, 2789+5G->A) were found in 2 and 6 patients, respectively. In all but one patient, IV-antibiotic therapy was conducted over 14 days. For clinical reasons this one patient underwent a 28-day treatment. However, the present trial mostly assessed changes within early treatment period by median after 7.5 days (range 3-28 days). Treatment was either started at our CF outpatient clinic, or in hospital and continued as home treatment for most of the patients (11 of 16). Therefore, median hospitalization time resulted in 4 days (range 0-14).

Therapy was directed against *P. aeruginosa* in 15 patients and additionally against *S. aureus* (6 patients), *Haemophilus (H) influenzae* (1 patient) and *Burkholderia (B) cepacia* (1 patient). In one patient it was directed only against *S. aureus*.

Most frequently patients were treated with a double antibio-

Table 3. Microbiological characteristics of included patients (metric and ordinal variables).

Nominal variables	n	Absolute frequency
UAW: detection of pathogenic bacteria and/or fungi before AB		13/16 (81.3%)
<i>P. aeruginosa</i>		9/16 (56.3%)
non-mucoid		3/16 (18.8%)
mucoid		6/16 (37.5%)
multiresistant		2/16 (12.5%)
intermittent		7/16 (43.8%)
permanent		6/16 (37.5%)
<i>S. aureus</i>	16	6/16 (37.5%)
intermittent		5/16 (31.3%)
permanent		3/16 (18.8%)
<i>Enterococcus faecalis</i>		2/16 (12.5%)
<i>Proteus mirabilis</i>		1/16 (6.3%)
<i>E. coli</i>		1/16 (6.3%)
<i>Citrobacter</i>		1/16 (6.3%)
<i>Sphingomonas</i>		1/16 (6.3%)
LAW: detection of pathogenic bacteria and/or fungi before AB		15/16 (93.8%)
<i>P. aeruginosa</i>		13/16 (81.3%)
non-mucoid		3/16 (18.8%)
mucoid		10/16 (62.5%)
multiresistant		4/16 (25%)
intermittent		1/16 (6.3%)
permanent		12/16 (75%)
<i>S. aureus</i>	16	7/16 (43.8%)
intermittent		5/16 (31.3%)
permanent		4/16 (25%)
Enterobacteriaceae		3/16 (18.8%)
<i>E. coli</i>		1/16 (6.3%)
Yeasts (<i>Candida</i> etc.)		4/16 (25%)
<i>Aspergillus fumigatus</i>		3/16 (18.8%)
<i>Aspergillus flavus</i>		1/16 (6.3%)
<i>Sphingomonas</i>		1/16 (6.3%)
Corynebacteriaceae		1/16 (6.3%)
<i>Haemophilus parainfluenza</i>		1/16 (6.3%)
<i>Burkholderia cepacia</i>		1/16 (6.3%)

According the criteria published by Lee et al. ⁽³⁴⁾ "permanent colonization" was defined as at least 50 % of cultures within the last year were found positive and "intermittent" as less than 50 % of cultures within the last year were found positive.

tic combination that contained meropenem and tobramycin (14/16). Adjusted to clinical conditions, infection status and resistance situation, IV-therapy was modified or supplemented with antibiotic agents as ceftazidim (1/16), ciprofloxacin (1/16), piperacillin-tazobactam (1/16) and vancomycin (1/16). 25% (4 patients) of our study population were affected by nasal polyps while 11 patients (68.8%) already had a history of sinonasal surgery (9 patients had a polypectomy, one patient an adenoidectomy and one patient surgery of the nasal septum done). Furthermore, CRS criteria according to the EPOS 2008

NL recovery volumes

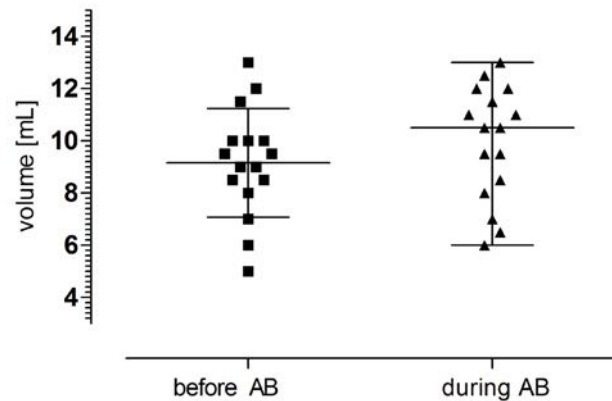


Figure 1. NL backflow volume after IV-antibiotic treatment was slightly higher than the retrieved volume of NL before therapeutic intervention (median 10.5 mL, range 6.00-13.00, and median 9.25 mL, range 5.00-13.00, respectively).

criteria ⁽³³⁾ were fulfilled by 7 patients (43.8%).

