

Long-term multicentre trial in chronic nonspecific lung disease: methodology and baseline assessment in adult patients

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ABSTRACT: Airways obstruction and airways hyperresponsiveness are two dominant features in patients with chronic nonspecific lung disease (asthma and chronic obstructive pulmonary disease (COPD)). We set up a study to determine whether long-term (3 yrs) therapeutic intervention directed at airways obstruction and hyperresponsiveness is superior to one directed at airways obstruction alone. Patients were selected on functional criteria (age, baseline forced expiratory volume in one second (FEV₁), and airways hyperresponsiveness) and, furthermore, extensively characterized by history, smoking habits, allergy, reversibility of airways obstruction and quality of life. The methodology and practical problems of setting up this large multicentre study are outlined, together with an analysis of baseline data.

Standardization of methods and techniques and recruitment of patients required much effort, recruitment taking about twice as long as expected. A 3 month feasibility study allowed us to eliminate minor problems in the protocol.

Over a 16 month period, 274 adult patients (18-60 yrs) from the out-patient clinics of six university centres entered the study; 99 met the diagnostic criteria for asthma, 51 for COPD, 88 for asthmatic bronchitis, and 36 could not be classified. Their mean (SD) FEV₁ % pred was 65.1 (15.2)%. Their geometric mean provoking concentration of histamine producing a 20% fall in FEV₁ (PC₂₀ histamine) was 0.28 mg·ml⁻¹. In a multiple regression analysis, more severe airways hyperresponsiveness was associated with lower prechallenge FEV₁ % pred (p<0.0001), higher pack-years of smoking (p=0.0099), blood eosinophil count (p=0.0004), skin test reactivity (p=0.0047) and with female sex (p=0.0302).

We conclude that setting up long-term multicentre trials in chronic nonspecific lung disease (CNSLD) is feasible and that these may offer valuable information on treatment and outcome of the disease.

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Chronic nonspecific lung disease (CNSLD) encompasses both asthma and chronic obstructive pulmonary disease (COPD) [1], which are characterized in most patients by airways obstruction and airways hyperresponsiveness (AH). Patients with CNSLD generally need drug therapy for years but until recently only relatively short-term (e.g. months) effects of drugs in CNSLD have been studied in clinical trials. Over the last few years, there has been increasing interest in prospective studies to analyse the efficacy of long-term therapeutic interventions on the course and prognosis of asthma and COPD. The first of such studies was the intermittent positive-pressure breathing trial in patients with COPD in the USA over a 3 yr period [2]. Another long-term trial has been designed

to determine whether smoking cessation and/or anti-cholinergic bronchodilator therapy can prevent the development of COPD in high-risk individuals [3]. Recently, results from a double-blind study in which patients with mild asthma were treated for one year with inhaled corticosteroids or bronchodilators were published [4].

Several long-term studies on the natural history of patients with asthma and COPD have suggested that the outcome of disease is related to both severity and reversibility of airflow obstruction and to severity of AH [5-10]. It has been proposed that the slower decline in forced expiratory volume in one second (FEV₁) in COPD patients with greater initial reversibility may result from the treatment with

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bronchodilators at follow-up [8–10], a hypothesis that is now being tested in the USA [3]. On the other hand, recent reports indicate that regular bronchodilator treatment in asthma may have detrimental effects on its course [11, 12]. The observation that in asthma as well as in COPD the presence of more severe AH predicts a bigger decline in FEV₁ at follow-up [5, 7, 8] also suggests that it is worthwhile investigating therapeutic intervention in this respect.

Therefore, a prospective multicentre drug intervention trial with three years of follow-up was set up in patients with CNSLD. The main objective of this study is to test the hypothesis that a medical intervention directed against both airways obstruction and AH (*i.e.* the combination of inhaled beta-agonists and corticosteroids [13]) is superior to an intervention directed against bronchial obstruction alone (*i.e.* inhaled beta-agonists with placebo or combined with inhaled anticholinergic agents). Moreover, we intend to investigate whether treatment effects differ between the various clinical entities of CNSLD.

Long-term studies of CNSLD are increasingly important. Performing such a study poses some specific practical and methodological problems, particularly when conducted as a multicentre trial. These will be highlighted in the present report, together with an analysis of baseline data of the study population.

Patients

At six university pulmonary out-patient clinics, chart records of all outpatients were checked to see if they fitted predefined functional inclusion criteria (FEV₁, level ranging between 4.5–1.64 standard deviations (SD) below the predicted value, and larger than 1.2 l, or FEV₁/inspiratory vital capacity (IVC) ratio lower than 1.64 SD below the predicted value, provided that total lung capacity was higher than 1.64 SD below the predicted level [14]. Another selection criterion was hyperresponsiveness to inhaled histamine (provoking concentration producing a 20% fall in FEV₁ (PC₂₀) <8 mg·ml⁻¹, see below)). After exclusion of subjects considered to be ineligible, patients fulfilling these criteria were asked to participate in the trial. Those who were willing entered the baseline period of the study, during which lung function was measured twice (see below) to determine whether the inclusion criteria were met under standardized conditions.

