

Jan Szczegielniak, Katarzyna Bogacz, Jacek Łuniewski, Edyta Majorczyk, Andrzej Tukiendorf, Marcin Czerwiński

Institute of Physiotherapy, Faculty of Physical Education and Physiotherapy, Opole University of Technology, Opole, Poland
 Head: J. Szczegielniak, MD, PhD, Associate Professor

The effect of physiotherapy on interleukin-8 levels in patients with chronic obstructive pulmonary disease

A research statutory project NBS 40/08 conducted by Marcin Czerwiński as the principal investigator

Topic: "Physical exercise and the expression of cytokines and chemokines in patients with chronic lung diseases"

Abstract

Introduction: Interleukin-8 (IL-8), a chemokine formed by macrophages and epithelial cells and a potent chemoattractant for neutrophils, plays the principal role in the activation of neutrophils and eosinophils in the airways of patients with chronic obstructive pulmonary disease (COPD) and may be used as a marker of inflammation. The inflammation in the course of COPD may be characterised by an increased neutrophil count in the sputum and elevated IL-8 levels. The aim of the study was to assess the effect of physiotherapy on IL-8 levels in induced sputum in patients with COPD.

Material and methods: The study included 44 patients (21 men and 23 women, mean age 56.47 ± 9.52) managed at the Ministry of Internal Affairs and Administration Specialist Hospital in Glucholazy, Poland, and undergoing rehabilitation at the Therapeutic Rehabilitation Facility, with the diagnosis of COPD ($FEV_1/VC < 65\%$), receiving stable drug treatment during physiotherapy. Before physiotherapy all the patients underwent treadmill exercise testing according to the Bruce protocol and had their dyspnoea assessed on the 20-point Borg scale as part of qualification for physiotherapy. All the patients participated a multidirectional three-week respiratory physiotherapy programme based on aerobic training performed on a cycloergometer. The training loads were selected on an individual basis depending on exercise tolerance. The standard physiotherapy programme also included respiratory muscle exercises with a particular emphasis on the abdominal muscles and the diaphragm, saline inhalations, postural drainage and percussion, relaxation and walks.

Results: IL-8 levels were significantly lower after completion of the three-week physiotherapy. IL-8 levels in the sputum decreased from 18.91 ± 25.2 to 9.69 ± 14.06 ng/ml ($p = 0.0215$). The highest reduction of IL-8 levels was observed in patients with the highest baseline values.

Conclusions: The decrease of IL-8 levels in induced sputum following comprehensive physiotherapy in patients with COPD suggests suppression of neutrophil activity, which may be one of the reasons for the improved clinical condition of the patients.

Key words: interleukin-8, chronic obstructive pulmonary disease, physiotherapy

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Introduction

Chronic obstructive pulmonary disease (COPD) is a condition characterised by a limited flow of air in the respiratory tract. Ventilation abnormalities in COPD patients are caused by airway obstruction resulting from chronic inflammation

in the bronchial wall. Interleukin-8 (IL-8, CXCL8), a chemokine produced by macrophages and epithelial cells and a potent chemotactic factor for neutrophils, plays the fundamental role in the activation of neutrophils and eosinophils in the airways of patients with COPD and may be used as a marker of inflammation. Inflammation in the case

Corresponding author: Prof. Jan Szczegielniak, MD, PhD, Ministry of Internal Affairs and Administration Specialist Hospital in Glucholazy, Karłowicza St. 40, 48–340 Glucholazy, Poland; tel.: +48 77 408 0165; fax: +48 77 439 3861; e-mail: dul@szpitalmsw-glucholazy.pl

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of COPD patients may be characterised by an increased neutrophil count in the sputum and elevated levels of IL-8 [1–3].

The aim of the study was to assess the effect of physiotherapy on IL-8 levels in induced sputum obtained from patients with COPD.

Material and methods

The study included 44 patients (21 men and 23 women, mean age 56.47 ± 9.52) managed at the Ministry of Internal Affairs and Administration Specialist Hospital in Głuchołazy, Poland, and undergoing rehabilitation at the Therapeutic Rehabilitation Facility, with the diagnosis of COPD. All the patients had moderate airway obstruction (according to the American Thoracic Society [ATS] and the European Respiratory Society [ERS] definition: $FEV_1/VC < \text{lower limit of norm}$, and $60\% \text{ predicted} \geq FEV_1 < 70\% \text{ predicted}$). The mean FEV_1 was 63% predicted.

The inclusion criteria were as follows: diagnosis of COPD, referral for physiotherapy, stable drug treatment during physiotherapy, patient consent. The exclusion criteria were as follows: oral glucocorticosteroid treatment, smoking, dry cough.

Before rehabilitation treatment all the patients underwent treadmill exercise testing according to the Bruce protocol and had their dyspnoea assessed on the 20-point Borg scale as part of qualification for physiotherapy.

