Iran Red Crescent Med J. 2015 December; 17(12): e20026. doi: 10.5812/ircmj.20026

Published online 2015 December 5. **Research Article**

Efficacy of Tiotropium Bromide and Rehabilitation Treatment on Pulmonary Function of Patients With Sulfur Mustard Lung Injury

Majid Shohrati,[1] Maryam Jalili,[2] Homa Afshar,[2] Bita Najafian,[3] Ali Qazvini,[1] Meysam Zaeri,[4] Ali Amini Harandi,[5] and Mostafa Ghanei[1,*]

[1]Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences, Tehran, IR Iran
[2]Department of Pharmacology, Faculty of Pharmacy, Islamic Azad University, Tehran, IR Iran
[3]Department of Pediatrics, Baqiyatallah University of Medical Sciences, Tehran, IR Iran
[4]Department of Pharmacology, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, IR Iran
[5]Department of Neurology, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran

*Corresponding Author: Mostafa Ghanei, Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences, Tehran, IR Iran. Tel: +98-2188600067, E-mail: mghaneister@gmail.com

Received 2014 May 6; **Revised** 2014 July 3; **Accepted** 2014 July 26.

Abstract

Background: Chronic pulmonary complication is the most common delayed toxic effect of sulfur mustard (SM) and it has no treatment so far.

Objectives: To evaluate short-term therapeutic effects of inhaled tiotropium bromide and pulmonary rehabilitation on pulmonary function of patients with SM induced lung injury.

Patients and Methods: In a randomized clinical trial, using convenient sampling method, 54 patients with chronic lung disease due to SM exposure were recruited in Baqiyatallah General Hospital, Tehran, Iran for a period of 2-month study. They were randomly divided into 3 groups of 18 participants each. Group 1 received routine drugs (Serevent, Flixotide), pulmonary rehabilitation 30 minutes/2 times a week, and tiotropium bromide 18 μg/day. Group 2 was treated with routine drugs and pulmonary rehabilitation and group 3 was only on the routine drugs. cardiopulmonary exercise test (CPET), plethysmographic measurements, and respiratory symptoms evaluation were performed before and after medical intervention.

Results: In group 1, compared to group 3, significant differences were found with regard to symptoms of cough ([difference between the first and last visit in group 1: Diff 1] = -1.6, Diff 3 = -0.3, P = 0.01) and nocturnal dyspnea (Diff 1 = -1.9, Diff 3 = 0.0, P = 0.01), likewise, compared to group 2, significant differences were found with regard to lung function parameters of forced vital capacity (Diff 1 = 3.0, Diff 2 = -3.5, P = 0.03), forced expiratory volume in one second (Diff 1 = 3.9, Diff 2 = -5.6, P = 0.009), maximal mid-expiratory flow rate 25% - 75% (Diff 1 = 1.5, Diff 2 = -3.2, P = 0.007) and peak expiratory flow (Diff 1 = -2.06, Diff 2 = -4.3, P = 0.04). Total lung capacity (Diff 2 = 9.28, Diff 3 = -12.07, P = 0.02) and residual volume (Diff2 = 32.1, Diff3 = -27.6, P = 0.04) were increased in group 2 compared to group 3. There were no significant differences with regard to CPET results among all groups (P > 0.05).

Conclusions: Inhalation of tiotropium bromide in combination with pulmonary rehabilitation could improve some plethysmographic lung volumes and clinical outcomes in patients with chronic pulmonary disease due to SM. Short-term prescription of pulmonary rehabilitation has no effect on CPET of patients.

Keywords: Bronchiolitis Obliterans, Exercise Test, Tiotropium, Plethysmography, Rehabilitation, Sulfur Mustard

1. Background

Sulfur mustard (SM) is a potent chemical weapon that was used widely by Iraqi military during Iraq-Iran war (1). Delayed toxic effects of SM involve eyes, skin, nervous system, immune system, and especially respiratory system (2). One of the most significant delayed toxicity of SM (causing disability) is pulmonary disorders in which the main underlying pathology is bronchiolitis obliterans (BO) (3). In the long-term, cough, sputum, and shortness of breath were reported in 80% of Iranian patients after exposure to SM (4). Hemoptysis, feeling of pressure in the thorax, chest pain, and nocturnal dyspnea are other common complications. Clinical findings are often generalized wheezing, crackles, clubbing, and cyanosis (1). Pulmonary function tests show that obstruction is the most common abnormal pattern and about half of the obstructive cases are reversible after using inhaled bronchodilators. Abnormal spirometry findings tend to increase by passing time (5).