Those excluded were pregnant women, patients with a history of occupational asthma or concomitant serious diseases (*e.g.* tuberculosis, myocardial infarction or malignancies), patients who used oral corticosteroids, beta blockers, nitrates, or anticoagulants, and patients who continuously used antibiotics. Atopy, smoking habits, and previous diagnosis of asthma or COPD were deliberately not used as selection criteria.

By using data from a standardized history on respiratory symptoms, we identified different clinical syndromes, closely adhering to the criteria proposed by the American Thoracic Society (ATS) [15]:

1. Patients reporting attacks of breathlessness and wheeze (asthmatic attacks) without chronic (*i.e.* for more than 3 months per year) cough or sputum production were labelled asthma (n=99, 36%).
2. Current or former smokers without a history of asthmatic attacks, reporting either chronic cough with or without sputum production or dyspnoea when walking quietly on level ground, or both, were included in the COPD group (n=51, 19%).
3. Patients with both asthmatic attacks or recurrent wheeze and chronic cough and sputum production were labelled asthmatic bronchitics (n=88, 32%).
4. In 36 subjects (13%), a clinical syndrome diagnosis could not be made from the history data because these were either incompletely or unreliably filled out ("no diagnosis" group).

Power calculations (see Appendix) were used to compute required sample sizes [16], and a 35% drop-out rate during follow-up was, rather arbitrarily, predicted. Rounding up the numbers, the aim was to recruit 300 adult patients for the study.

The study protocol was approved by the Medical Ethics Committees of all participating centres; all patients gave written informed consent. A full protocol is available by request.

Study design

This randomized, double-blind study uses three parallel treatment regimens with drugs in identical metered dose inhalers:

- 1) terbutaline (500 µg *q.i.d.*) plus placebo (*q.i.d.*);
- 2) terbutaline (500 µg *q.i.d.*) plus ipratropium bromide (80 µg *q.i.d.*);
- 3) terbutaline (500 µg *q.i.d.*) plus beclomethasone (200 µg *q.i.d.*).

Baseline data

Baseline data were acquired on two visits with an interval of 2–4 wks, after which patients were randomized. Before entering the baseline period of the study, patients discontinued their usual maintenance treatment for at least 1 month (ketotifen, anti-histamines), 2 wks (inhaled corticosteroids, cromolyn sodium), or 2 days (theophyllines). Only bronchodilators were used during the 14 days prior to the study and during the baseline period; these were withheld at least 8 h before lung function measurements. All measurements were performed during clinically stable periods (*i.e.* not within 3 weeks after an exacerbation or discontinuation of an oral corticosteroid course).

At both baseline visits, a standardized history regarding respiratory symptoms was obtained and a physical examination performed, in addition to spirometry, bronchodilator response, and histamine provocation test (see below). Furthermore, at the second baseline visit, intradermal skin testing was performed, as well as an extensive lung function assessment (volume-flow loops,

transfer factor for carbon monoxide, and static volumes). Blood was drawn for total leucocyte and eosinophil counts, and serum was stored at -20°C for later assays of cotinine levels. Total and house dust mite (HDM)-specific immunoglobulin E (IgE) concentrations were determined. A quality of life questionnaire was filled in by all patients [17]. Patients kept a diary with symptom scores and peak expiratory flow (PEF) measurements for 14 consecutive days prior to the second baseline visit.

Patient follow-up

Patients are to be seen every 3 months, for 3 yrs. At these follow-up visits lung function is measured, including alternate testing of reversibility and AH. PEF recordings, symptom score cards, and the technique of using the metered dose inhaler are checked. Therapy compliance is monitored by weighing the used aerosol canisters. In addition to their study medication, patients may inhale salbutamol (Rotacaps[®], 400 μg) on demand. No other pulmonary drugs are allowed. Exacerbations (defined as conditions with increased complaints of cough and/or wheezing and/or dyspnoea and a decreased response to inhaled beta-agonists, *i.e.* an increase in the required dosage of more than four additional Rotacaps a day) are treated with short courses of corticosteroids (30 mg of prednisolone orally per day, diminishing with 5 mg per 2 days over 12 days).

Methods

The entire study was performed according to a standardized protocol. This protocol was repeatedly tested in training sessions with technicians from all centres.

Spirometry was performed using calibrated water-sealed spirometers according to standardization guidelines [14]. FEV₁ and IVC were measured until three reproducible (less than 5% difference) recordings were obtained. Highest values were used for analyses. Reference values are those of the European Community for Coal and Steel (ECCS) [14].

Reversibility of airways obstruction was tested: FEV₁ measurements were carried out before and 20 min after four single inhalations of 250 μg of terbutaline sulphate from a metered dose inhaler, administered through a 750 ml spacer device (Nebuhaler, Astra Pharmaceuticals, Rijswijk, The Netherlands). Patients refrained from coffee, tea, and smoking between these measurements. Results are presented as postbronchodilator FEV₁ (% pred), and as ΔFEV_1 (% pred) [18].

