

**Title: Improved treatment response to dornase alfa in cystic fibrosis patients
using controlled inhalation**

Subtitle: A randomized controlled clinical trial

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Abstract

Rationale and objectives

Better treatment of obstructed small airways is needed in CF. This study investigated whether efficient deposition of dornase alfa in the small airways improves small airway obstruction.

Methods

In a multi-centre, double-blind, randomized controlled clinical trial, CF patients on maintenance treatment with 2.5 ml dornase alfa once daily were switched to a smart nebulizer and randomized to small airways deposition (n=24) or large airways deposition (n=25) for 4 weeks. The primary outcome parameter was Forced Expiratory Flow at 75% of Forced Vital Capacity (FEF₇₅).

Results

FEF₇₅ increased significantly by 0.7 SD (5.2% predicted) in the large airways group and 1.2 SD (8.8% predicted) in the small airways group. Intention to treat analysis did not show a significant difference in treatment effect between groups. Per protocol analysis, excluding patients not completing the trial or with adherence <70%, showed a trend (p = 0.06) in FEF₇₅ Z-score and a significant difference (p = 0.04) between groups in absolute FEF₇₅ (L/s) favouring small airways deposition.

Conclusions

Improved delivery of dornase alfa using a smart nebulizer that aids patients in correct inhalation technique resulted in significant improvement of FEF₇₅ in children with stable CF. Adherent children showed a larger treatment response for small airways deposition.

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Introduction

Cystic Fibrosis (CF) lung disease is characterized by chronic airway infection and inflammation that start early in life and cause progressive structural lung damage which eventually leads to respiratory failure and early death [1-3]. Small airways are involved early in the disease process [4], as indicated by trapped air on CT scans [5, 6] and by lung function tests [7, 8] of young children.

Sputum of CF patients is rich in leukocyte-derived DNA which contributes to its abnormal viscoelasticity, which in turn leads to small airways obstruction [9]. A major challenge in the treatment of CF is therefore to mobilize as much sputum as possible from the airways. Airway clearance techniques and nebulization of mucolytic drugs play an important role in mobilizing sputum.

Recombinant human DNase (rhDNase, dornase alfa) is a mucolytic drug that has been extensively investigated in CF and is part of current standard treatment. Dornase alfa cleaves extracellular DNA through hydrolysis and reduces the viscoelasticity of CF sputum in vitro [10]. Dornase alfa has been shown to improve lung function and reduce the number of pulmonary exacerbations in CF patients [11-13]. In a study in children with normal forced vital capacity (FVC) a treatment benefit of 7.9% predicted was observed for the Forced Expiratory Flow between 25% and 75% of FVC (FEF_{25-75}), compared to a benefit of only 3.2% predicted in the primary outcome parameter, Forced Expiratory Volume in one second (FEV_1) [13]. FEF_{25-75} and Forced Expiratory Flow at 75% of FVC (FEF_{75}) are considered sensitive to changes in the small airways [14].

Because most CF patients show lung function evidence of persistent small airways obstruction [13, 15] and patients with advanced lung disease have extensive destruction of the small airways [16, 17], more efficient treatment of

the small airways is still needed [18]. This could be achieved through improved delivery of dornase alfa to the small airways. Currently, dornase alfa is routinely administered with relatively inefficient nebulizers. These nebulizers deposit most of the inhaled drug in large airways and relatively little drug in small airways [19, 20]. This is primarily due to two nebulizer related factors: particle size of the inhaled aerosol and breathing pattern during inhalation. Standard jet nebulizers produce an aerosol with a wide range of particle sizes and a relatively large mean particle size. Large aerosol particles have a high probability to get deposited in the central airways. Furthermore, most patients perform tidal breathing while using a standard nebulizer. This leads to preferential deposition of particles in the large airways. In contrast, a slow and deep inhalation increases the probability of particle deposition in the small airways.

Recently, smart nebulizers have become available that coach patients in proper inhalation technique, are more efficient in drug delivery and can target more of the inhaled dose to small airways [21-23]. However, it is unknown whether preferential delivery of dornase alfa to small airways using a state of the art nebulizer actually improves small airway patency in children with CF. To answer this question we conducted a randomized controlled clinical trial comparing large airways and small airways deposition of dornase alfa in stable CF patients using a state of the art nebulizer.

