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Effect of tiotropium on sputum and serum inflammatory markers and exacerbations in chronic obstructive pulmonary disease

Duncan J Powrie MRCP[1]

Tom MA Wilkinson MRCP[2]

Gavin C Donaldson PhD[2]

Peter Jones BSc MSc[3]

Kenneth Scrine BSc[3]

Klaus Viel MD[3]

Steven Kesten MD[3]

Jadwiga A Wedzicha MD[2]

1. Southend University Hospital, Essex , UK
2. Academic Unit of Respiratory Medicine, Royal Free and University College Medical School, London, UK
3. Boehringer-Ingelheim, Bracknell, UK

Corresponding Author: Prof. JA Wedzicha

Academic Unit of Respiratory Medicine

Royal Free and University College Medical School

Rowland Hill St.

London

NW3 2PF. UK

Tel. 44 207 3177517 Fax. 44 207472614

e-mail j.a.wedzicha@medsch.ucl.ac.uk

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Running head: tiotropium and inflammation

Abstract

COPD patients experiencing frequent exacerbations demonstrate increased stable state airway inflammation. Tiotropium has been shown to reduce exacerbation frequency but its effect on airway inflammation is unknown. We studied the effect of tiotropium on sputum inflammatory markers and exacerbation frequency.

142 patients were randomized to receive tiotropium or placebo in addition to their usual medication for one year. Sputum and serum cytokines were assayed by ELISA and exacerbation frequency calculated using a symptom based diary.

There was no difference in area under the curve for sputum IL-6 ($p=0.324$) or MPO ($p=0.079$) between the groups but sputum IL-8 was increased in the tiotropium arm ($p=0.043$). There was no difference between start and study end in serum IL-6 ($p=0.691$) or CRP ($p=0.700$).

Tiotropium was associated with a 52% reduction in exacerbation frequency (1.17 v 2.46 per year, $p<0.01$). 43% of patients on tiotropium experienced at least one exacerbation compared to 64% on placebo ($p=0.01$). Total exacerbation days were reduced compared to placebo (17.3 v 34.5, $p<0.01$).

Tiotropium reduces exacerbation frequency in COPD but this effect does not appear to be due to a reduction in airway or systemic inflammation.

Key words- anticholinergic, cytokines, sputum

Introduction

COPD is an inflammatory disease of the airways resulting in a progressive irreversible decline in respiratory function. Patients with COPD experience acute exacerbations characterised by worsening symptoms, deterioration in lung function and increased airway inflammation [1, 2]. Exacerbations are a major cause of morbidity and mortality and have major resource implications for the treatment of COPD [3, 4].

Patients suffering more frequent exacerbations report worse quality of life, are more likely to become housebound and have increased mortality [3, 5, 6]. Frequent exacerbations are associated with a faster decline in lung function and it has been shown that patients experiencing more frequent exacerbations demonstrate increased airway inflammation in the stable state [7, 8]. Reducing exacerbation frequency is therefore clearly an important objective in the management of COPD.

Tiotropium is a once daily inhaled anticholinergic bronchodilator. Long term studies have demonstrated improvements in FEV_1, FVC, lung volumes, health-related quality of life and exercise capacity [9-13]. In addition, studies have reported reductions in exacerbation frequency [9-11]. Recent prospective, multi-centre studies have shown an increase in time to first exacerbation with tiotropium compared to placebo, as well as a reduction in exacerbation frequency, hospitalisations and healthcare utilisation [14, 15] however the mechanism of this reduction in exacerbations is unknown.

It has been shown that acetylcholine increases neutrophil chemotactic activity in COPD and that this effect is attenuated *in vitro* by tiotropium thus providing a

possible anti-inflammatory mechanism of action [16, 17].There is a close relationship between airway inflammation and exacerbation frequency and it might be postulated that a reduction in stable state airway inflammation would result in a reduction in exacerbation frequency.