Histamine provocation tests were performed using a 2 min tidal breathing method, adapted from COCKCROFT *et al.* [19]. Aerosols were delivered by a calibrated DeVilbiss 646 nebulizer (DeVilbiss Health Inc., Somerset, Pennsylvania, USA), connected to the central chamber of an inspiratory and expiratory valve box

with an expiratory aerosol filter (Pall BB50T, Pall Biomedical Ltd, Portsmouth, UK) and placed directly opposite the mouthpiece. Solution output was 0.13 ml·min⁻¹. After inhalation of phosphate-buffered saline (PBS), subjects inhaled doubling concentrations of histamine diphosphate diluted in PBS, ranging from 0.03–8 mg·ml⁻¹, at 5 min intervals. FEV₁ was measured 30 and 90 s after each inhalation; the lowest technically satisfactory FEV₁ was used. The challenge was discontinued if FEV₁ was less than 80% of mean prechallenge level, or when the highest concentration of histamine diphosphate had been administered. PC₂₀ histamine, was determined by linear interpolation between the last two data points on the log concentration-response curve.

Residual volume (RV), functional residual capacity (FRC) and total lung capacity (TLC) were performed by the closed circuit multibreath helium dilution method [14], results were expressed in litres. Carbon monoxide transfer factor (TLCO, expressed in ml·min⁻¹·kPa⁻¹) was recorded by a breathholding method [14]. Total and HDM-specific IgE concentrations were quantified using an enzyme immunoassay procedure (Pharmacia, Uppsala, Sweden), and expressed in international units (IU)·ml⁻¹, and Phadebas radioallergosorbent test (RAST) units (PRU)·ml⁻¹, respectively.

Intradermal skin tests to 12 common aeroallergens (*i.e.* HDM, several grass and tree pollens, *Aspergillus*, *Alternaria*, dog, cat, horse and birds) were applied using purified allergenic extracts (ALK Laboratory, Copenhagen, Denmark). Negative (PBS) and positive (histamine 0.03 mg·ml⁻¹) controls were also applied. For classification purposes, a skin test was considered positive if the mean wheal diameter (MWD) was larger than the MWD of the histamine skin test. The number of positive wheal reactions per patient was counted.

During 14 days prior to each follow-up visit, patients recorded daily symptom scores on a 4 point scale for wheeze, dyspnoea, cough, and phlegm, separately (0 = no symptoms; 3 = severe symptoms). Use of additional bronchodilator medication (in number of dosages per day) and number of days they were absent from work or school because of CNSLD symptoms were also recorded. After a standardized instruction at the out-patient clinic, patients used a Wright mini peak flow meter (Clement Clarke International Ltd, London, UK) to record PEF at home, again during 14 days prior to each visit. PEF is recorded twice daily: in the morning directly after rising, before and 10 min after usage of bronchodilator drugs, and late in the afternoon, before dinner and before usage of bronchodilators. The highest PEF value of three blows is recorded in the diary.

Allocation to treatment

After the baseline period, patients were randomly allocated to one of the treatment groups, with stratification for age, sex, FEV₁ % pred, reversibility, PC₂₀,

skin test reactivity, smoking habits, prior usage of inhaled steroids and participating centre [16, 20]. Stratification was performed using the minimization method [16] on a personal computer by an independent 24 h service telephone centre.

End-points

The primary end-points in the trial are: actual values and annual decrease of FEV₁, bronchodilator response assessed by FEV₁, and PC₂₀ histamine. Secondary end-points are: daily PEF rates, symptom scores, absence from school/work, hospitalization, and quality of life measurement.

Quality control

All data were recorded on standardized forms and submitted to a data centre where they were keyed into a data base. Missing and out-of-range data were noted and referred back immediately to the appropriate clinical centre for clarification. Data input into the computer was double-checked with data on the submitted forms. Twice yearly, the clinical centres were visited by independent lung function experts to check that the lung function measurements were performed according to protocol. The DeVilbiss nebulizers were centrally calibrated at one year intervals.

Feasibility study

The entire process of patient recruitment, data collection, and allocation to treatment was tested in a three months' feasibility study prior to the actual trial. Remaining problems in the protocol were identified and corrected.

Data analysis

All data for this report were analysed using the statistical package SPSS/PC+ [21]. Correlations between variables were expressed as Pearson's *r*. Differences between groups were analysed with Student's *t*-tests and one-way analysis of variance (ANOVA). The influence of several patient characteristics on the level of AH was assessed in a stepwise multiple linear regression model. For the measurement of AH, saline responders and patients responding with a >20% fall in FEV₁ after the first dose of histamine (0.03 mg·ml⁻¹) were attributed a PC₂₀ value of 0.015, being one doubling dose below the first actual dose. Calculations were performed with base 2 log-transformed PC₂₀ values because these values reflect the use of doubling doses in the histamine provocation test, and with base 10 log-transformed eosinophil counts and total serum IgE levels (in order to obtain Gaussian distributions for these variables). *P* values <0.05 were considered significant.

