

Efficacy of tiotropium bromide (Spiriva®) in patients with chronic obstructive pulmonary disease (COPD) of different severities

Autoren

K. M. Beeh^{1,2}, J. Beier^{1,2}, R. Buhl², P. Stark-Lorenzen³, F. Gerken⁴, N. Metzdorf⁴
for the ATEM [= Breath] Study Group

Institute

¹ insaf Institut für Atemwegsforschung, Wiesbaden, Germany
² III. Med. Klinik, Universitätsklinik Mainz, Germany
³ Pfizer GmbH, Karlsruhe, Germany
⁴ Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany

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Korrespondenzadresse

Kai Michael Beeh
insaf Institut für Atemwegsforschung
Biebricher Allee 34
65187 Wiesbaden
Germany
k.beeh@insaf-wi.de

Abstract

Background: Aim of this study was to evaluate the efficacy of inhaled tiotropium bromide in COPD patients of different severities in pneumological practices during a three month clinical trial.

Methods: A randomized, double blind, placebo-controlled study including COPD-patients (FEV₁/FVC < 70%, FEV₁ 70% predicted; age 40 years; smoking history 10 pack years) of different severities was performed. The efficacy of 18 µg tiotropium bromide once daily on lung function and exacerbations over 12 weeks was evaluated by respective pulmonary function tests (spirometry) before (trough value) and 2 hours after inhalation of study medication.

Results: 1639 patients (1236 tiotropium bromide, 403 placebo; FEV₁ reversibility after 200 µg ipratropium bromide + 200 µg fenoterol:

7.9 ± 7.5% predicted [mean ± sd]) were randomized. After 12 weeks of treatment tiotropium bromide led to significant increases of trough FEV₁ (23–24 h after last inhalation; + 79 ± 17 ml), and 2 h after tiotropium bromide inhalation (+ 128 ± 19 ml) (all values vs. placebo, adjusted mean ± se, p < 0.0001). FVC and IVC were also improved significantly. In mild COPD (FEV₁ 50 to 70%) improvements were most pronounced (trough FEV₁ + 113 ± 29 ml, 2 h post-inhalation + 181 ± 33 ml; all values vs. placebo, p < 0.0001). 14.6% of patients treated with tiotropium bromide had a COPD exacerbation vs. 19.9% of patients treated with placebo (p = 0.0151). The time to first exacerbation was prolonged (p = 0.0092 vs. placebo).

Conclusion: Tiotropium bromide 18 µg once daily led to a persistent improvement of lung function and a reduction of exacerbations in patients with COPD of different severities.

Introduction

In accordance with national [1] and international recommendations [2], bronchodilators constitute the mainstay of maintenance treatment for chronic obstructive pulmonary disease (COPD). The use of long-acting bronchodilators is recommended in patients with a forced expiratory volume in 1 second (FEV₁) < 80% of the predicted value [2]. Long-acting bronchodilators, such as anticholinergic and sympathomimetic agents, not only involve a simpler dosing regimen but also have a superior clinical profile compared with short-acting preparations. Clinical trials have confirmed the advantage of long-acting substances in respect to lung function, symptoms, quality of life, and frequency of exacerbations.

Together with the long-acting beta-sympathomimetics salmeterol and formoterol, the long-

acting anticholinergic agent tiotropium bromide is considered to be a bronchodilator of choice in the treatment of COPD [2]. In major randomized studies, tiotropium bromide produced an improvement in all clinically relevant end-points compared with both ipratropium and placebo [3,4]. However, these studies mainly investigated COPD patients with moderate or severe obstructive ventilatory dysfunction. The aim of the study described in this article was to investigate the efficacy and tolerability of tiotropium bromide in a large population of patients with COPD of different severities under “real life” conditions in German pulmonology practices. The primary end-points were the improvement in lung function at 2 hours after inhalation of the study medication and at 23–24 hours post inhalation (trough value). The reduction in COPD exacerbations was also investigated.