There have been several short-term studies of the effect of COPD therapies on airway inflammation [18-21]. We hypothesised that tiotropium may reduce airway inflammation and this would lead to a reduction in exacerbation frequency. Thus the aim of the trial was to study for the first time the effect of inhaled tiotropium on airway and systemic inflammatory markers in COPD in a one year, randomised, placebo-controlled study. We also studied the effect of tiotropium on exacerbation frequency using symptom-based diary cards. Results from this study have been presented in the form of abstracts [22, 23]

Methods

Study design

This was a one year, single centre, double-blind, randomised, placebo-controlled study to assess the effect of tiotropium on sputum inflammatory markers and exacerbation frequency. Ethics approval was obtained from East London and the City Research Ethics Committee, the study was registered on ClinicalTrials.gov (NCT00405236) and all patients gave written informed consent. The primary endpoint was the concentration of interleukin-6 (IL-6) in sputum. Secondary end points included sputum interleukin-8 (IL-8) and myeloperoxidase (MPO), serum IL-6 and C-reactive protein (CRP), sputum bacterial colonisation, FEV_1 and exacerbation frequency.

Patient selection

Patients aged ≥ 40 years with a diagnosis of COPD (FEV_1 < 80% predicted and FEV_1 /FVC < 70%) and a minimum 10 pack year smoking history were recruited from primary care or the outpatients department of the London Chest Hospital. Patients with a history of asthma or atopy were excluded, as were those on long term oxygen therapy or those with another clinically significant disease. Anticholinergics other than the study drug were not allowed during the course of the study.

Study Protocol

Subjects were randomised to tiotropium 18mcg once daily or placebo via the *HandiHaler* device. They were provided with diary cards for recording of daily symptoms, morning peak flow and drug compliance, and baseline sputum and blood samples were collected.

Patients were seen at weeks 4, 16, 32 and 52 after randomisation. Spirometry was performed, a sputum sample obtained and diaries were examined. Additionally at weeks 32 and 52, serum samples were collected and patients asked about any change in sputum production.

Laboratory analyses

Patients provided spontaneous or induced sputum samples as previously described [24]. Following processing, sputum IL-6, IL-8 and MPO and serum IL-6 and CRP were quantified by ELISA. A portion of the sputum sample underwent quantitative and qualitative bacteriological analysis [25, 26].

Exacerbations

Patients were asked to record any increase in respiratory symptoms above their normal, stable condition using a letter-annotated system in their diary. The diagnosis of an exacerbation was based on symptomatic criteria previously validated by our group [1, 5, 7]. An exacerbation was defined as the presence for at least two consecutive days of increase in any two "major" symptoms (dyspnoea, sputum purulence, sputum volume) or increase in one "major" and one "minor" symptom (wheeze, sore throat, cough, symptoms of a common cold).

Statistics

Analyses were carried out using the full analysis data set (all randomized, treated patients with efficacy data) using an analysis of covariance that adjusted for smoking status and exacerbation history over the previous year (<3 or >=3) recorded at recruitment. For sputum markers, the area under the curve (AUC) was calculated for each patient with missing data replaced by interpolation or the last observation carried forward. The model also included baseline inflammatory marker levels as a covariate. AUCs for IL-6 and MPO were skewed and therefore log10 transformed. For lung function, comparisons were between changes from start and end of the study. The pre-specified data analysis with AUCs was chosen for its ability to estimate the total effect of tiotropium on sputum and serum cytokines over one year rather than the effect at a single time point [27]. An additional analysis was also carried out using cross-sectional regression models (xtreg command in Stata 8). Systemic inflammatory markers were not sampled at weeks 4 and 16, so comparisons were made by Wilcoxon sign-rank test between changes from baseline to final sample.

The effect of tiotropium on individual annual rates (= the number of exacerbation divided by days on drug * 365) was tested using a Wilcoxon test. Differences in time to the next exacerbation were examined using a log-rank test.

Exacerbation recovery times were calculated as the time for a 3 day moving average of peak flow to return post-exacerbation to a baseline taken as the average on days 14 to 8 prior to exacerbation onset.