Results

Protocol and its feasibility

It took several sessions to discuss all measurements involved in the study protocol. Much energy was used in editing the respiratory symptom questionnaire. Several differences in methods and techniques were noted and resolved. For example, it was found that the composition of both the PBS and the histamine solutions varied considerably between the participating laboratories. For the PBS solution, different mixtures of NaH₂PO₄ and Na₂HPO₄ were used. All histamine solutions contained the same weight of histamine phosphate per litre of saline, but the dry histamine salts differed in the amount of molecular water, amounting to molecular weight differences up to 10%. Therefore, these solutions were standardized (table 1).

Table 1. - Preparation of histamine solution

Phosphate-buffered saline (PBS)			
Matter	Weight g	Equivalent weight	
NaH ₂ PO ₄	1.808	= NaH ₂ PO ₄ ·2H ₂ O	2.35 g
Na ₂ HPO ₄	7.576	= Na ₂ HPO ₄ ·12H ₂ O	19.11 g
NaCl	4.400		
H ₂ O (pH 7.40)	ad 1000 ml		
Histamine diphosphate (HDP) 32 mg·ml ⁻¹ = 104 mmol·l ⁻¹			
HDP	32 g	= HDP·1H ₂ O	33.88 g
PBS (see above)	ad 1000 ml		
Other dilutions of HDP			
Made by diluting the HDP 32 mg·ml ⁻¹ (104 mmol·l ⁻¹) solution with PBS			
Remarks			
Sterilization: 20 min at 120°C. No preservative added. Stored in dark place			

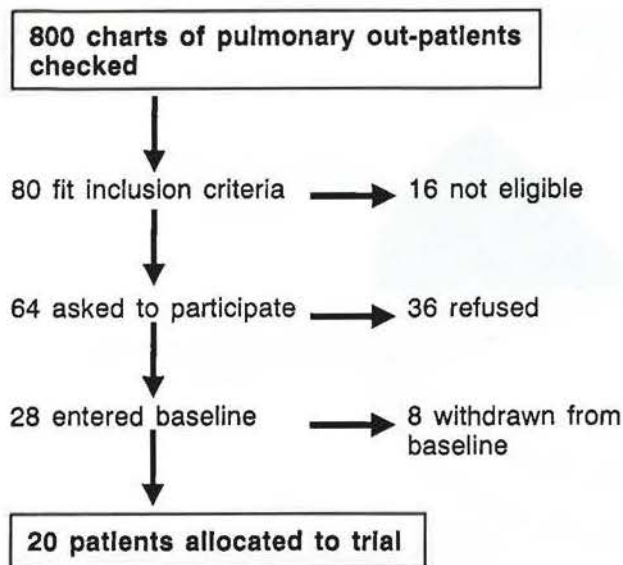


Fig. 1. - Example of the patient recruitment process in the Dutch chronic non-specific lung disease (CNSLD) study. The numbers give an impression of the effort involved in recruiting the required number of patients for a long-term clinical trial.

psychiatric illness, concomitant disease, or earlier refusal to participate in any study). The other patients were asked to participate. More than 50% of them refused, mainly because the long-term nature of the trial deterred them or because they were reluctant to have their pulmonary maintenance medication withdrawn. The patients who entered the baseline period were representative of the larger group who fitted the inclusion criteria in terms of age, sex, and lung function (t-tests, all p values >0.10). Among those who refused to participate in the trial, there was a significantly (chi square test, $p < 0.05$) larger proportion of patients who used inhaled corticosteroids than among those who entered the baseline period.

Of all patients who entered the baseline period, about 25% could not be allocated to treatment, because they failed to meet the entry criteria or because they had increased pulmonary symptoms after withdrawal of maintenance medication (fig. 1). Differences between baseline withdrawals and those patients who completed the baseline period are presented in table 2. Although small differences between the two groups were noted for FEV_1/IVC and for smoking habits, the

Table 2. - Comparison of patients who were withdrawn during baseline and those who completed baseline characterization.

	Withdrawals n=78		Non-withdrawals n=274		p*
	Mean	SEM	Mean	SEM	
FEV_1 prebronchodilator					
<i>l</i>	2.38	0.13	2.39	0.05	0.973
% pred	68	2.8	65	0.9	0.226
FEV_1 postbronchodilator					
<i>l</i>	2.77	0.13	2.84	0.05	0.563
% pred	79	2.8	77	0.9	0.593
FEV_1/IVC prebronchodilator					
%	60	2.0	57	0.7	0.058
% pred	75	2.4	71	0.8	0.058
$\log_2 PC_{20}$ mg·ml ⁻¹	-1.56	0.34	-1.80	0.15	0.499
Age yrs	41	1.4	40	0.7	0.352
Male sex %	60		64		0.520
Smoker: lifetime non %	31		32		
ex %	47		32		0.027
current %	22		36		

*: t-test; chi square test for male sex and smoking habits. FEV_1 : forced expiratory volume in one second; FEV_1/IVC : FEV_1 /inspiratory vital capacity ratio; PC_{20} : provoking concentration of histamine producing a 20% fall in FEV_1 .

Patients

It turned out to be relatively hard to recruit patients for this study (fig. 1). At participating centres, about 10% of all pulmonary out-patients appeared to fit the inclusion criteria mentioned above. Of these patients, 20% were considered not eligible for the trial (e.g.

two groups appeared fairly comparable with respect to lung function and PC_{20} histamine (table 2).