All the patients underwent a multidirectional physiotherapy programme [4]. Exercise load was set according to exercise tolerance. Target heart rate was calculated from the Karvonen formula. The patients were assigned an appropriate physiotherapy model according to exercise tolerance (A, B, C, D) [5].

Physiotherapy was based on aerobic training on a cycloergometer. The patients participated in continuous training on a cycloergometer according to the monitored training system conducted for 3 weeks, 6 days a week, for 30 minutes, with loads increased every 4 minutes.

The team respiratory exercises and general rehabilitation exercises included respiratory muscle training with a particular emphasis on the abdominal muscles and the diaphragm, relaxing exercises and exercises mobilising the chest, exercises increasing the respiratory movement of the lower chest and the training of diaphragmatic respiration. The exercises were performed in groups of uniform intensity, no more than 6–8 patients each [5]. The standard physiotherapy programme provided to all the patients also included drainage positions, inhalations, postural drainage and

percussion with evacuation of discharge from the bronchial tree, relaxation and walks [5].

The control group consisted of 30 healthy individuals — volunteers matched for age and sex. Sputum from healthy individuals was collected during standard prophylactic check-ups. All the patients had their IL-8 levels in induced sputum determined before and after the comprehensive physiotherapy programme. IL-8 level were determined by ELISA.

Analysis of interleukin-8

Sputum was collected on the first and twentieth day of physiotherapy cycle. The patients inhaled 5 ml of 0.9% aqueous solution of sodium chloride from an ultrasonic inhaler and expectorated sputum in accordance with the applicable standards. The sputum was then frozen. After freezing, the study material was diluted at a 1:4 ratio with buffered saline at pH 7.4, centrifuged at 13,000 g for 10 minutes and diluted at a 1:25 ratio with buffered saline. IL-8 levels were determined by ELISA (QuantiKine kit, R&D, Oxford, UK).

Statistical analysis

The analysis of the investigated variables in the aspect of single and combined effects of the independent variables was performed using the so-called hierarchical linear model, also called multilevel modelling [6]. The method enables the analysis of variance of dependent variables for various stratification level of independent variables and determination of linear regression coefficients for single and combined effects of these variables.

Results

The mean energy expenditure expressed in METs in the exercise test before physiotherapy was 6.08 ± 1.61 , the mean severity of dyspnoea on the 20-point modified Borg scale was 5.0 ± 4.32 . All the subjects in the control group had normal levels of IL-8. In the COPD group, IL-8 levels were significantly higher than in the group of healthy individuals. No relationship between FEV_1 and IL-8 levels or between FEV_1/VC and IL-8 levels was observed in COPD patients. There were also no sex-related differences (Table 1).

IL-8 levels were significantly higher after 3 weeks of physiotherapy in COPD patients (18.91 ± 25.2 ng/ml before vs 9.69 ± 14.06 ng/ml after physiotherapy, $p = 0.0215$). The highest reduction in IL-8 levels was observed in patients with the highest baseline levels (Table 2).

A statistical model was developed to depict the trends in IL-8 level changes allowing direct observation of the decreases in IL-8 levels (Figures 1 and 2).

Table 1. Concentration of IL-8 in induced sputum in control and chronic obstructive pulmonary disease (COPD) group before physiotherapy

	Concentration of IL-8 [ng/ml]
Control group	3.15 ± 1.9
COPD group	18.91 ± 25.2

Table 2. Concentration of IL-8 in induced sputum in patients with chronic obstructive pulmonary disease (COPD) before and after physiotherapy

	Concentration of IL-8 [ng/ml]
Before physiotherapy	18.91 ± 25.2
After physiotherapy	9.69 ± 14.06

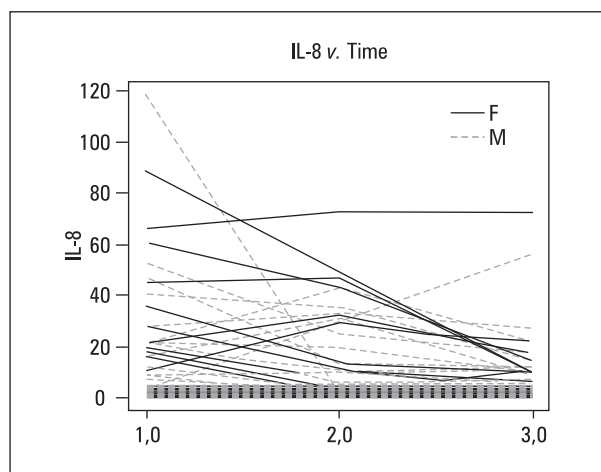


Figure 1. Decrease in the concentration of IL-8 in induced sputum during pulmonary physiotherapy — real data

