ORIGINAL PAPERS

Adv Clin Exp Med 2014, **23**, 3, 381–394
ISSN 1899–5276

Aleksander E. Makarevich[1, A, C–G], Svetlana S. Lemiasheuskaya[1, A–F], Aleksander J. Poctavcev[1, A, C, E, F], Aleksander I. Lemeschewskij[2, B–F], Michail Nedvedz[3, C, E, F]

The Dynamics of Respiratory Muscle Changes During the Progression of Chronic Obstructive Pulmonary Disease

[1] Department of Internal Medicine, Belarusian State Medical University, Minsk, Belarus
[2] Department of General Surgery, Belarusian State Medical University, Minsk, Belarus
[3] Department of Pathologic Anatomy, Belarusian State Medical University, Minsk, Belarus

A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. Pathological changes of the respiratory muscles (RM) during the development of chronic obstructive pulmonary disease (COPD) have not yet been studied in detail.
Objectives. The aim of the research was to assess RM status in COPD and the relationship between echodensitometric and morphological changes in the RM.
Material and Methods. The participants – 152 male COPD patients – were divided into three groups according to the severity of the disease: $COPD_1$ (mild), $COPD_2$ (moderate) and $COPD_3$ (severe). The status of the accessory RM in these groups was investigated using echodensitometry indices (echogenicity: IE; homogeneity: IH; and structural density: ISD) and the morphological material of the abdominal internal oblique muscle in 25 patients with $COPD_1$ and $COPD_2$.
Results. Considerable differences among the COPD groups were revealed. In mild COPD a tendency toward increased homogeneity was detected (due to RM hypertrophy), while echogenicity results varied. IH and ISD decreased in moderate COPD, while IE was increased due to RM fatty infiltration and sclerosis (according to the morphological data). In severe COPD, IH and ISD rose again against a background of IE decrease. The presence of myolysis, sclerotic changes and contractures of myofibrils in the RM was significantly more frequently observed in $COPD_1$ and $COPD_2$ compared with the controls (where these features were rare). The sensitivity and specificity of the morphological and ultrasonic methods were similar in assessing atrophic and sclerotic RM changes, while their accuracy was lower in analyzing other morphological signs.
Conclusions. The echodensitometric parameters investigated reflect, in a complex way, different dynamics of degenerative RM processes during the progression of COPD (**Adv Clin Exp Med 2014, 23, 3, 381–394**).

Key words: chronic obstructive pulmonary disease, sarcopenia, respiratory muscles, ultrasonic densitometry.

Sarcopenia (loss of muscular mass) is one of the processes accompanying advanced age. Sarcopenia is divided into two types: primary, when there are no other reasons for the decrease in muscle mass, and secondary, which is the loss of muscle mass as a result of some disease [1]. The incidence of primary sarcopenia fluctuates from 5% to 13% in persons aged 60–70 years [2]. A decrease in muscle mass is not an isolated process, because it develops simultaneously with fatty mass accumulation [3, 4]. There is a tendency to a higher incidence of fatty infiltration in satellite cells in sarcopenia, the consequence of which is fatty muscle infiltration [5].

Currently, increasing attention is being paid to disorders of the skeletal muscles and respiratory muscles (RM) among other extrapulmonary complications in COPD, which is characterized by the progressing atrophy of these muscles, with loss of muscular strength and mass, as well as subsequent development of physical exercise intolerance and chronic respiratory insufficiency [6–8].

The latter depends not only on the degree of severity of the pulmonary pathology [9, 10] but also on extrapulmonary disorders [11, 12]. Decreases in RM strength by 15–30% in 20–50% of COPD patients (depending on the severity of the disease and RM type) have been detected [13]. The loss of muscle strength results predominantly from RM atrophy and rather than a loss of RM contractile properties [14]. In addition, the functioning of the intercostal and sternocleidomastoid muscles is believed to have fewer disadvantages than the diaphragm in the presence of severe lung hyperinflation [15]. Insufficient RM inspiratory activity leads to hypoventilation, while expiratory RM weakness favors the development of dynamic hyperinflation which increases during exercise [16–18]. These increases in RM fatigability and atrophy are connected (in a complex manner) with the development of breathlessness on exertion and limitation of exercise.

Prolonged bronchial obstruction (which increases RM workload and oxygen demand) in COPD has been shown to lead to RM overload and reduction in RM ability to generate maximum respiratory effort [19, 20]. RM hypertrophy develops in response to increased RM functional load even in the initial stages of COPD [19]. Then, during the course of COPD development, RM strength decreases against the background increase of atrophic changes. The atrophy of muscles is only partially reversible in COPD patients [13].

The insidious development of flow limitation and hyperinflation in prolonged COPD enables several structural adaptive mechanisms of muscles to come into play in response to chronic mechanical loading to preserve the functional strength of overloaded RM [21–24]. These include a reduction in sarcomere length, an increase in the relative proportion of fatigue resistant fiber types and an increase in mitochondrial concentration, which improves oxidative capacity. Differences in morphological changes in damaged myofibrils have been detected in COPD patients [25]. One of the most common forms of muscular dystrophy is the formation of contractures when myofilaments become deregulated and displaced, while myofibrils lose cross-section striation [26]. In addition, reduced numbers of capillaries per unit of area have also been found in the muscular tissue of COPD patients [27].

Decreased body weight in COPD patients is commonly observed with age, primarily at the expense of muscles; the loss of muscle mass is aggravated by fatty mass accumulation. Fatty muscle infiltration leads to decreased muscle strength and inadequate functioning. Increasing hypodynamia, in turn, stimulates the growth of sarcopenia. Another mechanism of the decrease in muscle mass is an imbalance (tending toward catabolism) between synthesis and protein degradation. This is caused by suppressed formation of humoral factors that stimulate protein synthesis, with the simultaneous expression of subclinical inflammation factors (TNF-α, IL-6) and a decline in myocyte apoptosis [28, 29].

Compared with other muscles, RM have rather particular characteristics, due to the necessity of overcoming the elastic properties of the chest. The most important feature of RM is peak effort, in which type II muscle fibers play a more impotent role, whereas the endurance and relative strength are of greater importance for skeletal muscles. The peak expiratory flow rate is a basic RM characteristic, but its relationship to sarcopenia has not yet been fully established. In COPD patients, inspiratory RM have to generate more negative intra-thoracic pressure than is generally required for adequate alveolar ventilation. This is due to the presence of internal positive pressure at the end of the expiration phase as well as static and dynamic hyperinflation [17, 18].

The development of RM dysfunction in COPD occurs due to the complex interplay of several factors [7, 27, 30–34]: an increase in pro-inflammatory mediators (which plays a key role in the pathogenesis of these changes); decreased nutritional status and anabolic hormone levels; tissue hypoxia; oxidative stress; reduced capillarity and proportion of type II fibers; muscle apoptosis; and the use of oral or inhaled corticoids in high doses. RM are involved in this pathological process with some delay [25]. An increase in proinflammatory cytokine levels (IL-1, IL-2, IL-6; TNF-α, interferon-j) circulating in the blood flow leads to decreased muscular fiber synthesis. Indeed, TNF-α directly or indirectly promotes the development of a systemic inflammatory process and the proteolysis of myosin as well as an increase in catecholamin synthesis [35]. In addition, pathological changes in muscular fibers combined with decreased transverse section areas of muscle fibers are observed [36]. These RM changes decrease the efficacy of mechanical muscular effort.

The ultrasonic method provides direct detection of separate structures and early pathological changes in the RM of COPD patients, and their timely correction afterwards. Unfortunately, only the area of RM cross sections and their amplitude of movement are currently being analyzed by this method [37]. At present, investigations of morphometric changes in RM are scarce [38]. The relationship between these RM changes and other pathological processes occurring in COPD has not been studied in detail yet. Usually RM biopsies

with subsequent morphological and histochemical analysis are used only for scientific purposes. All this emphasizes the importance of developing new noninvasive methods for the dynamic assessment of functional and structural pathological changes of RM in COPD patients.

The aim of this study was to assess accessory RM status (dystrophic changes) by ultrasonic scanner and to compare them with the histological data from muscle biopsy material from males with different stages of COPD according to the Global Initiative for Obstructive Lung Disease (GOLD) grading system.