Tiotropium bromide is a long-acting anticholinergic agent, which can improve lung function and exercise tolerance. It also reduces dyspnea, mortality and exacerbations in chronic obstructive pulmonary disease (COPD) (6).

Additionally, respiratory rehabilitation programs are essential tools in the management of COPD (7). Pulmonary rehabilitation is now an established standard of care for patients with COPD who remain symptomatic de-

spite bronchodilators therapy (8) .It shows improvement of symptoms and exercise tolerance (9).

There are thousands of such victims without cure with severe respiratory symptoms. However, results of tiotropium bromide and respiratory rehabilitation on patients with isolated BO (as their main pulmonary delayed toxic effects following exposure to SM) have not been determined yet.

2. Objectives

This trial was designed to evaluate, for the first time, the role of tiotropium bromide in combination with pulmonary rehabilitation in improvement of pulmonary function of patients with chronic pulmonary lesions following exposure to SM.

3. Patients and Methods

This study was performed as a randomized clinical trial from March 2011 to January 2013 in pulmonary department of Baqiyatallah Hospital, Tehran, Iran. It is a general, referral, and university affiliated hospital. We enrolled volunteer patients with long-term obstructive respiratory disease compatible with BO following definite exposure to SM. Exclusion criteria were acute bronchiolitis and or pneumonia, history of pulmonary tuberculosis or resection of one or more lobes, history of smoking or exposure to other toxins, and participating in simultaneous clinical trials.

The study protocol and advantages and disadvantages of drugs were explained to patients and written consent form was obtained from all participants before recruitment. The consent forms tell the patients that they can leave the study at any time. This study was registered and confirmed in the Scientific and Ethics Committee of the Baqiyatallah university (the code and date of ethical approval: 2422, February 21, 2010). The study protocol followed the ethical guidelines of the (1975 revision) Declaration of Helsinki.

Considering α = 5%, statistical power of 90%, and d = 1.2, the sample size was calculated as 18 subjects in each group, using the following formula.

$$(1) \qquad n = \frac{2\left(Z_{\frac{\alpha}{2}} + Z_{\beta}\right)^2}{d^2} = \frac{2 \times 10.45}{1.44} = \frac{20.9}{1.44} = 14.51 = 15$$

Considering 20% drop up in sample, we add 3 more samples to the optimal sample size, so we totally considered 18 samples in each group. We used standard protocol for our study. Patients were selected using convenient sampling method from the whole population with medical records of chronic pulmonary diseases due to SM. History of medication, smoking, addiction, and current diseases were recorded. Then, they were randomly divided into 3 groups. All patients were on standard routine respiratory medication and there was not any washing period before study. Group 1 (n = 18) received routine drugs (Serevent, Flixotide), twice-weekly 30-minute pulmonary rehabilitation and tiotropium bromide 18 μg/day. Group 2 (n = 18) received routine drugs and pulmonary rehabilitation, and group 3 (n = 18) received just routine drugs. The patients were treated for 2 months and visited every 15 days for recording possible clinical adverse effects.

Only one observer was recruited for the study. Clinical symptoms assessed by visual analogue scale. Physical examinations, pulmonary function tests, and cardiopulmonary exercise test (CPET), were performed before and after the study period. The exercise test was performed according to the protocol of Wasserman on an electrically-braked cycle ergometer (Sensor Medics 2900). Before each test, calibration of gas analyzers was checked using standard gas provided by the manufacturer (Sensor Medics). Plethysmographic measurements were performed according to the American Thoracic Society and the European Respiratory Society recommendations (10, 11) with daily calibration (ZAN 500 Body II, Meβgrerate GmbH, Germany).

Data were analyzed using SPSS version 19 (SPSS Inc., Chicago, Ill., USA). Continuous variables are presented as the mean (SD), whereas categorical data are presented as frequency and percentages. Independent and paired sample t tests, ANOVA, Mann-Whitney Test, and Kruskal-Wallis Test were used for continuous variables. There was no missing value. Homogeneity of data was assessed using Levene's test, and normal distribution of data was checked using Kolmogorov Smirnov test. Also because of small sample size, we used Bootstrap method. In this study, the probability value of 0.05 or less ($P \leq 0.05$) was set the significance level.

