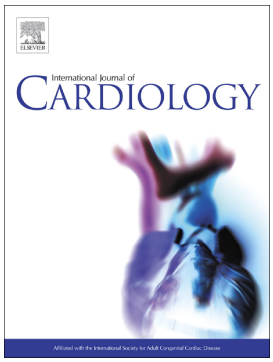

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Interaction Between Alveolar Diffusing Capacity and B Lines in Heart Failure with Preserved Ejection Fraction – A Non-Invasive Exercise Study

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Key words: Alveolar gas diffusion for carbon monoxide, HFpEF, B-lines, CPET

Abstract

Background: Patients with heart failure and preserved ejection fraction (HFpEF) present with elevated left atrial pressures. The elevation in pulmonary venous pressures and lung interstitial fluid accumulation is exacerbated by exercise. An analysis on the lung extravascular fluid (lung B-lines) combined with lung diffusing capacity for carbon monoxide (DLCO) with his subcomponents, alveolar-capillary membrane conductance (Dm) and capillary volume (Vcap) has not been performed.

Purpose: To assess the association between resting pulmonary diffusing capacity and exercise-induced pulmonary congestion in HFpEF patients. .

Methods: DLCO, Dm and Vcap were measured in 40 stable HFpEF subjects (age = 73 ± 8; BMI = 27,1 ± 4,3 kg/m2) and in healthy controls at rest with parallel assessment of B-lines during combined Stress Echocardiography and Cardiopulmonary Exercise Test (ESE-CPET). B-lines evaluation of both hemithorax was performed at rest and at peak of exercise.

Results: Compared to controls, HFpEF group exhibited lower DLCO and Dm at rest and a higher number of B-lines at peak of exercise with a significant correlation observed for HFpEF but not for controls (resting DLCO vs peak B-lines in HFpEF: R = - 0,66, P < 0,001; resting Dm vs peak B-lines in HFpEF: R = - 0,72, P < 0,001). We found also a significant negative correlation between Dm at rest with VE/VCO2 slope at CPET (R = - 0,51, P < 0,001), and Dm at rest with E/e' at peak of stress test in HFpEF group (R = - 0,59, P < 0,001), reflecting a ventilatory/perfusion mismatch along with an elevation of left ventricular filling pressures exacerbated by exercise.

Conclusions: In HFpEF altered pulmonary gas exchange capacity at rest, and an excessive lung extravascular water at rest and during effort are combinatory factors affecting exercise performance. The true cause-effect relationship between these variables needs to be tested by mechanicistic experimental studies.

Introduction

Heart failure with preserved ejection fraction (HFpEF) is a complex and heterogeneous syndrome that has garnered wide attention for the rising prevalence and significant morbidity[1]. HFpEF is characterized by impaired ventricular compliance, elevated left atrial (LA) pressures and abnormal diastolic function, leading to an inability to augment cardiac output during exercise pending an abnormal increase in left ventricular (LV) filling pressures[2,3] whose direct clinical manifestations are exertional dyspnea, exercise intolerance, and fluid retention[4].

Pulmonary congestion and impaired gas exchange play a crucial role in the pathophysiology of HFpEF[5,6]. The alveolar-capillary membrane is a key interface for pulmonary gas diffusion, and its function is often impaired in HF bearing relevant clinical and prognostic insights[7].

Especially, patients are at risk of developing exercise-induced pulmonary edema because of an increase in transcapillary fluid pressure which clearly depends on the level of exercise performed and the severity of heart failure[8].

Understanding the relationship between alveolar-capillary membrane function, exercise-induced hemodynamic changes, and pulmonary congestion may offer explanations for what mechanism drives dyspnea and functional limitations clarifying the role of congestion and its dynamic changes during a burst of maximal exercise.

Lung B-lines are hyperechoic artefacts perpendicular to the pleural line attributed to subpleural interlobular septal thickening due to the presence of liquid. In previous studies a clear correlation was found between the number of comets and both radiological and invasive signs of pulmonary congestion[9]. Patients with ≥30 B-lines during stress usually have higher functional impairment at baseline and during exercise and a higher chance of transition to acute decompensated HF and other events in the short-term follow-up[10].

We aimed at evaluating whether an altered pulmonary diffusing capacity at rest, with the assessment of lung diffusing capacity for carbon monoxide (DLCO) along with the membrane conductance (Dm), is correlated with exercise-induced pulmonary congestion, assessed by B-lines, in HFpEF patients and in control subjects.