Material and Methods

The study was undertaken at the Pulmonology Department of the 10th Minsk clinical hospital in the years 2009 to 2012. All participants gave their written informed consent. The study protocol was approved by the Human Studies Committee on Research Ethics at the Belarusian State Medical University. The inclusion criteria for the study were established before the trial and were strictly followed: Males aged 50–67 years with different degrees of severity of COPD, with FEV_1 increase < 15% during bronchodilation tests were qualified for the study. Exclusion criteria included substantial uncontrolled comorbidity.

The following accessory RM were studied:

- muscles of inspiration: sternocleidomastoid (SCM), scalenus anterior (SA), and external intercostals (ExI);
- muscles of expiration: abdominal internal oblique (AIO), abdominal external oblique (AEO), rectus abdominis (RA), transversus abdominis (TA) and internal intercostals (InI).

A HONDA Electronics HS-2000 ultrasonic scanner with 7.5 MHz/50 mm and 256 shades of grey color, providing high-quality histograms, was used in the study. The following quantitative indices of RM were investigated: homogeneity (IH, which characterizes the degree of muscle homogeneity); echogenicity (IE, the level of the shade of the grey scale that most often emerged in the outlined zone); structural density (ISD, which permitted a quantitative assessment of muscles of different size) and standard deviation (SD). The technical features of this scanner did not permit investigation of structural changes in the diaphragm (the main respiratory muscle), because of its subtlety and deep arrangement.

The study group consisted of 152 COPD patients (aged 51–67 years) with acute exacerbation (increased wheezing, dyspnea, sputum volume or sputum purulence one week prior to admission) of varying severity. These patients and a control group consisting of 34 healthy persons of comparable age, sex, smoking status and body mass index (BMI) were examined. Special emphasis was placed on the participants' history of chronic, progressive symptoms such as dyspnea, coughing and wheezing, and on their smoking history. Most of the patients were current smokers or ex-smokers (who had stopped smoking at least 12 months before evaluation); some of them were nonsmokers. The diagnosis of COPD and its severity was based on the GOLD guidelines. The expressiveness of dyspnea (as an indirect indicator of decreased exercise tolerance) in these COPD patients was defined according to a modified Medical Research Council (MRC) questionnaire [39].

The patients were divided into three groups according to the severity of their COPD, which reflected the evolution of disease (Table 1). The first group ($COPD_1$) consisted of 42 patients with mild COPD (median age and duration of disease: 55 and 4 years respectively; FEV_1: 83%; BMI: 27 kg/m^2; current smokers: 79%, ex-smokers 6%, and nonsmokers: 15%). The second group, with moderate COPD ($COPD_2$), was composed of 80 patients (median age and duration of disease: 57 and 10 years respectively; FEV_1: 55%; BMI: 30 kg/m^2; current, ex- and nonsmokers: 78%, 10% and 12% respectively). The third group, with severe COPD ($COPD_3$), was made up of 30 patients (median age and duration of disease: 60 and 13 years respectively; FEV_1: 33%; BMI: 25 kg/m^2; current, ex- and nonsmokers: 86%, 10% and 4% respectively).

As shown in Table 1, the control group (with normal pulmonary function) did not differ from $COPD_1$ patients in terms of median age, the percent of patients under 60 years, BMI and intensity of smoking. Compared with the controls, $COPD_1$ patients only had decreased ventilation parameters. $COPD_3$ patients a had significantly higher median age, disease duration, MRC breathlessness index and number of current smokers than the control group and $COPD_{1,2}$ patients, while their BMI was significantly lower than in the $COPD_2$ group. As Table 1 shows, a significant increase in the number of exacerbations (which are an important component of the clinical development of disease) over the past year was found in severe COPD compared with mild to moderate COPD.

A progressive decrease in ventilation parameters was noted as the disease developed: FVC dropped from 86% in $COPD_1$ to 38 % in $COPD_3$; FEV_1 dropped from 80% to 33%; and blood oxygen saturation dropped from 97% to 93%. The patients (mostly $COPD_{2-3}$) were treated in the hospital with a combination of inhaled long-acting

anticholinergic or β_2-agonists; inhaled and systemic steroids (5 days of dexametasone intravenously, 4 mg a day); and antibiotics if there was clinical evidence of Type 1 or 2 exacerbation according to Anthoninsen criteria.

Additionally, morphological examinations of AIO biopsy material from 25 stable $COPD_{1,2}$ patients were carried out (Table 2). The biopsies were taken during inguinal herniotomies in order to assess the accuracy of the proposed ultrasonic method in the diagnosis of dystrophic RM changes. Hematoxylin-eosin staining and the one by Van Gieson's method were the histological methods used. The following morphological signs in RM were assessed: atrophic and sclerotic changes, myolysis, fragmentations and contractures of myofibrils, the presence of granules in sarcoplasm, the proliferation of perimysium cells and fibroblasts, as well as the growth of fatty tissue into the muscular tissue.

The statistical analysis was performed using Statistica 8.0 software (2007, Statsoft Inc., USA). The preliminary analysis of the variables under consideration was a Shapiro-Wilk test of correspondence to normal distribution. The results of the analysis were shown as median and interquartile range (25–75%) because all the parameters differed from normal distribution. The comparison of non-parametric parameters in two independent groups was carried out using a Mann-Whitney test, while in three or more independent groups it was performed using the Kruskal-Wallis rank sum test. Fisher's exact test was used to detect statistically significant differences between independent groups according to the frequency characteristic of the investigated parameter. Spearman's rank correlation coefficient (r_s) was used to describe the relationship between the two quantitative variables that differed from normal distribution. The level of statistical significance was set at $p < 0.05$.

Results

The ultrasonic images of accessory RM in the control group and in all the patient groups were significantly different. RM tissue in COPD patients was represented as more echogenic and less homogenic compared with the control group. The peak histogram of the abdominal external and internal oblique muscles showed that echogenicity was more common in the initial part of the grey color scale (Fig. 1). Additionally, the low standard deviation value and the narrow basis of the graph denoting the uniformity of muscle tissue was due to a narrower spectrum of grey color gradation.

Various ultrasonic images denoting increased echogenicity and heterogeneity in the muscular tissue of $COPD_{1,2,3}$ patients were found. Increases in the basis of the peak histogram and value as well as changes in structural density, echogenicity and homogeneity were observed. Figure 2 shows a typical amplitude histogram of abdominal oblique muscles in mild to moderate COPD patients.

It was determined that the changes in structural density, echogenicity and homogeneity in RM on inspiration varied in a complex way during the

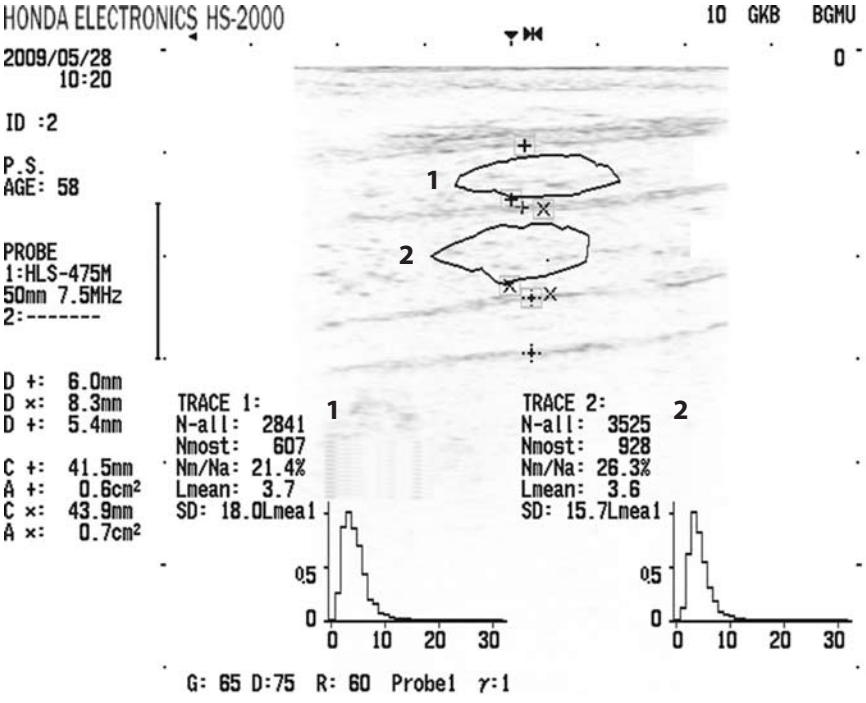

Fig. 1. The ultrasonic image of external (1) and internal (2) oblique abdominal muscles (without the tendon part) in a healthy 58-year-old man, and the peak histograms Homogeneity = N m/N all; Echogenicity = L mean (the most common shade of grey); Structural Density = N most/S (where S is the square of the interest zone)

