A Randomized Clinical Trial of α_1-Antitrypsin Augmentation Therapy

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We have investigated whether restoration of the balance between neutrophil elastase and its inhibitor, α_1-antitrypsin, can prevent the progression of pulmonary emphysema in patients with α_1-antitrypsin deficiency. Twenty-six Danish and 30 Dutch ex-smokers with α_1-antitrypsin deficiency of PI*ZZ phenotype and moderate emphysema (FEV_1 between 30% and 80% of predicted) participated in a double-blind trial of α_1-antitrypsin augmentation therapy. The patients were randomized to either α_1-antitrypsin (250 mg/kg) or albumin (625 mg/kg) infusions at 4-wk intervals for at least 3 yr. Self-administered spirometry performed every morning and evening at home showed no significant difference in decline of FEV_1 between treatment and placebo. Each year, the degree of emphysema was quantified by the 15th percentile point of the lung density histogram derived from computed tomography (CT). The loss of lung tissue measured by CT (mean $\pm$ SEM) was 2.6 $\pm$ 0.41 g/L/yr for placebo as compared with 1.5 $\pm$ 0.41 g/L/yr for α_1-antitrypsin infusion ($p = 0.07$). Power analysis showed that this protective effect would be significant in a similar trial with 130 patients. This is in contrast to calculations based on annual decline of FEV_1 showing that 550 patients would be needed to show a 50% reduction of annual decline. We conclude that lung density measurements by CT may facilitate future randomized clinical trials of investigational drugs for a disease in which little progress in therapy has been made in the past 30 yr. Dirksen A, Dijkman JH, Madsen F, Stoel B, Hutchison DCS, Ulrik CS, Skovgaard LT, Kok-Jensen A, Rudolphus A, Seersholm N, Vroman HA, Reiber JHC, Hansen NC, Heckscher T, Viskum K, Stolk J. A randomized clinical trial of α_1-antitrypsin augmentation therapy.

AM J RESPIR CRIT CARE MED 1999;160:1468–1472.

Pulmonary emphysema is highly associated with cigarette smoking, but it is estimated that only 15% of smokers develop symptomatic emphysema. In contrast, almost all smokers who have hereditary deficiency of α_1-antitrypsin of Z phenotype (PI*ZZ) will develop emphysema in early adult life (1–3). Their emphysema is mainly located in the lower lobes of the lung, whereas smokers with normal phenotype have predominantly upper lobe disease. The gene frequency in white individuals of this phenotype varies between countries, but in the United Kingdom it is approximately 0.03 (4). α_1-Antitrypsin is the principal serum inhibitor of proteolytic enzymes and its function is believed to be the protection of the pulmonary elastic tissue against the destructive activity of elastase (5). This enzyme can be released by neutrophils when they penetrate into the alveolar wall by cigarette smoke–induced chemotaxis. For more than 30 yr it was hypothesized that restoration of the balance between elastase and its inhibitor, α_1-antitrypsin, could prevent the progression of emphysema in deficient patients (5). α_1-Antitrypsin concentrate is purified by fractionation of normal human plasma and has been administered without proven efficacy to a large number of patients in several countries at an annual individual cost of approximately 25,000 Euros. No controlled trial of this product has yet been carried out. We consider this mandatory in view of the arduous and expensive nature of the treatment regimen.

The progression of emphysema is clinically assessed by the decline in pulmonary function tests, i.e., FEV_1 and carbon monoxide diffusion. The main objective of this study was to compare the rate of change in FEV_1 in PI*ZZ patients receiving augmentation therapy with that of control subjects receiving placebo. A similar comparison of other pulmonary function indices and quantitation of emphysema by computed tomography (CT) was made.

(*Received in original form January 15, 1999 and in revised form April 22, 1999*)

Supported by The Danish State Serum Institute, Laboratoire Français du Fractionnement et des Biotechnologies, The National Danish Research Council for Public Health, The Danish Lung Foundation, and The Netherlands Asthma Foundation N.A.F. 93.21.