The statistical analysis was performed with SAS 8.2 and Stata 8.

Results

Figure 1 shows the trial profile of the 237 COPD patients who were enrolled into the study. One hundred and forty two patients were randomized (69 patients on tiotropium and 73 on placebo). The two treatment groups were well matched (Table 1). Ninety nine patients had a history of less than 3 exacerbations per year (41 non-smokers and 58 current smokers) and 43 patients more than 3 exacerbations per year (18 non-smokers and 25 current smokers; chi-squared p=0.960).

Sputum and serum inflammatory markers

As a percentage of patients active in the study, 91%, 57%, 70%, 78%, 67% of patients on tiotropium and 90%, 60%, 66%, 62% and 63% on placebo produced a sputum sample at baseline, 1, 3, 6 and 12 months respectively. Figure 2 shows the results for the area under the curve (AUC) for the sputum inflammatory markers. Table 2 shows that there was no difference in the $\log_{10}$ transformed AUC sputum IL-6 on tiotropium

compared with placebo after adjustment for baseline values, exacerbation frequency and smoking status (p=0.324). The AUC for sputum IL-8 was 15.4% higher (p=0.043) on tiotropium than placebo. In the analysis of sputum IL-8, smokers had significantly higher AUC (p=0.029). There were no significant differences in sputum MPO levels on tiotropium compared with placebo (p=0.079). Inclusion in the analysis of whether the patients were taking inhaled steroids or not did not alter the findings. There was still no effect of tiotropium on sputum IL-6 (p=0.327) or sputum MPO (P=0.082). The AUC for sputum IL-8 remained higher on tiotropium (p=0.047).

There was no significant difference between the groups in the change from baseline to week 52 in serum IL-6 (Wilcoxon; p = 0.691) or CRP (p=0.700) (Table 2).

Further analysis using cross-sectional regression models, with similar allowance for exacerbation frequency, smoking habits and baseline levels, gave similar results. Tiotropium did not increase $\log_{10}$ sputum IL-6 (p=0.540). The effect of tiotropium on sputum IL-8 was not significant (P=0.055) whilst for $\log_{10}$ MPO tiotropium caused it to rise by 0.156 $\log_{10}$ IU/L (p=0.006). No significant differences were seen in $\log_{10}$ CRP (P=0.456) or $\log_{10}$ serum IL-6 (p=0.058).

Lung function

During the trial, the FEV_1 of patients taking tiotropium rose from 1.35 l (SD 0.47) at baseline to 1.39 l (0.55) at 1 year whereas patients taking placebo experienced a fall from 1.26 l (0.49) at baseline to 1.20 l (0.49) at 1 year (p=0.027).

The FVC of patients on tiotropium was unchanged between baseline and the end of treatment, at 2.26 l (0.79), whereas patients in the placebo group experienced a decrease in FVC of 0.20 l from 2.21 l (0.81) at baseline to 2.01 l (0.81) at the end (p=0.003).

Exacerbations

The patients in the tiotropium treated group experienced 60 exacerbations compared to 134 in the placebo arm. Patients on tiotropium had fewer exacerbations per year (1.17 (SD 2.25); median 0 (IQR 0 – 1.0)) compared to (2.46 (3.82); median 1.6 per year (IQR 0 – 3.0)) for those on placebo (Wilcoxon; p=0.001) representing a 52% reduction in exacerbation frequency on tiotropium. Exacerbations treated with antibiotics or steroids were also reduced; 0.98 (SD 2.11) in the tiotropium arm compared to 1.73 (2.46) on placebo (p=0.007). Forty three per cent of the patients on tiotropium had at least one exacerbation during the study period compared to 64% on placebo (p=0.01) (Figure 3). Survival time analysis indicated the mean time to first exacerbation was 236 days (SD 143; SE 17.2) with tiotropium compared to 157 days (SD 124; SE 14.6) days with placebo (logrank test; p=0.0092,). There were significantly fewer exacerbation days with tiotropium (17.3 days/year (SD 33.6); median 0 days) than placebo (34.5 days/year (47.5); median 21.7 days) (Wilcoxon p=0.002). This was due to a reduction in the number of exacerbations, as there was no