Methods

We prospectively enrolled 40 ambulatory patients, referred to San Paolo Hospital in Milan, with known or suspected HFpEF diagnosis, that had H_2FPEF score ≥ 5 or HFA-PEFF score = 5 or 6 (at rest or after exercise) and 26 controls. The experimental protocol was approved by the local Ethics Committee (CET 160-2023; company authorization No. 241 of 12/02/2024) in accordance with the Declaration of Helsinki and all participants provided written informed consent. Patients with other causes of HF such as significant valvular heart disease, pericardial disease, infiltrative, restrictive, or hypertrophic cardiomyopathy were excluded. Patients with ejection fraction < 50% were also excluded. Furthermore, since most pulmonary pathologies, such as chronic obstructive or interstitial diseases, lead to an impaired gas transfer function, all patients with a known medical history for these conditions or with altered values of spirometry performed in our study protocol, were not considered in our analysis. All patients underwent blood testing with NT-proBNP at rest, at peak of maximal effort and at early recovery, 2 minutes after peak.

Pulmonary gas exchange evaluation. All participants performed pulmonary function measurements before exercise including spirometry with assessment of forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV1). Patients underwent also to DLCO evaluation using the single breath technique in which the breath-holding time, as recommended, was 10 s and gas mixtures were 0.3% CO, 0.3%, methane, 21% oxygen (O2) balanced with nitrogen (N2). For calculations of Dm and Vcap (with Roughton-Forster multiple FiO2 method), subjects inhaled, in three separate maneuvers, a gas mixture containing 0.3% CH4, 0.3% CO, with three different O2 concentrations of 20, 40 and 60% respectively, balanced with N2[11].

Rest and Exercise Stress Echocardiography. Trained personnel conducted echocardiographic assessments at rest, during the entire exercise and recovery: the evaluations were performed using a specialized 58 device equipped with a 3-D sector array probe (Philips Epiq 7) with a frequency range of 1–5 MHz. Throughout the exercise session, imaging complete views were recorded every 2 minutes.

Doppler measurements represent the average of two beats in sinus rhythm and ≥3 beats in atrial fibrillation. Stroke volume (SV) was assessed applying the equation $SV = VTI_{LVOT} \times CSA_{LVOT}$, where VTI_{LVOT} is the velocity-time integral of pulsatile Doppler obtained at the level of the LV outflow tract (LVOT), and CSA_{LVOT} is the cross-sectional area of the LVOT, determined using the circumference area formula. Cardiac output (CO) was obtained as SV

× heart rate (HR). Averaged septal and lateral E/e' ratio (E/e'_{avg}) was determined to estimate LV filling pressure. The systolic tissue velocity at the lateral tricuspid annulus (TV S'), and tricuspid annular plane systolic excursion (TAPSE) were used as measures of right ventricular (RV) systolic function. Tricuspid regurgitation (TR) peak velocity at rest and during exercise, was recorded in the best view (apical 4-chamber view or RV-focused or fore shortened 4-chamber view).

Pulmonary artery systolic pressure (PASP) was calculated as 4 × (the highest averaged TR velocity)^2 + estimated right atrial pressure (RAP) with vena cava dimension and collapsibility. In addition, RV afterload was assessed by TAPSE/PASP ratio.

Cardiopulmonary Exercise Test. A symptom-limited CPET was executed using COSMED Omnia equipment on a tiltable cycle ergometer by trained staff, alongside exercise echocardiography, employing a standardized left-sided approach. Customized exercise ramp protocols, varying from 8 to 20 W/min, were set based on predicted exercise capacity. Ventilatory expired gas analysis was performed using a metabolic cart (COSMED QuarkCPET). During the exercise test, 12-lead ECG and heart rate were recorded continuously, while blood pressure was measured every two minutes, and oxygen saturation was monitored using a pulse oximeter.

Breath-by-breath oxygen consumption (VO2), carbon dioxide production (VCO2) and minute ventilation (VE) were analyzed at rest and throughout exercise[12]. VE efficiency was calculated as VE vs VCO2 slope by least squares linear regression (y = mx + b, where m is slope). The VO2/work rate (WR) slope was automatically calculated using the software program, defining the start and the end of the linear relationship by the operator. The anaerobic threshold (AT) was calculated with the standard technique[13].

