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Heart Failure Results in Inspiratory Muscle Dysfunction Irrespective of Left Ventricular Ejection Fraction

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Keywords
Heart failure · Muscle strength · Diaphragm · Interleukin-6 · Tumor necrosis factor-α

Abstract
Background: Exercise intolerance in heart failure with reduced ejection fraction (HFrEF) or heart failure with preserved ejection fraction (HFpEF) results from both cardiac dysfunction and skeletal muscle weakness. Respiratory muscle dysfunction with restrictive ventilation disorder may be present irrespective of left ventricular ejection fraction and might be mediated by circulating pro-inflammatory cytokines. ***Objective:*** To determine lung and respiratory muscle function in patients with HFrEF/HFpEF and to determine its associations with exercise intolerance and markers of systemic inflammation. ***Methods:*** Adult patients with HFrEF (n = 22, 19 male, 61 ± 14 years) and HFpEF (n = 8, 7 male, 68 ± 8 years) and 19 matched healthy control subjects underwent spirometry, measurement of maximum mouth occlusion pressures, diaphragm ultrasound, and recording of transdiaphragmatic and gastric pressures following magnetic stimulation of the phrenic nerves and the lower tho-

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racic nerve roots. New York Heart Association (NYHA) class and 6-min walking distance (6MWD) were used to quantify exercise intolerance. Levels of circulating interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α) were measured using ELISAs. ***Results:*** Compared with controls, both patient groups showed lower forced vital capacity (FVC) ($p < 0.05$), maximum inspiratory pressure (PI_{max}), maximum expiratory pressure (PE_{max}) ($p < 0.05$), diaphragm thickening ratio ($p = 0.01$), and diaphragm strength (twitch transdiaphragmatic pressure in response to supramaximal cervical magnetic phrenic nerve stimulation) ($p = 0.01$). In patients with HFrEF, NYHA class and 6MWD were both inversely correlated with FVC, PI_{max}, and PE_{max}. In those with HFpEF, there was an inverse correlation between amino terminal pro B-type natriuretic peptide levels and FVC ($r = -0.77$, $p = 0.04$). In all HF patients, IL-6 and TNF-α were statistically related to FVC. ***Conclusions:*** Irrespective of left ventricular ejection fraction, HF is associated with respiratory muscle dysfunction, which is associated with increased levels of circulating IL-6 and TNF-α.

Introduction

Heart failure (HF) is a highly prevalent disease that is associated with significant morbidity and mortality and therefore places a major financial burden on public health systems [1–4]. Approximately half of the patients have HF with reduced ejection fraction (HFrEF), while the remainder have HF with preserved ejection fraction (HFpEF) [3]. Daily symptoms of HF are mainly driven by exercise intolerance and dyspnea [4].

Initial description of respiratory muscle involvement in HFrEF dates back to the 1990s [5]. Using invasive measurement of transdiaphragmatic pressure following stimulation of the phrenic nerves (referred to as "twitch" Pdi or twPdi when magnetically stimulated), it was shown that patients with HFrEF show impaired contractility of the diaphragm [6]. Measurement of twPdi is considered the gold standard of respiratory muscle strength testing because it overcomes most of the technical flaws associated with volitional tests such as forced vital capacity (FVC) or maximum inspiratory pressure (PI_{max}) [7, 8]. Despite progress in the understanding of both the prevalence and clinical significance of diaphragm involvement in HFrEF, underlying mechanisms are still not well understood [9–13].

Chronic HF is associated with morphologic, histologic, and metabolic alterations of skeletal muscle. Loss of muscle mass is present even in non-cachectic patients with HF [9–13]. Probably dependent on disease severity, histologic abnormalities may include decreased capillary density, muscle fiber atrophy, and reduced electromyographic activity [9–13]. Metabolic changes include hypoperfusion of muscle tissue and impairment of oxidative and glycolytic pathways [9–13].

Circulating pro-inflammatory cytokines have been hypothesized as a potential mechanistic link between HFrEF and skeletal muscle dysfunction, and diaphragm weakness in particular [13]. Patients with severe HFrEF show increased levels of circulating pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), which relate to impaired functional status and worse prognosis [14–18]. These cytokines have been shown to directly impair muscle function in animal models [19]. To date, inspiratory muscle dysfunction in HFrEF patients has not been linked with immunological markers of the systemic inflammatory response.

In HFpEF, the understanding of respiratory muscle involvement and its underlying mechanisms is currently poor. Whereas skeletal muscle involvement has been reported to be less severe in patients with HFpEF than in those with HFrEF [18], recent animal studies have shown significant alterations of limb and respiratory muscle tissue in rat models of HFpEF [20, 21].

Little is known about expiratory muscle involvement in both HFrEF and HFpEF. While it has been shown to occur in HFrEF, expiratory muscle weakness has not yet been shown to contribute to exercise intolerance or dyspnea and has never been comprehensively studied in HFpEF using invasive non-volitional tests, as recently reviewed [13]. As an innovative diagnostic method, magnetic stimulation of the thoracic expiratory nerve roots with recording of twitch gastric pressure (twPgas) has been introduced for non-volitional assessment of expiratory muscle function [5, 13, 22]. This case-control study investigated the extent and pathophysiological characteristics of respiratory muscle dysfunction in patients with HFrEF and HFpEF, including assessment of exercise performance and serum levels of circulating IL-6 and TNF-α.

Methods

Study Design and Participants

This cross-sectional case-control study was conducted from November 2017 to May 2019. Ethical approval was obtained from the local Ethics Committee (Ethikkommission der Ärztekammer Westfalen-Lippe und der Westfälischen Wilhelms-Universität Münster, Az. 2016-072-f-S). The study was registered and updated prospectively online (German Clinical Trial Register Identifier: DRKS00015912).