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METHODS

Patient Population

From 1991 to 1995, 26 patients from the Danish Alpha$_1$-Antitrypsin Deficiency Registry, and from 1993 to 1997, 32 patients from a similar Dutch Registry participated in the study. All patients had α_1-antitrypsin deficiency of PI*ZZ phenotype, verified by isoelectric focusing (6) and moderate to severe emphysema (FEV_1 between 30% and 80% of predicted). All refrained from smoking for at least 6 mo before entering the study, and urinary cotinine was checked every 4 wk during the trial. Two Dutch subjects dropped out of the study during the first 2 yr because they resumed smoking. Their data were omitted from further analyses. The study was approved by the ethics committee of both participating hospitals and all patients gave informed consent.

Design

The study was performed at two centers (Copenhagen, Denmark and Leiden, The Netherlands) as a randomized, parallel, double-blind, and placebo-controlled trial. The number of patients required for the study was based on statistical calculations of lung function data from PI*ZZ subjects in the United Kingdom (2) and Denmark (3), which indicated that a significant effect of intravenous α_1-antitrypsin augmentation on FEV_1 could be reached in a trial with 50 patients, provided FEV_1 was measured daily over a period of 3 yr and assuming a treatment effect of at least 50% (7).

Patients were stratified by age, level of FEV_1, and nationality and randomized by the minimization method (8) to receive infusions every 4 wk of either α_1-antitrypsin (250 mg/kg body weight) or placebo (human albumin Ph.Eur. [625 mg/kg body weight] in an isotonic solution) both from Laboratoire Français du Fractionnement et des Biotechnologies, Lille, France (9). α_1-Antitrypsin has been administered previously to a number of patients in France without adverse effects (10). The study was terminated after 5 yr. All subjects were treated for at least 3 yr.

Respiratory Laboratory Testing

At inclusion and every 3 mo throughout the study, the patient visited the respiratory laboratory in the morning. Pulmonary function testing was performed according to European Respiratory Society (ERS) recommendations (11, 12). A constant-volume body plethysmograph and a dry rolling seal spirometer (SensorMedics 2800 and 2450, Anaheim, CA and Morgan, Haverhill, MA) were applied. Fifteen minutes after bronchodilatation (nebulized terbutaline, 5 mg), with the patient seated, and with a noseclip in place, a slow vital capacity (VC) maneuver was performed, followed by an FVC maneuver from which the maximal flow–volume loop and FEV_1 were derived. Carbon monoxide diffusing constant (K_{CO}) was measured by the single-breath technique, and because the hemoglobin was always within normal limits, the values were not corrected for hemoglobin. The diffusion capacity ($D_{L_{CO}}$) was calculated as the product of K_{CO} and the alveolar volume. The latter was obtained from the dilution of helium during the single-breath maneuver. All measurements were performed in triplicate except for the He dilution. Gas volumes are reported with body temperature and pressure saturated (BTPS) corrections, and results are expressed in absolute values and as percentage of predicted values, calculated according to European reference equations (11, 12).

Patient-administered Serial Spirometry (PASS)

At inclusion the patients were carefully instructed in spirometry for about an hour and they received written information on how to perform spirometry at home (PASS). The patients performed spirometry every morning and evening throughout the study. Technical details and quality control of the PASS data have been reported elsewhere (13).

Computed Tomography

Annual CT was performed in Copenhagen on a Siemens Somatom DRG scanner (Siemens, Erlangen, Germany) or in Leiden on a Philips SR7000 scanner (Best, The Netherlands). The scanners were calibrated regularly using water and air phantoms to allow for comparison between examinations. Subjects were scanned through the chest in the supine position. No contrast medium was injected. The Danish subjects performed tidal breathing, at a lung volume close to the functional residual capacity in the sitting position (14). With the Siemens scanner, slices of 8-mm collimation at 8-mm intervals were obtained, the scanning parameters being 125 kVp, 88 mA, and 4-s scanning time. For the Philips scanner, spiral scans of the entire lung were acquired in approximately 35 s, while the subjects ventilated through a pneumotachograph containing a valve (Masterscreen; Jaeger, Hoechberg, Germany) that was closed at 75% of the total lung capacity at the time of the spiral scan (15). For the Dutch patients the parameters were 120 kVp, 250 mA, 10-mm collimation, 1 pitch, and $<$ 5/10 mm reconstruction. From the scans of the entire lung, slices 5 cm below the carina were selected and analyzed separately (14, 15).