The total recruitment period required 16 months, which was about twice as long as was expected. In the end, 274 patients were allocated to blind treatment at six centres (fig. 2). Well-balanced treatment groups were formed for all stratification factors (fig. 3).

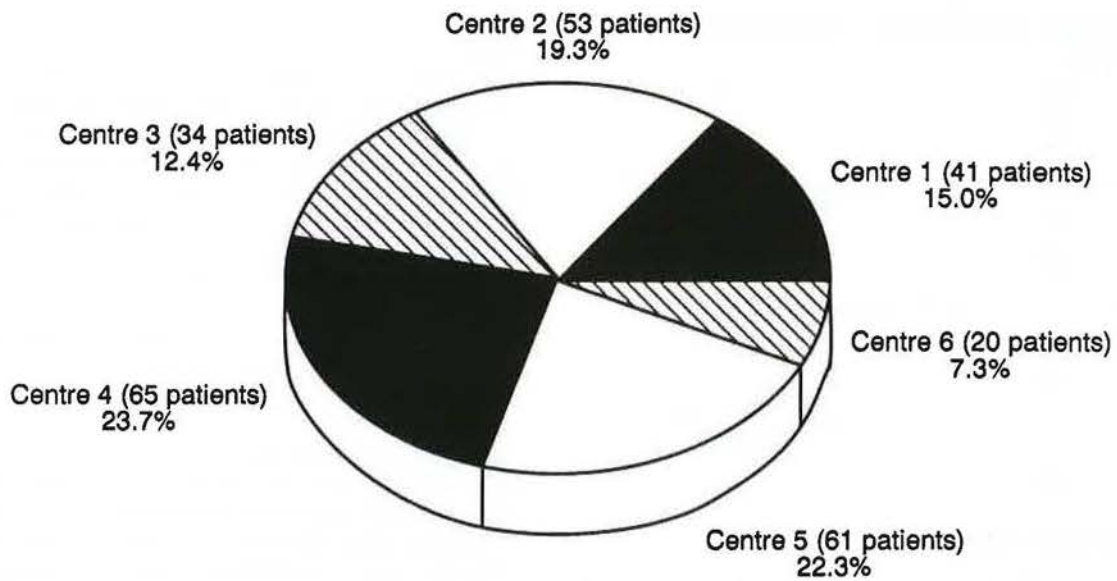


Fig. 2. - Number of patients per participating centre. Total 274 patients.

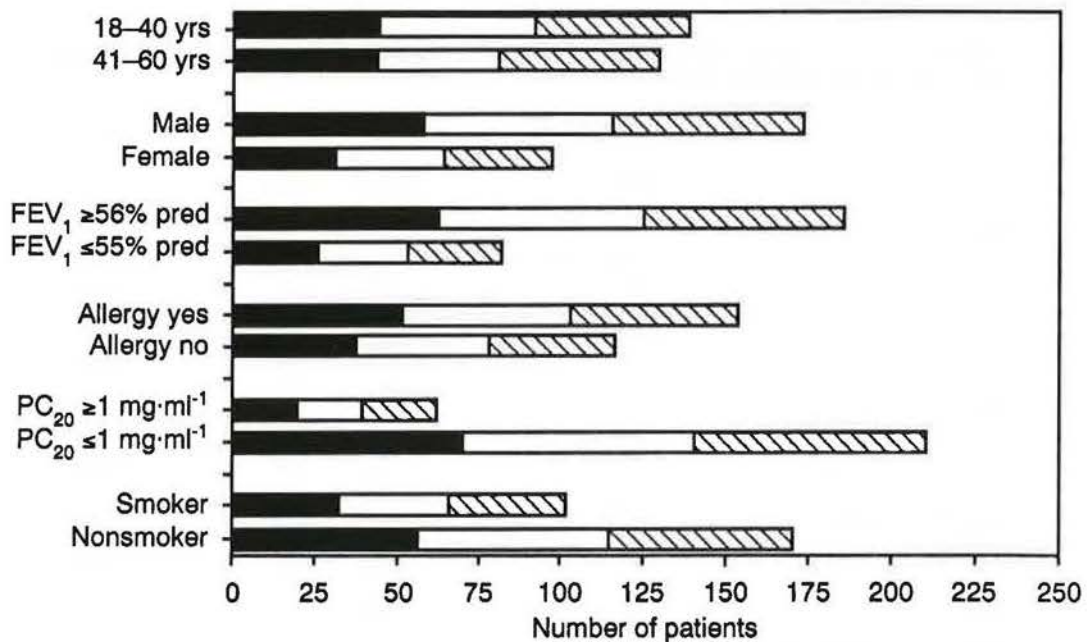


Fig. 3. - Stratification of patients using the minimization method. Total 274 patients. Nine stratification factors were used, six of which are displayed in this graph. For each stratification factor, nicely balanced treatment groups were formed. FEV₁: forced expiratory volume in one second; PC₂₀: provoking concentration of histamine producing a 20% fall in FEV₁; ■: Therapy A; □: Therapy B; ▨: Therapy C.