Table 1. Demographic data and clinical characteristics of the study population

	HFrEF (n = 22)	HFpEF (n = 8)	p value
Male, *n* (%)	19 (87)	7 (88)	ns
Age, years	60.9±13.7	67.8±8.5	ns
BMI, kg/m^2	29.5±5.0	27.6±4.2	ns
BSA, m^2	2.1±0.2	2.0±0.1	ns
Ischemic heart disease, *n* (%)	14 (64)	2 (25)	0.04
Valvular disease, *n* (%)	6 (27)	4 (50)	ns
Hypertension, *n* (%)	13 (59)	4 (50)	ns
Diabetes, *n* (%)	7 (32)	3 (38)	ns
Atrial fibrillation, *n* (%)	5 (23)	4 (50)	0.05
6-min walking distance	385±104	433±84	ns
NYHA class I, *n* (%)	4 (18)	0 (0)	ns
NYHA class II, *n* (%)	10 (45)	4 (50)	ns
NYHA class III, *n* (%)	7 (32)	3 (38)	ns
NYHA class IV, *n* (%)	1 (5)	1 (13)	ns
Laboratory values			
Hemoglobin, g/dL	13.4±1.7	12.6±2.4	ns
Cr, mg/dL	1.5±0.7	1.2±0.2	ns
NT-proBNP, pg/mL	770 (392–1,914)	461 (299–840)	ns
Pro-inflammatory cytokines, pg/mL			
Interleukin-6	4.0 (3.0–6.0)	7.0 (4.5–11.3)	ns
Tumor necrosis factor-α	10.5 (8.9–11.4)	11.2 (9.7–12.1)	ns
Echocardiography			
LVD, *n* (%)	16 (73)	1 (13)	0.01
LVEF, %	36.5±6.1	53.3±2.0	<0.01
Diastolic dysfunction (grades II–III), *n* (%)	3 (14)	2 (25)	ns
Mitral regurgitation (grades II–III), *n* (%)	3 (14)	3 (38)	0.05
LAE, *n* (%)	15 (71)	5 (63)	ns
LVH, *n* (%)	9 (41)	6 (75)	0.04
TAPSE <17 mm, *n* (%)	5 (23)	2 (25)	ns
RAE, *n* (%)	4 (18)	3 (38)	ns
Medication, n (%)			
ACEI/ARB	12 (55)	5 (63)	ns
ARNI	9 (41)	0 (0)	0.03
β-Blocker	22 (100)	7 (88)	ns
Diuretics	18 (82)	6 (75)	ns
Aldosterone antagonists	16 (73)	1 (13)	0.01

Values are mean±standard deviation, median (interquartile range), or number of patients (%). ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; ARNI, angiotensin receptor-neprilysin inhibitor; BSA, body surface area; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; LAE, left atrial enlargement; LVD, left ventricular dilatation; LVEF, left ventricular ejection fraction; LVH, left ventricular hypertrophy; ns, not statistically significant ($p > 0.05$); NT-pro BNP, N-terminal pro brain natriuretic peptide; NYHA, New York Heart Association; RAE, right atrial enlargement; TAPSE, tricuspid annular plane systolic excursion (~right ventricular function).

Consecutive patients with chronic HF were recruited from the HF specialty outpatient clinic at Muenster University Hospital. Inclusion criteria were >18 years of age and diagnosis of HFrEF or HFpEF in accordance with the current European Society of Cardiology guidelines [4]. Patients were excluded if they met any of the following criteria: change in pharmacological and/or device-based therapy within the preceding 60 days; hospital admission requiring the administration of intravenous diuretics and/or invasive testing (coronary catheterization) within the preceding 30 days; intake of muscle relaxants; BMI >35 kg/m^2; chronic obstructive pulmonary disease with GOLD class >II; significant lung emphysema; interstitial lung disease; precapillary pulmonary hypertension confirmed

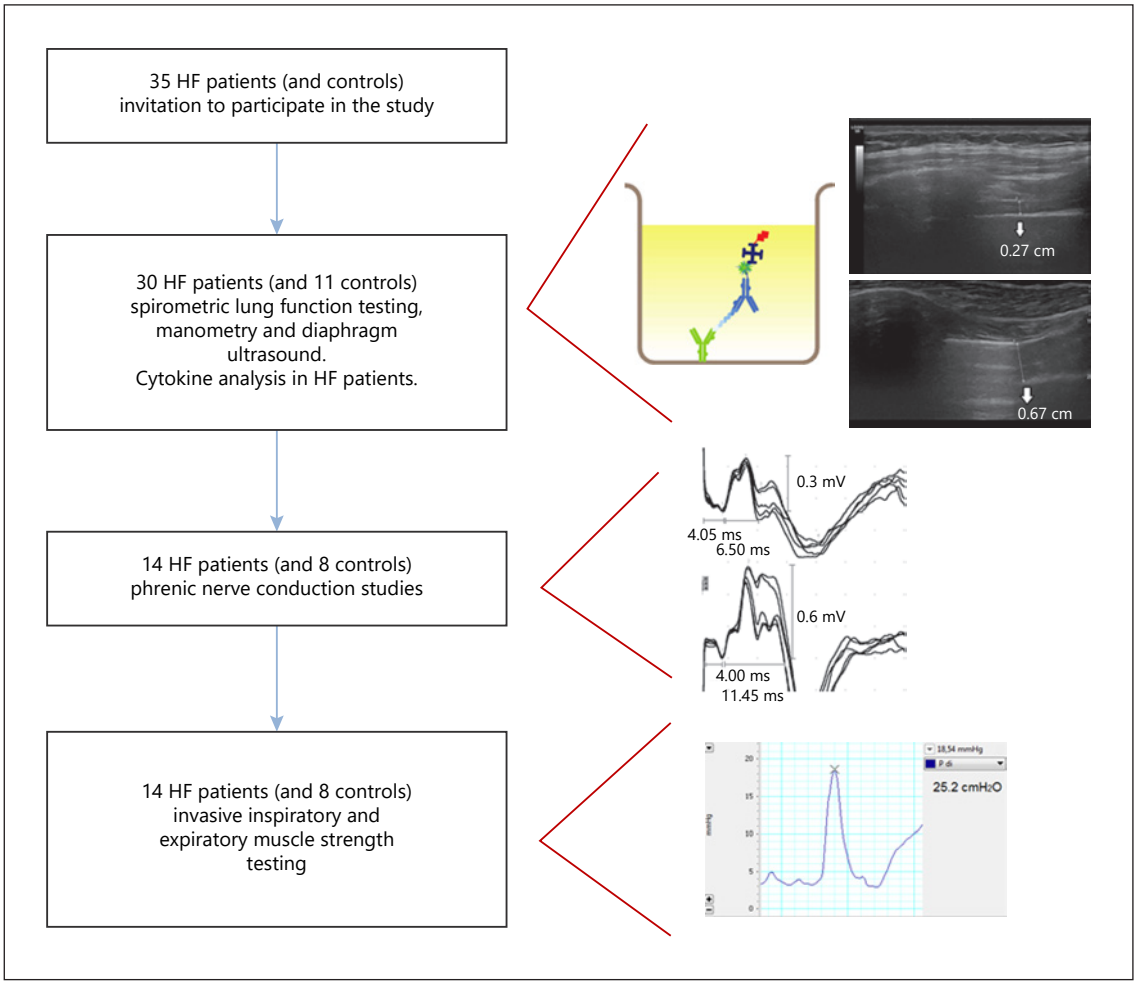

Fig. 1. Study flowchart and methodology.

by right heart catheterization; concomitant neuromuscular, phrenic nerve, or thoracic wall disease; epilepsy; insulin-dependent diabetes mellitus; and active cardiac pacemaker disease. Healthy control subjects were consecutively recruited and matched for age, gender, and BMI (1:2 for HFrEF patients and 1:1 for HFpEF patients). All subjects gave written informed consent to participate in the study.