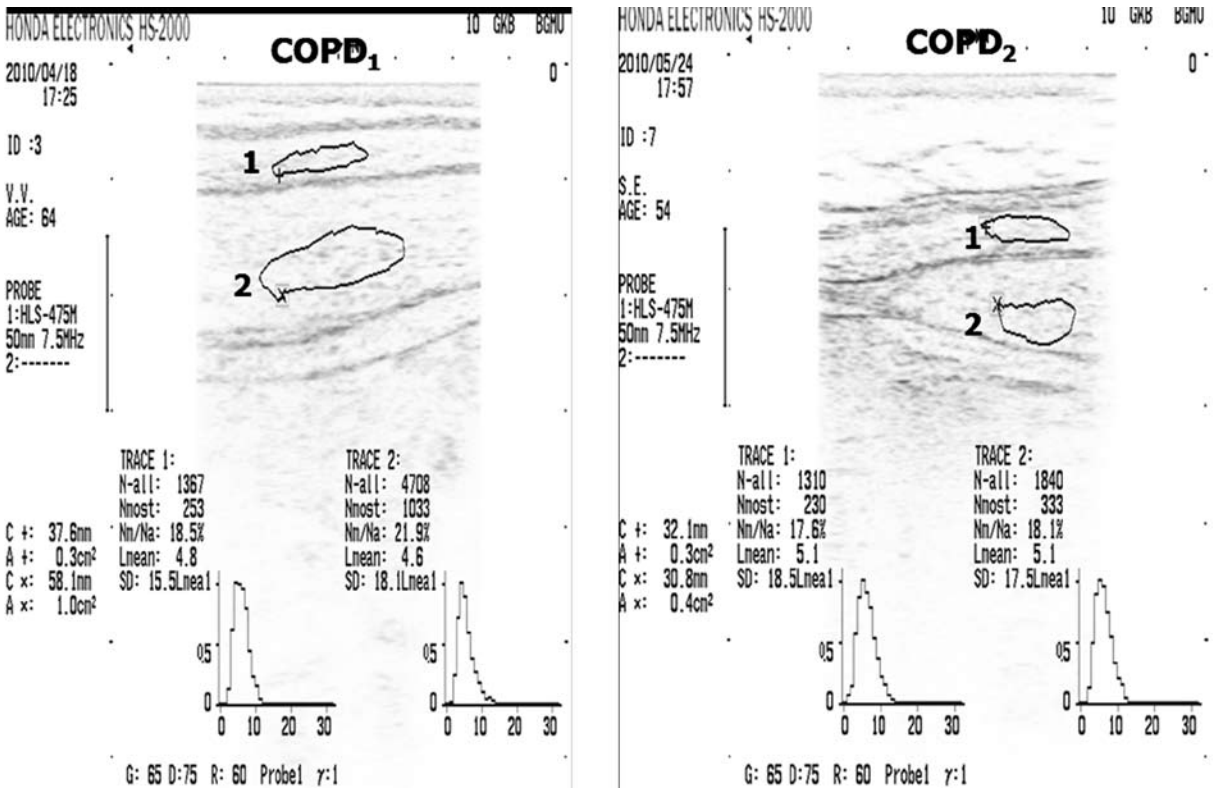

Fig. 2. Echodensitometric data of external (1) and internal (2) oblique abdominal muscles in patients with $COPD_{1,2}$

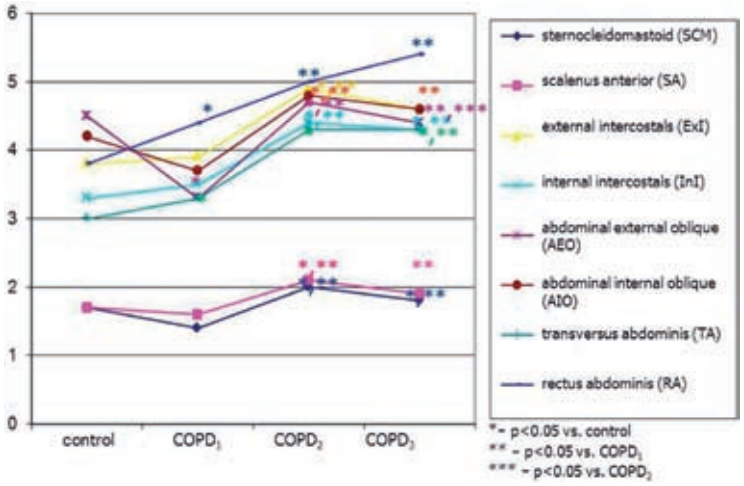

Fig. 3. Dynamics of echogenicity index in RM in the groups of patients with different severity of airflow limitation (GOLD spirometric levels) and the control group. * – compared with the control group, $p < 0.05$; ** – compared with patients of $COPD_1$ group, $p < 0.05$; *** – compared with patients of $COPD_2$ group, $p < 0.05$

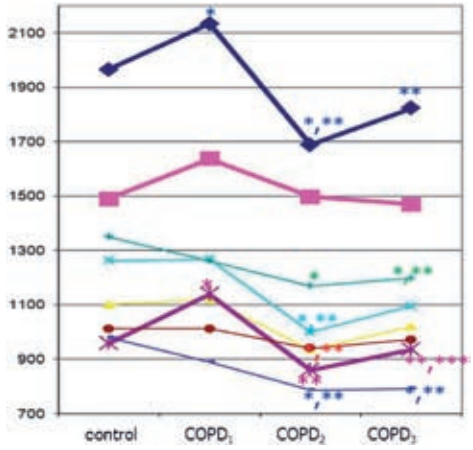

Fig. 4. Dynamics of structural density index changes in RM in the groups of patients with different severity of airflow limitation (GOLD spirometric level) and the control group. * – compared with the control group, $p < 0.05$; ** – compared with patients of $COPD_1$ group, $p < 0.05$; *** – compared with patients of $COPD_2$ group, $p < 0.05$

course of COPD development (Table 3; Fig. 3, 4). A significant increase in homogeneity and structural density against a background decrease of echogenicity was revealed. The structural density of SCM significantly increased in $COPD_1$ (by 9% compared to the control group), while it decreased in $COPD_2$ (by 14% compared to the controls). Echogenicity decreased in $COPD_1$ and increased in $COPD_2$ (both by 18% in comparison to the control group). The opposite was that case with the

Table 1. Baseline characteristics and the results of comparisons between the control group and the groups of patients with different degrees of severity of airflow limitation (GOLD spirometric level) on admission

Parameters	**Control n = 15**	**$COPD_1$ n = 42**	**$COPD_2$ n = 80**	**$COPD_3$ n = 30**
Median age (years): < 60/ > 60 years (%)	56 (54; 59) 73/27	55 (51; 59) 79/21	57 (53; 60)* 66/34	60 (55; 67)*,**,*** 50/50
Body mass index (kg/m^2)	26.0 (23.5; 30)	27.0 (24; 30)	29.7 *(24.8; 33)	25.1*** (20.8; 32.1)
Present smokers (%)	60	79	78	86*
Smoking history index packs/years	17 (10; 30)	20 (10; 30)	20 (10; 30)	28 (15; 40)
Median duration of COPD (years)	–	4 (2; 6)	10 (4; 14)	13**,*** (8; 19)
Number of exacerbations in the last year	–	1 (0.1; 2)	2 (1; 3)	3**,*** (2; 4)
MRC index dyspnea (score)	0	0 (0; 1)	2 (1; 3)	3**,*** (2; 3)
FVC (% pred.)	102 (98; 112)	86 (75; 90)*	55 (48; 66)*,**	38 (28; 46)*,**,***
FEV_1 (% pred)	88 (77; 94)	80 (75; 87)*	55 (50; 64)*,**	33 (22; 40)*,**,***
FEV_1/FVC (%)	98 (89; 104)	70 (66; 71)*	67 (57; 70)*	51 (39; 57)*,**,***
psO_2 (%)	97 (96; 97)	97 (96; 97)	96 (95; 98)	93 (90; 95)*,**,***
C-reactive protein (mg/dL)	0.4 (0.1; 2.0)	0.6 (0.1; 2.8)	3.3*,** (1; 5.6)	2.9 *,** (0.9; 5.4)
Home treatment: corticoids (%); inhaled/intermittently systemic; with inhaled long active anticholinergic (%); with inhaled long active β_2-agonists (%)		– –	35/2 20 1	43/13*** 7 17***