As concomitant CF manifestation of other organ systems exocrine pancreatic insufficiency was present in 13 patients (81.3%), diabetes mellitus and hepatopathy in 9 (56.3%) and 3 (18.8%) patients, respectively. Allergic sensitization was frequent in our study population, mostly to *Aspergillus fumigatus* (11 patients, 68.8%). Moreover, house dust mite (1/16, 6.3%), grass pollen (2/16, 12.5%), pet hair (2/16, 12.5%) and cows' milk protein (1/16, 6.3%) were represented as allergens. Detailed clinical and serological data are presented in Tables 1 and 2.

Serological data

Systemic inflammation parameters like erythrocyte sedimentation rate (ESR) were elevated before the start of IV antibiotic treatment in 76.9% (10/13, median 1h 45, range 1h 2-80), CRP in 62.5% (10/16, median 8.85, range 2.0-190.6), and leucocytes in 43.8% (7/16, median 10.8, range 6.6-21.7) of patients. IgG was found elevated in 61.5% of patients (8/13, median 18.00 g/l, range 10.5-26.40 g/l), IgA in 25% (3/12, median 2.91 g/l, range 1.54-6.58 g/l), and IgE in 58.3% (7/12, median 145.00 kU/l, range 7.17-672.0 kU/l). IgM was within the normal range in all 16 patients (median 1.59 g/l, range 1.09-2.22 g/l).

Methodological issues

NL backflow volume after IV-antibiotic treatment was slightly higher than NL recovery before therapeutic intervention (median 10.5 mL, range 6.00-13.00, respective median 9.25 mL, range 5.00-13.00, (Figure 1), but differences did not reach statistical significance ($p = 0.49$).

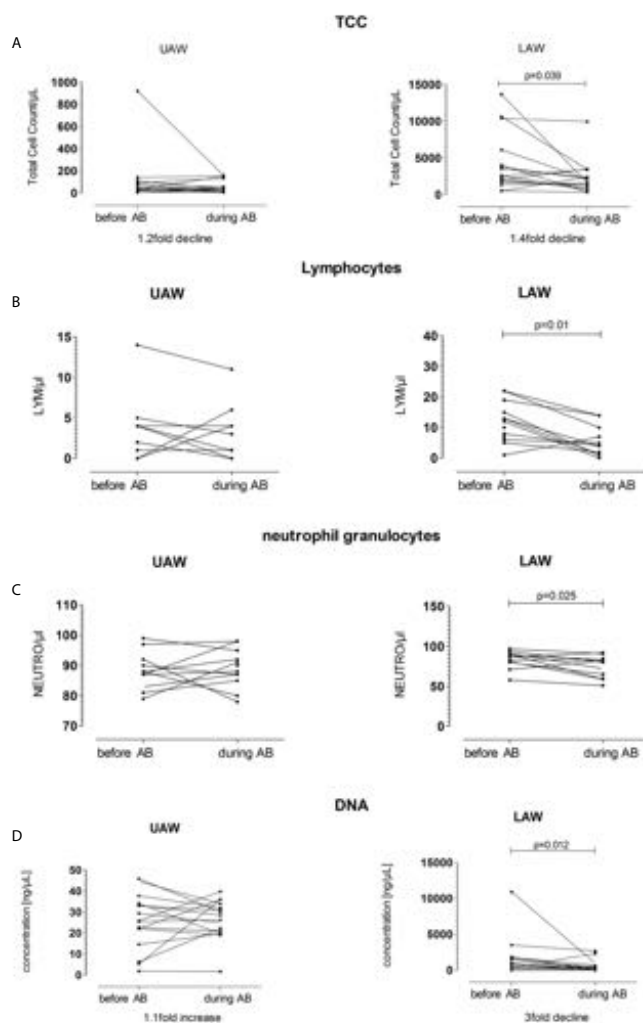


Figure 2. Differences in changes of total cell count and DNA concentration in UAW and LAW. In UAW declines of TCC did not reach statistical significance. Median values declined 1.2-fold during IV therapeutic intervention (A). TCC in LAW declines significantly ($p = 0.039$) during IV-antibiosis. Median values declined 1.4-fold (B). No significant changes of DNA concentration in UAW during IV-antibiosis. Median values increased slightly (1.1-fold, C). Significant decline of DNA concentrations in LAW during IV-antibiosis ($p = 0.012$), median values decline 3-fold (D).