Materials and methods

Patients

This 12-week placebo-controlled study enrolled patients with COPD of different severities. The multicentre study involved 294 pulmonology trial centres in Germany. These centres were pulmonology practices or hospital pulmonology outpatient departments, together with a small number of general medical practices specialising in respiratory disorders (<10). Patients were required to have stable COPD with FEV₁ 70% of the predicted value and an FEV₁/FVC ratio of <0.7. Additionally, they were required to have a smoking history of at least 10 pack years and be at least 40 years of age. Patients with a history of asthma or atopic conditions suggesting the existence of an asthmatic clinical picture were excluded from participation in the study. Other exclusion criteria included the use of long-term oxygen therapy, a respiratory infection in the 6 weeks prior to screening, or significant concomitant illnesses. Long-acting beta-mimetic agents and other (short-acting) anticholinergic agents were discontinued at the start of the study as these constituted non-permitted concomitant medications. Short-acting beta-mimetics for inhalation were replaced by a fenoterol metered dose inhaler for use as required.

Approvals were obtained from the relevant Ethics Committees for all participating centres prior to the start of patient enrolment.

Clinical trial schedule

Screening

A bronchospasmolysis test with ipratropium bromide 200 µg plus fenoterol 200 µg was conducted on the first day of the study. This test was designed to determine the maximum possible bronchodilatory potential of patients before admission to the study; however, the result of this test was not an inclusion criterion.

Randomization and treatment phase

After a one-week run-in phase patients were randomized and assigned to one of two treatment groups (tiotropium bromide or placebo, ratio 3 : 1). The first dose was given in the test centre. Lung function (FEV₁, FVC and IVC) was measured 30 and 10 minutes before (baseline), and 2 hours after the inhalation of the study medication. During the subsequent 12-week treatment phase, patients inhaled each morning one capsule of tiotropium bromide or placebo via a HandiHaler®. At the end of the treatment phase, the study medication was administered in the test centre in the morning and lung function was measured using the same procedures employed at the start of the treatment phase.

The following wash-out periods for bronchodilator preparations were observed before the lung function tests. (a) Screening: short-acting inhaled beta-mimetics, 8 hours; short-acting anticholinergic agents, 12 hours; long-acting inhaled and oral beta-mimetics, 24 hours; non-sustained-release theophylline, 12 hours; and sustained-release theophylline, 24 hours. (b) Treatment phase: short-acting inhaled beta-mimetics, 8 hours; non-sustained-release theophylline, 12 hours; and sustained-release theophylline, 24 hours. The spirometric measurements were recorded according to the criteria of the American Thoracic Society [5].

Statistical methods

Unless otherwise specified, all data are stated as arithmetic means with standard deviations. The primary end-points of the study were the initial morning (trough) FEV₁ value 23–24 hours after the preceding inhalation of the study medication and the FEV₁ 2 hours after inhalation following 12 weeks of treatment with either tiotropium bromide 18 µg or placebo. Statistical analysis of the primary outcome measures was performed using an analysis of covariance (ANCOVA) model with terms for treatment and centre and baseline as a covariate. In order to keep the overall type I error below a significance level of 5%, the Bonferroni-Holm correction was applied to the ANCOVA of both primary end-points. A sample size of at least 612 tiotropium bromide treated patients and 204 placebo treated patients was required to provide a 90% power to detect a difference of 0.1 litre in both primary outcome measures with a standard deviation of 0.35 litres. The number of exacerbations was subjected to the chi-squared test and the time to onset of the first exacerbation was subject to the log-rank test. An exacerbation was defined as a respiratory event lasting for three or more days that required treatment with, or a significant increase in the dosage of, COPD drugs (bronchodilators and/or systemic corticosteroids) or treatment with antibiotics.

Data were analysed according to the intent-to-treat principle (ITT, as randomized) with the full-analysis-set (FAS) for the parameters of lung function and for the recording of exacerbations for all treated patients (safety set). Other adverse events were assigned to the treatment groups as treated and likewise analysed for all treated patients.