Data are presented as n (%) or median (ranges), unless otherwise stated. GOLD – global initiative for chronic obstructive lung disease; COPD – chronic obstructive pulmonary disease; MRC – modified medical research council questionnaire for assessing the severity of breathlessness; FVC – forced vital capacity; % pred.: % predicted; FEV_1 – forced expiratory volume in one second; FEV_1/FVC – ratio of these two measurements; psO_2 – % blood oxygen saturation by pulse oximetry; * – compared with the control group, $p < 0.05$; ** – compared with the $COPD_1$ group, $p < 0.05$; *** – compared with patients of $COPD_2$ group, $p < 0.05$

Table 2. Characteristics and the results of comparisons between the control group and the groups of patients who underwent morphological examinations

Parameters	**Control n = 11**	**$COPD_1$ n = 12**	**$COPD_2$ n = 13**	**Kruskal-Wallis ANOVA, p**
Age in years	55 (48; 60)	56 (51; 60)	57 (56; 59)	> 0.05
BMI (kg/m^2)	24 (23; 28)	24 (22; 26)	24 (24; 26)	> 0.05
FEV_1 (% pred.)	94 (85; 98)	79 (75; 87)	54 (49; 64)	< 0.05
Present smokers (%)	6 (55)	8 (66)	10 (77)	< 0.05
Index packs/years	12 (4; 20)	14 (5; 20)	29 (22; 40)	< 0.05

Data are presented as n (%) or median (ranges), unless otherwise stated. COPD – chronic obstructive pulmonary disease; BMI – body mass index; FEV_1 – forced expiratory volume in one second; % pred. – % predicted; $p < 0.05$ – significant difference between these groups

homogeneity of SCM, which increased in $COPD_1$ (by 6% as compared to the controls) and decreased in $COPD_2$ (by 13% compared to the controls).

An 8% increase in the homogeneity of SCM was observed in $COPD_3$ in comparison with $COPD_2$ ($p < 0.05$), but this parameter remained below that

Table 3. Echodensitometric parameters of respiratory muscles and the results of comparisons between the control group and the groups of patients with different degrees of severity of airflow limitation (GOLD spirometric level)

Parameters	**Control n = 43**	**$COPD_1$ n = 42**	**$COPD_2$ n = 80**	**$COPD_3$ n = 30**
RM of inspiration**:** sternocleidomastoid				
IH	41 (30.8; 45.9)	43.4* (38.1; 52.1)	35.7*,** (27.6; 43.4)	38.5** (33; 41.9)
IE	1.7 (1.4; 2.1)	1.4* (1.0; 1.7)	2.0** (1.3; 2.8)	1.8** (1.4; 2.3)
ISD	1965 (1620; 2183)	2133* (1794; 2638)	1688*,** (1327; 2204)	1823** (1613; 2058)
External intercostals				
IH	24.5 (21.2; 25.1)	23.4 (19.6; 29.1)	21.4*,** (18.8; 24.2)	20.8*,** (16.7; 23.6)
IE	3.8 (3.1; 4.8)	3.9 (3.0; 5.1)	4.9*,** (4.0; 5.6)	4.8*,** (3.4; 5.3)
ISD	1100 (980; 1221)	1120 (940; 1380)	986 (780; 1120)	1020 (816; 1290)
Scalenus anterior				
IH	37.9 (27.8; 45)	38.8 (34.5; 46.6)	33.4*,** (27.2; 40.9)	37.4 (25.4; 45)
IE	1.7 (1.3; 2.5)	1.6 (1.2; 1.9)	2.1*,** (1.4; 3.0)	2.0*** (1.2; 2.9)
ISD	1490 (1175; 1975)	1637 (1280; 2180)	1497 (1105; 2065)	1470 (1190; 1770)
RM of expiration: abdominal external oblique				
IH	19.1 (16.1; 28.6)	22.9 (18.8; 27.9)	18.0 (15.7; 20.4)	19.2 (16.7; 22.3)
IE	4.5 (2.6; 5.4)	3.8 (2.5; 4.2)	4.7 (4.1; 5.9)	4.4 (3.7; 5.3)
ISD	958 (805; 1331)	1139 (901; 1400)	860*,** (722; 976)	936 (821; 1032)
Rectus abdominis				
IH	19.6 (15.6; 25.1)	18.4 (14.8 26.8)	16.1*,** (13.6; 19.0)	15.7*,** (14.5; 17.9)
IE	3.8 (2.9; 4.8)	4.4 (2.6; 5.2)	5.0*,** (4.0; 6.3)	5.4*,** (4.3; 5.9)
ISD	981 (778; 1221)	890 (761; 1180)	787*,** (669; 917)	792*,** (716; 897)
Internal intercostals				
IH	30.1 (23; 31)	27.8 (23.7; 30.9)	24.4*,** (20.5; 29.4)	24.5*,** (20.4; 27.3)
IE	3.3 (2.5; 4.5)	3.5 (2.8; 4.6)	4.4*,** (3.7; 5.4)	4.3*,** (3.3; 5.8)
ISD	1262 (1015; 1403)	1267 (930; 1555)	1000*,** (770; 1310)	1095 (910; 1380)
Abdominal internal oblique				
IH	20.9 (18.2; 27.5)	20.9 (18.3; 23.3)	19.3*,** (17.4; 22)	19.3*,** (17.3; 22.7)
IE	4.2 (3.0; 5.4)	3.7 (3.4; 4.7)	4.8*,** (4.1; 5.8)	4.6** (3.7; 5.5)
ISD	1013 (893; 1325)	1012 (872; 1195)	941*,** (836; 1085)	972 (826; 1171)
Transversus abdominis				
IH	29.5 (26.5; 36.4)	26.7 (22.3; 33.3)	24.0*,** (18.9; 27.6)	25.1* (20.8; 29.6)
IE	3.0 (2.5; 3.7)	3.3 (2.6; 4.2)	4.3*,** (3.4; 5.2)	4.3*,** (3.5; 4.7)
ISD	1350 (1163; 1695)	1263 (1015; 1660)	1170* (937; 1335)	1179* (1013; 1395)

Data are presented as median (ranges), unless otherwise stated. GOLD – global initiative for chronic Obstructive lung disease; COPD – chronic obstructive pulmonary disease; RM – respiratory muscles; IH – index of homogeneity, which characterized the degree of muscle homogeneity; IE – index of echogenicity (the level of the shade of the grey scale that most often emerged in the outlined zone); ISD – index of structural density, which permitted a quantitative assessment of muscles of different size; * – compared with the control group, $p < 0.05$; ** – compared with the $COPD_1$ group, $p < 0.05$; *** – compared with the $COPD_2$ group, $p < 0.05$.

Table 4. Comparison of echogenicity index values in RM between groups of patients with different degrees of severity of breathlessness

RM	MRC_0 n = 26	MRC_1 n = 20	MRC_2 n = 83	MRC_3 n = 24
AEO	3.0 (2.1; 3.5)	4.3* (3.0; 5.3)	4.7 *,** (4.1; 5.6)	4.3*,▼ (3.6; 5.6)
AIO	3.5 (3.3; 4.5)	4.2 (3.6; 5.2)	4.8*,** (4.1; 5.8)	4.8*,** (3.6; 5.5)
TA	3.2 (2.4; 3.7)	3.7 (2.7; 4.7)	4.3* (3.4; 5.1)	4.1* (3.4; 4.7)
ExI	3.8 (2.9; 5.2)	4.2 (3.5; 5.2)	4.8* (3.8; 5,5)	4.6* (4.0; 5.6)
InI	3.4 (2.4; 4,0)	3.9* (3.1; 5.5)	4.4* (3.6; 5.3)	4.2* (3.4; 5.9)
SCM	1.4 (0.9; 1.7)	1.5 (1.2; 2.5)	1.9* (1.3; 2.6)	2.0* (1.6; 2.3)
SA	1.4 (1.1; 1.8)	1.9* (1.7; 3.0)	1.9* (1.3; 2.8)	2.3* (1.4; 3.2)
RA	3.4 (2.5; 5.1)	5.1* (4.5; 5.6)	5.3* (4.0; 6.5)	4.9* (4.2; 5.7)

Data are presented as median (ranges), unless otherwise stated. RM – respiratory muscles; MRC – modified Medical Research Council questionnaire for assessing the severity of breathlessness; AEO – abdominal external oblique muscle; AIO – abdominal internal oblique muscle; TA – transversus abdominis muscle; ExI – external intercostal muscle; InI – internal intercostal muscle; SCM – sternocleidomastoid muscle; SA – scalenus anterior muscle; RA: rectus abdominis muscle; * – compared with patients with MRC_0, $p < 0.05$; ** – compared with patients with MRC_1, $p < 0.05$; *** – compared with patients with MRC_2, $p < 0.05$; ▼– compared with patients with MRC_3, $p < 0.05$

of the control group. A similar picture was detected in the structural density, which increased by 21% in $COPD_3$ compared with $COPD_2$ ($p < 0.05$) and almost reached the control value. The dynamics of these indices for SA were similar to SCM, but were less pronounced during the progression of COPD. The homogeneity of ExI was significantly decreased in $COPD_{2,3}$ (by 13% and 15% as compared to the control group), while the echogenicity of ExI was increased in $COPD_{2,3}$ (by 29% and 21% compared to the controls). The structural density of ExI did not significantly differ from the control value. The changes in the echogenicity index in developing COPD are quite evident in Fig. 3.