Data Analysis

As previously reported (14), CT densitometric parameters were standardized by log-transformed lung volume in order to correct for (residual) differences in lung volume between scans. In preliminary analyses, percentile parameters in the range from 1% to 50% were evaluated, and percentiles in the range from 10% to 20% were found most pertinent because they showed the strongest time trend (14). Before the treatment code was broken, the 15th percentile point was chosen as the effect variable of the present study for the whole lung and for a single slice 5 cm below the level of the carina. The 15th percentile point is extracted from the frequency histogram of lung pixels as the density value (g/L) at which 15% of the pixels have lower densities.

The effect of augmentation therapy was evaluated by a random effects regression model (16) with pulmonary function measurements and CT densitometric parameters as effect variables and time, nationality, and treatment group as explanatory variables. Lung volume was

TABLE 1

PATIENT CHARACTERISTICS AT ENROLLMENT*

	Danish		Dutch	
	Absolute	% pred	Absolute	% pred
Sex, male/female	14/12		20/10	
Age, yr	50.4 ± 1.62		45.1 ± 1.17	
Height, cm	173 ± 1.90		175 ± 1.32	
Smoking, pack-years	20.0 ± 2.39		17.1 ± 1.90	
FEV_1, ml	1,570 ± 94.9	49.4 ± 2.75	1,660 ± 102	47.1 ± 2.58
FVC, ml	4,420 ± 285	110 ± 3.53	4,330 ± 189	101 ± 2.92
VC, ml	5,040 ± 303	123 ± 2.86	4,660 ± 192	105 ± 2.92
$D_{L_{CO}}$, mmol/min/kPa	5.67 ± 0.36	59.5 ± 3.28	6.03 ± 0.30	61.2 ± 2.98
$K_{L_{CO}}$, mmol/min/kPa/L	0.86 ± 0.05	55.5 ± 3.36	1.03 ± 0.05	52.5 ± 2.48
CT, whole lung, g/L	76.7 ± 6.15		70.5 ± 2.14	
CT, slice 5 cm below carina, g/L	72.8 ± 6.17		73.9 ± 2.25	
Treatment, active/placebo	13/13		15/15	

* Results are expressed as mean ± SE.