Results

A total of 1838 patients were enrolled, of whom 1639 were randomized to the double-blind treatment phase (n = 1236 tiotropium bromide and n = 403 placebo). The patients' baseline characteristics and demographic details are listed in **Table 1**. Patients were uniformly distributed over three levels of severity accord-

Table 1 Demographic and lung function data at the start of the study. The findings are stated as means (standard deviation)

	Total (n = 1639)	Tiotropium bromide (n = 1236)	Placebo (n = 403)
Age (years)	62.2 (8.7)	62.1 (8.6)	62.2 (8.7)
Male (%)	75.5	75.1	76.7
Smoking history (pack years)	35.8 (19.5)	36.1 (19.5)	35.0 (19.4)
Duration of COPD (years)	9.7 (7.8)	9.7 (7.9)	9.9 (7.6)
FEV ₁ (L)	1.33 (0.49)	1.32 (0.48)	1.36 (0.50)
FEV ₁ (% of pred.)	45.4 (14.9)	45.3 (14.9)	45.7 (15.0)
FVC (L)	2.35 (0.74)	2.33 (0.74)	2.41 (0.76)
FEV ₁ /FVC (%)	57.1 (12.1)	57.2 (12.2)	56.8 (12.0)
IVC (L)	2.50 (0.77)	2.49 (0.76)	2.55 (0.77)
Inhaled steroids (%)	42.2	41.9	43.7
BMI	25.9 (4.6)	25.9 (4.7)	25.7 (4.3)
▶ mild	26.8 (4.5)	26.9 (4.6)	26.5 (4.2)
▶ moderate	25.9 (4.4)	26.0 (4.5)	25.7 (4.1)
▶ severe	24.4 (4.5)	24.4 (4.6)	24.6 (4.0)

ing to the classification recommended by the American Thoracic Society [6]: severe ($FEV_1 < 35\%$ of predicted), 439 patients; moderate ($FEV_1 35 - < 50\%$ of predicted), 586 patients; mild ($50 - 70\%$ of predicted), 530 patients (81 patients with an $FEV_1 > 70\%$ of predicted were evaluated separately as patients with very mild COPD; the measurements required for severity classification were lacking in three patients).

The reversibility in the initial bronchospasmolysis test with $200 \mu\text{g}$ ipratropium bromide plus $200 \mu\text{g}$ fenoterol, as determined according to the ERS criteria [7], was $7.9 \pm 7.5\%$ (mean \pm sd) of predicted for all patients (severe COPD: 6.6%, moderate: 7.5%, mild: 9.6% and very mild: 11.8%).

After 12 weeks, the once-daily treatment with tiotropium bromide $18 \mu\text{g}$ produced significant increases in both trough FEV_1 (23–24 hours after the last inhalation of the study medication) and FEV_1 2 hours post-inhalation, compared with placebo. The trough FEV_1 values increased by an average of $79 \pm 17 \text{ ml}$ versus placebo ($p < 0.0001$). At 2 hours post-inhalation FEV_1 increased by $128 \pm 19 \text{ ml}$ versus placebo ($p < 0.0001$). By comparison, on the first day of treatment, tiotropium bromide produced an increase in FEV_1 of $122 \pm 14 \text{ ml}$ ($p < 0.0001$) compared with placebo, i.e. the response to the repeated inhalation of tiotropium bromide was fully maintained even after 12 weeks of continuous treatment. The improvement in lung function, stratified according to the severity of the COPD is presented in **Fig. 1**.

A similar trend in results was observed for both forced vital capacity (FVC) and inspiratory vital capacity (IVC). The trough FVC increased by $116 \pm 27 \text{ ml}$ compared with placebo ($p < 0.0001$). Additionally, the value for FVC 2 hours post-inhalation increased by $176 \pm 29 \text{ ml}$ compared with placebo ($p < 0.0001$). The corresponding figures for IVC were $92 \pm 27 \text{ ml}$ ($p = 0.0008$) and $196 \pm 30 \text{ ml}$ ($p < 0.0001$) for trough IVC and 2 hours post-inhalation, respectively. Improvements in lung function were observed on the first day of treatment – improvements 2 hours after inhalation were, for FVC, $186 \pm 22 \text{ ml}$ ($p < 0.0001$) and, for IVC, $190 \pm 22 \text{ ml}$ ($p < 0.0001$).