Methods

We conducted a multi-center, double-blind, randomized controlled clinical trial in stable pediatric CF patients who were on maintenance treatment with 2.5 mg dornase alfa once daily, to compare the efficacy of small airways versus large airways deposition of dornase alfa using a breath-controlled nebulizer.

Patient recruitment and randomization

Study participants were recruited from the outpatient clinics of 3 participating hospitals: Erasmus MC – Sophia Children’s Hospital (Rotterdam, The Netherlands), Radboud University Nijmegen Medical Centre (Nijmegen, The Netherlands) and the Cystic Fibrosis Center (Verona, Italy).

CF patients were eligible to participate in the study if they were: aged 6 – 18 years, in stable condition, on maintenance treatment with 2.5 mg dornase alfa once daily, and had small airways obstruction present on spirometry (defined as $FEF_{75} < 70\%$ predicted). A full checklist of inclusion, exclusion, and study stop criteria used in the study is available in the online supplement.

Patients were randomly assigned to the small airways deposition group or the large airways deposition group, according to the randomization schedule prepared by the study statistician. The hospital pharmacy was responsible for the administration of randomization data and guaranteed 24-hour availability of randomization information.

This trial was registered in the Dutch trial register (<http://www.trialregister.nl>) and in the International Standard Randomised Controlled Trial Number Register (number: 64225851). The local ethics committee approved the study.

Parental written informed consent and children's assent were obtained prior to the first study measurement.

Study treatment

Study treatment consisted of targeted peripheral or central deposition of 2.5 mg (2.5 ml) dornase alfa. Central deposition simulated conventional nebulizers and deposited most of the drug in the large airways (large airways group), while peripheral deposition optimized deposition in the small airways (small airways group) [21, 24-26]. Dornase alfa was inhaled once daily using the Akita²® APIXNEB device (Activaero technologies, Gemuenden, Germany). This device consists of a nebulizer handset (APIXNEB) that uses vibrating mesh technology (Touchspray; PARI GmbH) and an electronic unit (Akita²). In this publication we will refer to this device as Akita². The Akita² deposits approximately 70% of the loaded dose in the lung [26], compared to 10-20% for conventional jet nebulizer systems [27-30]. Therefore total lung dose of dornase alfa during this study was estimated to be approximately 3-5 times higher than with standard jet nebulizers. The Akita² can be programmed to preferentially target specific regions of the lung [21, 24-26]. The Akita² directs the flow and depth of each inhalation, coaches the patient on correct inhalation technique and controls the fraction of the inspiration time during which aerosol is generated. These settings are optimized for each patient based on individual inspiratory capacity using a microchip-containing smartcard inserted in the device.

In order to generate the two different lung deposition patterns we adjusted three characteristics of the nebulizer treatment: particle size, breathing pattern and timing of aerosol bolus (Figure 1).

The investigator carefully instructed each patient on the use of the Akita² at the start of the study. Patients in both treatment arms received instructions

and feedback from the display of the Akita² during each nebulization to ensure that the correct breathing pattern was performed. Additional details on feedback to the patient and inhalation settings of the device, as well as technical details can be found in the online supplement.

Study endpoints

The primary endpoint of this study was change in FEF₇₅ (Z-score) after 4 weeks of targeted treatment. Secondary endpoints were change in FEF₇₅ (L/s), change in FEV₁, FVC, FEF₂₅₋₇₅ (Z-score and absolute values), Lung Clearance Index (LCI), and diary symptom scores.

Study visits were scheduled at t = 0, 2, and 4 weeks. At each study visit, spirometry tests were performed. Spirometry was measured on a Masterscreen electronic spirometer (Jaeger/Viasys, Würzburg, Germany) according to ERS/ATS guidelines. LCI measured by a MBW test (Exhalyzer®D Ecomedics, Duernten, Switzerland) was performed at each visit only in the Sophia Children's Hospital because this test was not available in the other two centers.

Adverse events during the study were recorded in an updated version of a diary used in previous studies [31, 32] (see Table E2 of the online supplement). Patients were asked to daily record any unusual symptoms and a symptom score containing items on cough, mucus and shortness of breath. The possible total symptom score for one day ranged from 0 to 18 points.

Adherence

The Akita² monitors adherence by recording on the smartcard the date, time and number of breaths of each nebulization treatment. For each patient we calculated daily dose adherence which expresses the percentage of days a

patient adhered correctly to the prescribed once daily nebulization of dornase alfa.