Cytological analysis in NL and sputum

Total cell counts (TCC) and distinction of polymorphonuclear neutrophils PMN and monomorphonuclear leucocytes (MN, resp. non-PMN) was performed for all 16 patients on both dates, and differential cell count for 11 patients. Changes for UAW cytology and significant declines for LAW are shown in Table 4 and Figure 2. Further on, changes correlated with changes of IL-8 LAW ($r = 0.547$, $p = 0.028$) and with the decline ($r = 0.507$, $p = 0.045$).

DNA

During the therapeutic intervention, DNA concentration decre-

ased significantly in LAW ($n = 16$, before IV-AB: median: 817.99 ng/ μ l; during IV-AB: median: 275.14 ng/ μ l; $p = 0.012$), whereas only a slight, but not significant, increase was found in UAW samples ($n = 16$, before IV-AB: median: 25.75 ng/ μ l; during IV-AB: median: 26.45 ng/ μ l; $p = 0.86$).

Microbiological analysis

Before AB treatment pathogenic bacteria and fungi were detected in 13/16 (81.3%) and 15/16 (93.8%) patients for UAW resp. LAW. Distributions and details are shown in Table 3. The most frequently detected pathogen was *P. aeruginosa* (UAW: 56.3% LAW: 81.3%) followed by *S. aureus* (UAW: 37.5%, LAW: 43.8%). Permanent colonization of *P. aeruginosa* for UAW resp. LAW was found in 37.5% and 75% and of *S. aureus* in 18.8% and 25% of patients, respectively. In our cohort, the risk of permanent *P. aeruginosa* colonization in LAW was increased by a factor of 6.6 (CI = 95%: 0.2908-149.7819, $p = 0.236$) if UAW were also chronically infected. Odds for consistent *S. aureus* infection in LAW in case of permanently infected UAW are increased by factor 58.3 (CI = 95%: 1.9215-1770.9393, $p = 0.019$). Further on, in LAW overall concentrations of MPO ($p = 0.025$, mean rank 9.77) and IL-8 ($p = 0.039$, mean rank 9.65) revealed to be significantly higher in *P. aeruginosa* positive samples than in non-colonized patients (MPO: mean rank 3.0; IL-8: mean rank 3.5) whereas median values of IL-8 (*P. aeruginosa* positive 16287.03 pg/ml, *P. aeruginosa* negative 7419.14 pg/ml) and MPO (*P. aeruginosa* positive 4359.95 ng/ml, *P. aeruginosa* negative 3079.42 ng/ml) showed only a trend toward significance (MPO: $p = 0.055$, IL-8: $p = 0.055$).

Changes of inflammatory markers during intravenous anti-biotic therapy

Concentrations of MPO and IL-8 significantly decreased in both, the UAW and LAW after antibiotic therapy of 7.5 days (MPO-UAW: $p = 0.011$, IL-8-UAW: $p = 0.008$, respectively MPO-LAW: $p = 0.003$, IL-8-LAW: $p = 0.003$). Interestingly, the decline was much less pronounced in the UAW compared to the LAW: median values for IL-8 declined 1.9-fold in UAW, respectively, 3-fold in LAW (Figure 3). MPO in NL declined 1.8-fold, respectively, 9.8-fold in LAW (Figure 3). The same tendency applies to IL-13 ($p = 0.285$) in UAW, while IL-4 showed a trend to increase ($p = 0.625$). In LAW detection frequencies were below the detection limit for IL-17A and sICAM while RANTES could be detected only in very low frequencies being insufficient for any statistical assessment. During antibiotic intervention, decreases of IL-8-UAW and neutrophil granulocytes UAW correlated significantly ($r = 0.653$, $p = 0.029$, $n = 11$) as well as the dynamics of IL-13-UAW and IL-4-UAW ($r = 0.816$, $p < 0.001$). An inverse correlation was found between the declines of TCC-UAW and IL-13-UAW ($r = -0.592$, $p = 0.016$). In addition, we detected a significant correlation between the decreases of MPO-LAW and IL-8-LAW ($r = 0.579$, $p = 0.019$). Moreover, we observed a correlation between declines of