Baseline assessment

During the baseline period, a significant decrease in measures of airway calibre was observed, together with an increase in AH which did not reach statistical significance (table 3), probably as a result of prolonged withdrawal of pulmonary maintenance medication. We therefore felt that our original objective of using mean values of both baseline visits as the baseline levels of lung function would not be justified. Instead, we

chose to use only the results of the second visit as the baseline values. These values are used for the subsequent analyses in this report.

Syndrome diagnosis

Results of various baseline measurements in the different syndrome diagnosis groups are shown in table 4. Patients with a history compatible with

asthma had significantly lower mean \log_2 PC₂₀ values than asthmatic bronchitics and COPD patients. Patients in the group with no conclusive diagnosis had intermediate mean \log_2 PC₂₀ values. Prechallenge FEV₁ levels were not significantly different between the groups. Postbronchodilator FEV₁, however, was significantly lower in COPD than in the other three groups. Further between-group differences were noted in static lung volumes, TLC₀, age, pack-years of smoking, and indices of atopy (table 4).

Relationship of patient characteristics to level of AH

A small, but statistically significant positive correlation existed between age and \log_2 PC₂₀ ($r=0.168$, $p=0.0054$). There was also a positive correlation between \log_2 PC₂₀ and pack-years of smoking ($r=0.240$, $p=0.0001$). Current smokers had higher \log_2 PC₂₀ values (mean \pm SEM; -1.15 ± 0.21) than former (2.30 ± 0.24) or lifetime nonsmokers (-2.48 ± 0.24) (oneway ANOVA,

Table 3. - Comparison of lung function at the two baseline visits of the study for those patients who completed baseline characterization (n=274)

	First visit		Second visit		p*
	mean	SEM	mean	SEM	
FEV ₁ prebronchodilator					
<i>l</i>	2.39	0.05	2.33	0.05	0.022
% pred	65	0.92	64	0.93	0.017
FEV ₁ postbronchodilator					
<i>l</i>	2.84	0.05	2.77	0.05	0.001
% pred	78	0.95	76	0.99	0.001
FEV ₁ /IVC %	57	0.67	55	0.67	<0.001
\log_2 PC ₂₀ mg·ml ⁻¹	-1.78	0.14	-1.95	0.14	0.097

*: paired t-test. For further abbreviations see legend to table 2.

Table 4. - Patient characteristics and results of baseline assessment in different syndrome diagnosis groups

	Asthma mean (SEM)	AB mean (SEM)	COPD mean (SEM)	No D mean (SEM)	p*
Age yrs	36 (1.2)	40 (1.3)	46 (1.3)	38 (1.9)	<0.0001
Male %	62	69	69	53	0.2873
Pack-years	4.1 (0.77)	12.3 (1.94)	24.1 (2.96)	8.7 (2.09)	<0.0001
FEV ₁ prebronchodilator					
% pred	64 (1.5)	65 (1.7)	61 (2.3)	64 (2.7)	0.5053
FEV ₁ postbronchodilator					
% pred	79 (1.6)	76 (1.7)	69 (2.3)	76 (2.4)	0.0048
Δ FEV ₁ % pred	9.2 (0.93)	8.1 (0.86)	7.1 (1.00)	11.7 (1.51)	<0.0001
Δ FEV ₁ % init	26 (1.79)	18.6 (1.72)	14.9 (1.99)	19.4 (2.84)	0.0007
FEV ₁ /IVC %	55.8 (1.00)	55.6 (1.27)	52.6 (1.74)	56.7 (1.53)	0.2779
% pred	69 (1.2)	69 (1.5)	66 (2.1)	70 (1.8)	0.4713
FRC <i>l</i>	3.49 (0.09)	3.72 (0.10)	3.93 (0.15)	3.43 (0.14)	0.0181
TLC <i>l</i>	6.56 (0.14)	6.89 (0.15)	7.04 (0.21)	6.47 (0.23)	0.1061
\log_2 PC ₂₀ mg·ml ⁻¹	-2.83 (0.18)	-1.06 (0.23)	-0.87 (0.31)	-1.94 (0.38)	<0.0001
Tlco ml·min ⁻¹ ·kPa ⁻¹	10.2 (0.26)	10.1 (0.30)	8.8 (0.36)	9.2 (0.47)	0.0103
\log_{10} eosinophils $\times 10^6$ ·l ⁻¹	2.40 (0.03)	2.31 (0.05)	2.16 (0.06)	2.35 (0.09)	0.0159
\log_{10} IgE IU·ml ⁻¹	2.22 (0.06)	2.11 (0.09)	1.79 (0.11)	2.24 (0.11)	0.0038
EAST HDM·PRU·ml ⁻¹	5.1 (0.55)	4.1 (0.59)	2.1 (0.59)	4.1 (0.96)	0.0184
HEWS of HDM skin test	1.06 (0.06)	0.74 (0.07)	0.57 (0.08)	0.69 (0.10)	<0.0001
Wheal size of histamine skin test mm	11.6 (0.35)	11.8 (0.21)	11.3 (0.25)	11.8 (0.35)	0.5768