Clinical Evaluation

All study participants underwent a clinical examination with assessment of current New York Heart Association (NYHA) functional class and standard transthoracic 2-dimensional Doppler echocardiography (LOGIQ S8-XD clear; GE Healthcare, London, UK) performed according to current recommendations [23]. Serum levels of brain natriuretic pro-peptide hormone (NT-proBNP) were also assessed.

Exercise Testing

The 6-min walking distance (6MWD) was measured in all HF patients under standardized conditions; heart rate and oxygen saturation were recorded before and after the 6MWD test, as recommended [24].

Spirometry and Maximum Inspiratory and Expiratory Pressures

Lung function tests were carried out according to current guidelines using an electronic spirometer (Vitalograph 3000™; Vitalograph, Hamburg, Germany) [7, 25]. FVC was obtained in the upright sitting position [7]. At least 5 attempts were performed until the highest value was achieved and varied from the preceding test by <10%. FVC was expressed as percentage of the predicted value based on gender, age, and height [7, 25]. Maximum expiratory pressure (PE_{max}) and PI_{max} were obtained using a handheld electronic manometer (MicroRPM™; Care Fusion, Baesweiler, Germany) according to standard recommendations [7, 26]. Peak cough flow was measured using a standard peak flow meter [7]. For all measurements, a nasal clip was used to prevent air leakage [7].

Diaphragm Ultrasound

Diaphragm ultrasound was performed on the right hemidiaphragm, as previously described [27–30]. Briefly, a portable ultrasound device (LOGIQ S8-XD; GE Healthcare, London, UK) with a 5-MHz and a 10-MHz linear transducer was used for evaluation

Table 2. Lung function data and diaphragm ultrasound measures for patients with heart failure and controls

	HFrEF (n = 22)	Controls (n = 11)	HFpEF (n = 8)	Controls (n = 7)
Male, n (%)	19 (87)	8 (73)	7 (88)	6 (86)
Age, years	60.9±13.7*	55.0±11.1	67.8±8.5*	59.1±10.0
BMI, kg/m^2	29.5±5.0	24.5±2.2	27.6±4.2	24.4±2.5
BSA, m^2	2.3±0.2	2.1±0.2	2.2±0.1	2.0±0.1
Lung function data				
FVC, L	3.4±1.1*	4.7±1.2	3.2±1.0*	4.5±0.9
FVC, % predicted	79.1±20.1*	109.6±16.4	80.8±22.6*	106.6±19.5
FEV_1, L	2.7±1.0*	3.6±1.0	2.4±0.6*	3.5±0.8
FEV_1, % predicted	80.8±20.1*	104.7±15.0	79.1±23.0*	102.6±18.4
FEV_1/VC, %	78.9±7.6	77.5±3.4	82.9±11.1	76.8±3.9
PEF, L/s	6.9±2.5*	9.0±2.2	6.1±1.9*	8.6±1.9
PEF, % predicted	85.8±20.7*	109.2±21.1	76.3±20.4*	104.1±22.0
PCF L/min	425.5±170.7*	618.2±128.2	377.1±126.1*	627.1±101.9
PI_{max}, cmH_2O	69.2±21.2*	100.0±26.5	53.0±22.7*	86.4±12.8
PI_{max}, % predicted	74.2±21.7*	112.5±35.5	60.7±26.3*	95.9±26.6
PE_{max}, cmH_2O	102.1±34.1*	137.4±16.1	94.8±26.4*	133.9±14.5
Diaphragm ultrasound				
Diaphragm excursion				
Amplitude during tidal breathing, cm	1.6 [1.2–3.0]	1.5 [1.2–2.0]	1.6 [1.6–2.9]	1.3 [1.1–1.7]
Velocity during tidal breathing, cm/s	1.3 [0.9–1.9]	1.3 [0.7–1.7]	1.7 [1.5–2.1]	1.3 [1.1–1.7]
Amplitude during voluntary sniff, cm	2.6 [2.0–3.3]	2.1 [1.9–2.8]	2.9 [1.6–4.8]	2.1 [1.9–3.4]
Velocity during voluntary sniff, cm/s	6.7±2.4	7.1±2.1	7.0±2.6	6.4±1.4
Amplitude during max. inspiration, cm	5.0±1.6*	8.1±2.7	5.0±2.3	7.5±2.8
Diaphragm thickness				
FRC, cm	0.21 [0.16–0.25]	0.21 [0.20–0.27]	0.19 [0.16–0.21]	0.21 [0.20–0.28]
TLC, cm	0.40 [0.39–0.53]*	0.65 [0.48–0.83]	0.33 [0.29–0.41]*	0.65 [0.44–0.92]
Thickening ratio	1.9 [1.8–2.1]*	3.1 [2.1–3.3]*	1.8 [1.4–2.0]*	3.1 [2.1–3.3]

Data are presented as mean±standard deviation, number of patients, or percentage as indicated. BSA, body surface area; FEV_1, forced expiratory volume after 1 s; FRC, functional residual capacity; FVC, forced vital capacity; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; max., maximum; PE_{max}, maximum expiratory pressure; PI_{max}, maximum inspiratory pressure; PCF, peak cough flow; PEF, peak expiratory flow; TLC, total lung capacity. * $p \leq 0.05$ versus controls.

of diaphragm excursion (amplitude and velocity) and diaphragm thickness in the zone of apposition [27]. The diaphragm thickening ratio (DTR) was calculated as thickness at total lung capacity divided by thickness at functional residual capacity (FRC) ([27], see online suppl. Fig. 1; for all online suppl. material, see www.karger.com/doi/10.1159/000509940).