Estimate of sample size

We aimed to detect a 10% difference in FEF₇₅ at the 5% significance level for a two-sided test with 80% power. Based on previous data [33], this would require 44 patients in total, 22 patients in each group. To anticipate possible dropouts, we aimed to include 25 patients per study arm.

Statistical analysis

Data were analyzed on an intention-to-treat basis. Analyses of between-group comparisons regarding change in lung function variables (FVC, FEV₁, FEF₇₅, FEF₂₅₋₇₅, LCI) were performed using repeated measurements ANOVA (analysis of variance) with adjustment for baseline values, center and use of tobramycin solution for inhalation. Changes are reported as mean ± SEM. Analyses were performed with SPSS software (version 15.0). For all analyses, two-tailed p-values of <0.05 were considered to indicate statistical significance. A per-protocol analysis was conducted in which patients who violated the study protocol were excluded. Protocol violations were defined as patients who did not complete the trial, had adherence < 70% or for whom adherence data could not be retrieved (Figure 2).

Patients who improved by 10% predicted or more for FEF₇₅ were defined as excellent responders. We calculated whether there was a significant difference between the groups in number of excellent responders using a Chi-Square test with continuity correction.

Spirometry variables were expressed as Z-scores and % predicted using the reference values by Stanojevic et al.[34] for FVC, FEV₁ and FEF₂₅₋₇₅. Since these reference equations do not include data for FEF₇₅, we used the

reference values by Zapletal et al.[35] for FEF₇₅. Changes in absolute values of spirometry variables (L and L/s) were expressed as percentage of baseline values.

For each patient mean daily symptom scores per week were computed. When less than 4 out of 7 days of a week were recorded, scores for that week were regarded as not evaluable. Differences in number of adverse events and changes in diary scores were analyzed using a Mann-Whitney test and Chi-Square test.

Results

49 patients were included in the study, 44 of whom completed the trial (22 patients in each group). All patients were enrolled between September 2007 and March 2010.

Baseline characteristics of patients in the two treatment groups were similar (Table 1).

Mean treatment time for the high lung dose of dornase alfa that was delivered during this study was 11 min. 51 s. for the large airways group and 9 min. 27 s. for the small airways group. Difference in treatment time between the groups was not significant, $p = 0.13$.

Variables	Large airways (N = 25)	Small airways (N = 24)
Gender (M/F)	14/11	12/12
Age (yrs)	13.3 (3.4)	12.7 (3.5)
Height (cm)	153.5 (16.5)	153.4 (18.1)
BMI (m/kg ²)	18.7 (2.7)	18.2 (2.2)
Pancreatic insufficiency (Y/N)	22/3	20/4
Chronic Pseud. Aer. (Y/N)	10/15	9/15
Maintenance inhaled tobramycin (Y/N)	9/16	8/16
CF mutation (homozygous delF508 / other)	15/10	10/14
FVC (Z-score)	-0.5 (1.2)	-0.7 (1.5)
FVC (% predicted)	94.6 (13.1)	91.7 (17.1)
FEV ₁ (Z-score)	-1.6 (1.1)	-1.7 (1.3)
FEV ₁ (% predicted)	81.8 (13.1)	80.4 (15.6)
FEF ₂₅₋₇₅ (Z-score)	-2.2 (1.2)	- 2.3 (1.2)
FEF ₂₅₋₇₅ (% predicted)	55.5 (19.6)	54.7 (19.9)
FEF ₇₅ (Z-score)	-5.2 (2.7)	- 5.5 (2.7)
FEF ₇₅ (%predicted)	39.7 (17.4)	37.2 (16.0)
LCI (absolute values)	8.7 (1.6) n = 15	9.9 (1.8) n = 14

Table 1. Baseline characteristics of patients in each treatment group. Data are reported as numbers of patients or mean (SD).

Lung function (intention to treat analysis)

FEF₇₅ improved substantially and statistically significantly in both groups after 4

weeks of treatment (Table 2 and Figure 3a): by 0.7 SD ($p = 0.03$) in the large airways group and by 1.2 SD ($p = 0.001$) in the small airways group. This corresponds to an improvement of 5.2 and 8.8 % predicted, respectively. The difference in mean FEF₇₅ between both groups was 0.4 SD (95% CI -0.4 to 1.2; $p = 0.31$) for small airways versus large airways deposition.