The changes in the echodensitometric parameters of expiratory RM were similar to those in the inspiratory RM. The homogeneity and structural density of AIO were significantly lower in $COPD_2$ (both by 7% in comparison with the controls) and in $COPD_3$ (by 7% and 5% in comparison with the controls), while increased echogenicity was observed in $COPD_{2,3}$ (by 14% and 10% as compared with the controls). The homogeneity and echogenicity of AEO did not differ from the control group in $COPD_{1,2,3}$, while the structural density was lower only in $COPD_2$ (by 10% in comparison with the control group). Decreased homogeneity and structural density of RA were also noted in $COPD_2$ (by 18% and 20% in comparison with the controls) and $COPD_3$ (by 20% and 13% in comparison with the controls), while significantly increased echogenicity was registered in $COPD_{2,3}$ (by 31% and 42% in comparison with the controls). The dynamics of the changes in structural density in RM were noticeably different from the dynamics of echogenicity during the progression of COPD (Fig. 4).

Additionally, the study investigated the relationship between the patients' physical activity (approximately, in accordance with the severity of breathlessness) and the echodensitometric parameters of RM. All the patients were divided into four subgroups according to the degree of dyspnea expressiveness on the MRC scale. In these subgroups, significant differences in homogeneity were revealed for AEO, ExI, InI, SCM, SA and RA. Changes in echogenicity were expressed to a greater extent than changes in homogeneity in these COPD subgroups. Significant echogenicity differences between the MRC_0 subgroup and the $MRC_{1,2,3}$ subgroups were revealed for all the studied RM (Table 4).

The morphological study of RM in $COPD_{1,2}$ patients provided the following picture (Table 5): Structural abnormalities – atrophic changes – were found in 25 % of the $COPD_1$ patients compared with 69% of $COPD_2$ patients, and in 45% of subjects from the control group ($\chi^2 = 4.91$; $p = 0.085$). Additionally, in the $COPD_2$ group, the proportion

Table 5. Distribution of $COPD_{1,2}$ patients according to the presence of some morphological signs in RM

Morphological sign	**Control n = 11**	**$COPD_1$ n = 12**	**$COPD_2$ n = 13**
Atrophy of myofibrils	5 (45)	3 (25)	9 (69)**
Myolysis	1 (9)	11 (92)*	12 (92)*
Proliferation of perimysium cells	4 (36)	8 (67)	8 (62)
Proliferation of fibroblasts	0	3 (25)	9 (69)*,**
Intergrowth of fatty tissue into muscular tissue	4 (36)	3 (25)	9 (69)**
Sclerotic changes: moderate	1 (9)	7 (58)	3 (23)*
pronounced	0	2 (17)	10 (77)*,**
Contractures of myofibrils	0	8 (67)*	11 (85)*

Data are presented as n (%), unless otherwise stated. COPD – chronic obstructive pulmonary disease; RM – respiratory muscles; * – compared with the control group, $p < 0.05$; ** – compared with patients of $COPD_1$ group, $p < 0.05$.

of patients with atrophic changes in muscle tissue was higher than in the $COPD_1$ group (69% and 25% respectively; $\chi^2 = 4.89$; $p = 0.047$). It is necessary to note that this morphological sign was not specific to COPD patients, because it was often detected in the control group as well.

As it can be seen from Table 5, the presence of myolysis in RM (Fig. 5A) was observed in 92% of the $COPD_{1,2}$ patients), but in only 9% of subjects from the control group ($\chi^2 = 23.6$; $p = 0.00001$). In some cases the presence of myolysis zones with a proliferation of perimysium cells arranged into a continuous series was detected in these COPD patients. Additionally, the presence of granules in sarcoplasm (a sign of severe dystrophic changes) was revealed. Deep dystrophic albuminous granules of various sizes were also noted in relatively unaffected muscular fibers. The fragmentation of myofibrils was also present in all the groups: 82% in the control group; 83% and 85% in $COPD_1$ and $COPD_2$ respectively ($\chi^2 = 0.01$; $p = 0.99$).

No significant differences were detected between the control group and the $COPD_{1,2}$ groups regarding the presence of perimysium cell proliferation (36%, 67% and 72% respectively; $\chi^2 = 2.43$; $p = 0.29$); nor were significant differences observed between the $COPD_{1,2}$ groups (Fig. 5B). Round-cell infiltrates around microcirculation vessels, perivascular and intramuscular fatty tissue along the myofibrils were found, with no significant differences between the control group and $COPD_{1,2}$ patients ($\chi^2 = 6.08$; $p = 0.06$). Deposits of lymphocytes

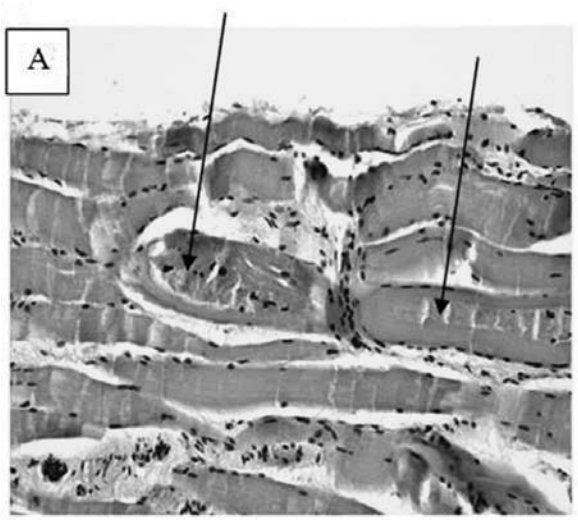

Fig. 5A. Fragmentation and destruction of sarcoplasm in the center of myofibrils and proliferation of perimysium cells and fibroblasts

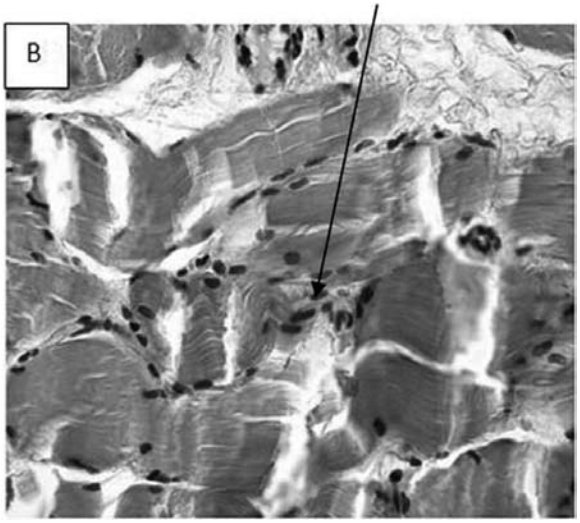

Fig. 5B. The presence of contractures in $COPD_1$ patients

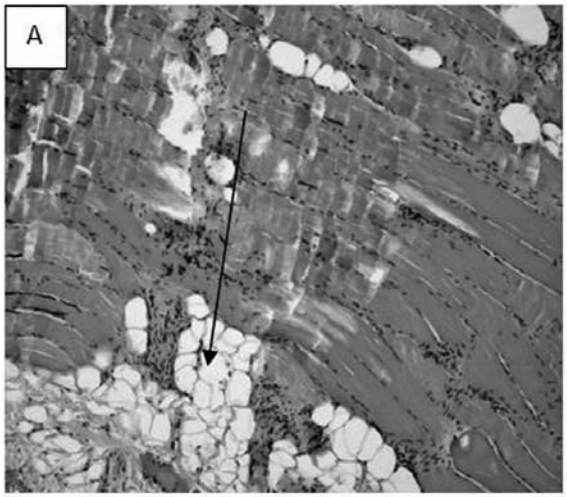

Fig. 6A. Intergrowth of fatty tissue into the muscular tissue and proliferation of peremysium cells