Overall there was a positive correlation between initial reversibility in the bronchospasmolysis test and the improvement observed during treatment with tiotropium bromide. The increases in the post-inhalation FEV_1 values versus baseline for patients with reversibility results in the initial reversibility test of $< 10\%$, $10 - < 20\%$ and 20% were 88, 103 and 187 ml, respectively ($p < 0.05$ for all versus placebo). For the trough values 41, 84 and 56% of the post-inhalation values were reached.

Exacerbations

Tiotropium bromide $18 \mu\text{g}$ significantly reduced the number of exacerbations and significantly prolonged the time to the onset of the first exacerbation compared with placebo. In the placebo group, 19.9% of patients suffered an exacerbation. In comparison, the corresponding figure in the tiotropium bromide group was 14.6% (**Fig. 2**), which corresponds to a reduction of 26% ($p = 0.0151$). The time to the first COPD-exacerbation was also significantly prolonged in the patients treated with tiotropium bromide $18 \mu\text{g}$ ($p = 0.0092$). **Fig. 3** shows a Kaplan-Meier plot for the probability of not suffering an exacerbation during the course of the study. The profiles for placebo and tiotropium bromide show that the difference relating to the onset of the first exacerbation increases steadily over time between tiotropium bromide and placebo. Thus, the first exacerbations were not just postponed across the whole study period, but their occurrence was actually prevented.

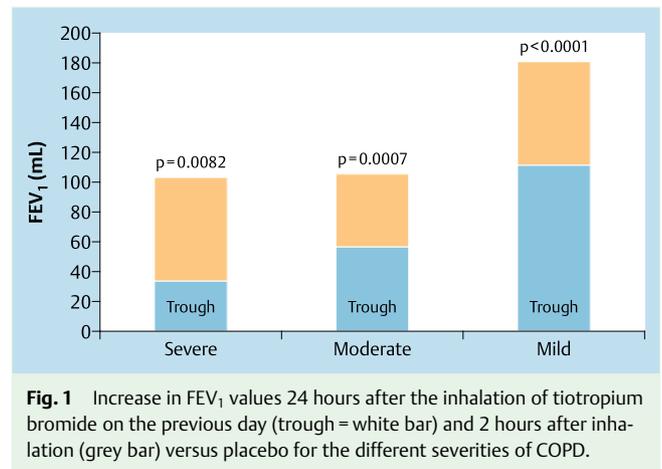


Fig. 1 Increase in FEV_1 values 24 hours after the inhalation of tiotropium bromide on the previous day (trough = white bar) and 2 hours after inhalation (grey bar) versus placebo for the different severities of COPD.

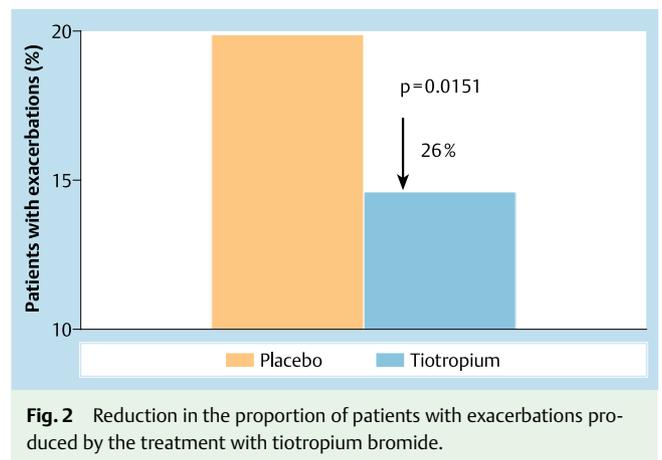


Fig. 2 Reduction in the proportion of patients with exacerbations produced by the treatment with tiotropium bromide.