4. Results

The data were normally distributed. All patients were male and nobody was excluded from the study. The mean (SD) age of the patients were 46.2 (4.3), 44.5 (2.78), and 45.5 (5.9) years in groups 1, 2, and 3, respectively ($P > 0.05$). Comparison between groups 1 and 2 showed that tiotropium significantly improved forced vital capacity (FVC) (P = 0.03), forced expiratory volume in one second (FEV1) (P = 0.009), peak expiratory flow (PEF) (P = 0.04), and maximal mid-expiratory flow rate (MMEF) 25% - 75% (P = 0.007) (Table 1). Residual volume (RV) was increased in group 2, compared with group 1 (P = 0.15) and group 3 (P = 0.04) during study (Table 1). Using routine drugs and or pulmonary rehabilitation did not produce significant improvement in any of plethysmographic lung volumes (Table 1). Comparison between group 1 and group 3 showed that tiotropium and pulmonary rehabilitation significantly improved cough (P = 0.01) and nocturnal dyspnea (P = 0.01) (Table 2).

There were no significant differences between the groups over time regarding CPET indexes (Table 3).

Table 1. Comparison of the Plethysmographic Profiles Before and After Study in 3 Groups of Patients[a]

Group	First Visit	Last Visit	PV	$PV_{1\&2}$	$PV_{1\&3}$	$PV_{2\&3}$
	Mean ± SD	Mean ± SD				
FVC				0.035	0.079	0.874
1	70.17 ± 17.09	73.17 ± 21.86	0.169			
2	62.94 ± 17.33	59.39 ± 15.26	0.349			
3	68.93 ± 18.57	64.53 ± 20.72	0.255			
FEV1				0.009	0.133	0.634
1	54.36 ± 24.17	58.28 ± 25.71	0.163			
2	44.39 ± 18.28	38.78 ± 15.49	0.100			
3	63.27 ± 22.38	60.07 ± 25.51	0.425			
FEV1/FVC				0.116	0.384	0.559
1	81.17 ± 21.01	80.89 ± 20.14	0.931			
2	72.28 ± 16.53	68.95 ± 24.16	0.531			
3	94.73 ± 17.43	95.67 ± 20.08	0.850			
MMEF 25% - 75%				0.007	0.676	0.685
1	37.00 ± 23.77	38.50 ± 22.79	0.593			
2	22.28 ± 17.07	19.06 ± 17.60	0.301			
3	55.67 ± 32.50	54.80 ± 36.58	0.861			
PEF				0.044	0.317	0.488
1	53.28 ± 23.52	51.22 ± 25.13	0.366			
2	41.72 ± 11.89	37.39 ± 12.42	0.292			
3	66.00 ± 29.03	56.53 ± 28.18	0.188			
TGV				0.095	0.910	0.147
1	123.00 ± 31.74	115.94 ± 45.22	0.608			
2	121.17 ± 53.70	140.67 ± 40.99	0.185			
3	115.40 ± 52.58	106.20 ± 41.85	0.477			
TLC				0.568	0.209	0.022
1	96.50 ± 17.40	94.72 ± 21.43	0.766			
2	89.28 ± 24.93	98.56 ± 18.31	0.189			
3	90.47 ± 22.95	78.40 ± 19.35	0.037			
RV				0.158	0.500	0.047
1	157.11 ± 51.29	148.39 ± 76.87	0.688			
2	150.17 ± 80.72	182.33 ± 63.45	0.172			
3	143.60 ± 98.03	115.93 ± 66.42	0.112			
RV/TLC				0.078	0.812	0.147
1	155.61 ± 32.70	147.33 ± 41.43	0.373			
2	153.33 ± 50.36	173.00 ± 43.24	0.166			
3	140.07 ± 56.15	134.80 ± 45.50	0.539			

[a]Abbreviations: FVC: Forced Vital Capacity, FEV1: Forced Expiratory Volume in one second, MMEF: Maximal Mid Expiratory Flow Rate, PEF: Peak Expiratory Flow, SD: Standard Deviation, TGV: Thoracic Gas Volume, TLC: Total Lung Capacity, RV: Residual Volume.