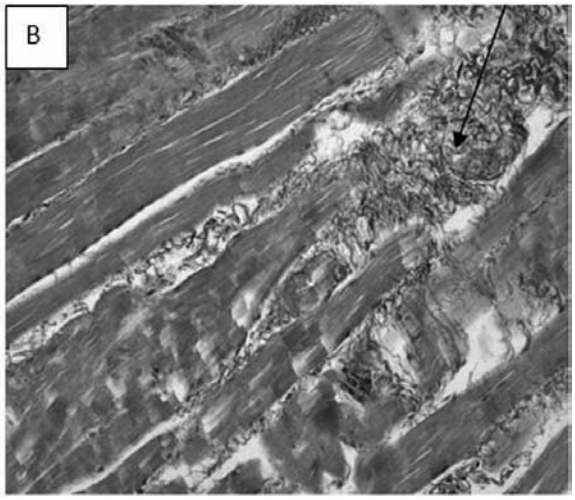

Fig. 6B. The presence of zones with interstitial scleroses and a large number of collagen fibers in $COPD_1$ patients

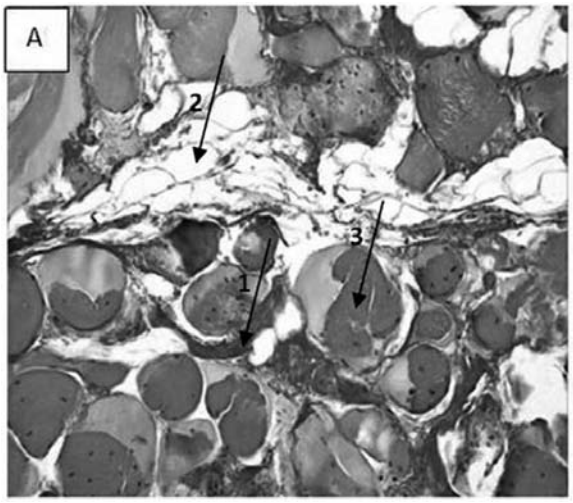

Fig. 7A. Foci of sclerosis in intramuscular regions (1), increase in adipose cells (2) and destruction of myofibrils (3) in $COPD_2$ patient

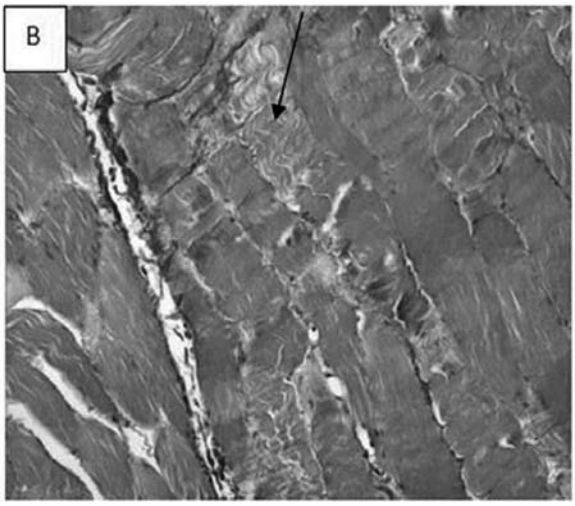

Fig. 7B. Contractures of myofibrils in $COPD_1$ patient

in separate fibers with fusion of sarcolemma and bordering sarcoplasm were noted in $COPD_{1,2}$ patients; there were no significant differences between these groups ($\chi^2 = 0.99$; $p = 0.43$). No proliferation of fibroblasts was detected in the control group, but it was observed in 25% and 69% of the $COPD_1$ and $COPD_2$ patients respectively. There were significant differences between the control group and the study groups ($\chi^2 = 13.4$; $p = 0.0012$) as well as between $COPD_1$ and $COPD_2$ ($\chi^2 = 4.89$; $p = 0.047$). Intergrowth of fatty tissue into muscle tissue (Fig. 6A) was observed in 25% of $COPD_1$ patients and in 69% of $COPD_2$ patients, as well as in 36% of the control group. This shows the nonspecific character of these changes, although there was a significant difference between the COPD groups and the controls ($\chi^2 = 12.3$; $p = 0.002$) as well as between the $COPD_1$ and $COPD_2$ groups ($\chi^2 = 11.54$; $p = 0.0012$). Marked focal growth of fatty tissue between muscular fibers and in damaged muscle fibers may be considered a sign of "false hypertrophy" of muscle tissue.

Sclerotic changes in RM muscle tissue were observed in 75% of the $COPD_1$ patients and in all $COPD_2$ patients (Fig. 6B, 7A) compared with only 9% of the healthy controls. This also indicates nonspecificity of these changes, although there was a significant difference between these three groups ($\chi^2 = 20.6$; $p = 0.00038$) as well as between the $COPD_1$ and $COPD_2$ groups ($\chi^2 = 5.16$; $p = 0.039$) in the combination of expressed and insignificant sclerotic manifestations. Expressed interstitial sclerosis that extended to certain areas of the damaged muscle fibers was revealed.

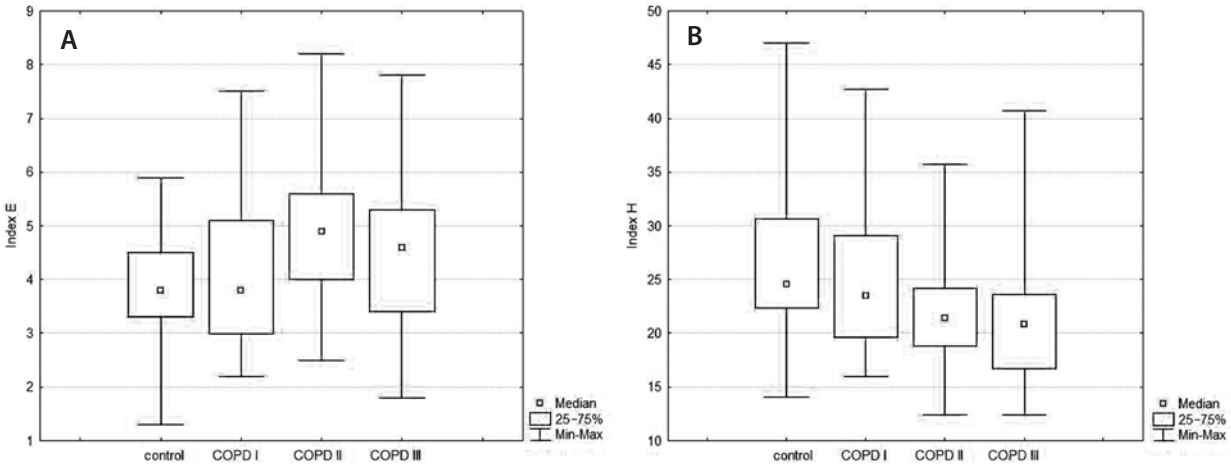

Fig. 8. Comparisons of echodensitometry parameters: indices of echogenicity (A) and homogeneity (B) of the external intercostals muscle in the groups of patients with different degrees of severity of airflow limitation (GOLD spirometric level) and the control group (results of the Kruskal-Wallis test)