Phrenic Nerve Conduction Studies following Cervical Magnetic Stimulation

Phrenic nerve conduction studies were conducted as previously described [31]. Diaphragm compound muscle action potentials (CMAPs) were recorded using a Dantec 2000™ electromyography device (Dantec Medical, Skovlunde, Denmark) and surface electrodes. Posterior cervical magnetic stimulation was performed with the subject in the seated position. Stimuli were delivered using a MagPro Compact™ magnetic stimulator equipped with a 2 T 12-cm C-100 circular coil (MagVenture, Willich, Germany) [31, 32]. The coil was placed at the seventh cervical vertebra and then moved up toward the sixth cervical vertebra until the highest reproducible diaphragm CMAP was obtained. At least 5 stimuli were delivered to achieve the highest possible diaphragm CMAP showing <10% variation from the preceding 2 stimulations. In order to avoid twitch potentiation, stimuli were separated by at least 40 s [11, 25, 26]. Stimulation at FRC was determined by visual observation of abdominal movements [31].

Invasive Inspiratory Muscle Strength Measurements

Twitch esophageal pressure (twPes) and twPgas were simultaneously recorded using balloon catheters (Cooper Surgical, Trumbull, CT, USA) transnasally inserted into the stomach and the distal esophagus, as previously described [8, 33]. Balloon catheters were connected to a differential pressure transducer (DPT-100™; UT Medical Products, Athlone, Ireland) and an amplifier (ADInstruments, Oxford, UK) [7, 8, 33]. Pressure data for twPgas, twPes, and twPdi (defined as twPes – twPgas) were continuously displayed using LabChart™ software (ADInstruments, Oxford, UK) [11, 27] (online suppl. Fig. 2). Subjects were also instructed to repeatedly perform a maximum sniff maneuver (a short, sharp in-

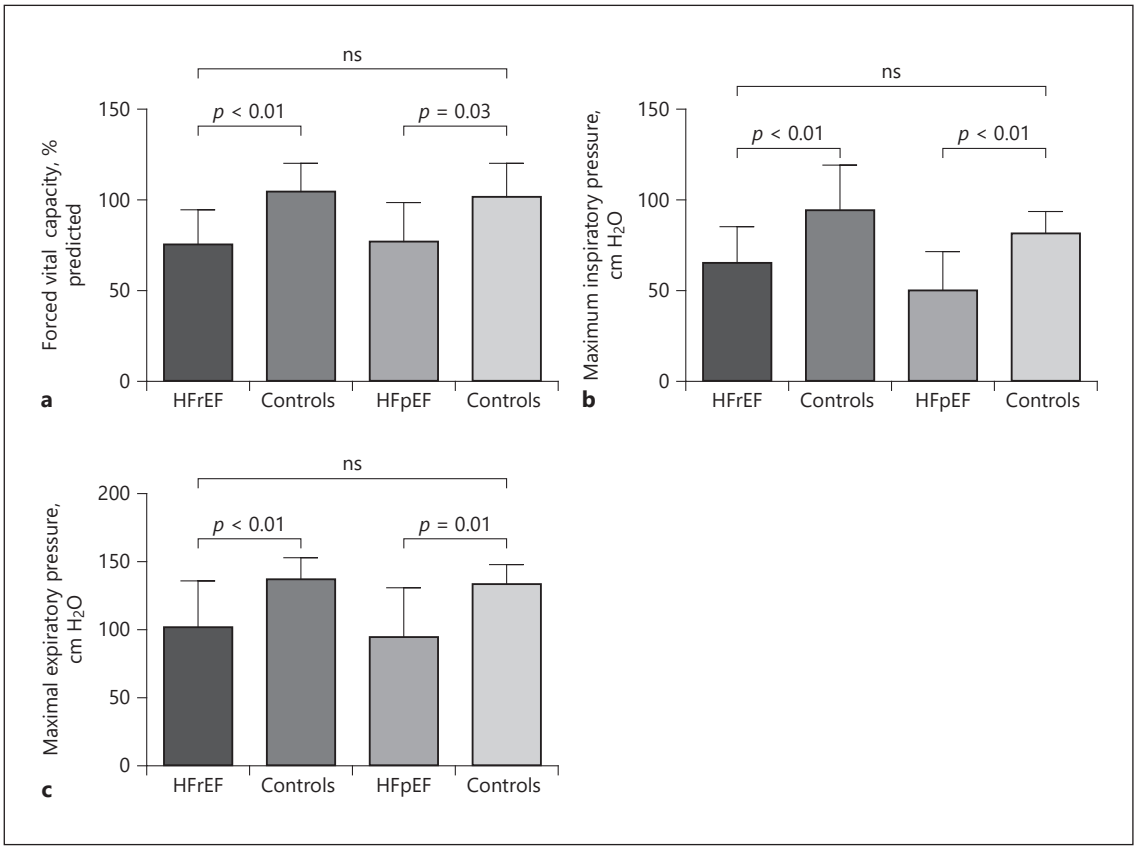

Fig. 2. Forced vital capacity (**a**), maximum inspiratory pressure (**b**), and maximum expiratory pressure (**c**) in patients with heart failure and controls. HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; ns, not statistically significant ($p > 0.05$).

spiratory maneuver). The best of 5 consecutive efforts was used for analysis [7, 8, 33].

Invasive Expiratory Muscle Strength Measurements

The lower thoracic nerve roots were magnetically stimulated at the tenth vertebra with rostrocaudal adjustment of the coil position (by no more than 2 vertebrae) in order to achieve the highest reproducible CMAP [34, 35]. For bilateral recording of abdominal CMAPs, surface electrodes were placed in the anterior axillary line close to the lower costal margin [34, 35]. Stimulation intensity was set at 100% of the maximum magnetic output (2 T). Stimulation was performed at FRC with recording of twPgas through the gastric balloon catheter [34, 35]. In addition, subjects were instructed to repeatedly perform a maximum cough maneuver for recording of cough Pgas.

Measurement of Circulating Pro-Inflammatory Cytokines

Fasting venous blood samples (30 mL) were collected after supine rest for 30 min. Blood samples were centrifuged immediately. Plasma aliquots were stored at −70°C and analyzed using automated assays or commercially available ELISAs. Analytical quality was monitored according to national regulations (Rili-BÄK 2014), including regular external quality assessments. Quantification of IL-6 was performed using an electrochemiluminescence immunoassay on a Cobas e802™ automated analyzer (Roche, Mannheim, Germany). The proposed reference value and limit of detection was 1.5 pg/mL, and intra- and inter-assay variabilities were <5.2 and <3.9%, respectively, at high analyte concentrations, and <4.9 and <5.1%, respectively, at low analyte concentrations (as specified in the manufacturer's user manual). Plasma levels of TNF-α were determined using an ELISA assay (Thermo Fisher Scientific, Oberhausen, Germany). The limit of detection and proposed reference value was 5.6 pg/mL, and intra- and inter-assay variabilities were <1.4 and <1.8%, respectively, at high analyte concentrations, and <5.9 and <8.5%, respectively, at low analyte concentrations (as specified in the manufacturer's user manual).