difference in the length of the exacerbations, as indicated by symptom count recovery times; 9.89 days (6.94) on tiotropium and 9.54 days (6.96) on placebo (p=0.748). There were no differences in the symptoms recorded at exacerbation onset in the 60 exacerbations of the tiotropium group and 134 exacerbations of the placebo group. Tiotropium was associated with a subjective reduction in sputum production in 33% of patients compared to 7.9% on placebo (chi^2, p=0.001).

There was no difference in the proportion of treated exacerbations in the tiotropium group 50/60 (83.3%) compared to 108/134 (80.6%) in the placebo group (chi-squared; p=0.650). Two patients who experienced exacerbations on tiotropium were hospitalized compared to 3 patients on placebo (p=0.657).

Bacteria

There were no differences in the proportion of any bacterial species identified at any time point between tiotropium and placebo. The commonest species isolated was *Haemophilus influenzae*, present in 10-20% of patients at each time point. Bacterial counts (mean) were similar at all time points ($10^{6.6}$ colony forming units (cfu)/ml for patients on tiotropium at baseline and $10^{6.9}$ cfu/ml for those on placebo and at week 52, $10^{7..2}$ and $10^{6..9}$ respectively).

Study compliance and withdrawals.

82.6% of patients on tiotropium took more than 90% of their medication compared to 90.4% of patients on placebo (p=0.172). Compliance with diary cards was also high. Patients on tiotropium recorded diary data for 94.5% of the time, whilst patients on placebo recorded diary card data for 94.1% of the time.

Figure 1 shows that during the study 21/69 (30.4%) of patients on tiotropium withdrew before one year compared to 21/73 (28.8%) patients on placebo (p=0.828). There were no significant differences in withdrawals at any other time points. Seven (10.1%) patients on Tiotropium and 14 (19.2%) patients on placebo withdrew due to an adverse event (p=0.130).

Adverse events

Table 4 shows the adverse events during treatment excluding exacerbations. Most adverse events were mild or moderate in intensity. None of the adverse events were considered therapy related, with the exception of one episode of pruritus in the tiotropium group and one incidence of palpitations in the placebo group.

Discussion

This is the first study to assess the effect of an inhaled anticholinergic therapy on sputum and systemic inflammatory markers and exacerbations in patients with COPD using a symptom-based exacerbation definition and followed over 12 months. There were no significant differences in sputum IL-6 and MPO or systemic inflammatory markers between the groups, but sputum IL-8 was significantly higher in the tiotropium group at the end of the study. We demonstrated a significant reduction of 52% in exacerbation frequency, together with a reduction in total exacerbation days and the proportion of patients suffering at least one exacerbation in the tiotropium group compared to placebo.

COPD is associated with progressive airway and systemic inflammation and exacerbations are associated with increases in these inflammatory markers [8, 28, 29]. These exacerbations are important events in the natural history of the disease, but the mechanisms of reduction of exacerbation frequency with therapies are not clearly understood. We have previously demonstrated that there are increased levels of sputum IL-6 and IL-8 in COPD patients experiencing frequent exacerbations thus suggesting that frequent exacerbators have increased airway inflammation in the stable state [8]. It may therefore be postulated that a reduction in airway inflammation might lead to a reduction in exacerbation frequency.

Short term biopsy studies have demonstrated a reduction in epithelial mast cells and CD8+ cells after therapy with fluticasone and salmeterol/fluticasone [19, 21]. However, no studies have demonstrated an effect of inhaled therapy on sputum cytokines. It has recently been shown that acetylcholine increases neutrophil chemotactic activity and production of LTB4 in COPD and this action is attenuated *in vitro* by tiotropium thus indicating a potential anti-inflammatory action of tiotropium [16, 17]. However in our study, no effect of tiotropium on sputum IL-6 or MPO was demonstrated and thus we cannot relate the reduction of exacerbation frequency to changes in airway inflammation.