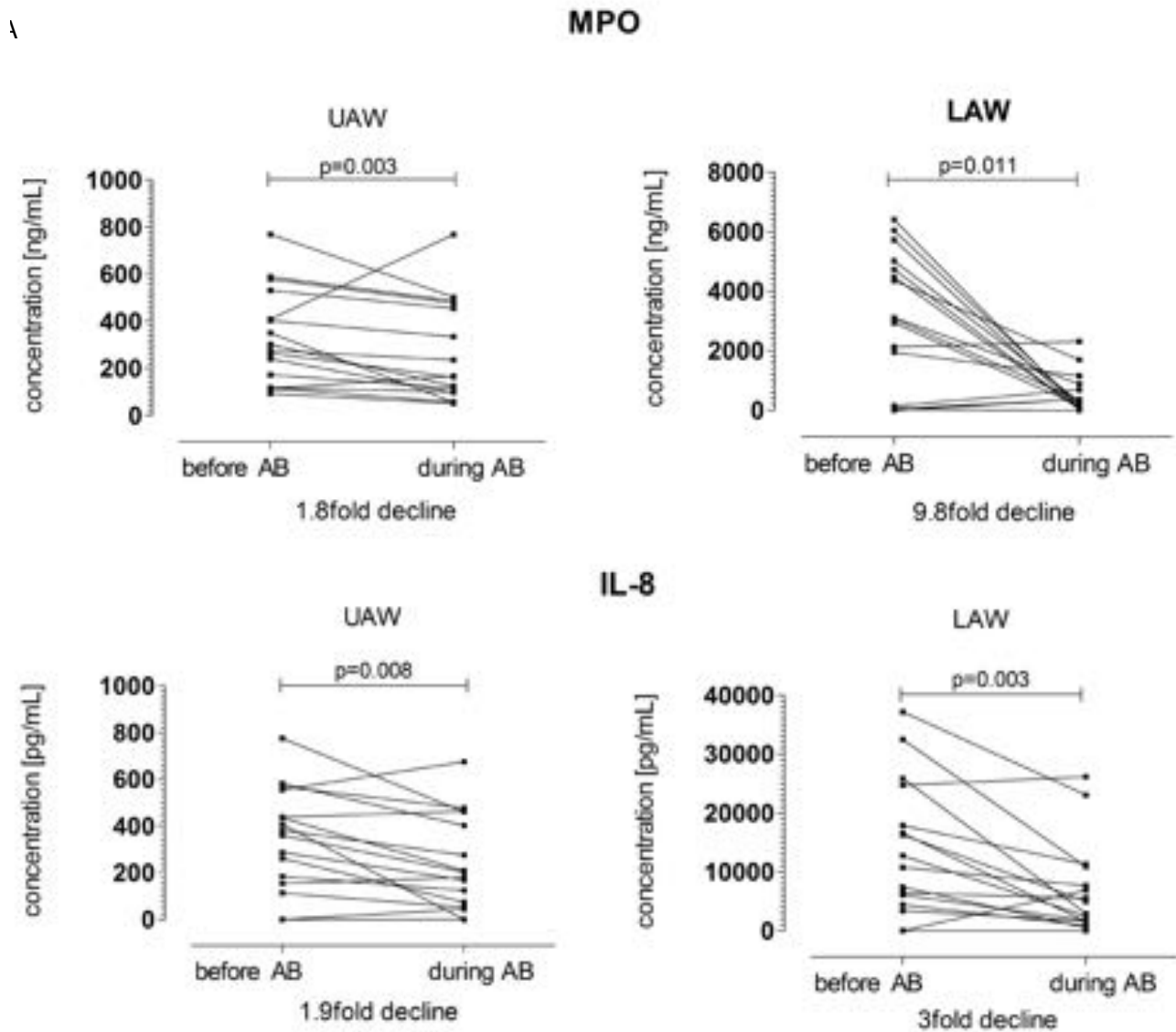


Figure 3. Differences of IL-8 and MPO in upper and lower airways before and during IV-antibiotic treatment. A) MPO declines significantly in the UAW ($p = 0.003$) and in the LAW ($p = 0.011$). However, median decline only results 1.8-fold in the UAW but 9.8-fold in the LAW. B) IL-8 also decreases significantly in UAW ($p = 0.008$) and in the LAW ($p = 0.003$). Thereby, median values decline 1.9-fold in the UAW and 3-fold in the LAW.