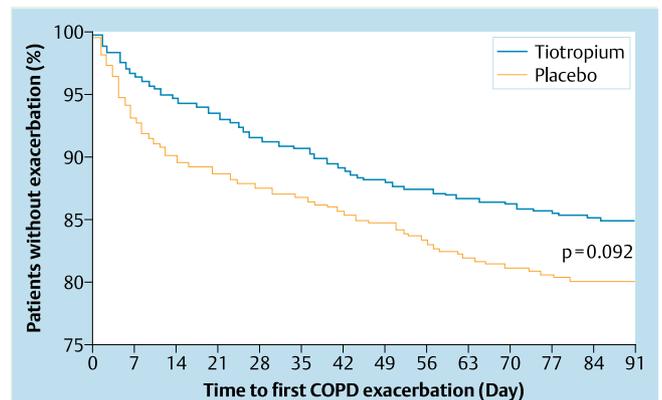


Fig. 3 Kaplan-Meier plot for the probability of remaining free of COPD exacerbations.

The proportion of patients who discontinued the study due to a worsening in their COPD was lower in the tiotropium bromide group. In the placebo group, 13.6% of patients withdrew due to a worsening of their COPD, compared with only 8.8% in the tiotropium bromide group.

Tolerability

Adverse events occurred in 29.9% of patients receiving placebo, compared with 26.0% of patients in the tiotropium bromide group. The frequency of serious adverse events was comparable

in both treatment groups (tiotropium bromide 5.3%, placebo 5.9%). Fewer patients in the tiotropium bromide group (11.4%) withdrew from the study due to adverse events compared with the placebo group (16.4%).

Adverse effects over and above the hitherto known extent of side effects were not observed in this study.

Discussion

Tiotropium bromide is an inhaled anticholinergic agent that produces its effect by means of sustained binding to the M₃ receptor [8]. The bronchodilation induced by this drug is sustained over a 24 hour period, making tiotropium bromide suitable for once-daily administration. Clinical studies [9,10] have confirmed, by means of lung function tests, that the efficacy is in fact sustained for more than 24 hours. The peak bronchodilatory effect of tiotropium bromide in these studies was reached during the first 3 hours after inhalation. The bronchodilation produced by tiotropium bromide gradually declined over the course of the following day and night until the next morning inhalation, after 24 hours. However, comparison of the maximum (peak) bronchodilation with the effect after 24 hours (trough) shows that tiotropium bromide in the long-term therapy of COPD possesses an excellent “peak-to-trough” ratio [3,4], i.e. the improvement in lung function is maintained over a sustained period.

To date, long-term studies have primarily enrolled patients with moderate or severe COPD. Consequently, limited data are available on patients with mild COPD. In order to confirm the efficacy of tiotropium bromide under “real life” conditions, this multicentre study was conducted in 294 German pulmonology trial centres. The improvements observed in trough FEV₁ and FEV₁ at 2 hours post-inhalation confirm that tiotropium bromide produces sustained bronchodilation at a high level, even in a large population of patients with COPD of different severities, including mild COPD (defined using ATS criteria [6]). The results also demonstrate that the individual response to repeated tiotropium bromide inhalation was fully maintained after continuous treatment over 12 weeks. The peak bronchodilation measured 2 hours post-inhalation at the end of the study did not differ from that measured on the first day of treatment. This observation concurs with the results of previously published long-term studies relating to the efficacy of tiotropium bromide, in which no decline in response was observed during continuous treatment over 6 months [11] and 1 year [3,4].

As with the results for FEV₁, improvements were also noted for forced vital capacity (FVC) and inspiratory vital capacity (IVC). This is of particular interest given the increasing attention paid in recent years to the significance of alternative lung function parameters for the evaluation of treatment effects in COPD [12,13]. For example, several studies have demonstrated that inspiratory parameters such as IC play an important role in the evaluation of the therapeutic effect of drugs in COPD, since they appear to correlate more closely than FEV₁ with the dominant symptom of dyspnoea [14,15]. A distinct reduction in dyspnoea has been demonstrated in previous studies during treatment with tiotropium bromide [3,4,11,16], which may be attributable to the increase in inspiratory reserve. This hypothesis is supported by more recent studies that have investigated the effects of bronchodilators on exercise tolerance and hyperinflation in COPD [17,16].