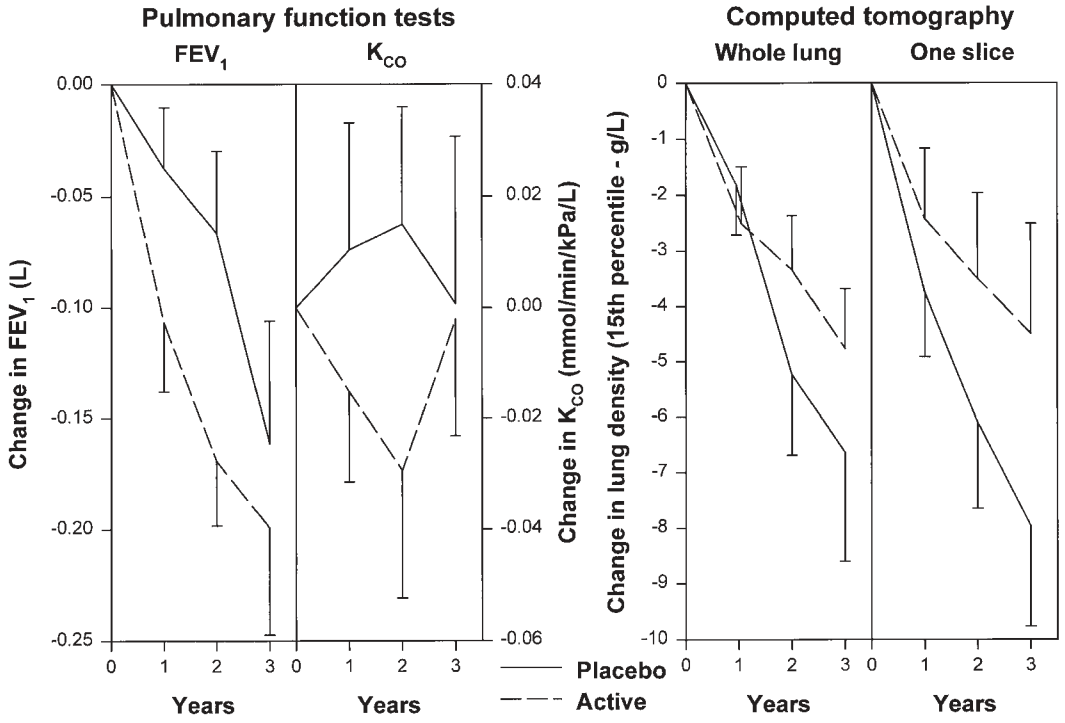

Figure 1. Annual mean change from baseline (at enrollment) in pulmonary function tests and lung densities by CT divided by treatment group. *Error bars* indicate standard error of mean (SE).

a covariate. The random effects were taken as level and rate of decline for each single individual.

Pearson correlations between pulmonary function parameters and CT densitometric parameter of patients who received placebo were calculated. From the same group the sensitivities of these parameters for detecting progress of emphysema were calculated as the mean difference in annual decline (slope) between normal and emphysematous subjects divided by the standard error of the slopes. Previously published cross-sectional data of lung density measurements in normal individuals showed no decline of density with age (17, 18).

RESULTS

Patient characteristics at enrolment are shown in Table 1. The female/male ratio differed between the centers and the Danes were on average 5 yr older. Results of pulmonary function tests

TABLE 2

EFFECT OF α_1-ANTITRYPSIN AUGMENTATION THERAPY IN 56 PATIENTS WITH SEVERE α_1-ANTITRYPSIN DEFICIENCY AND EMPHYSEMA

	Baseline		Annual Change		Effect of Treatment	
Effect Variable	Placebo*	Active*	Placebo*	Active*	Active − Placebo*	p Value
PASS,† ml	1,500 ± 118	1,340 ± 93.2	−25.2 ± 22.0	−26.5 ± 15.1	−1.3 ± 26.7	0.96
FEV_1						
ml	1,650 ± 107	1,600 ± 94.6	−59.1 ± 11.9	−78.9 ± 12.0	−19.8 ± 16.9	0.25
% pred	50.0 ± 3.01	46.2 ± 2.25	−1.47 ± 0.35	−2.11 ± 0.35	−0.64 ± 0.50	0.20
FVC						
ml	4,350 ± 209	4,380 ± 249	−8.1 ± 27.0	−33.1 ± 27.1	−24.9 ± 38.3	0.52
% pred	108 ± 3.10	102 ± 3.42	0.08 ± 0.63	−0.55 ± 0.63	−0.63 ± 0.90	0.48
VC						
ml	4,770 ± 223	4,870 ± 260	−49.9 ± 23.2	−77.4 ± 23.3	−27.5 ± 32.9	0.41
% pred	115 ± 3.58	110 ± 3.28	−0.80 ± 0.54	−1.46 ± 0.54	−0.66 ± 0.77	0.40
$D_{L_{CO}}$						
mmol/min/kPa	5.82 ± 0.35	5.93 ± 0.31	−0.16 ± 0.04	−0.19 ± 0.04	−0.03 ± 0.06	0.60
% pred	60.7 ± 3.20	60.8 ± 3.06	−1.34 ± 0.43	−1.76 ± 0.43	−0.42 ± 0.61	0.50
$K_{L_{CO}}$						
mmol/min/kPa/L	0.94 ± 0.05	0.98 ± 0.06	−0.0162 ± 0.004	−0.0168 ± 0.004	−0.0006 ± 0.01	0.92
% pred	52.1 ± 2.80	55.4 ± 2.89	−0.69 ± 0.26	−0.68 ± 0.26	−0.01 ± 0.37	0.98
CT, whole lung, g/L	73.0 ± 4.78	67.7 ± 4.17	−2.57 ± 0.41	−1.50 ± 0.41	1.07 ± 0.58	0.07
CT, slice 5 cm below the carina, g/L	72.8 ± 4.56	66.7 ± 4.31	−2.74 ± 0.46	−1.90 ± 0.47	0.83 ± 0.66	0.21

* Results are expressed as mean ± SE.