A similar effect was observed for FEF₂₅₋₇₅ (Table 2). FVC and FEV₁ did not change significantly in both groups, and the difference in FEV₁ or FVC between the groups after 4 weeks of treatment was not significant.

FEF₇₅ improved by $\geq 10\%$ predicted in 4 of 22 patients (18%) in the large airways group and in 10 of 22 patients (45%) in the small airways group. The difference in number of excellent responders between the groups was not significant ($p = 0.10$).

LCI was measured in 29 patients: 15 in the large airways and 14 in the small airways group. LCI did not change significantly in either group over 4 weeks, nor was there a significant difference between the groups in the change in LCI (Table 2).

Variables	Large airways ITT (n = 25)		Small airways ITT (n = 24)		Mean difference ITT
	Z-score	L/s % BL	Z-score	L/s % BL	Z-score (95% CI)
FEF ₇₅	0.7 (0.3)*	24.1 (10.3)*	1.2 (0.3)*	35.3 (10.1)*	0.4 (-0.4 – 1.2)
FVC	0.0 (0.1)	1.4 (1.5)	0.1 (0.1)	2.4 (1.5)	0.0 (-0.2 – 0.3)
FEV ₁	0.2 (0.1)	3.6 (2.1)	0.1 (0.1)	3.5 (2.1)	-0.1 (-0.4 – 0.3)
FEF ₂₅₋₇₅	0.4 (0.1)*	14.8 (6.4)*	0.3 (0.1)*	16.1 (6.3)*	-0.1 (-0.5 – 0.3)
LCI	-0.1 (0.5) (n = 15)		-0.3 (0.5) (n = 14)		0.5 (-0.7 – 1.7)
	Large airways PP (n = 17)		Small airways PP (n = 17)		Mean difference PP
	Z-score	L/s % BL	Z-score	L/s % BL	Z-score (95% CI)
FEF ₇₅ PP	0.7 (0.3)*	17.3 (7.5)* [†]	1.4 (0.3)*	37.5 (7.5)* [†]	0.7 (-0.1 – 1.5)

Table 2. Lung function after 4 weeks of study treatment. Data are presented as mean change from baseline \pm SEM (Anova estimates). Difference is shown for small airways minus large airways group (Z-score and 95% CI). ITT = intention to treat analysis. PP = per protocol analysis. % BL = Percentage change from baseline. PP analysis was performed only for the primary endpoint, FEF₇₅.

* P-value \leq 0.05 for change from baseline within treatment group.

[†] P-value \leq 0.05 for difference between treatment groups.

Symptom scores

Mean symptom score per day in week 1 was 1.8 ± 1.9 points for the large airways group and 2.9 ± 2.3 points in the small airways group. Symptom scores were slightly lower in the first week of the study in the large than the small airways group (p =

0.05). Mean symptom scores during the rest of the study did not significantly differ between treatment groups and there were no significant changes within either group (see Table E3 of the online supplement).

FEF₇₅ (per protocol analysis)

Fifteen of the 49 patients were excluded from the per protocol analysis: 5 patients did not complete the trial, 5 patients had a daily dose adherence below 70% and for 5 patients data on adherence could not be retrieved (Figure 2). As a result, 17 patients in each group were analyzed for the primary endpoint (FEF₇₅).

FEF₇₅ improved significantly in both groups after 4 weeks of treatment: by 0.7 SD (95% CI 0.1–1.2; $p = 0.02$) in the large airways group, and by 1.4 SD (95% CI 0.8–1.9; $p < 0.001$) in the small airways group (Table 2). These increases in Z-scores correspond with improvements of 5.6% and 10.7% predicted, respectively. Analysis of change in absolute values of FEF₇₅ (L/s expressed as percentage of baseline) demonstrated that FEF₇₅ improved by 17.3% (95% CI 2.1–32.6; $p < 0.001$) in the large airways group and by 37.5% (95% CI 22.2–52.8; $p < 0.001$) in the small airways group.

After 2 weeks of treatment the difference in FEF₇₅ between the groups was 1.2 SD (95% CI 0.1–2.2; $p = 0.03$) in favor of the small airways group. After 4 weeks the difference was 0.7 SD (95% CI -0.1–1.5; $p = 0.06$) in favor of the small airways group (Figure 3b). Expressed as absolute values the difference in FEF₇₅ in favour of small airways deposition was 32.4% (95% CI 3.4–61.4; $p = 0.03$) after 2 weeks and 21.3% (95% CI 1.4–41.2; $p = 0.04$) after 4 weeks of treatment (data shown in Figure E1 of the online supplement).