A predictable finding in this 12-week study was a correlation between the improvements in FEV₁ values and the degree of reversibility in the initial reversibility test. An analysis of the corresponding patients from 1-year studies with tiotropium bromide has shown, however, that even patients with an initially low level of reversibility can achieve significantly better lung function results during long-term treatment with tiotropium bromide [18].

In addition to improvements in pulmonary function, a reduction in the number of exacerbations was observed despite the relatively short treatment period. Furthermore, the time to the onset of the first exacerbation was prolonged in the tiotropium bromide group. This effect increased steadily during the course of treatment. A comparable phenomenon has also been observed in other studies with tiotropium bromide conducted over 6 months [11] and 1 year [3,4], indicating that exacerbations were not only postponed but actually prevented.

An interesting aspect of this study is the fact that patients with mild COPD (according to ATS criteria, [6]) or mild-to-moderate COPD (according to GOLD [2]) also suffered COPD exacerbations even within a relatively short observation period and that these patients profited from treatment with tiotropium bromide in respect to exacerbations [19]. This observation supports the early use of long-acting bronchodilators in COPD proposed by more recent treatment recommendations [2,20] and calls into question the idea of demand-based bronchodilator treatment, as in the placebo group, at least for patients with a FEV₁ value of < 70% of predicted.

The mechanisms whereby bronchodilators are able to prevent COPD exacerbations are still not fully understood. Studies investigating lung function during exacerbations have shown that tiotropium bromide reduces the decline of peak flow values during a COPD exacerbation [21,22]. Given the effect of tiotropium bromide on both static and dynamic pulmonary hyperinflation in COPD [16,17], it is conceivable that the sustained bronchodilation provided by tiotropium bromide reduces dyspnoea, the key symptom associated with a COPD exacerbation. Recent *in vitro* studies have also indicated that tiotropium bromide may down regulate cholinergically-mediated inflammatory activity in some cell types [23]; human neutrophils express muscarinic receptors, the stimulation of which leads to the release of proinflammatory mediators [24]. However, whether these mechanisms are also relevant in COPD patients in the context of exacerbations has not been adequately investigated up to now.

As in other studies, the question of a universally accepted definition of an exacerbation also arises in this case. The definition used in this investigation was essentially based on symptoms and interventions (respiratory event lasting for 3 or more days and requiring treatment or a significant increase in the dosage of COPD drugs, i.e. bronchodilators and/or systemic corticosteroids or treatment with antibiotics) and also concurs with the popular definitions by GOLD, the German Airways League (Atemwegsliga) and consensus guidelines of the ATS and ERS [1,2,25].

This study, using over 1200 patients with COPD of different severities and treated with tiotropium bromide (including over 300 patients with severe COPD), supports the results on tolerability reported in previously published 6-month [11] and 1-year studies [3,4]. Specific adverse events over and above the hitherto known extent of side effects were not documented in this large-scale investigation.

In our view, the new aspects of this study, compared to the published data, are a) the demonstration of an exacerbation-preventing effect of tiotropium bromide after just a few weeks' treatment, b) the wide spread of patients (from mild COPD to very severe COPD), and c) the large number of enrolled patients under "real life" conditions.

In conclusion, this study confirms that the once-daily administration of tiotropium bromide 18 µg produces a significant improvement in inspiratory and expiratory lung function parameters, both 2 and 24 hours after inhalation. The effect is largely maintained over the 24-hour dosage interval. Tiotropium bromide also reduces the number of COPD exacerbations and significantly prolongs the time to the onset of the first exacerbation.