MPO-LAW and MPO-LAW and activated monocytes LAW, respectively, IL-8-LAW and activated lymphocytes LAW ($r = 0.679$, $p = 0.022$, $n = 11$, respectively, $r = 0.632$, $p = 0.037$, $n = 11$).

Correlation of inflammatory markers before and during AB

Before and during AB treatment IL-8-UAW correlated significantly with MPO-UAW ($n = 16$, $r = 0.64$, $p = 0.008$, respectively, $r = 0.646$, $p = 0.007$). IL-8-UAW correlations with TCC-UAW ($n = 16$, $r = 0.52$, $p = 0.039$) and eosinophil granulocytes UAW ($n = 12$, $r = 0.777$, $p = 0.003$) could be shown only prior to therapy. Further on, correlations between IL-4-UAW and IL-13-UAW were found before, respectively, during AB therapy ($n = 16$, $r = 0.806$, $p = 0.00016$, respectively, $r = 0.715$, $p = 0.002$). Changes of IL-8-LAW

did correlate with changes of MPO-LAW ($r = 0.741$, $p = 0.001$) and DNA-LAW ($r = 0.693$, $p = 0.003$).

Discussion

The relevance of pathogen related rhinosinusitis in general health and its financial consequences have been massively underestimated. Additionally, resulting antibiotic treatments are related to complications and relevantly contribute to development of antibiotic resistances. Thereby antibiotic treatment of bacterial rhinosinusitis empirically was shown to require prolonged periods of therapy, but this issue has not been understood sufficiently.

Our results from a cohort of cystic fibrosis patients receiving

Table 4. Cytological changes and DNA-Concentration in UAW and LAW prior and after IV AB therapy.

		before IV-antibiotic therapy		during IV-antibiotic therapy		
Sputum (LAW)	n	median	range	median	range	p-value
TCC [cells/ml]	16	2571.5	488-13651	1875	307-9923	0.039
LYM [%]	11	12.5	1-36	4	0-14	0.01
NEUTRO [%]	11	87	58-97	81	51-92	0.025
DNA [ng/μl]	16	818.0		275.1		0.012
NL (UAW)	n	median	range	median	range	p-value
TCC [cells/ml]	16	40.5	5-922	33.5	7-161	0.336
LYM [%]	11	4	0-14	3	0-11	0.539
NEUTRO [%]	11	88	79-99	89	78-98	0.650
DNA [ng/μl]	16	25.8		27.5		0.860

systemic intravenous antibiotic treatment can add knowledge to this topic. To some extent, CF could serve as a model for pathogen-related CRS as the patients almost regularly reveal upper and lower airway colonization with pathogenic bacteria and rhinosinusitis is frequent in the inherited disease⁽⁶⁻¹⁰⁾. However, it has to be taken in mind, that CF regularly is associated to impaired mucociliary clearance, unlike CRS in primary healthy individuals.

We assessed changes in cytokine expression, extracellular DNA concentration and cytological dynamics in NL and sputum of 16 CF patients after a median period of 7.5 days of intravenous antibiotic treatment directed against pulmonary colonization with pathogens like *P. aeruginosa*, *S. aureus* and/or *H. influenzae*. Most interestingly, inflammatory markers in the upper airways showed a reduced decline under this treatment, in contrast to markers in lower airway secretions. IV-antibiotic treatment significantly reduced total cell counts (TCC) in induced sputum and the proportion of neutrophils, known as the major player in LAW inflammation (p TCC = 0.039, p neutrophils = 0.025). In contrast, this therapy did not affect such a reduction in upper airway secretions (p TCC = 0.336, p neutrophils = 0.650). Whereas for the LAW our findings are in good accordance to published studies^(28, 35, 36), previously only a few investigations were performed to assess effects of IV-antibiotics on inflammation in the UAW^(29, 37). This to our knowledge is the first study to compare changes of inflammatory markers in UAW and LAW during IV-antibiotic treatment in CF.

MPO and IL-8 play an important role in CF patients' immune response to pathogen colonization in both airway compartments.