Adverse events

A total of 31 adverse events (AEs) were reported during the study, 18 in the large airways group and 13 in the small airways group. There was no statistically significant difference in number of AEs between the groups ($p = 0.90$).

In general, AEs were either self-limiting symptoms (e.g. runny nose, headache) or symptoms consistent with CF (e.g. respiratory infections, bowel problems). Three AEs were considered to be possibly related to the study intervention: three patients in the large airways group experienced hoarseness for one or two days during the first 2 weeks of the study. Temporary hoarseness is a known side effect of dornase alfa inhalation. In these patients the symptoms resolved spontaneously. No serious adverse events (SAEs) were reported in either group

Discussion

To our knowledge, this is the first randomized double-blind controlled trial comparing large airways versus small airways deposition of inhaled dornase alfa using a smart nebulizer system. We observed a substantial and statistically significant improvement in spirometry indicators of small airway patency in both groups. The per protocol analysis, but not the intention to treat analysis, demonstrated better treatment response in the small airways group.

The most striking finding in this study is that both treatment groups showed considerable improvement in FEF_{75} and FEF_{25-75} , indicating improved patency of the small airways, within 4 weeks of switching from a conventional nebulizer to a smart nebulizer for their daily dornase alfa inhalation. Mean improvement in FEF_{25-75} after 4

weeks of treatment for all patients, independent of deposition group, was 5.9% predicted. This is remarkable because all patients were on maintenance treatment with the same nominal dose of dornase alfa prior to the study. It is worthwhile to realize that this improvement is relatively large compared to the 8.4% improvement in the PEIT study [13], that included dornase alfa-naive patients who were randomized to 2.5 mg dornase alfa or placebo once daily with a conventional jet nebulizer. In our study we included only patients who were already on maintenance dornase alfa treatment and simply changed the nebulizer. Hence, the present study suggests that the efficacy of the same medication can be improved by another 70% simply by optimizing adherence and inhalation technique.

There are a few explanations for this improvement of small airway function. One likely explanation is that adherence to dornase alfa treatment was higher during the study than before it. Overall adherence to once daily dornase alfa nebulization was 85% during the study and thus probably above daily routine. It is well-known that adherence to chronically inhaled medication is in the order of 40-60% in CF patients and is higher during clinical trials [36-38]. However, we feel that the improvement in FEF₇₅ is too large to be caused by improved adherence only.

A second likely explanation for the improvement in FEF₇₅ in both groups is the controlled inhalation technique of the smart nebulizer. The Akita² device directs inspiratory flow and inhaled volume during each breath and coaches patients to perform the correct breathing maneuver. Consequently, the use of this device increases total lung dose of dornase alfa, improves small airways deposition and reduces loss of aerosol into the environment [21, 24-26].

A third explanation is the higher lung dose delivered during study treatment. Before enrolment in this study 84% of the patients used a conventional jet nebulizer for their

maintenance treatment (see also table E4 of the online supplement). These nebulizers have poor efficiency, delivering only 10-20% of the loaded dose to the lungs [27-30]. Up to 50% of the drug is retained in the nebulizer at the end of nebulization, and 20% of the nominal dose is lost to the environment [28]. The smart nebulizer used in our study delivers up to 70% of the nominal dose to the lungs [24, 26].

At the time of the registration studies of dornase alfa several dose finding studies were performed. Daily doses varying between 2.5 and 20 mg were given to patients in phase II and III trials without observing a dose-dependent response on FEV₁ or FVC [11, 39]. All doses were safe. Unfortunately, these studies did not report the effect of the various treatment regimens on FEF₇₅ or FEF₂₅₋₇₅.

The other important new finding in this study is that increased delivery of dornase alfa to small airways is possible, as demonstrated by greater FEF₇₅ and FEF₂₅₋₇₅ responses in both treatment groups, and that doing so seems to be safe. Small airways deposition was accomplished by reducing aerosol particle size and nebulizing drug early during deeper inhalation, but even the large airways group benefitted from an increased peripheral deposition. The intention to treat analysis did not show a difference between the two deposition groups. This might be explained by the fact that the use of a breath actuated and efficient nebulizer in this population with relatively mild airways obstruction resulted in substantially more drug reaching the large surface area of the small airways than when using a conventional jet nebulizer, even for the central airways deposition settings. This might have resulted in reaching the plateau of the dose-response curve in both groups and thus not finding a significant difference between the two deposition groups.