Statistical Analysis

Statistical analyses were performed using Sigma Plot™ software (Version 13.0; Systat, Erkrath, Germany). The primary endpoint was a reduction in twPdi. Assuming a 2-sided significance level of 0.05 (α) and 80% power (β), a sample size of 11 subjects per group was calculated to be sufficient to detect a 25% difference in twPdi. This was considered clinically relevant based upon previous studies, indicating that a 25% decrease in twPdi was usually associated with significant and clinically relevant diaphragm weakness [36, 37]. Based on our own observations regarding invasive pressure measurements in patients with HF, the dropout

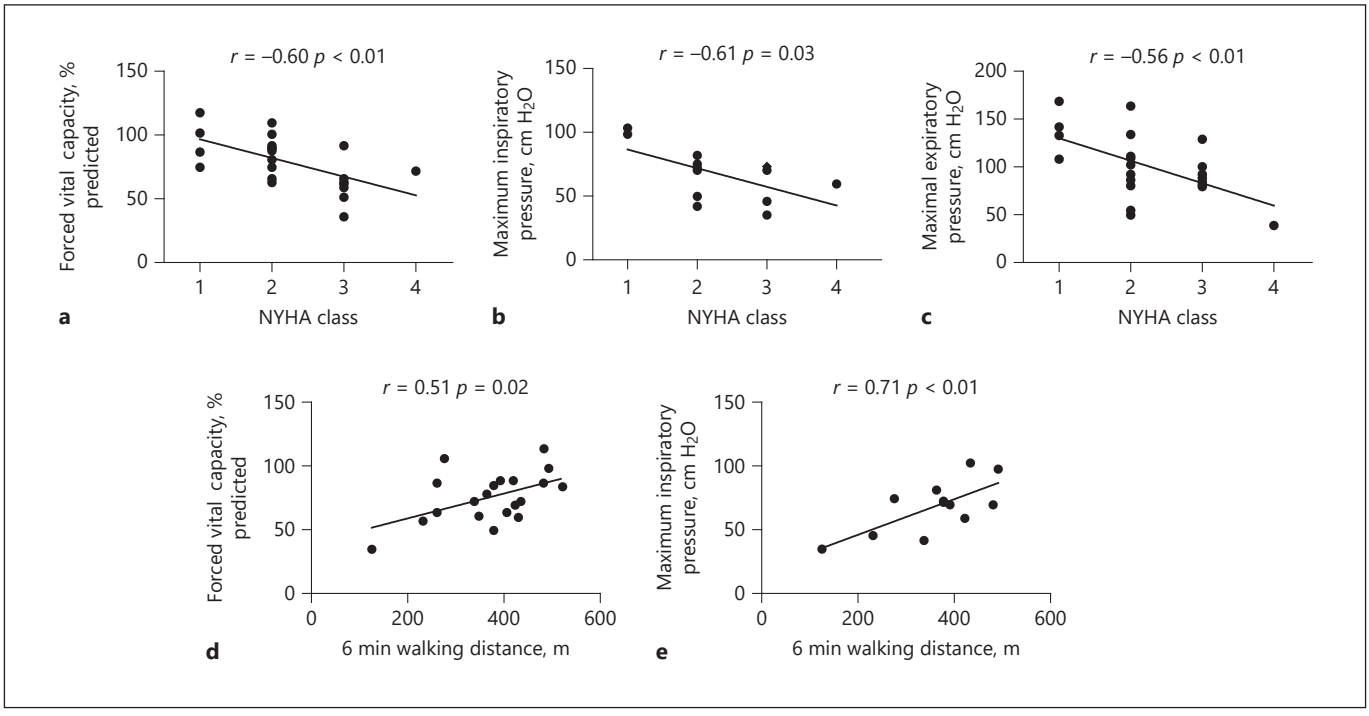

Fig. 3. Association between New York Heart Association (NYHA) class and forced vital capacity (**a**), maximum inspiratory pressure (**b**), and maximum expiratory pressure (**c**), and association between 6-min walking distance and forced vital capacity (**d**) and maximum inspiratory pressure (**e**) in patients with heart failure with reduced ejection fraction.

rate was assumed to be up to 40% [5, 7, 11]. For twPdi, normal values in healthy individuals were derived from our previous data [8]. For normally distributed data, results are expressed as mean and standard deviation, and the *t* test for independent samples was used for group comparisons. For non-parametric variables, median and interquartile ranges or the Mann-Whitney *U* test was used as appropriate. Pearson's correlation coefficient was used to test for correlations between continuous variables. Bonferroni's post hoc correction was applied to adjust for multiple testing. For all analyses, a p value of ≤0.05 was considered statistically significant.

Results

Subjects

Thirty patients with HF were enrolled in the study (age 63 ± 13 years, 26 male, BMI 29.0 ± 4.8 kg/m^2, 22 with HFrEF and 8 with HFpEF); all patients presented with exertional dyspnea and had elevated NT-proBNP levels (Table 1; Fig. 1). Eleven matched control subjects (age 55 ± 11 years, 8 male, BMI 24.5 ± 2.2 kg/m^2) were recruited for the HFrEF group, and 8 controls were enrolled for the HFpEF subgroup. Patients with HF showed moderate exercise intolerance (6MWD 130–570 m; median 414 m, interquartile range [IQR] 355–448), and 20/30 (66%) had a 6MWD of <450 m.

Spirometry and PI_{max} and PE_{max}

FVC (by ~30%) and PE_{max} (by ~25%) were significantly lower in patients with HFrEF versus controls (Table 2; Fig. 2). Clinical severity of HF (based on NYHA class) showed a moderate inverse correlation with FVC ($r = -0.60$, $p < 0.01$), PI_{max} ($r = -0.61$, $p = 0.03$), and PE_{max} ($r = -0.56$, $p < 0.01$) (Fig. 3a–c). In addition, the 6MWD was significantly correlated with FVC ($r = 0.51$, $p = 0.02$) and PI_{max} ($r = 0.71$, $p < 0.01$) (Fig. 3d, e). Patients with HFpEF had significantly lower FVC (by ~25%), PI_{max} (by ~40%), and PE_{max} (by ~30%) compared with matched controls (Table 2; Fig. 2). There were no differences between the 2 types of HF with respect to FVC and mouth occlusion pressures (all $p > 0.05$).