Lung Ultrasound. All patients underwent lung ultrasound (LUS) for quantification of B-lines in a semi-recumbent position on the cycle ergometer both at rest and immediately after the peak of the exercise during combined exercise stress echocardiography and cardiopulmonary testing (ESE-CPET).

When integrating LUS during stress echocardiography, a simplified four-zone scanning protocol is often used, placing the probe at the third intercostal space along the anterior axillary and mid-axillary lines, in both hemithorax[14,15]. For each zone, the number of B-lines was counted, and for each patient the total number of all B-lines from all 4 chest zones was added together.

Statistical analysis

The distributions of continuous variables were evaluated using the one-sample Kolmogorov-Smirnov test. Continuous variables are presented as mean and standard deviation for data with a normal distribution, as median and percentile for data with a non-Gaussian distribution. Categorical variables are expressed as numbers and proportions. Between-group differences were compared by the chi-square test for categorical variables, analysis of variance for normally distributed continuous variables, and by Wilcoxon rank sum or Kruskal-Wallis tests for non-normally distributed continuous variables. Pearson and Spearman coefficients were employed for testing correlation between alveolar diffusing capacity (DLCO and its subcomponent Dm), and peak exercise capacity, ventilatory function, echocardiographic parameters and brain natriuretic peptides.

The potential selection bias related to the observational nature of the study was mitigated with a propensity score approach[16]. The probability of being diagnosed with HFpEF was generated by a non-parsimonious logistic regression model including age, gender, BMI, height, and weight. Propensity scores were employed for calculating the inverse probability of treatment weight (IPTW), defined as the inverse of the probability of receiving the treatment that the patient received, and estimates the average treatment effect on the treated. The balance between the weighted groups was evaluated with standardized mean differences[16]. Propensity score weighting gains the advantage of using all the subjects in the two treatment groups for the outcome analysis compared to other balancing methods based on propensity score, such as propensity score matching.

The relationship between Dm/DLCO and the study outcomes was then evaluated with IPT-weighted longitudinal data analyses. The endpoints (lung ultrasound B-lines and exercise-related hemodynamic and ventilatory parameters in patients with HFpEF) were analyzed with multivariable generalized linear models (GLM) and multivariable generalized linear mixed effect models (GLMM), with fixed effects for population-level trends and random effects to capture between-subjects variability. The variables' selection for the models was performed by a back-and-forward stepwise regression (probability of stay = 0.10, probability of entry = 0.05), considering Dm and the group (HFpEF vs control).

Two-sided statistics were performed with a significance level of 0.05. Analyses were performed with SPSS version 29.0.1 (SPSS Inc., Chicago, IL, USA), GraphPad Prism 6 (GraphPad Software, La Jolla, California) and R language (R 4.3.3; R Development Core Team (2023). R:

A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/)

Results

Patient Characteristics. HFpEF patients were older (mean age 75 ± 8 vs 61 ± 13 years; p < 0,001), with higher body mass index (27,1 ± 4,3 vs 25 ± 4; p = 0,047), prevalence of diabetes (32% vs 0%; p < 0,001) and atrial fibrillation (25% vs 0%; p < 0,001) than controls (**Table 1**). The two groups had similar haemoglobin values, smoking history and arterial hypertension prevalence. Patients with HFpEF were in New York Heart Association (NYHA) functional class II to III symptoms and displayed NT-proBNP levels higher than controls [median values 52,5 (20-296) pg/ml vs. 362 (30-2940) pg/ml; p < 0,001] along with lower eGFR (52 ± 16,6 vs 78 ± 12,6 ml/min per m^2; p = 0,02). They also exhibited a higher use of loop diuretic agents, mineralocorticoid receptor agonist (MRA) and sodium-glucose transport protein 2 inhibitors (SGLT2i).

The distribution of baseline characteristics was balanced after IPTW, as demonstrated by the mean standardized differences (**eTable 1** and **eFigure 1**). It is worth noting that the overall number of patients in the two groups is not integer because of the IPTW.

Relationship between B-lines (at rest and at peak) and Dm (at rest). Patients with HFpEF had lower levels of DLCO and Dm at rest compared with control subjects (16,7 ± 5,7 vs 19,7 ± 5,5 ml/mmHg/min; p = 0,04 and 19,3 ± 7,6 vs 23,1 ± 7,9 ml/mmHg/min; p = 0,048, respectively), and higher lung B-lines (2,7 ± 2,7 vs 0,77 ± 0,8; p < 0,001) (**Table 2**).