AB: asthmatic bronchitis; COPD: chronic obstructive pulmonary disease; No D: no conclusive diagnosis; Δ FEV₁: change in forced expiratory volume in one second (FEV₁) after inhalation of a bronchodilator; Δ FEV₁ % pred: Δ FEV₁, expressed as a percentage of predicted FEV₁; Δ FEV₁ % init: Δ FEV₁, expressed as a percentage of prebronchodilator FEV₁; FEV₁/IVC: FEV₁/inspiratory vital capacity ratio; FRC: functional residual capacity; TLC: total lung capacity; Tlco: transfer factor of lungs for carbon monoxide; IgE: immunoglobulin E; EAST: enzyme-linked allergosorbent test for determination of anti-HDM specific IgE; HDM: house dust mite; HEWS: histamine equivalent wheal size; PRU: Phadebas radioallergosorbent test units; *: oneway analysis of variance (ANOVA) (except for male sex: chi square test).

$p=0.0001$). A negative association was observed between $\log_2 PC_{20}$ on the one hand and \log_{10} eosinophil counts ($r=-0.303$, $p<0.0001$), \log_{10} IgE levels ($r=-0.197$, $p=0.0013$), and the number of positive skin tests (one-way ANOVA, $p=0.0005$) on the other hand.

Women had lower $\log_2 PC_{20}$ values (mean \pm SEM -2.43 ± 0.23) than men (-1.68 ± 0.17) (t-test, $p=0.010$).

A positive correlation existed between prechallenge FEV₁ level (as a percentage of predicted FEV₁) and $\log_2 PC_{20}$ values ($r=0.267$, $p<0.0001$).

A stepwise multiple linear regression model was built with $\log_2 PC_{20}$ value as dependent variable and \log_{10} eosinophil count, \log_{10} IgE level, the number of positive skin tests, the histamine equivalent wheal size (HEWS) of the HDM skin test, age, sex, FEV₁, % pred, smoking status (current, former, or lifetime nonsmoker), and pack-years of smoking as independent variables. Results are presented in table 5. FEV₁, % pred was the most important predictor of $\log_2 PC_{20}$ values. Pack-years of smoking, eosinophil count, the number of positive skin tests, and female sex entered the model at subsequent steps. The other variables were not significantly ($p>0.05$) related to $\log_2 PC_{20}$ levels (table 5).

Table 5. - Stepwise multiple regression analysis of $\log_2 PC_{20}$ values

Variable	B	SE	P
Intercept	-1.883	0.943	0.0469
FEV ₁ % pred	0.041	0.008	<0.0001
Pack-years	0.021	0.008	0.0099
Log ₁₀ eosinophils	-1.046	0.291	0.0004
Number of positive skin tests	-0.171	0.060	0.0047
Female sex	-0.579	0.266	0.0302
Smoking (lifetime non, ex, or current)	0.109	0.618	0.1133
Age	0.034	0.672	0.6128
log ₁₀ IgE	-0.109	0.702	0.0894
HEWS of HDM skin test	-0.890	0.553	0.2332

B: regression coefficient. For further abbreviations see legend to table 4.

When syndrome diagnosis was added as an independent variable, it contributed to the model ($p<0.0001$), taking the place of smoking status which was no longer significantly related to $\log_2 PC_{20}$ ($p=0.1376$). The relationship of other independent variables with $\log_2 PC_{20}$ levels remained more or less the same.

Discussion

This report had two main objectives. Firstly, reporting our experience in setting up and conducting a long-term multicentre trial may be useful to others considering setting up such studies. Secondly, our baseline data allowed for a multivariable analysis of airway hyperresponsiveness in patients with CNSLD.

Protocol

In this multicentre trial, standardization of methods required much effort, but it was valuable because lung function tests were thoroughly re-evaluated. Because the use of weight-based solutions is recommended in histamine provocation testing [22] and large differences may exist between laboratories in the dry weight of histamine salts (as was found in our study), we feel that the standardization of histamine solutions (table 1) may be important. Further problems in the protocol were effectively eliminated by a feasibility study and by frequent site visits by lung function experts. Such procedures should be incorporated in any long-term trial in CNSLD. Although one might be tempted to design one's own way of recording a respiratory history, the most reliable method of recording symptoms is to use validated questionnaires [23, 24]. Because we used drugs from three different manufacturers in our study, considerable effort of the companies involved was necessary to guarantee identical canisters.

To achieve balance between treatment arms with respect to several risk factors, nine stratification factors were used, and a simple computer programme was designed for the randomization procedure. For this purpose, a central allocation centre, accessible by telephone, is required in a multicentre trial. We used an independent round-the-clock telephone service. After careful testing during the feasibility study, no problems were encountered during the randomization phase. Well balanced treatment groups with respect to a number of important risk factors were thus formed (fig. 3).