Diaphragm Ultrasound

Diaphragm excursion and DTR (both by ~40%) were lower in patients with HFrEF than in controls (Table 2; Fig. 4a). DTR was also lower in HFpEF patients than in

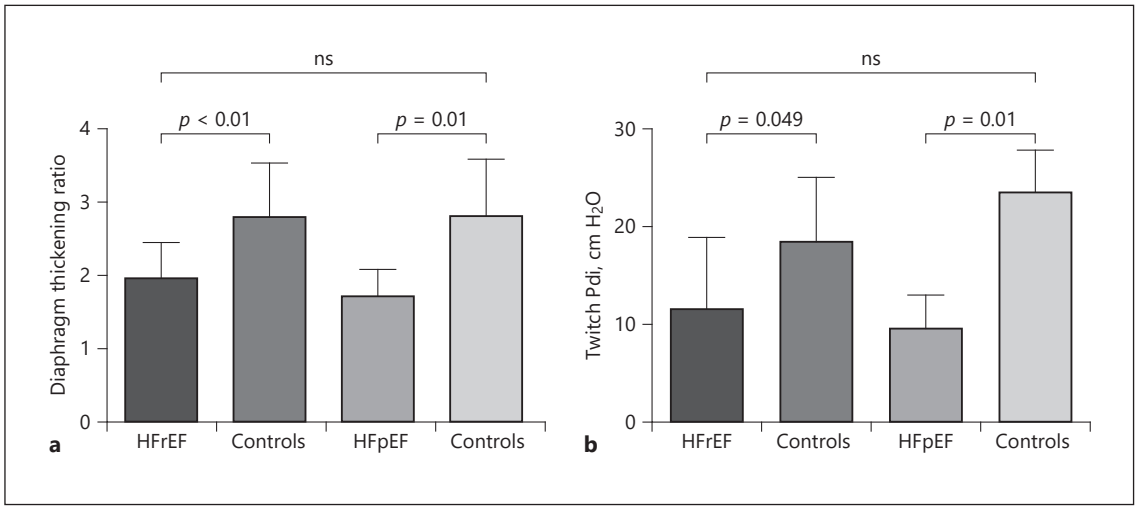

Fig. 4. Diaphragm thickening ratio (derived from diaphragm ultrasound) (**a**) and twitch transdiaphragmatic pressure (**b**) in patients with heart failure with reduced ejection fraction (HFrEF) or preserved ejection fraction (HFpEF) and controls.

controls (by ~40%) (Table 2; Fig. 4). Diaphragm thickness at FRC was normal in both HF subgroups (Table 2). There was no correlation between ultrasound data and NYHA class or NT-proBNP levels in both HFrEF and HFpEF patients (all $p > 0.05$, Fig. 4a). Diaphragm ultrasound parameters did not significantly differ between HF groups (all $p > 0.05$).

Phrenic Nerve Conduction Studies

Reproducible diaphragm CMAP and twPdi readings were obtained in 11 patients with HFrEF, 3 patients with HFpEF, and all controls. In all groups, no side-to-side difference was found, and neither CMAP latency (HFrEF 5.5 [4.9–6.8], HFpEF 5.5 [5.1–6.7] vs. control 5.0 [4.4–5.3] ms, $p > 0.05$) nor amplitude (HFrEF 0.15 [0.10–0.25], HFpEF 0.20 [0.10–1.43] vs. control 0.10 [0.05–0.33] mV, $p > 0.05$) was abnormal in either of the 2 patient subgroups, indicating normal phrenic nerve conduction properties.

Invasive Inspiratory Muscle Strength Measurements

Transnasal insertion of balloon catheters was refused by 16 patients, leaving 11 patients with HFrEF, 3 patients with HFpEF, and all control subjects for group comparison of invasive pressure recordings. The twPdi was significantly lower in patients with HFrEF (by ~35%) or HFpEF (by ~55%) than in controls (Table 3; Fig. 4b). No statistically significant correlations were found in any of the groups between invasively obtained inspiratory muscle strength measures and NYHA class, NT-proBNP levels, or 6MWD (all $p > 0.05$). Furthermore, twPes, twPgas, and twPdi did not differ significantly between patient subgroups (all $p > 0.05$).

Invasive Expiratory Muscle Strength Measurements

twPgas following magnetic stimulation of the abdominal muscles did not differ between HF subgroups or for either HF group compared with controls ($p > 0.05$). This was also the case for Pgas values following maximum voluntary cough (cough Pgas) in all HF patients (Table 3).

Circulating Pro-Inflammatory Cytokines

Data on IL-6 and TNF-α serum levels were available for all patients with HFpEF and for 20/22 patients with HFrEF. IL-6 serum levels exceeded the reference value in 19/20 HFrEF patients and in 6/8 HFpEF patients. Levels of TNF-α were elevated in 19/20 patients with HFrEF and in all patients with HFpEF (Table 1). Levels of IL-6 and TNF-α did not differ significantly between HF subgroups (all $p > 0.05$).

When dichotomized into 2 groups based on the median FVC (% predicted) as a cutoff, HFrEF patients with FVC <78% had higher IL-6 levels compared to those with FVC ≥78% (6.0 [4.0–12.0] vs. 3.0 [3.0–5.0] pg/mL, $p = 0.04$; Fig. 5a). In addition, patients with higher IL-6 levels (≥7 pg/mL) had significantly lower FVC compared to those with IL-6 <7 pg/mL (60.1 ± 22.8 vs. 86.1 ± 16.4 cm-H_2O, $p = 0.02$; Fig. 5b). Levels of TNF-α or IL-6 levels did not differ in HFrEF subgroups dichotomized by PI_{max}, PE_{max}, DTR, or TwPdi (all $p > 0.05$). In patients with HF-

Table 3. Invasively obtained inspiratory and expiratory muscle strength data in patients with heart failure (n = 14) and matched control subjects (n = 8)