Surprisingly tiotropium therapy was associated with a significant increase in the concentration of sputum IL-8. Tiotropium could potentially inhibit cholinergic stimulation of mucus secreting airway goblet cells thus causing a reduction in airway mucus and increasing the concentration of cytokines. This is supported by the

subjective reporting of reduced sputum production by patients in this study. In common with previous studies of COPD therapies, no reduction in sputum inflammatory markers was demonstrated and it is possible that measurement of sputum cytokines is not the optimal way of assessing airway inflammation.

It is well known that systemic inflammation is a factor in COPD. Inhaled fluticasone has been shown to reduce levels of serum CRP thus indicating a reduction in systemic inflammation [30]. We studied serum CRP and IL-6 to assess systemic inflammation, but found no differences between tiotropium and placebo.

This trial demonstrated a significant reduction in exacerbation frequency in the tiotropium group in keeping with previous studies, though we found a larger (52%) reduction in exacerbation frequency than previously reported. This is the first intervention trial to use our previously validated daily diary card over one year in order to detect exacerbations [1, 5]. There has been much debate about what constitutes a COPD exacerbation and several definitions have been proposed [5, 31, 32]. Some have included an increase in respiratory symptoms from baseline requiring a change in usual therapy. This relies on the patient seeking healthcare assistance and as such has some associated difficulties. Factors other than the severity of symptoms may prevent the patient from seeking input from a healthcare practitioner. We have previously shown that that 50 per cent of exacerbations go unreported and these unreported events also impact on quality of life and are associated with disease progression [1, 5, 7]. The advantage of our diary cards is that we can detect all exacerbations, whether reported or unreported. Compliance using the diary card in

this trial was extremely good indicating their utility as a tool for detecting exacerbations.

Patients in the tiotropium arm demonstrated an improvement in FEV_1 and FVC over the year of the study whilst patients on placebo demonstrated a decline in lung function. Tiotropium has been shown to improve inspiratory capacity, thus reducing dyspnoea which is an important symptom of a COPD exacerbation [12, 13]. It is this reduction in dyspnoea that is probably a major factor in the reduction of exacerbation frequency that was demonstrated in the tiotropium group.

Mucus secretion is frequently increased in patients with COPD. This mucus hypersecretion might be associated with airway obstruction, disruption of the mucociliary escalator and provide an attractive milieu for airway bacterial colonisation. These changes might be expected to be associated with increased inflammation and exacerbation frequency. Secretion from mucus glands is mediated by cholinergic pathways and potentially inhibited by anticholinergics. Tamaoki and colleagues showed a reduction in sputum volume using the anticholinergic agent, oxitropium [33]. Thus, a reduction in mucus hypersecretion is a further potential mechanism by which tiotropium might reduce exacerbation frequency. Although we did not perform quantitative measurements of sputum in this study, patients on tiotropium reported a subjective reduction in sputum volume suggesting that tiotropium might indeed have an effect on mucus secretion.

Patients with COPD may demonstrate lower airway bacterial colonisation (LABC) which is associated with increased airway inflammation, disease progression and

increased exacerbation frequency [26,34]. We therefore studied the rates of LABC in the trial, but no differences were found between the tiotropium and placebo arms of the study.