At peak of exercise, the number of B-lines was higher in HFpEF group compared to controls (5,4 ± 3,3 vs 1,1 ± 1; p < 0,01). As shown in **Central Illustration** (**Panel A**), there is a significant negative correlation between B-lines at peak and Dm at rest in patients with HFpEF (R = -0,72, p < 0,001).

The IPT-weighted GLM (**eTable2**) confirmed the negative relationship between Dm at rest and B-lines at peak (B = -0,13 ± 0,03, p < 0.001) and also showed that HFpEF is associated with an increased number of B-lines at peak (B = -3,38 ± 0,49, p < 0,001).

Figure 2 (**Panel A**) showed a negative correlation between B-lines trend and Dm in HFpEF (R = -0,52, p < 0,001). B-lines trend was negatively associated to Dm at rest (B = -0,14 ± 0,03, p < 0,001) and was steepened by HFpEF (B = 1,83 ± 0,49, p < 0,001), as resulted by IPT-weighted GLMM.

The increase of B-lines from baseline to peak was wider in HFpEF (**Figure 3, Panel A**).

Hemodynamic and echocardiographic data at rest and peak and relationship with Dm.

Echocardiography at rest showed higher left ventricular mass index, LA volumes, estimated LA pressures by E/e' ratios and PASP estimates in HFpEF than in control group (**Table 2**); TAPSE/PAPS ratio, a marker of right ventricle-pulmonary artery coupling, was significantly lower in HFpEF group. Left Ventricular Ejection Fraction (LVEF) was similar by definition, and also non-invasive estimated stroke volumes and cardiac output at rest did not differ between groups.

Exercise stress echocardiography demonstrated higher left ventricular filling pressures, pulmonary arterial systolic pressures and left atrial volumes in HFpEF (**Table 3**). Unlike resting data, HFpEF had worse cardiac output, a reduced heart rate response and lower systolic blood pressure. The slope of the ratio between mean PAP and CO (mPAP/CO slope), an index of pulmonary vascular limitation in patients with HFpEF, was significantly increased in the HFpEF compared to controls.

Central Illustration (**Panel B**), shows a negative correlation between E/e' ratio at peak and Dm at rest in the HFpEF group (R = -0,59, p < 0,001) but not in the control group.

In the IPT-weighted GLM (**eTable2**), the E/e' ratio at peak was inversely related to Dm at rest (B = -0,36 ± 0,07, p < 0,001) and increased by HFpEF (B = 6,86 ± 1,08, p < 0,001). **Figure 2** (**Panel B**) showed a negative correlation between E/e' ratio trend and Dm in HFpEF (R = -0,32, p < 0,01). In the IPT-weighted GLMM, the E/e' ratio trend was negatively related to Dm at rest (B = -0,22 ± 0,05, p < 0,001) and was steepened by HFpEF (B = 2,74 ± 0,84, p < 0,002).

The increase of E/e' ratio from baseline to peak was wider in HFpEF (**Figure 3, Panel B**).

Cardiopulmonary function and its relationship with Dm at rest.

Vcap, VO2 and V_E at rest were not different between the two groups (**Table 2**).

HFpEF had a lower peak VO2, peak workload and peak respiratory exchange ratio with a higher VE/VCO_2 slope, V_E and $PetCO_2$ (**Table 3**), consistent with echocardiographic data of an underlying pulmonary vascular limitation.

Central Illustration (Panel C) shows a negative correlation between VE/VCO_2 slope and Dm at rest in the HFpEF group (R = -0,51, p = 0,001) but not in the control group. In the IPT-weighted GLM (**eTable2**), the VE/VCO_2 slope was increased by HFpEF (B = 6,73 ± 1,67, p < 0,001) while the relationship with Dm (B = -0,18 ± 0,11, p = 0,09) was not significant.

Relationship between NT-proBNP (at rest and at peak) and Dm at rest.

At peak, NT-proBNP was significantly higher in HFpEF [median values 386 (31-3130) pg/ml vs. 60,5 (20-328) pg/ml; $p < 0,001$].

As shown in **Central Illustration** (**Panel D**), NT-proBNP at peak was negatively correlated to Dm at rest in patients with HFpEF ($R = -0,34$, $p < 0,03$). In the final IPT-weighted GLM (**eTable2**), NT-proBNP at peak was decreased by Dm at rest ($B = -41,84 \pm 12,8$, $p = 0,002$) and increased by HFpEF ($B = 1240,9 \pm 198,2$, $p < 0,001$).