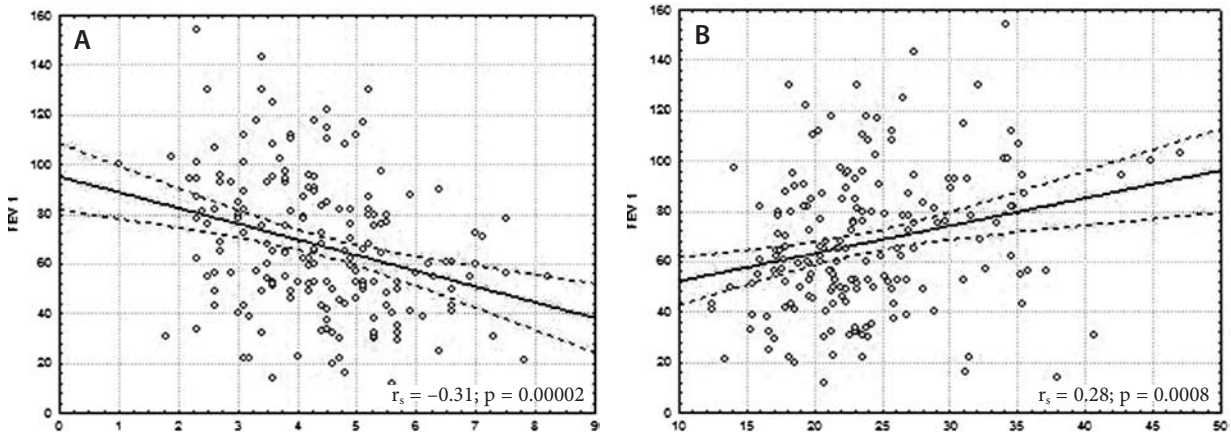

Fig. 9. Correlations between FEV_1 and the echodensitometric parameters: indices of echogenicity (A) and homogeneity (B) of the external intercostals muscle (–) with 95% confidence intervals (––). FEV_1 – forced expiratory volume in one second

Contractures of myofibrils (Fig. 7B) were observed in 67% of the $COPD_1$ patients and in 85% of the $COPD_2$ patients, while these changes were not found in the control group. There was a significant difference between these COPD groups and the control group, but no significant difference was noted between the COPD groups ($\chi^2 = 1.1$; $p = 0.37$). A combination of contracture foci and interstitial sclerosis around separate muscular fibers was found.

Discussion

Muscular dysfunction often occurs in COPD and may involve both respiratory and peripheral muscles. Decreased strength and endurance in RM can lead to clinical symptoms and ventilatory insufficiency, whereas in the peripheral muscles it limits exercise capacity and daily activities. The structural changes of accessory RM that were revealed by ultrasonic densitometry consisted of echogenicity phase changes, the cause of which may be multifactorial. A relationship between the severity of COPD and the dystrophic changes in RM according to the data from the ultrasonic densitometry was established. This is illustrated in Fig. 8.

It was determined that the changes in the echodensitometry parameters in isolated RM correlate with decreased FEV_1 and increased breathlessness expressiveness in COPD patients. The correlation between the echodensitometric parameters of external intercostal muscle and FEV_1 as well as breathlessness can be seen in Fig. 9 and Fig. 10. Additionally, a simultaneous increase in the severity of dyspnoea and the echogenicity of isolated RM in the course of COPD development was detected against a background of decreasing homogeneity and structural density due to the expressed atrophy of muscular fibers and the expansion of connective and fatty tissue.

The dynamics of these ultrasonic parameters precisely reflects the course of the dystrophic

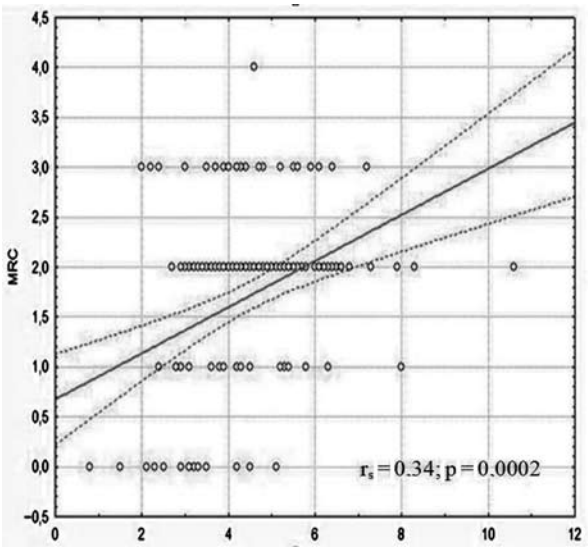

Fig. 10. Correlations between severity of breathlessness (MRC) and index of echogenicity of the external intercostal muscle. MRC – modified medical research council questionnaire for assessing the severity of breathlessness

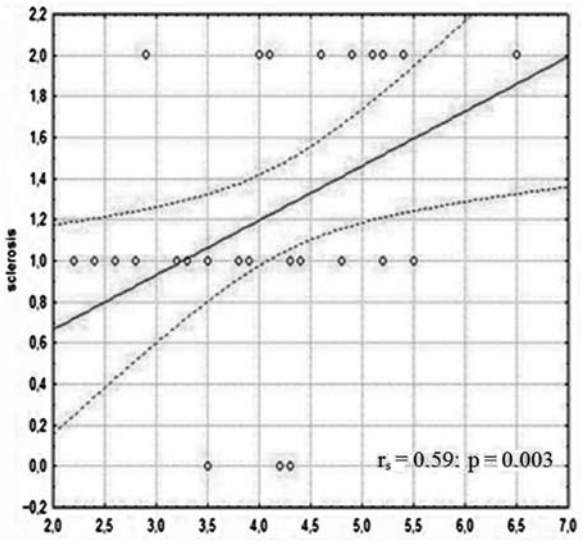

Fig. 11. Correlations between sclerosis manifestations and index of echogenicity of abdominal internal oblique muscle

process in RM during the progression of COPD: from compensatory hypertrophy of RM in response to the increase in RM functional load, through their dystrophy in the phase of substituting muscle growth with fatty and connective tissue, to severe RM atrophy in severe COPD. Thus, each subsequent stage of COPD severity leads to increasing atrophic and degenerative RM changes. This fact provides the basis for a conclusion about a relationship between RM structural changes (revealed during ultrasonic examination) and functional changes of the respiratory system (manifested by decreased FEV_1 and increased dyspnea expressiveness) in severe COPD. The significance of these phase changes in RM echodensitometric parameters is not fully understood. This dynamics of echodensitometric parameters in $COPD_1$ could be explained to a certain degree by the increase in echogenicity due to substituting muscle growth with fatty and connective tissue. Thus, a significant positive correlation was determined between sclerotic manifestations of RM and their echogenicity index (Fig. 11) in COPD patients.

Homogeneity and structural density increased slightly in $COPD_3$ compared with $COPD_2$, while echogenicity decreased slightly. It can be assumed that the development of RM fatigability in severe COPD was caused by several factors (a decrease in their mass, sclerotic changes and a loss of fatty mass) that reduced the echogenicity of RM. These morphological changes could be primarily due to the expressed systemic inflammation, as well as (to a lesser extent) changes in the hormonal status and the high sensitivity of patients who had received long-term high doses of glucocorticoids during COPD exacerbation.

The study has demonstrated the advantages of the ultrasonic method in the diagnosis of sclerotic changes, the detection of intergrowth of fatty tissue into the muscle tissue and atrophic changes. The method is a major source of information for assessing structural RM changes. This was probably connected with the use of the ultrasonic method to examine the muscles throughout their full thickness. These morphological data suggest that the alternating increases and decreases in the ultrasonic parameters could be caused by changes in the fatty tissue volume in the muscle tissue in different stages of COPD development.

The morphological method of studying RM is invasive and inconvenient, as it is associated with possible complications, whereas the proposed ultrasonic method for examining RM is simple and safe. In light of this, the authors compared the possibilities of these 2 diagnostic methods for the assessment of RM status in COPD patients with different degrees of severity. The sensitivity and specificity of the ultrasonic method were confirmed in 55% and 59% of cases for the estimation of sclerotic changes in RM, which was comparable to the results of morphological study. Both the prognostic positive and prognostic negative results of the ultrasonic method approached 57%. Thus, the likelihood ratio for a positive result was 1.34. No significant differences between the ultrasonic and morphometric methods were detected while evaluating sclerotic changes in COPD patients ($\chi^2 = 0.82$; $p = 0.37$).

The sensitivity and specificity of the ultrasonic method of determining the expressiveness of fatty tissue intergrowth into the muscle tissue were confirmed in 55% of the cases, including both prognostic positive and prognostic negative results. Thus, the likelihood ratio for a positive result was 1.20. No significant differences between the ultrasonic and morphological methods were found in relation to these morphological phenomena ($\chi^2 = 0.36$; $p = 0.55$).

The sensitivity and specificity of the ultrasonic method for estimating atrophic muscular tissue expressiveness were confirmed in 55% and 59% of cases. The prognostic importance of both positive and negative results was 57%. Thus, the likelihood ratio for a positive result was 1.33. No significant differences were detected comparing the results from COPD patients who underwent examinations by both the ultrasonic and morphological methods to evaluate atrophic muscular tissue expressiveness ($\chi^2 = 0.82$; $p = 0.37$). The accuracy of the ultrasonic method was lower than that of the morphological method in diagnosing RM dystrophic changes in relation to other morphological signs analyzed.