† FEV_1 obtained by PASS.

TABLE 3

SENSITIVITY OF VARIOUS PARAMETERS FOR MONITORING THE PROGRESS OF EMPHYSEMA

Parameter	Annual Change: Normal Subjects According to Reference Values*	Annual Change: Emphysematous Subjects (Placebo Group)	Difference between Emphysema and Normal (mean ± SE)†	Sensitivity (mean/SE)
PASS, ml	−27	−25	2 ± 22	0.1
FEV_1, ml	−27	−59	−32 ± 12	2.7
FVC, ml	−26	−8	18 ± 27	0.7
VC, ml	−26	−50	−24 ± 23	1.0
D_{LCO}, mmol/min/kPa	−0.06	−0.16	−0.10 ± 0.04	2.5
K_{LCO}, mmol/min/kPa/L	−0.01	−0.02	−0.01 ± 0.004	2.5
CT, whole lung, g/L	0‡	−2.57	−2.57 ± 0.41	6.3
Single slice 5 cm < carina, g/L	0	−2.74	−2.74 ± 0.46	6.0

* References 11 and 12.
† SE: standard error from Table 2.
‡ References 17 and 18.

were similar in the two countries, as were lung densities by CT. Participants were equally allocated to active treatment and placebo. No adverse effects of treatment or placebo were observed.

The annual mean changes from baseline (at enrollment) of three monthly FEV_1, KCO, and CT lung densities are shown in Figure 1. Baseline values of effect variables, the time trend, and the differences between active and placebo-treated patients are summarized in Table 2. The primary parameter of this study, daily FEV_1 measured at home, showed an annual decline in the placebo group of 25.2 ± 22.0 ml, which was not significantly different from the treatment (26.5 ± 15.1 ml, $p = 0.96$). The secondary parameter of the study, the 15th percentile point of the lung density distribution of the whole lung measured by CT scanning, suggests that treatment inhibited the annual loss of lung tissue by 1.07 g/L compared with placebo ($p = 0.07$).

The correlation between decline of FEV_1 and change in the CT 15th percentile point for the whole lung in patients who received placebo was 0.18 ($p = 0.39$), whereas the change in KCO correlated significantly with the change in the CT 15th percentile point ($r = 0.47$, $p = 0.02$). For patients receiving placebo, the sensitivity to detect progression of emphysema was more than twice as great for CT (whole lung: 6.3) as for pulmonary function (FEV_1: 2.7) (Table 3).

DISCUSSION

This is the first randomized placebo-controlled trial of α_1-antitrypsin augmentation therapy in patients with emphysema. The results demonstrate no significant effect of α_1-antitrypsin augmentation on pulmonary function in patients with moderate to severe emphysema. However, analysis of the CT scans showed a trend toward a favorable effect of protease inhibitor treatment, suggesting some protection against loss of lung tissue. When placebo-treated patients were analyzed as a group, CT lung density measurement proved to be more than twice as sensitive for detecting the progress of emphysema as pulmonary function tests.

The main concept of this study was close monitoring by PASS, i.e. twice daily FEV_1 measurements at home. However, the potential gain produced by frequent tests in each subject proved spurious because measurements at short intervals were heavily interdependent, and therefore added little extra information. Technical aspects of this result have been presented and discussed in more detail elsewhere (13).

So far, only two surveillance studies regarding the efficacy of intravenous augmentation therapy have been reported, neither of which were randomized trials. In a German–Danish study (19) of 198 German patients on weekly infusions with α_1-antitrypsin, a subgroup with moderate emphysema (FEV_1 between 31% and 65% of predicted) showed a significant decline in FEV_1 slope when compared with similar untreated Danish patients. A beneficial effect of the therapy was one possible explanation, but the effect was small (21 ml/yr reduction in a decline of FEV_1 of 83 ml/yr) and could be explained by factors in time, smoking habits, and nationality (20). In the American Alpha-1-Antitrypsin Deficiency Registry Study (21) the mortality of subjects who never received augmentation therapy was twice that of subjects with similar lung function, who did receive augmentation. However, this was not a randomized trial, and the difference may have been due to smoking habits (more current smokers) and social factors (lower income and less insurance coverage). As in the German–Danish study there was only a significant difference in decline in FEV_1 (27 ml/yr reduction in a decline of 93 ml/yr) in