The principal limitation of the study was in the difficulty in the use of sputum inflammatory markers as sputum samples were difficult to obtain although there was no significant difference between the groups. In addition there was marked variability in levels of sputum cytokines in the trial, thus reducing the power of the study to detect a significant difference. This may also explain the failure of previous studies to demonstrate an effect of COPD therapies on airway inflammatory markers. Many patients in the study were taking inhaled steroids and it might be argued that this was a potentially confounding factor,however,, no significant differences in inflammatory markers were demonstrated between those taking inhaled steroids and those not. This was a cohort of patients with moderate to severe disease and as it has been shown that steroid withdrawal results in a deterioration in lung function and increase in exacerbation frequency [35] it was decided that patients should remain on inhaled corticosteroids

In summary, this was the first year-long trial using our symptom-based exacerbation definition and daily diary card monitoring. Results of the study showed a significant reduction in exacerbation frequency in COPD patients treated with tiotropium, compared to placebo. However this effect does not seem to be related to a reduction in airway or systemic inflammation and is likely to be related to reductions in dynamic hyperinflation or airway mucus production. Further studies are now required to evaluate the mechanisms for this effect of inhaled tiotropium on exacerbation frequency in COPD.

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Table 1. Patient physical, smoking and exacerbation characteristics and pre-study medication

Patient Characteristics	Tiotropium N=69	Placebo N=73	P-value
Male (%)	69.6%	56.2%	0.099
>=3 exacerbations last year (%)	30.4%	30.1%	0.969
Current smoking (%)	59.4%	57.5%	0.820
	Mean (SD)	Mean (SD)	
Age (years)	66.3 (8.1)	66.4 (9.8)	0.947
Body Mass Index (kg/m2)	27.1 (6.71)	27.1 (6.15)	0.993
Pack years (years)	54.6 (25.5)	55.7 (28.0)	0.807
FEV1 (l)	1.35 (0.47)	1.23 (0.51)	0.148
FEV1% predicted (%)	50.9 (14.8)	49.2 (15.6)	0.507
FVC (l)	2.29 (0.75)	2.16 (0.79)	0.317
PEFR weekly mean (l/min)	261.6 (82.5)	236.4 (84.5)	0.075
Pulmonary medications at baseline			
Daily inhaled steroids	73.9%	76.7%	0.699
Daily oral steroids	4.3%	1.4%	0.284
Short acting anticholinergics	44.9%	45.2%	0.973
Short acting beta-adrenergics	94.2%	93.2%	0.797
Long acting beta-adrenergics	42.0%	43.8%	0.828

Table 2. Areas under the curve (AUC) for sputum inflammatory markers for tiotropium and placebo treatment arms

Sputum Markers (Area under Curve)	Tiotropium	Placebo	ANCOVA (p-value)
IL-6† (week.pg/mL)	4898.5 (3894.0, 6162.3) (n=55)	4198.1 (3326.7, 5297.7) (n=58)	0.324
IL-8 (week.pg/mL)§	143325 (129623, 157027) (n=55)	124223 (110331, 138116) (n=58)	0.043
MPO (week.IU/L)†	1512.5 (1255.5, 1822.0) (n=51)	1210.0 (1006.0, 1455.3) (n=58)	0.079
Serum markers (Change baseline to week 52)	Mean (SE)	Mean (SE)	Wilcoxon (p-value)
IL-6 (pg/ml)	-20.4 (6.6) (n=50)	-3.3 (6.3) (n=58)	0.691
CRP (mg/l)	-0.80 (1.2) (n=34)	-0.05 (1.8) (n=37)	0.700

† Geometric mean (95% confidence interval) adjusted for baseline, exacerbation frequency and smoking status.

§ Mean (95% confidence interval) adjusted for baseline, exacerbation frequency and smoking status.