The authors concluded that the proposed method of assessing echodensitometric parameters showed the heterogeneity of RM pathological changes and reflected the dynamics of the degenerative processes in RM during the development of COPD in a complex way. More dystrophic changes were detected in the following RM: the sternocleidomastoid, external intercostal, abdominal internal-external oblique and rectus. In COPD[1] a tendency to increased homogeneity and structural density along with decreased echogenicity was observed, whereas in $COPD_2$ there was a tendency to decreased homogeneity and structural density, while echogenicity increased. In $COPD_3$ a tendency to return to increased homogeneity and structural density against the background of decreased echogenicity was detected. In general, greater differences were detected between COPD groups in the echogenicity index.

- Increased breathlessness in COPD patients positively correlated with echogenicity and negatively correlated with the structural density of the sternocleidomastoid, internal intercostal, abdominal external oblique and rectus muscles.
- The high informative value of the ultrasonic method for evaluating dystrophic changes in RM was shown, providing a basis for considering this method a first-line choice for monitoring pathological RM changes during the progression of COPD.

References

[1] **Franssen FM:** Sarcopenia in COPD: functional and metabolic implications. Eds.: Schols A., Wouters E. Maastricht University 2009, 248.

[2] **Bezdenezhnykh AV, Sumin AN:** Sarcopenia: prevalence, detection, clinical significance. Clin Med (Russia) 2012, 10, 16–22.

[3] **Cesari M, Pahor M, Lauretani F:** Skeletal muscle and mortality results. In CHIANTI Study. J Gerontol 2009, 64A, 377–384.

[4] **Santanasto AJ, Glynn NW, Newman MA:** Impact of weight loss on physical function with changes in strength, muscle mass, and muscle fat infiltration in overweight to moderately obese older adults: A randomized clinical trial. J. Obesity 2011, article ID 516576, doi:10.1155/2011/516576.

[5] **Stenholm S, Harris TB, Rantanen T:** Sarcopenic obesity: definition, cause and consequences. Curr Opin Clin Nutr Metab Care 2008, 693–700.

[6] **Agusti AG:** Systemic effects of chronic obstructive pulmonary disease. Eur Respir J 2003, 21, 347–360.

[7] **Fitting JW:** Respiratory muscles in chronic obstructive pulmonary disease. Swiss Medical Weekly 2001, 131, 483–486.

[8] **Avdeev SN:** COPD as systemic disease. Pulmonology (Russia) 2007, 2, 104–116.

[9] **Garcia-Rio F:** Daily physical activity in patients with COPD is mainly associated with dynamic hyperinflation. Am J Respir Crit Care Med 2009, 180, 506–512.

[10] **Watz H:** Physical activity in patients with COPD. Eur Respir J 2009, 33, 262–272.

[11] **Watz H:** Extrapulmonary effects of chronic obstructive pulmonary disease on physical activity: a cross-sectional study. Am J Respir Crit Care Med 2008, 177, 743–751.

[12] **Waschki B:** Physical activity is the strongest predictor of all-cause mortality in patients with COPD. Chest 2011, 140, 331–359.

[13] **Perzeva TA:** Muscle's dysfunction in COPD: overestimate of this problem, new possibilities of therapy. Health of Ukraine (electronic resource) 2008.

[14] **Bernarg S:** Peripheral muscle weakness in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1998, 158, 629–634.

[15] **Sharp JT:** The respiratory muscles in COPD. Am Rev Respir Dis 1986, 134, 1089–1091.

[16] **O'Donnell DE, Laveneziana P:** Physiology and consequences of lung hyperinflation in COPD. Eur Respir Rev 2006, 15, 61–67.

[17] **Avdeev SN:** Pulmonary hyperinflation in COPD patients. Pulmonol Allergol (Russia) 2006, 2, 11–16.

[18] **Laghi F:** Disorders of the respiratory muscles. Am J Respir Crit Care Med 2003, 168, 10–48.
[19] **Chuchalin AG, Ajsanov ZR:** Disorders of respiratory muscles function in COPD. Therap. Archive (Russia) 1988, 8, 126–132.
[20] **Surkova EG:** The estimation of diaphragm function in COPD according to the data of ultrasound methods. In: Scientific proceedings of St.-Petersburg Academy. Ed.: Jaickij NA, St-Petersburg (Russia) 2009, 16, 28–32.
[21] **Similowski T, Yan S, Gauthier AP:** Contractile properties of the human diaphragm during chronic hyperinflation. N Engl J Med 1991, 325, 917–923
[22] **Orozco-Levi M, Gea J, Lloreta JL:** Subcellular adaptation of the human diaphragm in chronic obstructive pulmonary disease. Eur Respir J 1999, 13, 371–378.
[23] **Levine S, Kaiser L, Leferovich J, Tikunov B:** Cellular adaptations in the diaphgram in chronic obstructive pulmonary disease. N Engl J Med 1997, 337, 1799–1806.
[24] **Mercadier JJ, Schwarz K, Schiaffino S:** Myosin heavy chain gene expression changes in the diaphragm of patient with chronic lung hyperinflation. Am J Physiol 1998, 274, 527–534.
[25] **Orozco-Levi M:** Structure and function of the respiratory muscles in patients with COPD: impairment or adaptation? Eur Respir J 2003, 22, Suppl. 46, 41–51.
[26] **Cullen MJ:** Morphological changes in dystrophic muscle. British Medical Bulletin 1980, 36, 145–152.
[27] **Jobin J, Maltais F, Doyon JF:** Chronic obstructive pulmonary disease: capillarity and fiber-type characteristics of skeletal muscle. J Cardiopulm Rehabil 1998, 18, 432–437.
[28] **Petrella JK, Kim JS, Tuggle SC:** Age differences in knee extension power, contractile velocity, and fatigability. J Appl Physiol 2005, 98, 211–220.
[29] **Schertzer JD, Van der Poel C, Shavlakadze T:** Muscle-specific overexpression of IGF-I improves E-C coupling in skeletal muscle fibers from dystrophic mice. Am J Physiol Cell Physiol 2008, 294, 161–168.
[30] **Ambrosimov VN:** Dysfunction of respiratory muscles and their diagnostics in COPD patients. Clin Gerontology (Russia) 2008, 6, 38–43.
[31] **Wouters EF:** Nonpharmacological modulation of dynamic hyperinflation. Eur Respir Rev 2006, 15, 90–95.
[32] **Casaburi R, Bhasin S, Cosentino L:** Effects of testosterone and resistance training in men with chronic obstructive disease. Am J Respir Crit Care Med 2004, 170, 870–878.
[33] **Casaburi R:** Impacting patient-centered outcomes in COPD: deconditioning. Eur Respir Rev 2006, 15, 42–46.
[34] **Tkác J:** Systemic consequences of COPD. Therap Advances in Respir Dis 2007, 1, 47–59.
[35] **Van Eeden SF, Sin DD:** COPD: A Chronic Systemic Inflammatory Disease. Respiration 2008, 75, 224–238.
[36] **Mador MJ, Bozkanat E:** Skeletal muscle dysfunction in chronic obstructive pulmonary disease. Respiratory Research 2001, 2, 216–224.
[37] **Shrikrishna D:** Ultrasound measurement of quadriceps wasting in patients with GOLD stage II COPD and its relationship to physical activity. Eur Respir J (Abstracts; 21th ERS annual Congress), Amsterdam, Netherlands 2011, 38, Suppl. 55, 889.
[38] **Platonova IS:** The morphologic changes of respiratory muscles in COPD patients with different degree of respiratory insufficiency. Author's abstract of dissertation on scientific degree of candidate of medical sciences. St.-Petersburg Med. University, St.-Petersburg (Russia), 2003, 18.
[39] Global Initiative for Chronic Obstructive Pulmonary Disease (GOLD). Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease, Global Initiative for Chronic Obstructive Lung Disease NHLBI/WHO workshop report. Last updated 2011. http://www.goldcopd.org.

Address for correpondence:

Aleksander E. Makarevich
Department of Internal Medicine
Belarusian State Medical University
83 Dzerzhinsky Ave.
Minsk
Belarus
E-mail: makae@bsmu.by

Conflict of interest: None declared

Received: 16.01.2013
Revised: 2.10.2013
Accepted: 9.06.2014