Table 2. Patients' Clinical Symptoms Before and After Study in 3 Groups of Patients[a]

Group	First Visit	Last Visit	PV	$PV_{1\&2}$	$PV_{1\&3}$	$PV_{2\&3}$
	Mean ± SD	Mean ± SD				
Cough				0.09	0.01	0.30
1	6.7 ± 1.80	5.1 ± 1.75	0.001			
2	7.0 ± 1.63	6.1 ± 1.92	0.053			
3	7.2 ± 1.79	6.9 ± 1.83	0.265			
Dyspnea				0.21	0.09	0.73
1	6.8 ± 1.72	5.3 ± 2.30	0.005			
2	7.0 ± 1.59	6.3 ± 2.14	0.126			
3	7.6 ± 1.64	7.0 ± 2.15	0.072			
Sputum				0.10	0.19	0.63
1	6.1 ± 1.94	5.0 ± 2.44	0.164			
2	6.5 ± 2.25	6.3 ± 2.22	0.521			
3	6.1 ± 1.51	6.1 ± 1.85	1.00			
Hemoptysis				0.69	0.21	0.12
1	2.1 ± 2.94	1.2 ± 2.76	0.080			
2	2.6 ± 2.93	1.5 ± 2.33	0.044			
3	1.0 ± 2.48	0.73 ± 2.09	0.104			
Nocturnal Dyspnea				0.36	0.01	0.67
1	6.0 ± 2.10	4.1 ± 3.03	0.005			
2	5.3 ± 2.97	5.0 ± 2.75	0.610			
3	4.4 ± 2.50	4.4 ± 2.80	1.00			

[a]Abbreviation: SD: Standard deviation.

Table 3. Results of Cardiopulmonary Exercise Test Before and After Study in 3 Groups[a]

Group	First Visit	Last Visit	PV	$PV_{1\&2}$	$PV_{1\&3}$	$PV_{2\&3}$
	Mean ± SD	Mean ± SD				
VO_2 (Max/Pred)				0.175	0.962	0.304
1	38.18 ± 18.96	46.36 ± 14.91	0.188			
2	42.42 ± 11.95	37.42 ± 15.61	0.152			
3	51.47 ± 16.28	60.40 ± 49.83	0.493			
VO_2-AT				0.201	0.373	0.454
1	0.74 ± 0.41	0.83 ± 0.50	0.613			
2	0.82 ± 0.28	0.57 ± 0.44	0.093			
3	0.85 ± 0.34	0.78 ± 0.52	0.503			
VCO_2				0.224	0.148	0.339
1	38.00 ± 21.01	46.00 ± 16.57	0.181			
2	41.92 ± 12.09	38.08 ± 13.73	0.294			
3	46.40 ± 13.55	56.13 ± 49.90	0.477			
O_2-Pulse				0.099	0.564	0.536
1	50.091 ± 24.566	59.00 ± 28.046	0.190			
2	49.92 ± 7.609	43.58 ± 12.638	0.123			
3	70.27 ± 31.719	71.13 ± 42.02	0.937			
VE				0.110	0.167	0.718
1	46.91 ± 22.33	49.82 ± 23.73	0.701			
2	45.92 ± 9.66	36.42 ± 13.95	0.028			
3	55.47 ± 26.02	42.53 ± 24.70	0.121			
EQO_2 (AT)				0.103	0.306	0.355
1	30.55 ± 3.86	30.45 ± 8.48	0.973			
2	34.00 ± 15.08	25.50 ± 5.20	0.046			
3	23.47 ± 10.99	19.27 ± 10.10	0.148			
$EQCO_2$ (AT)				0.162	0.679	0.162
1	29.27 ± 5.46	28.55 ± 5.01	0.607			
2	32.58 ± 10.65	25.42 ± 5.32	0.021			
3	23.27 ± 9.27	21.27 ± 8.51	0.417			

[a]Abbreviations: SD: Standard Deviation, VO_2: Oxygen Consumption (l/min), VCO_2: Carbon Dioxide production; AT: Anaerobic Threshold, VE: Minute Ventilation, EQO_2: Equivalent Ventilator for O_2, $EQCO_2$: Equivalent Ventilator for CO_2.

5. Discussion

Our findings show that tiotropium bromide with pulmonary rehabilitation could improve clinical symptoms and some plethysmographic lung volumes of patients with SM lung injury. Although tiotropium has lots of advantages in patients with COPD, it has not been tested for BO patients after exposure to SM. Despite exercise intolerance, results of this study showed that combination of tiotropium bromide with pulmonary rehabilitation had considerable effect in this setting. Bronchodilators are the first line of treatment in COPD. After adding pulmonary rehabilitation, better treatment results could be achieved (12). Bronchodilators reduce dynamic hyperinflation and shortness of breath and improve air flow. Inhaled anticholinergic drugs like ipratropium are considered as an effective treatment of COPD (13). Tiotropium is a new anticholinergic drug and because of its prolonged blockage of muscarinic receptors, has a long duration of action and can be used only once a day (14).