Table 3. Exacerbation parameters in tiotropium and placebo groups

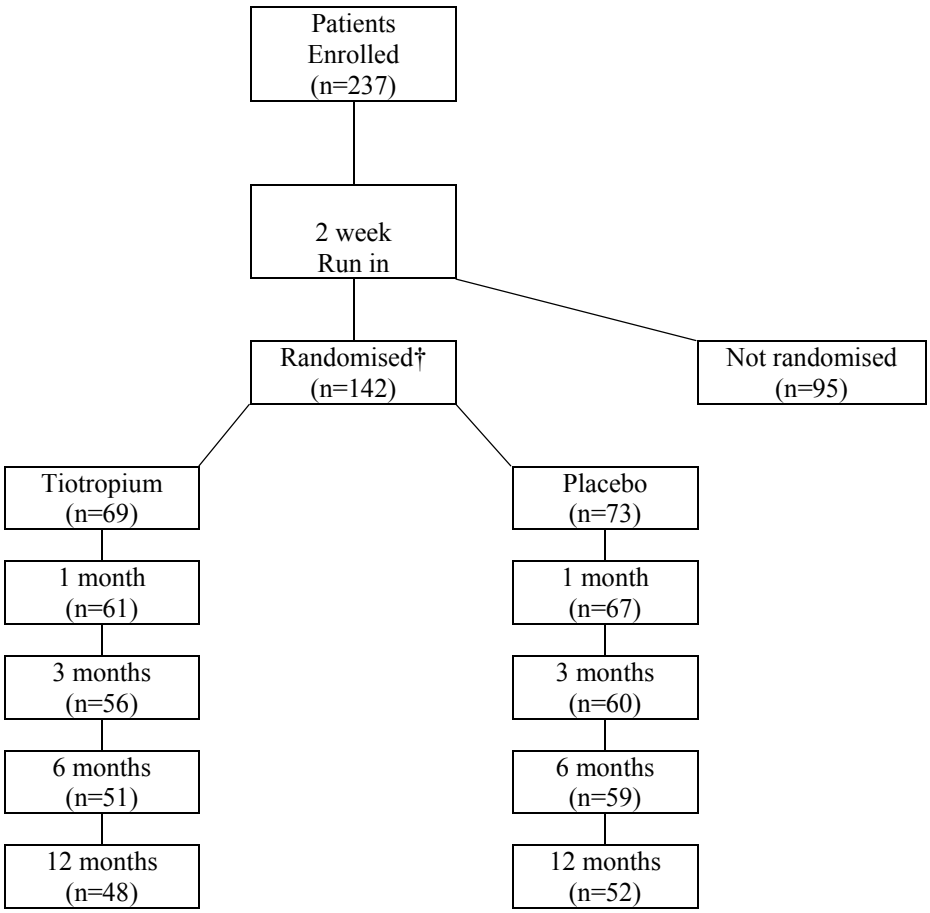
	Tiotropium N=69 (mean ± SD)	Placebo N=73 (mean ± SD)	p-value
Exacerbation rate (per year),	1.17 (2.25)	2.46 (3.82)	0.001
Treated exacerbations (per year)	0.98 (2.11)	1.73 (2.46)	0.007
Patients with no exacerbations over study period	56.5%	35.6%	0.012
Time to 1st exacerbation (days)	236 (143)	157 (124)	0.009
Days involving exacerbation (days/year)	17.3 (33.6)	34.5 (47.5)	0.002
	Exacerbations N=54 (mean ± SD)	Exacerbations N=125 (mean ± SD)	
Symptom recovery time (days)	9.89 (6.94)	9.54 (6.96)	0.748

Table 4. Adverse events occurring in more than 3% of patients during the treatment period, excluding exacerbation of COPD

EVENT	**Tiotropium** N=69	**Placebo** N=73
Hypertension	5 (7.2%)	1 (1.4%)
Myocardial infarction	3 (4.3%)	1 (1.4%)
Urinary tract infection	3 (4.3%)	1 (1.4%)
Pharyngitis	3 (4.3%)	1 (1.4%)
Pneumonia	2 (2.9%)	3 (4.1%)
Ear infection	1 (1.4%)	3 (4.1%)
Tooth extraction	0 (0.0%)	3 (4.1%)

The treatment period was defined as the period between the start (start date) of the treatment and end of treatment (stop date) plus 30 days post end of treatment.

Figure 1 Trial profile of patients in the study.



† All randomised patients received at least one dose of study treatment.