References

- 1 Worth H, Buhl R, Cegla U et al. Leitlinie der Deutschen Atemwegsliga und der Deutschen Gesellschaft für Pneumologie zur Diagnostik und Therapie von Patienten mit chronisch obstruktiver Bronchitis und Lungenerkrankung (COPD). *Pneumologie* 2002; 56: 704–738
- 2 GOLD-Workshop. Report. www.goldcopd.com, 2003
- 3 Vincken W, Noord JA van, Greefhorst APM et al. Improved health outcomes in patients with COPD during 1 yr's treatment with tiotropium. *Eur Respir J* 2002; 19: 209–216
- 4 Casaburi R, Mahler DA, Jones PW et al. A long-term evaluation of once-daily inhaled tiotropium in Chronic Obstructive Pulmonary Disease. *Eur Respir J* 2002; 19: 217–224
- 5 American Thoracic Society. Standardisation of spirometry – 1994 update. *Am J Respir Crit Care Med* 1995; 152: 1107–1136
- 6 American Thoracic Society. Standards for the diagnosis and care of patients with Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med* 1995; 152: S77–S120
- 7 Siafakas NM, Vermeire NB, Pride P et al. Optimal assessment and management of chronic obstructive pulmonary disease (COPD). *Eur Respir J* 1995; 8: 1398–1420
- 8 Disse B. Antimuscarinic treatment for lung diseases. From research to clinical practice. *Life Sci* 2001; 68: 2557–2564
- 9 Calverley PMA, Lee A, Towse J et al. Effect of tiotropium bromide on circadian variation in airflow limitation in Chronic Obstructive Pulmonary Disease. *Thorax* 2003; 58: 855–860
- 10 Noord JA van, Aumann J, Janssens E et al. Comparison of once-daily tiotropium, twice-daily formoterol and the free combination once-daily, in patients with COPD. *Eur Respir J* 2005; 26: 214–222
- 11 Brusasco V, Hodder R, Miravittles M et al. Health outcomes following treatment for six months with once daily tiotropium compared with twice daily salmeterol in patients with COPD. *Thorax* 2003; 58: 399–404
- 12 Nosedà A. Dyspnoea and perception of airway obstruction. *Revue des Maladies Respiratoires* 2003; 20 (3 Pt1): 311–314
- 13 Donohue JF. Therapeutic responses in asthma and COPD. *Chest* 2004; 126: 12S–137S
- 14 O'Donnell D, Lam M, Webb KA. Measurement of symptoms, lung hyperinflation and endurance during exercise in Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med* 1998; 158: 1557–1565
- 15 Taube C, Lehnigk B, Paasch K. Factor analysis of changes in dyspnea and lung function parameters after bronchodilation in Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med* 2000; 162: 216–220
- 16 O'Donnell D, Flüge T, Gerken F et al. Effects of tiotropium on lung hyperinflation, dyspnoea and exercise tolerance in COPD. *Eur Respir J* 2004; 23: 832–840
- 17 Celli B, Zu Wallack R, Wang S et al. Improvement in resting inspiratory capacity and hyperinflation with tiotropium in COPD patients with increased static lung volumes. *Chest* 2003; 124: 1743–1748
- 18 Tashkin D, Kesten K. Long-term treatment benefits with Tiotropium in COPD-Patients with and without short-term bronchodilator responses. *Chest* 2003; 123: 1441–1449
- 19 Beeh KM, Beier J, Buhl R et al. Efficacy of tiotropium in patients with mild-to-moderate COPD. *Am J Respir Crit Care Med* 2004; 169: A519
- 20 O'Donnell D, Hernandez P, Shawn E et al. Canadian Thoracic Society COPD guidelines: summary of highlights for family doctors. *Can Respir J* 2003; 10 (4): 183–185
- 21 Friedman M, Korducki L, Kesten S. Recovery of PEFr in COPD exacerbations in 1. year clinical trials. *Am J Respir Crit Care Med* 2002; 165: A270
- 22 Friedman M, Menjoge SS, Kesten S. An evaluation of PEFr as a predictor of COPD exacerbations in large 1-year COPD clinical trials. *Am J Respir Crit Care Med* 2001; 163: A768
- 23 Buehling F, Lieder N, Reisenauer A et al. Anti-inflammatory function of tiotropium mediated by suppression of acetylcholine-induced release of chemotactic activity. *Eur Respir J* 2004; 24: 318s
- 24 Bany U, Gajewski M, Ksiezopolska-Pietrzak K et al. Expression of mRNA encoding muscarinic receptor subtypes in neutrophils of patients with rheumatoid arthritis. *Ann. NY Acad Sci* 1999; 876: 301–304
- 25 Rodriguez-Roisin R. Toward a consensus definition for COPD exacerbations. *Chest* 2000; 117: 398S–401S