Up to now, many studies have been carried out on the effectiveness of either tiotropium or pulmonary rehabilitation alone. In their 1-year study, Vincken et al. have shown that tiotropium improves dyspnea and pulmonary function. In this study, tiotropium led to better results compared to ipratropium in the treatment of COPD (15). After 4 years study on the effects of tiotropium in COPD, Tashkin et al. have found that treatment with this drug improves pulmonary function and reduces the exacerbations (16). A 1-year study on the comparison of tiotropium and salmeterol effects showed that tiotropium was better than salmeterol in the management of COPD exacerbations (17).

Exercise practices are the main parts of pulmonary rehabilitation in patients with chronic pulmonary problems and improve exercise tolerance of these patients (7). Many studies have been performed on the effectiveness of pulmonary rehabilitation in patients with COPD. Porszasz et al. (18), Plankeel et al. (19), Farid et al. (20), Casaburi et al. (21) and Puente-Maestu et al. (22) all reported the positive effect of pulmonary rehabilitation on lung volumes and improvement in exercise tolerance in chronic pulmonary diseases. In another study, Casaburi et al. evaluated the exercise tolerance changes by using tiotropium and pulmonary rehabilitation in pulmonary obstructive diseases. Their results revealed significant improvements in shortness of breath and health condition of the patients compared to use of pulmonary rehabilitation alone (23).

In our study, for the first time, we focused on the effectiveness of tiotropium and pulmonary rehabilitation together and also the effectiveness of pulmonary rehabilitation alone on pulmonary function and symptoms of the patients exposed to SM. Our results of using combination of tiotropium and pulmonary rehabilitation are in consistent with the study of Casaburi et al. on patients with COPD (23). Similar to report of Pitta et al. (24), pulmonary rehabilitation practices alone did not show significant changes in lung volumes. Our results on rehabilitation could be explained by dynamic hyperinflation and increase of air trapping related to exercise in BO. Dynamic hyperinflation reduces expiratory flow limitation during exercise but increases the inspiratory loading. It also has effects on hemodynamic and results in shallow and rapid breathing and progressive drop in dynamic lung compliance. This process finally leads to exercise intolerance (25). As it is shown in Table 1, even residual volume has been increased in group 2 after rehabilitation compared to group 3 without exercise program. It should be considered that identification of patients with COPD who develop dynamic hyperinflation is important, because of the association between dynamic hyperinflation, dyspnea, and exercise limitation.

However, unchanged plethysmographic lung volumes in this study is related to underlying physiopathology of lung after exposure to SM that leads to a group of irreversible and progressive pulmonary diseases (26) as well as irreversible anatomical changes in the lung tissue. Consequently the possibility of reversibility and improvement in indexes and pulmonary capacities in these patients is low (27, 28). Furthermore, it has been shown that improvement in exercise tolerance and dyspnea was not accompanied by significant changes in FEV1. That may be related to the fact that rehabilitation intervenes with other outcomes of COPD patients such as muscle dysfunction, reduced physical activities, and depression (29). The slight changes in plethysmographic lung volumes and pulmonary volumes in some findings (FVC, FVE1, MMEF 25%-75%, and FEV1/FVC) may be due to the strength and endurance of respiratory muscles and as a result reduction in resistance of airways.

Our study has some limitations. The sample size was small and there was not placebo control for comparing the results. Performing the molecular and cellular assays were not feasible in this study, as well.

Tiotropium bromide in combination with pulmonary rehabilitation could improve clinical symptoms and some plethysmographic lung volumes of patients with sulfur mustard lung injury. However, the patients with dynamic hyperinflation had a low cardiopulmonary response to rehabilitation alone in our protocol. Further studies are warranted addressing improvements with long-term and different exercise protocols.

Acknowledgments

We would like to appreciate Baqiyatallah university of Medical Sciences, Tehran, Iran for financial support.