According to previous studies levels for both cytokines are increased in CF upper and lower airways, correlating significantly among each other. While IL-8 is the major neutrophil chemo-attractant derived from innate immune cells and Th1 lymphocytes after stimulation through different pathways, the neutrophil-released MPO is a well-established marker for neutrophil activity^(6, 38, 39). Despite reaching statistical significance in both airway levels, declines of IL-8 and MPO after a median of 7.5 days of IV-antibiotic treatment were much lower in the sinonasal compartment than in the LAW (MPO LAW 9.8-fold respective UAW 1.8-fold, IL-8 LAW 3-fold, respectively, UAW 1.9-fold). In accordance with previous studies of Hentschel et al.⁽²⁹⁾, respectively, Fischer et al.⁽³⁰⁾ a correlation of IL-8 and MPO levels was seen prior to and during IV-therapy in UAW. Further on, TCC in LAW correlated with IL-8 and extracellular DNA during antibiotic IV-treatment. Both, IL-8 and extracellular DNA correlated also among each other.

Extracellular DNA measured in NL and sputum originates predominantly from necrotic neutrophils and conforms neutrophil extracellular traps (NETs)⁽⁴⁰⁾. This composition of decondensed chromatin and neutrophil antimicrobial proteins degrades virulence factors and plays a role in killing of bacteria⁽⁴¹⁾. Interestingly, we observed a significant decline of DNA in sputum (p = 0.012) during antibiotic treatment, while in NL even a slight increase of DNA was measured, again supporting the hypothesis of reduced therapeutic success in the sinonasal compartment. Altogether, the present study reveals different changes of IL-8, MPO, DNA, neutrophils, and the TCC affected by IV-antibiotic therapy among the upper and lower airway levels. In the ongoing process aiming to understand similarities and differences

of both airway levels' immune responses toward colonization and infection with pathogens ^(8, 22) these results can give further hints.

Inflammatory responses in the sinonasal area appear to be reached with delayed or even with reduced effects by a systemic, intravenous antibiotic treatment compared to a systemic pulmonary therapy. Possible reasons are differences in the immune response between UAW and LAW in CF patients as reported previously ^(8, 22). Additionally, the anatomical character of paranasal sinuses leads to insufficient reachability by systemic antibiotics: the hollow organs are not as vascularised as the parenchymal lungs, and, similar to abscesses, they are often filled with purulent secretions or mucocoeles as a result of remittent or chronic pathogen colonization.

Assessment of UAW colonization has not been part of routine CF care during the last decades, according to most international consensus statements. Only recently, the UAW role in pathogen acquisition and persistence is coming into the clinical and scientific focus ^(8, 9, 17, 18, 20, 22, 30, 42). Thereby, findings in CF-related rhinosinusitis, which regularly is associated with pathogen colonization, are not only relevant for the care of patients with the inherited disease. Moreover, these trials give essential new insights for the large number of patients with acute and chronic bacterial rhinosinusitis of other origins. In this regard, the detected insufficiency of systemically applied antibiotics in CF patients colonized with pathogens on sinonasal inflammatory markers reveal the following: approaches to apply antibiotics at the site of bacterial infection within the sinuses require further assessment. For the lungs, topical inhalation of antipseudomonal antibiotics is a golden standard, besides IV-antibiotic therapy. Bronchial inhalation provides the highest concentrations of antibiotics within sputum together with low side effects as no relevant blood levels are reached.

As the paranasal sinuses are not reached by conventional inhalation ⁽⁴³⁾, our studies' results may indicate, that sinonasal surgery with widening of sinonasal ostia can be required in some chronic bacterial rhinosinusitis to allow nasal lavages with addition of antibiotics reach the paranasal sinuses. This approach recently has been taken in a large Danish cohort, postoperatively flushing the sinonasal space with colomycin ⁽²²⁾. On the other hand case reports ⁽¹⁸⁾ and a very recent pilot study on topical antibiotic administration via pulsating sinonasal inhalation could be a promising alternative for reducing respectively eradicating pathogen colonization in UAW ^(44, 45). In CF both approaches could help to prevent chronic sinonasal infection with *P. aeruginosa* and a consecutive expansion to the lungs.

Regarding anatomical conditions our publication is relevant to

any other bacterial related infection of the paranasal sinuses. Findings in the present study are in good accordance with empirical observations showing the need to treat bacterial sinusitis for much longer periods with systemic antibiotics than required for other infections of parenchymal organs. Consistently, there is a lack of evidence reported for the short-term use (< 4 weeks) of systemic antibiotics, whereas patients with CRSsNP may benefit from a long-term therapy with macrolides in low doses ^(3, 5, 46). The beneficial effect of this low-dose-therapy, that takes at least 12 weeks, harks back to macrolides' immune-modulatory properties, but again is linked to the risk of increasing antibiotic resistances ^(3, 46).