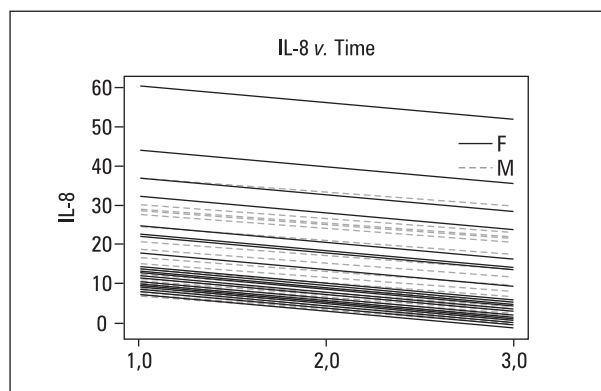


Figure 2. Decrease in concentration of IL-8 in induced sputum during pulmonary physiotherapy — statistical model

Discussion

It is currently believed that there are associations between the physiological changes in the body secondary to physical exercise and the immune system [7]. Cytokines, such as IL-8, seem to play a significant role in the association between physical exercise and the immune system.

According to the published studies, half an hour after completion of an intensive exercise (a marathon) elevated levels of plasma IL-8 were observed in the participants [8]. Ultrarunners had a 2.5-fold increase in IL-8 after running 90 km [9], while after running a distance of 160 km this increase exceeded 6-fold the baseline values [10]. A significant increase in plasma IL-8 levels was also observed after exercise in 12-14-year-olds undergoing a 60-minute exercise (pedalling) at VO_{2max} of 70% [11]. IL-8 levels and the expression of IL-8 mRNA are also increased in the cytoplasm of myocytes, which suggests that muscle activity stimulates myocytes to produce IL-8 [12]. This is consistent with the previous findings of increased expression of IL-8 mRNA in skeletal muscles after a 3-hour run on a mechanical treadmill [2]. Less pronounced changes, 10-fold increases in IL-8 levels, were observed following submaximal concentric exercises of up to 2 hours [13]. One of the factors responsible for the formation of cytokines turned out to be the energetic crisis which develops during long exertion [8].

Other studies showed that systematic physical activity significantly reduces the levels of proinflammatory cytokines. However, the quantity of pro- and anti-inflammatory cytokines released during one-off exercise depends on its intensity and duration and on the groups of active muscles [14–17]. The most pronounced changes were observed in response to performance training of more than 2 hours.

IL-8 is the best studied chemotactic factor for neutrophils. It has been shown to play the principal role in the activation of neutrophils and eosinophils in the airways of patients with COPD [18, 19]. Sputum levels of IL-8 are one of the markers of inflammation [21–23]. Our study has shown that IL-8 levels in induced sputum from COPD patients were significantly higher than those in the group of healthy individuals.

An analysis of the available literature suggests a possibility to reduce IL-8 levels through drug treatment [21]. It is believed that physiotherapy

should hold an important place in the management of COPD [24]. Studies have shown that physiotherapy in patients with COPD improves exercise tolerance, relieves dyspnoea and reduces the frequency of exacerbations. No comprehensive studies have been conducted so far to assess the reduction of IL-8 levels in response to physical exercise used as part of physiotherapy in patients with COPD.

Our study has demonstrated a statistically significant reduction of IL-8 levels in COPD patients. Sugawara et al. obtained similar results showing a reduction of the levels of inflammatory cytokines, including IL-8, following low-intensity exercise in combination with supplementation [25]. Our results confirm the hypothesis according to which physiotherapy improves the patients' condition. This improvement does not only involve an increased exercise tolerance and a reduced severity of dyspnoea, but also indicates that during physical exercise a new balance is established between the released proinflammatory cytokines, anti-inflammatory cytokines, inhibitors of proinflammatory cytokines and the TNF superfamily receptors. The favourable effect of moderate exercise would therefore involve a suppressed release of proinflammatory cytokines shifting the balance towards anti-inflammatory cytokines [8].

It seems that the possibility of reducing the activity of inflammation is evidenced in our study by the highest relative reduction of IL-8 levels in patients with the highest baseline levels. Our analysis of the dynamics of IL-8 changes during physiotherapy is quite interesting and requires further studies. The effects of individual elements of comprehensive physiotherapy, including inhalations, postural drainage and percussion, and respiratory muscle training, on the possibility to reduce IL-8 levels in induced sputum in COPD patients also merits further analysis.

Our study shows that the reduction of IL-8 levels in induced sputum as a result of physiotherapy in patients with COPD suggests suppression of neutrophil activity, which may be one of the reasons for the improved clinical condition of the patients.

Conclusions

1. IL-8 levels in induced sputum from patients with COPD was significantly higher than those in the control group of healthy individuals.

2. The decrease of IL-8 levels in induced sputum following comprehensive physiotherapy in patients with COPD suggests suppression of neutrophil activity, which may be one of the reasons for the improved clinical condition of the patients.

3. The highest decrease of IL-8 levels was found in patients with the highest baseline levels of this cytokine.

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