Footnotes

Authors' Contribution: Study concept and design: Mostafa Ghanei, Majid Shohrati, Bita Najafian, Ali Qazvini, Meysam Zaeri; analysis and interpretation of data: Ali Amini Harandi; drafting of the manuscript: Majid

Shohrati, Bita Najafian; critical revision of the manuscript for important intellectual content: Mostafa Ghanei, Ali Amini Harandi statistical analysis: Ali Amini Harandi; administrative, technical, and material support: Maryam Jalili, Homa Afshar, Meysam Zaeri; study supervision: Mostafa Ghanei.

Funding/Support: This study was financially supported by Baqiyatallah university of Medical Sciences, Tehran, Iran.

References

1. Ghanei M, Harandi AA. Long term consequences from exposure to sulfur mustard: a review. *Inhal Toxicol.* 2007;**19**(5):451–6. doi: 10.1080/08958370601174990. [PubMed: 17365048]
2. Khateri S, Ghanei M, Keshavarz S, Soroush M, Haines D. Incidence of lung, eye, and skin lesions as late complications in 34,000 Iranians with wartime exposure to mustard agent. *J Occup Environ Med.* 2003;**45**(11):1136–43. doi: 10.1097/01.jom.0000094993.20914.d1. [PubMed: 14610394]
3. Ghanei M, Tazelaar HD, Chilosi M, Harandi AA, Peyman M, Akbari HM, et al. An international collaborative pathologic study of surgical lung biopsies from mustard gas-exposed patients. *Respir Med.* 2008;**102**(6):825–30. doi: 10.1016/j.rmed.2008.01.016. [PubMed: 18339530]
4. Ghasemi H, Owlia P, Jalali-Nadoushan MR, Pourfarzam S, Azimi G, Yarmohammadi ME, et al. A clinicopathological approach to sulfur mustard-induced organ complications: a major review. *Cutan Ocul Toxicol.* 2013;**32**(4):304–24. doi: 10.3109/15569527.2013.781615. [PubMed: 23590683]
5. Ghanei M, Fathi H, Mohammad MM, Aslani J, Nematizadeh F. Long-term respiratory disorders of claimers with subclinical exposure to chemical warfare agents. *Inhal Toxicol.* 2004;**16**(8):491–5. doi: 10.1080/08958370490442421. [PubMed: 15204740]
6. Burgel PR, Paillasseur JL, Dusser D, Roche N, Liu D, Liu Y, et al. Tiotropium might improve survival in subjects with COPD at high risk of mortality. *Respir Res.* 2014;**15**:64. doi: 10.1186/1465-9921-15-64. [PubMed: 24913266]
7. Troosters T, Demeyer H, Hornikx M, Camillo CA, Janssens W. Pulmonary rehabilitation. *Clin Chest Med.* 2014;**35**(1):241–9. doi: 10.1016/j.ccm.2013.10.006. [PubMed: 24507849]
8. Nici L, ZuWallack RL. Pulmonary rehabilitation: future directions. *Clin Chest Med.* 2014;**35**(2):439–44. doi: 10.1016/j.ccm.2014.02.015. [PubMed: 24874138]
9. Jacome C, Marques A. Impact of pulmonary rehabilitation in subjects with mild COPD. *Respir Care.* 2014;**59**(10):1577–82. doi: 10.4187/respcare.03091. [PubMed: 24803497]
10. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. *Eur Respir J.* 2005;**26**(2):319–38. doi: 10.1183/09031936.05.00034805. [PubMed: 16055882]
11. Wanger J, Clausen JL, Coates A, Pedersen OF, Brusasco V, Burgos F, et al. Standardisation of the measurement of lung volumes. *Eur Respir J.* 2005;**26**(3):511–22. doi: 10.1183/09031936.05.00035005. [PubMed: 16135736]
12. Troosters T, Casaburi R, Gosselink R, Decramer M. Pulmonary rehabilitation in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 2005;**172**(1):19–38. doi: 10.1164/rccm.200408-1109SO. [PubMed: 15778487]
13. Hasegawa M, Makita H, Nasuhara Y, Odajima N, Nagai K, Ito Y, et al. Relationship between improved airflow limitation and changes in airway calibre induced by inhaled anticholinergic agents in COPD. *Thorax.* 2009;**64**(4):332–8. doi: 10.1136/thx.2008.103671. [PubMed: 19074932]
14. Montuschi P, Macagno F, Valente S, Fuso L. Inhaled muscarinic acetylcholine receptor antagonists for treatment of COPD. *Curr Med Chem.* 2013;**20**(12):1464–76. [PubMed: 22963553]