(n= no. of patients active in the study)

Figure 2. Areas under the curve (AUC) of sputum inflammatory markers (mean ± SE bars). AUCs for IL-6 and MPO were log10 transformed. P-values from ANCOVA with adjustment for exacerbation frequency and smoking.

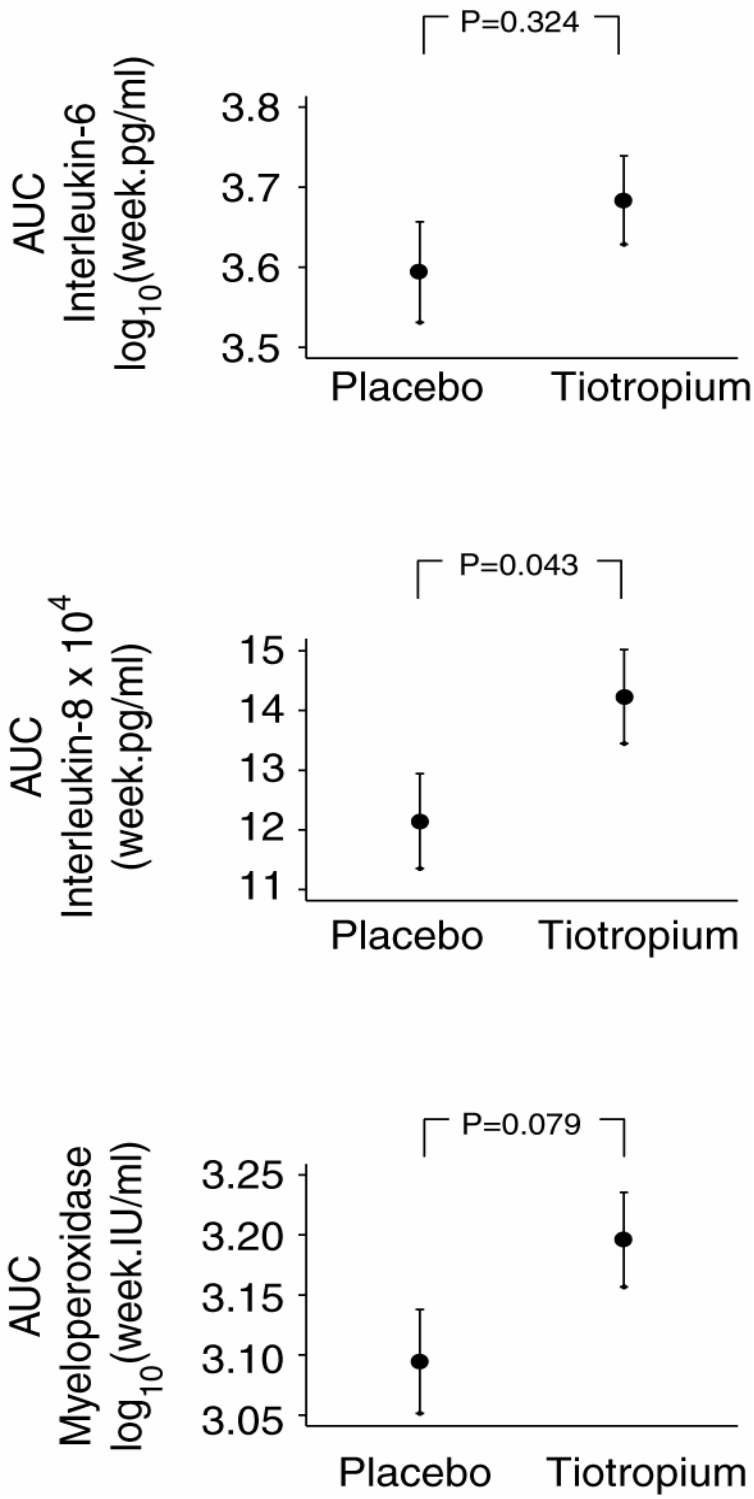


Figure 3. Proportion of patients on tiotropium (hatched bar) or placebo (solid bar) during the study, by annual exacerbation rate