There was a little NT-proBNP increase from baseline to peak in HFpEF (**Figure 3, Panel C**) while no changes were observed in the control group. NT-proBNP curve was significantly increased by HFpEF ($B = 280,04 \pm 123,23$, $p = 0,03$), but not Dm ($B = -13,25 \pm 7,69$, $p = 0,09$), as resulted by IPT-weighted GLMM.

Discussion

This prospective study approaches the context of lung fluid congestion and gas diffusion for carbon monoxide interaction in HFpEF syndrome with the gas analysis response during exercise as measure of exercise capacity. Especially, this is the first study addressing the link between lung B-lines and Dm, the membrane component of DLCO, expression of alveolar-capillary membrane conductance, and findings suggest that an impaired gas exchange at rest and lung fluid redistribution induced by exercise are tightly related. Actually, we found that a lower DLCO and Dm at rest correlate with lung B-lines augmentation during exercise. The lower gas diffusion and the higher lung extravascular water seem synergic in triggering a reduced exercise maximal performance and a worse ventilation efficiency.

In agreement with previous studies[17-19], mechanisms responsible for a reduced DLCO and Dm are an increase in pulmonary venous and capillary pressures both at rest and during effort triggering a long-term unfavourable evolution in the physical properties of the alveolar capillary membrane through a progressive thickening of pulmonary capillaries due to vascular remodelling.

If we consider the two components of DLCO, membrane conductance (Dm) is predominantly involved in reduction of lung diffusing capacity rather than capillary volume (Vcap). Indeed, Dm is reflective of the total pulmonary surface area where gases exchange takes place, matching ventilation and perfusion. The dependency of so this parameter on both interstitial or alveolar edema and membrane structural remodelling is quite compelling.

At rest HFpEF patients exhibited a significantly increase in left ventricular filling pressures estimated by E/e'_{avg} ratio, combined with more interstitial fluid accumulation due to elevated pulmonary hydrostatic pressures yielding to a reduced Dm and DLCO compared with controls.

Present findings are also reflective of an altered right ventricular-arterial coupling represented by low TAPSE/PASP ratio and of increased cardiac overload highlighted by the natriuretic peptide NT-proBNP, as typically happens in HFpEF group, especially when pulmonary hypertension overlaps[20,21].

For obvious reasons, we were unable to measure the thickness of alveolar-capillary membrane, but there is clear evidence that in HFpEF, membrane remodelling consists of endothelial dysfunction, myofibroblast proliferation, alveolar and pulmonary vascular disease that lead to an increased diffusion distance and a decreased distensibility of pulmonary vessels[22]. No differences in capillary volume (Vcap) were observed in the two

groups because Vcap may be compensatory or not and its changes may be independent on membrane function and structure reflecting the capacity of carrying oxygen in the capillary blood stream.

We therefore observed that patients with HFpEF and a reduced membrane conductance (Dm), developed more extravascular interstitial lung fluid and also higher left ventricular filling pressure leading a worse ventilatory/perfusion mismatch and impaired VE/VCO2 slope during exercise. A weaker but significant correlation was found with NT-proBNP levels and slightly significative differences were found in NT-proBNP concentration from rest to peak of exercise in HFpEF, unlike what other studies have shown in which it was hypothesized that probably BNP (rather than NT-proBNP) is a more sensitive marker during exercise[23,24].

Overall, our findings align with a previous study by Fermoyle et al.[6] simultaneous measures of lung diffusing capacity and invasive hemodynamic evaluations were performed showing that patients with HFpEF increase DLCO and Dm relative to mean pulmonary artery pressure (mPAP) and pulmonary capillary wedge pressure (PCWP) from rest to 20 W exercise and then reached a plateau till peak of exercise, while controls exhibited a linear increase of gas diffusion parameters relatively to the haemodynamic ones. The authors hypothesized that this behaviour is due both to the augmentation of interstitial lung fluid that limits gas diffusion and to a limited CO increase during exercise in HFpEF patients above the threshold of 20 W which would otherwise allow a more favourable capillary distension improving membrane conductance and gas exchange.

Also, in another study performed in HFrEF[23] patients exhibited a Dm and DLCO reductions 2 minutes after exercise, compared with rest condition. This has been interpreted with lung fluid accumulation during the early recovery phase, in contrast with what expected in normal conditions during effort, i.e. improvement in diffusing capacity due to distension of alveolar septa and opening new capillaries with increased cardiac output.