Possible limitations

A direct comparison of mediator concentrations in NL and sputum is difficult. Thereby, we added no dilution factor to NL but for sputum samples a dilution factor 10 was used for sample processing. However, by comparing changes in upper- and lower airway concentrations of inflammatory mediators during therapy a correction e.g. to the backflow volume and the protein content did not influence our results ⁽⁶⁾.

For future work it would be very helpful to administer a best practice guideline for sputum preparation to achieve greater comparability of different studies. Further on, for NLF as well as for BALF and sputum no suitable 'housekeeping' markers have been identified for normalization to improve standardization, which hampers the inter-study comparability ⁽⁴⁷⁾.

Following trials should include assessment of changes in microbiological findings and antibiotic resistances after IV-antibiotic treatment in CF. Also monitoring of rhinoscopic changes before and after finalisation of a 14 days standard IV-antibiotic treatment in CF would be interesting. These issues have not been included in the present trial which focuses on changes in inflammatory mediators during early phases of IV-AB therapy.

Conclusion

The present study identifies for the first time a substantially reduced effect of IV-antibiotic treatments on sinonasal- compared to pulmonary inflammatory markers. Accordingly, our results reveal a limited potential of systemic antibiotic treatment to control sinonasal bacterial infections. We contribute this to the anatomical situation of paranasal sinuses, which are not reached effectively by systemic antibiotics. We suppose that our findings do not only apply to CF related chronic rhinosinusitis, but it can be taken for bacterial CRS of other origin as well.

Abbreviations/Glossary

AB (antibiotic), CF (Cystic Fibrosis), CFTR (Cystic Fibrosis Transmembrane Conductance Regulator), CRS (Chronic Rhino-

sinusitis), NP (nasal polyps), NLF (Nasal lavage fluid), NL (Nasal lavage), BALF (bronchoalveolar lavage fluid), IS (induced sputum), UAW (upper airways), LAW (lower airways), TCC (total cell count), PMN (polymorphonuclear), MN (mononuclear), NE (neutrophil elastase), IL (interleukin), MPO (myeloperoxidase), RANTES (regulated on activation, normal T cell expressed and secreted), IV (intravenous), soluble intercellular adhesion molecule-1 (sICAM), AB (antibiotic), LTx (Lung transplantation)

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Author contributions

FD, JH and JGM elaborated the study concept and were the principal investigators, writing the manuscript. FD conducted the study with support of NF. TL contributed to the analysis and interpretation of data together with FD, JH and NF. URM, KB, and WP were our partners in the participating laboratories. WP and MWP supported the study regarding microbiological and infectiological issues and OGL regarding specific ENT issues.

Conflict of interest

We confirm that there are no known conflicts of interest associated with this publication. Neither there have been personal relationships with other people or organizations nor significant financial support for this work that could have influenced its outcome.

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Table S1. Medians and ranges of relevant measured inflammatory mediators.

upper airways			before IV-AB			during IV-AB		
mediator	unit	DL	median	range	frequency	median	range	frequency
MPO	ng/mL	0.02	288.85	92.98 - 769	16/16	165.02	51.5 - 768.05	16/16
IL-8	pg/mL	0.5	372.08	0.4 - 775.54	14/16	192.31	0.4 - 676.83	14/16
IL-4	pg/mL	20.8	20.7	12.88 - 752.45	8/16	20.7	16.78 - 369.37	6/16
IL-13	pg/mL	4.5	230.78	4.4 - 893.33	10/16	175.39	4.4 - 659.97	11/16
lower airways			before IV-AB			during IV-AB		
mediator	unit	DL	median	range	frequency	median	range	frequency
MPO	ng/mL	0.02	3095.4	0.02-6412.13	16/16	316.58	0.02-2315.44	16/16
IL-8	pg/mL	0.5	11754.91	0.4-37169.74	16/16	3936.805	0.4-25160.17	16/16
IL-4	pg/mL	20.8	20.7	20.7-20.7	16/16	20.7	20.7-148.83	16/16
IL-13	pg/mL	4.5	4.40	4.4-2538.55	16/16	4.4	4.4-2705.36	16/16