Power calculations for required sample sizes [16] depend heavily on the desired treatment effect and on its standard deviation (sd) which is often unknown (see appendix). Expected drop-out rates must also be taken into account as the calculated numbers refer to those subjects completing the study. Since no results of comparable intervention studies were available as a guideline, an estimation of treatment effect and its sd was derived from natural history studies (2, 25, 26). Our choice of 30 ml differences in FEV₁ being clinically relevant between treatment groups is comparable to that of another long-term trial [3]. This leads, by extrapolation, to a difference of almost a litre over 30 yrs. Recruiting the required number of patients for long-term trials may be difficult (fig. 1); it is a common experience that centres tend to overestimate the number of patients that they feel they can enter into the trial [16]. Motivation of trial staff and patients, therefore, is of vital importance in a long-term study, both during the recruitment period and especially during long-term follow-up.

Baseline data analysis

After withdrawal of pulmonary maintenance treatment, lung function parameters deteriorated slightly but significantly during the baseline period (table 3). Therefore, we chose not to use mean baseline values

as starting levels for comparing treatment effects. The data in table 3 may give an impression of the deterioration in lung function in CNSLD after withdrawal of anti-inflammatory treatment. Although this deterioration might lead to selective drop-out during baseline in our study, this does not appear to be serious as lung function was very similar in baseline withdrawals compared to those patients who completed the baseline period (table 2). Altogether, the population allocated to blind treatment appeared to be representative of the CNSLD "root" population fitting our inclusion criteria.

Subgroup analysis. Most studies of obstructive lung disease are performed in subgroups of patients, usually defined by a clinical diagnosis of asthma or COPD. Because the definitions of these syndromes are subject to various interpretations [15, 27-30], results of different studies are hard to compare. Therefore, we used functional inclusion criteria, which guarantee an unbiased description of the study population [29]. Subsequently, subgroup analyses can be performed. One approach is to divide patients into clinical syndromes based on history data alone, adhering to ATS guidelines [15]. The results of this analysis (table 4) reflect the concept that a history of asthma is generally encountered in young, atopic patients with largely reversible airways obstruction, whilst a history of COPD is most commonly found in elderly, non-atopic smokers who reveal at best partial reversibility [15]. Nevertheless, it should be noted that all variables in table 4 have unimodal and continuous distributions; despite significant differences, none of these variables allows for a complete distinction between asthma and COPD.

Determinants of airways hyperresponsiveness. To eliminate the influence of respiratory infections on AH [31], histamine provocation tests were only performed during clinically stable episodes. In our patient population where AH itself was a selection criterion, several factors were found to be related to $\log_2 PC_{20}$ level but only a few of them showed independent influence in multiple regression analysis (table 5). The most important determinant of AH was found to be prechallenge FEV_1 , a finding which has been reported in numerous clinical and epidemiological studies in asthma and COPD [32-35]. However, other factors were also found to be important. The relationship of eosinophil count and skin test reactivity with AH confirms the important association between atopy and AH [32, 36-38]. The dose-dependent effect of smoking on AH observed in epidemiology [39] is commonly obscured in clinical populations by a self-selection away from smoking of patients with severe AH, called the "healthy smoker effect" [32]. This appears also to be the case in our study, because a decrease in AH with increasing cumulative smoke exposure was found (table 5) and current smokers were less likely to drop out after withdrawal of maintenance treatment (table 2). Curiously, female sex was an independent

determinant of AH in this study. We have no explanation for this finding other than selection factors.

To our knowledge, comparison of our multivariable analysis of AH with other clinical investigations is not possible as no data are available. In one population-based studies of AH, sex was unrelated to PC_{20} [40]; in another, men had lower PC_{20} values than women [41]. Selection factors may be largely responsible for these differences. The other results of both studies were comparable to ours [40, 41].

Conclusion

Although setting up and conducting a long-term trial in CNSLD requires much effort, it is feasible and it may offer valuable information on mechanisms and therapy of the disease. In our baseline data, AH was related to prechallenge airway calibre, indices of atopy, smoking habits, and female sex.

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Appendix

Power calculations [16]

The three main end-points of the study are quantitative measurements. These allow estimation of how many patients are required in each group to obtain results which allow the assessment of whether differences in treatment are due to chance or represent real differences. The equation to compute the number is as follows:

$$n = \frac{2 \times SD^2}{(\mu_2 - \mu_1)} \times f(\alpha, \beta)$$

Where n = required number of patients per treatment group;
 μ = mean treatment response per group; $\mu_2 - \mu_1$ = difference

in treatment response between two groups; SD = standard deviation (of mean treatment response); α = type I error (risk of a false-positive result); β = type II error (risk of a false-negative result); $1 - \beta$ = "power" to detect a difference of magnitude $\mu_2 - \mu_1$; $f(\alpha, \beta)$ = function of α, β . May be calculated but is most conveniently obtained from statistical tables.

Assumptions: Assumptions underlying this equation are that we are dealing with approximately normal distributions of data in each group, and that the standard deviations are approximately equal. In this study we have adopted $\alpha = 0.05$ and $\beta = 0.10$. For FEV_1 the required number of patients was computed as follows. Let μ be the annual rate of change of FEV_1 . We have estimated that the spread (SD) in the rate of change over a three year period would be 50 ml, and that the clinically relevant difference in the annual rate of change of between the "best" and the "next best" group which we would want to detect would be 30 ml, e.g. 180 ml decline in one group and 210 ml in the other group. Then the required number of patients per treatment group is 60.