15. Vincken W, van Noord JA, Greefhorst AP, Bantje TA, Kesten S, Korducki L, et al. Improved health outcomes in patients with COPD during 1 yr's treatment with tiotropium. *Eur Respir J.* 2002;**19**(2):209–16. [PubMed: 11871363]
16. Tashkin DP, Celli B, Senn S, Burkhart D, Kesten S, Menjoge S, et al. A 4-year trial of tiotropium in chronic obstructive pulmonary disease. *N Engl J Med.* 2008;**359**(15):1543–54. doi: 10.1056/NEJMoa0805800. [PubMed: 18836213]
17. Vogelmeier C, Hederer B, Glaab T, Schmidt H, Rutten-van Molken MP, Beeh KM, et al. Tiotropium versus salmeterol for the prevention of exacerbations of COPD. *N Engl J Med.* 2011;**364**(12):1093–103. doi: 10.1056/NEJMoa1008378. [PubMed: 21428765]
18. Porszasz J, Emtner M, Goto S, Somfay A, Whipp BJ, Casaburi R. Exercise training decreases ventilatory requirements and exercise-induced hyperinflation at submaximal intensities in patients with COPD. *Chest.* 2005;**128**(4):2025–34. doi: 10.1378/chest.128.4.2025. [PubMed: 16236851]
19. Plankeel JF, McMullen B, MacIntyre NR. Exercise outcomes after pulmonary rehabilitation depend on the initial mechanism of exercise limitation among non-oxygen-dependent COPD patients. *Chest.* 2005;**127**(1):110–6. doi: 10.1378/chest.127.1.110. [PubMed: 15653970]
20. Farid R, Azad FJ, Atri AE, Rahimi MB, Khaledan A, Talaei-Khoei M, et al. Effect of aerobic exercise training on pulmonary function and tolerance of activity in asthmatic patients. *Iran J Allergy Asthma Immunol.* 2005;**4**(3):133–8. [PubMed: 17301436]
21. Casaburi R, Porszasz J, Burns MR, Carithers ER, Chang RS, Cooper CB. Physiologic benefits of exercise training in rehabilitation of patients with severe chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 1997;**155**(5):1541–51. doi: 10.1164/ajrccm.155.5.9154855. [PubMed: 9154855]
22. Puente-Maestu L, Sanz ML, Sanz P, Cubillo JM, Mayol J, Casaburi R. Comparison of effects of supervised versus self-monitored training programmes in patients with chronic obstructive pulmonary disease. Eur Respir J. 2000;15(3):517–25. doi: 10.1002/cne.901830305. [PubMed: 10759446]
23. Casaburi R, Kukafka D, Cooper CB, Witek TJ, Kesten S. Improvement in exercise tolerance with the combination of tiotropium and pulmonary rehabilitation in patients with COPD. *Chest.* 2005;**127**(3):809–17. doi: 10.1378/chest.127.3.809. [PubMed: 15764761]
24. Pitta F, Brunetto AF, Padovani CR, Godoy I. Effects of isolated cycle ergometer training on patients with moderate-to-severe chronic obstructive pulmonary disease. *Respiration.* 2004;**71**(5):477–83. doi: 10.1159/000080632. [PubMed: 15467325]
25. O'Donnell D E. [Dynamic lung hyperinflation and its clinical implication in COPD]. *Rev Mal Respir.* 2008;**25**(10):1305–18. [PubMed: 19107020]
26. American College of Sports Medicine Position Stand. The recommended quantity and quality of exercise for developing and maintaining cardiorespiratory and muscular fitness, and flexibility in healthy adults. *Med Sci Sports Exerc.* 1998;**30**(6):975–91. [PubMed: 9624661]
27. Thomason JW, Rice TW, Milstone AP. Bronchiolitis obliterans in a survivor of a chemical weapons attack. *JAMA.* 2003;**290**(5):598–9. doi: 10.1001/jama.290.5.598. [PubMed: 12902361]
28. Ghanei M, Mokhtari M, Mohammad MM, Aslani J. Bronchiolitis obliterans following exposure to sulfur mustard: chest high resolution computed tomography. *Eur J Radiol.* 2004;**52**(2):164–9. doi: 10.1016/j.ejrad.2004.03.018. [PubMed: 15489074]
29. Nici L, ZuWallack RL. Pulmonary rehabilitation: today and tomorrow. *Breathe.* 2010;**6**(4):305–11.