Subject no. (age, gender)	Inspiratory muscle strength tests			Expiratory muscle strength tests		
	twPdi, cmH_2O	sniff Pdi, cmH_2O	PI_{max}, cmH_2O	twPgas, cmH_2O	coughPgas, cmH_2O	PE_{max}, cmH_2O
HFrEF						
1 (78, M)	13.3	106.5	75	29.9	94.7	89
2 (52, F)	27.4	63.6	*	3.8	92.6	85
3 (49, M)	11.1	94.7	*	45.2	225.3	87
4 (54, M)	13.6	103.1	*	88.3	205.4	134
5 (48, M)	12.7	102.9	*	4.9	114.4	112
6 (80, M)	13.3	61.8	77	*	78.3	165
7 (31, M)	4.1	63.3	101	27.0	111.9	109
8 (67, M)	6.1	89.3	74	18.9	165.1	135
9 (56, M)	1.3	40.0	36	3.4	111.4	80
10 (63, M)	7.0	36.3	84	5.5	93.5	110
11 (60, M)	20.1	85.8	72	44.1	156.6	103
Mean	11.8	77.0	74.1	27.1	131.8	109.9
SD	7.4	25.3	19.5	26.9	49.2	26.0
Controls						
1 (36, M)	7.8	106.6	106	12.8	60.8	145
2 (49, M)	20.9	72.2	78	9.3	131.7	129
3 (49, M)	14.6	64.9	88	17.8	116.8	155
4 (54, F)	22.5	116.4	131	9.2	146.2	139
5 (55, M)	12.7	67.8	89	53.7	65.3	121
6 (60, M)	27.1	77.9	68	19.8	116.4	115
7 (64, M)	25.5	113.7	81	*	164.2	130
8 (78, M)	18.9	87.8	92	*	99.9	138
Mean	18.7	88.4	91.6	19.6	112.7	134.0
SD	6.6	21.1	19.4	15.5	36.4	13.0
p value	0.049	ns	ns	ns	ns	0.03
HFpEF						
1 (81, M)	11.6	42.0	28	30.1	52.4	57
2 (60, M)	12.0	67.0	64	*	109.4	53
3 (60, M)	5.9	67.8	89	18.9	214.4	129
Mean	9.8	58.9	60.3	24.5	125.4	79.7
SD	3.4	14.7	20.7	7.9	82.2	42.8
Controls						
1 (78, M)	18.9	87.8	92	17.8	99.9	138
2 (64, M)	25.5	113.7	81	(53.7)	164.2	130
3 (60, M)	27.1	77.9	68	19.8	116.4	115
Mean	23.8	93.1	80.3	18.8	126.8	127.7
SD	4.4	18.5	12.0	1.4	33.4	11.7
p value	0.01	0.07	ns	ns	ns	ns

CoughPgas, gastric pressure following a cough; F, female; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; M, male; ns, not statistically significant ($p > 0.05$); PE_{max}, maximum expiratory pressure; PI_{max}, maximum inspiratory pressure; SD, standard deviation; sniff Pdi, transdiaphragmatic pressure following maximum voluntary sniff; twPdi, transdiaphragmatic pressure following cervical stimulation of the phrenic nerve roots; twPgas, gastric pressure following magnetic stimulation at the tenth vertebra. * Missing value due to technical issues and/or poor cooperation.

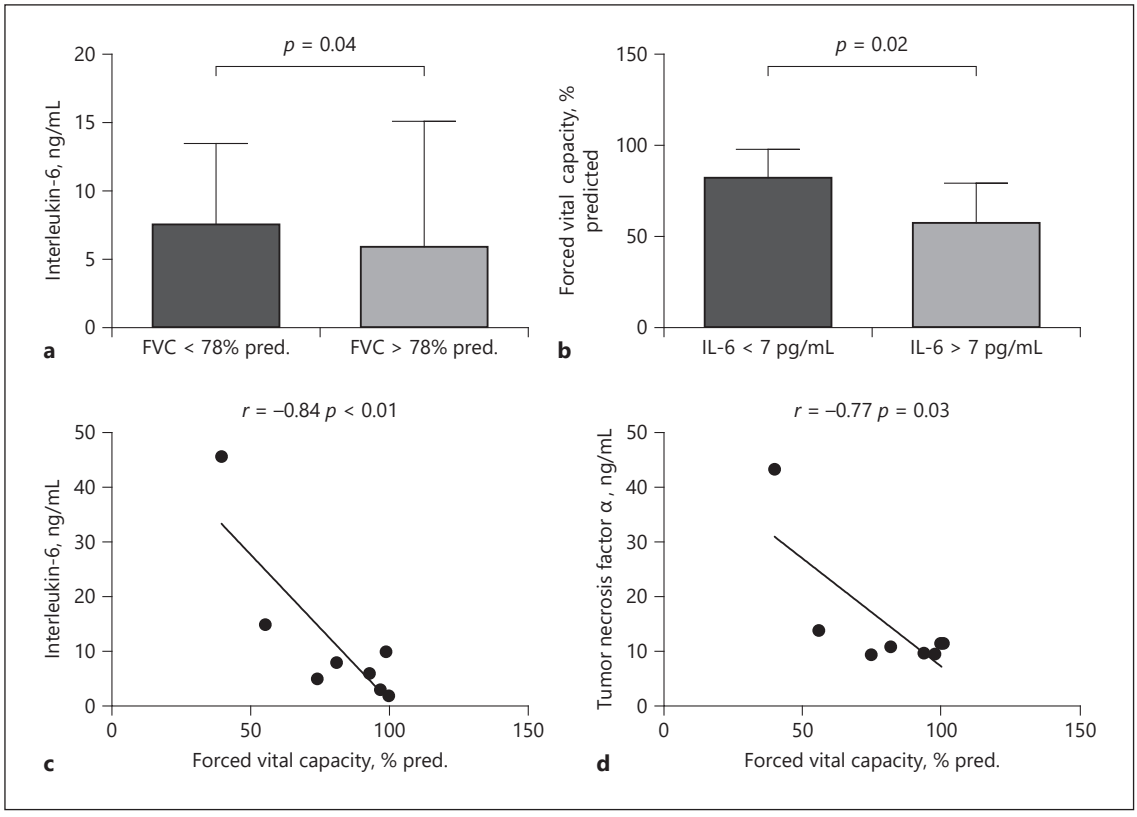

Fig. 5. Association between interleukin-6 (IL-6) levels and forced vital capacity (FVC) in patient with heart failure with reduced ejection fraction (**a**, **b**) (dichotomization of patients according to FVC and IL-6 using their median values), and between IL-6 (**c**) or tumor necrosis factor alpha (TNF-α) (**d**) levels in patients with heart failure with preserved ejection fraction.

pEF, levels of IL-6 and TNF-α were inversely correlated with FVC ($r = -0.84$, $p < 0.01$ and $r = -0.77$, $p = 0.03$, respectively) (Fig. 5c, d).

Discussion

This study is the first in human controlled trial to demonstrate inspiratory muscle dysfunction with restrictive ventilation disorders in patients with HF, as assessed by means of a multimodal diagnostic approach including spirometry, mouth occlusion pressures, diaphragm ultrasound, and standardized invasive measurement of the Pdi following magnetic stimulation. Respiratory muscle dysfunction was closely linked to exercise intolerance, and FVC correlated with serum levels of circulating IL-6 and TNF-α, suggesting an association between lung restriction and systemic inflammatory response in chronic HF.