However, the per protocol analysis demonstrated greater improvement and a significant difference between the groups for FEF₇₅ (L/s) in favor of the small airways

group. This ‘best case scenario’ analysis illustrates the potential of optimizing every possible aspect of dornase alfa therapy, i.e. optimal delivery to the small airways and optimal adherence. The clinical relevance of our study is that it underlines that both adherence and inhalation competence are important determinants to optimize treatment effect of dornase alfa. Clearly, the use of a breath actuated efficient nebulizer for maintenance treatment of dornase alfa should be considered ‘off label treatment’. However, nowadays most patients use ‘off label’ nebulizers because these are different from the ones used in the registration studies for dornase alfa almost two decades ago. We feel that a breath actuated efficient nebulizer is a promising treatment option that should be considered especially for those patients with progressive small airways disease or possible poor competence and/or adherence.

Two previous large open label studies in dornase alfa-naive patients compared the efficacy of small versus larger particles and showed a similar trend as in this study, but were not conclusive [40, 41]. The most likely explanation for these negative findings was the use of conventional jet nebulizer systems. Standard jet nebulizers produce an aerosol with a wide range of particle sizes and a relatively large mean particle size. Large aerosol particles have a high probability to get deposited in the central airways. Furthermore, most patients perform tidal breathing while using a standard nebulizer. This leads to preferential deposition of particles in the large airways. In contrast, a slow and deep inhalation increases the probability of particle deposition in the small airways. Therefore, the conditions in these two studies probably were not optimal to deliver drug to the small airways. An open label single arm study on bolus inhalation of dornase alfa by Geller et al. showed the importance of breath control during nebulization [42]. In this study, CF patients were treated with

a device that was breath actuated, delivered an aerosol bolus when the optimal inspiratory flow rate was achieved and generated visual cues for the subject to inhale deeply within the optimum range of inspiratory flow rates. FEV₁ and FEF₂₅₋₇₅ significantly improved after two weeks of dornase alfa treatment using the study device. Our study supports the conclusions by Geller et al, that controlled and deep inhalation is important to achieve optimal treatment effect.

It is likely that the optimized inhalation technique used in our study could also benefit patients with other respiratory diseases, such as COPD or asthma. However, this should be confirmed by clinical trials conducted in these patient groups.

In conclusion, our study shows that 4 weeks of dornase alfa nebulized using a state of the art nebulizer device resulted in a significant improvement in FEF₇₅ and FEF₂₅₋₇₅ in children with stable CF who were on prior maintenance treatment with dornase alfa.

In children with good adherence (per protocol analysis) greater treatment response was observed for small airways deposition compared with large airways deposition.

CF is a fatal disease and its main cause of death is loss of lung function with respiratory failure. Every option to improve lung function and to reduce its rate of decline should be utilized to improve its long-term prognosis. This study suggests that better treatment response and further improvement of small airway function is indeed feasible by optimizing the inhalation technique for administration of a known and effective mucolytic drug, using an efficient nebulizer and breath control technology.