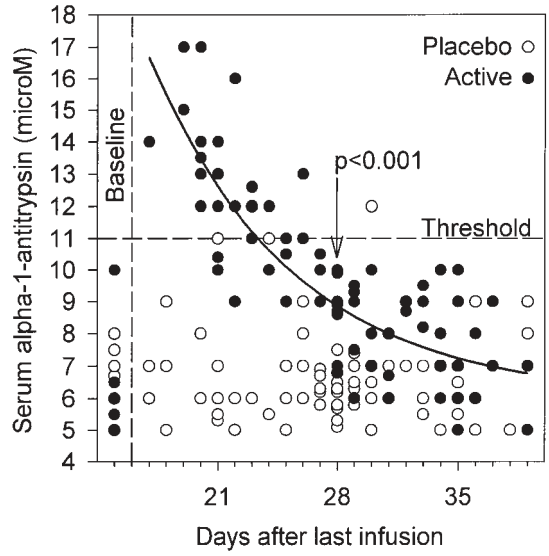

Figure 2. Serum levels of α_1-antitrypsin (μM) by days after last infusion. Baseline indicates levels at randomization. *Open circles* are levels after placebo infusions, and *closed circles* are levels after α_1-antitrypsin infusions (250 μg/kg). The threshold is an arbitrary "protective threshold" (11 μM), and the *solid line* indicates mean levels after α_1-antitrypsin infusions.

a subgroup with moderate emphysema (FEV_1 between 35% and 49% of predicted). No difference in either mortality or FEV_1 slope was observed between subjects receiving augmentation therapy continuously and those who received it intermittently.

For the actively treated group in our study, the 95% confidence interval of loss of lung tissue quantified with CT was 0.7 to 2.3 g/L, implying that α_1-antitrypsin infusions did not stop the progression of emphysema. However, this may be the result of our treatment regimen with relatively long intervals between infusions. For logistic reasons, the infusions were given every 4 wk and not on a weekly basis as recommended by most centers. Levels of α_1-antitrypsin were routinely measured just before each infusion (trough levels), which usually was 4 wk after the last infusion. Sometimes, for example, around holidays, infusions were given at shorter or longer intervals, and in Figure 2 trough levels are plotted against days after last infusion. Mean levels of α_1-antitrypsin at 28 d after the last infusion were 6.2 μM for placebo and 8.8 μM for the actively treated group ($p < 0.001$). It appears from Figure 2 that the infusions result in α_1-antitrypsin levels above a "protective threshold" of 11 μM for an average of 23 to 24 d after the infusion. Others have found that monthly infusions result in α_1-antitrypsin levels above the arbitrary threshold of 11 μM for an average of 25 d after the infusion (22).

What are the implications of our findings for clinical research? While pulmonary function tests have been used for many years for monitoring the progress of emphysema, measuring lung density is a novel concept. However, there is a growing body of evidence from cross-sectional studies showing that lung densities correlate well with microscopically detected emphysema as well as with CO diffusion. The latter two correlate significantly, with correlation coefficients between 0.71 and 0.77 (23, 24). These cross-sectional data and our present findings suggest that longitudinal CT studies can detect progression of emphysema. A statistical power calculation based on our study shows that a significant protection against the loss of 1.07 g/L of tissue owing to the treatment can be detected in a placebo-controlled trial over a period of 3 yr with 130 patients. This is much more feasible than a trial based on the demonstration of a corresponding (i.e., 50%) correction of the FEV_1 slope. For such a study, 550 patients would be needed.

Hence, provided that the lung density decline measured by CT can be generally accepted as an alternative parameter of the progress of emphysema, such measurements will have important implications for the required number of subjects needed in future randomized clinical trials. Several pharmaceutical companies have developed orally active synthetic elastase inhibitors as potentially valuable new drugs. These represent attractive alternatives for plasma-derived α_1-antitrypsin infusions for patients with α_1-antitrypsin deficiency-associated emphysema.

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