Initial description of inspiratory muscle impairment in HFrEF dates back to the 1990s [5]. Mancini and colleagues [5] showed that PI_{max} is reduced in patients with HFrEF ($n = 10$) compared with healthy controls. Notably, a strong correlation was found between ratings of perceived dyspnea and PI_{max} values, underlining the clinical importance of inspiratory muscle weakness in HFrEF [5].

Diaphragm ultrasound has emerged as a novel tool for assessing diaphragm function [38]. Specifically, the DTR may reflect diaphragm strength [38] and has been shown to be impaired in patients with HFrEF [11, 39]. Subsequently, in a larger cohort of HFrEF patients ($n = 244$), Meyer and colleagues [9] reported that approximately every third patient showed reduced PI_{max} values, which were associated with worse overall prognosis. Notably, this study showed that PI_{max} adds prognostic value beyond known risk factors of clinical deterioration, including peak oxygen consumption, left ventricular ejection fraction, and norepinephrine plasma concentration [9]. Furthermore, the clinical significance of diaphragm dysfunction was underlined by close correlation of PI_{max} with NYHA functional class and peak oxygen consump-

tion [9]. In this regard, the present study is confirmatory by showing that inspiratory muscle dysfunction relates to exercise intolerance in HFrEF patients while further strengthening this concept by application of diaphragm ultrasound.

In contrast to HFrEF, less is known about diaphragmatic dysfunction and its pathophysiological role and clinical implications in patients with HFpEF. Recent animal and clinical studies have shown that molecular, mitochondrial, histologic, and functional alterations in the diaphragm may also be present in HFpEF, and that these may be partly reversible by exercise training [20, 39, 40]. DTR is an intuitive ultrasound-derived measure reflecting the diaphragm's capacity to contract. As in HFrEF patients, DTR was reduced in patients with HFpEF and showed close association with exercise intolerance (based on the 6MWD) [41].

The diagnostic gold standard of diaphragm force generation (i.e., twPdi) has not yet been applied in HFpEF patients outside animal models [20, 21]. Its application in the current study objectively confirmed that diaphragm dysfunction is present in HFpEF. Of note, the extent and characteristics of diaphragm involvement appear to be comparable in patients with HFpEF and HFrEF. Thus, it can be assumed that respiratory muscle weakness is a consequence of HF per se rather than being caused by impairment of left ventricular function alone.

It has been hypothesized that circulating pro-inflammatory cytokines provide a mechanistic link between HF and diaphragm dysfunction [13]. Levels of circulating pro-inflammatory cytokines are increased in both HFrEF and HFpEF, possibly impacting overall functional status and prognosis [14–17, 19]. Of note, pro-inflammatory cytokines (TNF-α and IL-6 in particular) have been shown to directly impair muscle function in animal models [19, 42]. Furthermore, Gosselink and coworkers [42] showed that genetic abolition of TNF-α improves diaphragm function in a mouse model of dystrophin-related muscular dystrophy. Diaphragm isometric force was enhanced, and in the long run, alteration of the myosin heavy chain isoform profile was detectable [42]. Notably, it has been further highlighted that different intracellular pathways regulate expression of myosin heavy chain isoforms, including the mitogen-activated protein kinase family and the extracellular signal regulated kinase 1/2, which plays a decisive role in myosin heavy chain isoform maintenance [13, 42, 43]. Moreover, mitogen-activated protein kinase can be modulated in vitro by inflammatory cytokines such as TNF-α and oxidative stress [13, 42, 43]. For this reason, it can be hypothesized that chronic elevation of pro-inflammatory cytokines in patients with HF may contribute to long-term alterations of both limb and respiratory muscle composition on a molecular level [13].

Spirometric and manometric findings from this study suggest that expiratory muscle dysfunction is present in patients with HF, whether or not left ventricular function is impaired. This is consistent with the limited body of data previously published on this subject [5, 9]. The current study adds to this knowledge because expiratory muscle function in HF was specifically assessed by invasive measurement of cough Pgas and twPgas following magnetic abdominal muscle stimulation. Both measures showed no difference between HF patients and controls. This finding suggests that impairment of expiratory force generation is accountable to other muscles than the abdominal wall muscles (e.g., internal intercostal muscles) and that detailed assessment of expiratory muscle function in patients with HF requires both volitional and non-volitional tests.

This study has several limitations, which need to be taken into account. First, inter- and intra-observer variabilities may have affected magnetic stimulation data. To minimize this bias, extensive training and repetitive stimulations with maximum magnetic output were performed until variability of the twPdi and diaphragm CMAP amplitude was <10%, as previously published by our group [44–48]. Second, this study focused on ambulatory patients in a stable clinical condition, and findings may be different in patients with worse functional status. Third, a clear distinction between lung restriction due to gas trapping and ventilatory restriction because of inspiratory muscle dysfunction cannot be made as body plethysmography was not performed. Although entrapment of gas appears less likely in patients with HF, this aspect should be acknowledged as a limitation to this study. Finally, serum cytokine levels were not measured in control subjects, but reference values were provided by the manufacturer and compared with previously published normative data. Statistical correlations between cytokine levels and FVC (Fig. 5) should be interpreted with caution, with special regard to the small sample size. Since the current study aimed to be mainly hypothesis-generating, future studies in larger patient cohorts are needed to confirm an association between measures of respiratory muscle strength and circulating levels of proinflammatory cytokines in patients with HFrEF and HFpEF.

Conclusions

Respiratory muscle dysfunction is present in patients with chronic HF, irrespective of left ventricular ejection fraction. It contributes to exercise intolerance and is associated with increased serum levels of circulating pro-inflammatory cytokines, possibly reflecting that systemic inflammation mediates long-term structural impairment of respiratory muscles. Future studies should further investigate both the complex pathophysiology and clinical significance of respiratory muscle weakness in HF. Furthermore, interventional studies that evaluate the effects of respiratory muscle strength training on respiratory muscle function and exercise tolerance are desirable.

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Statement of Ethics

Ethical approval was received from the local Ethics Committee (Ethikkommission der Ärztekammer Westfalen-Lippe und der WWU Münster Reference Number: AZ 2016-072-f-S).

Conflict of Interest Statement

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Author Contributions

J.S. and M.B. designed the study. J.S. and C.H. were responsible for data collection. J.S., P.B., and S.O. performed the statistical analyses. J.S., H.-J.K., M.B., and I.T. prepared the manuscript, which was critically revised and amended by P.B., K.S., J.-R.N., M.S., G.P.D., J.-K.S., A.G., and M.D.

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