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Figure legends

Figure 1: Breathing patterns on smartcard

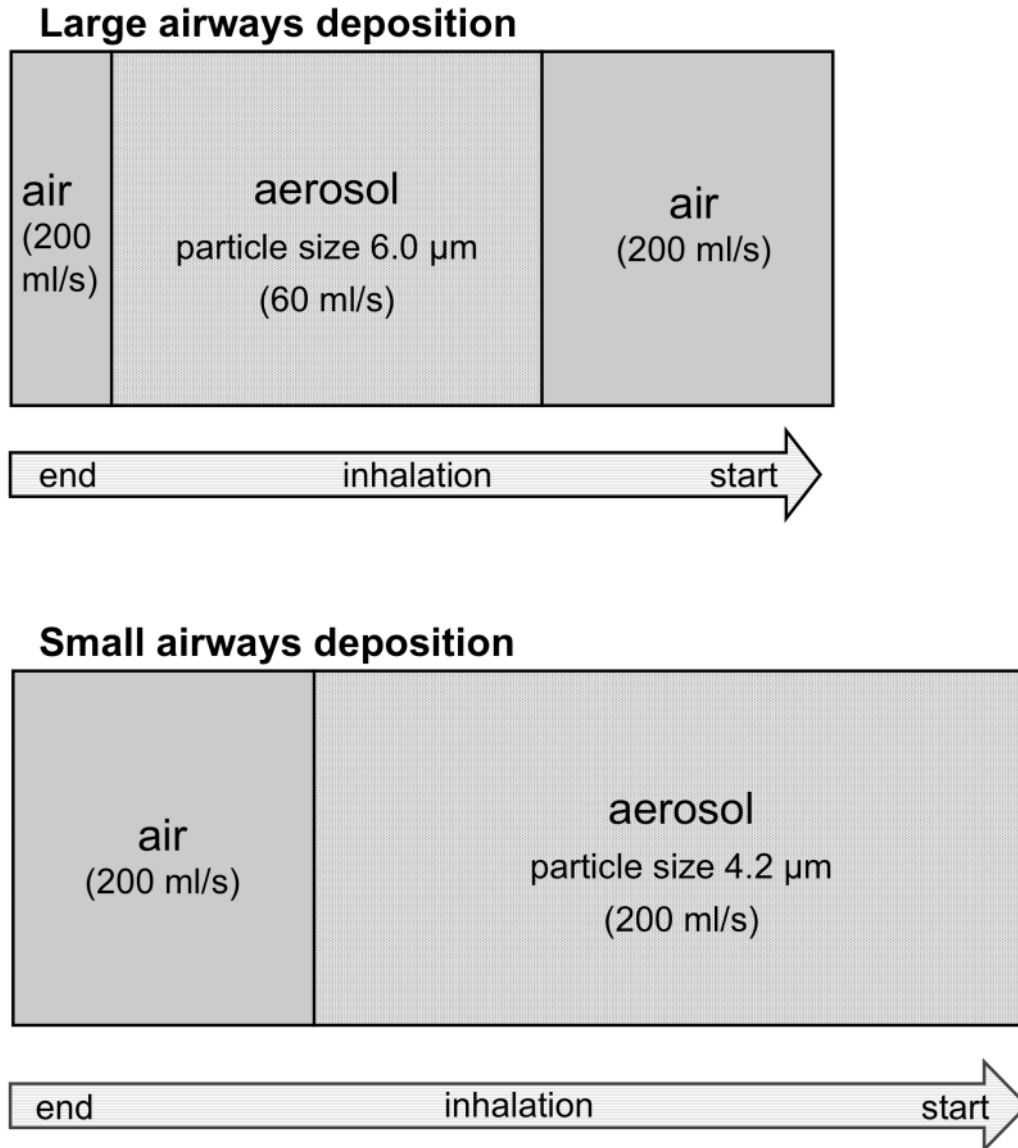


Figure 2. Enrollment, random assignment, follow-up and analysis.

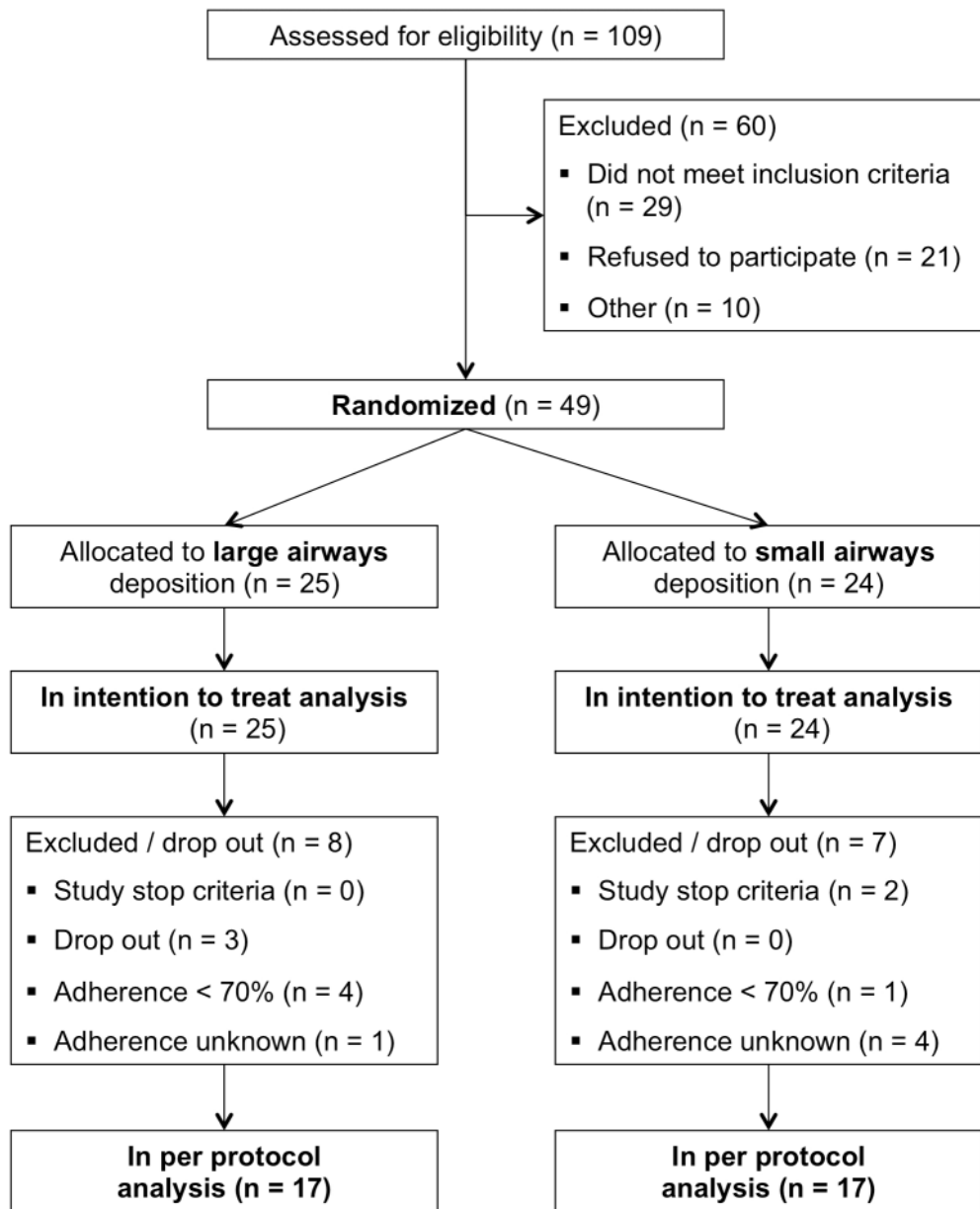
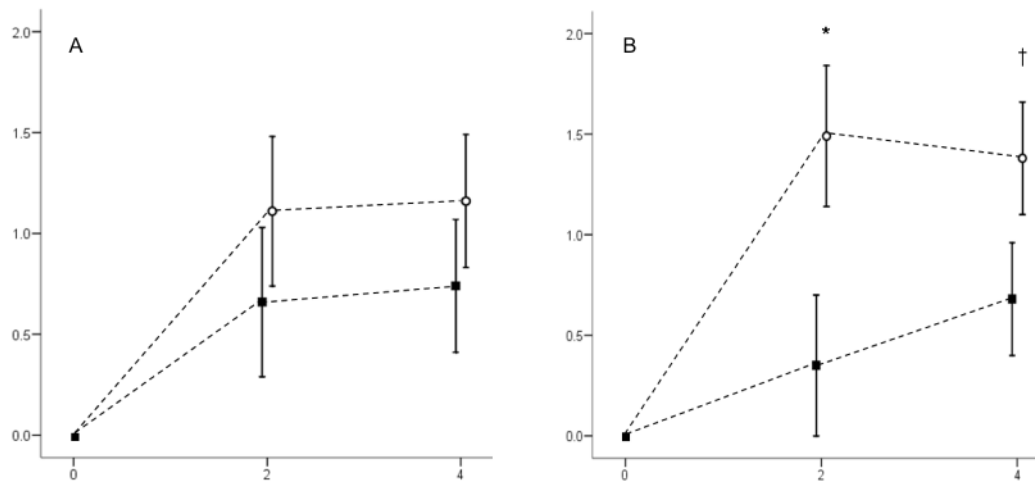


Figure 3a and 3b. Mean changes from baseline for FEF₇₅ (Z-score) after 2 and 4 weeks of treatment. Figure 3a shows intention to treat analysis, figure 3b shows per protocol analysis. Data are presented as mean ± SEM (Anova estimates). On the X-axis are weeks of treatment, on the Y-axis change in FEF₇₅ (Z-score). Closed

squares represent large airways deposition, open circles represent small airways deposition. * $p = 0.03$, † $p = 0.06$ for small airways vs large airways deposition.



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