Study limitations

There are several limitations that we have to be taken into account.

We were unable to measure DLCO and its subcomponents during effort and in early recovery because of the intrinsic difficulties of the Roughton-Forster technique and the impossibility for patients to holding breath for 8-10 seconds during and soon after maximal exercise.

For calculations of B-Lines we have utilized a simplified 4-zone scan protocol, as suggested in previous echo-stress studies[14,25], instead of a more precise 28-site scan, to save time during our exams. Due to logistical and time-consuming issues, unfortunately we have no data about a late recovery-phase lung ultrasound B-lines and lung diffusion evaluation, but of course this analysis could provide valuable insight into the dynamics of pulmonary congestion and its relationship with diffusing capacity, as other study have demostrated[23]. Lastly, a diagnosis of HFpEF was performed with probabilities scores and no hemodynamic invasive data were collected and compared with non-invasive ones.

Conclusions

Our study is the first evaluating the gas exchange response in HFpEF in a parallel assessment of B-lines and gas diffusing capacity. We found a tight relationship between DLCO, Dm at rest and the exercise induced extravascular fluid accumulation. A routine-based assessment of the gas exchange components may be viewed as additional target of therapeutic interventions from early to late stages of HFpEF clinical syndrome.

Further data are necessary to discover the true cause effect relationship between structural changes in alveolar surface, its dysfunction and the extent of exercise-induced fluid augmentation during exercise.

References

1. Hamo CE, DeJong C, Hartshorne-Evans N, Lund LH, Shah SJ, Solomon S, Lam CSP. Heart failure with preserved ejection fraction. Nat Rev Dis Primers. 2024 Aug 14;10(1):55. doi: 10.1038/s41572-024-00540-y. PMID: 39143132.
2. Pieske B, Tschope C, De Boer RA, De, Fraser AG, Anker SD, Donal E, Edelmann F, Fu M, Guazzi M, Lam CSP, Lancellotti P, Melenovsky V, Morris DA, Nagel E, Pieske-Kraighe E, Ponikowski P, Solomon SD, Vasan RS, Rutten FH, Voors AA, Ruschitzka F, Paulus WJ, Seferovic P, Filippatos G. How to diagnose heart failure with preserved ejection fraction: The HFA-PEFF diagnostic algorithm: a consensus recommendation from the Heart Failure Association (HFA) of the European Society of Cardiology (ESC). Eur Heart J. 2019; 40:3297–3317. https://doi.org/10.1093/eurheartj/ehz641.

3. Borlaug BA, Nishimura RA, Sorajja P, Lam CS, Redfield MM. Exercise hemodynamics enhance diagnosis of early heart failure with preserved ejection fraction. Circ Heart Fail. 2010; 3:588–595. https://doi.org/10.1161/CIRCHEARTFAILURE.109.930701.
4. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: Developed by the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) With the special contribution of the Heart Failure Association (HFA) of the ESC. Theresa A McDonagh, Marco Metra, Marianna Adamo, Roy S Gardner, Andreas Baumbach, Michael Böhm, Haran Burri, Javed Butler, Jelena Čelutkienė, Ovidiu Chioncel, John G F Cleland, Andrew J S Coats, Maria G Crespo-Leiro, Dimitrios Farmakis, Martine Gilard, Stephane Heymans, Arno W Hoes, Tiny Jaarsma, Ewa A Jankowska, Mitja Lainscak, Carolyn S P Lam, Alexander R Lyon, John J V McMurray, Alexandre Mebazaa, Richard Mindham, Claudio Muneretto, Massimo Francesco Piepoli, Susanna Price, Giuseppe M C Rosano, Frank Ruschitzka, Anne Kathrine Skibelund, ESC Scientific Document Group. European Heart Journal. 2021; 42: 3599–3726. https://doi.org/10.1093/eurheartj/ehab670.
5. Thomas P. Olson, PHD, Bruce D. Johnson, PHD, Barry A. Borlaug, MD. Impaired Pulmonary Diffusion in Heart Failure With Preserved Ejection Fraction. JACC Heart Fail 2016 Jun; 4(6):490-8. doi: 10.1016/j.jchf.2016.03.001.
6. Fermoyle CC, Stewart GM, Borlaug BA, Johnson BD. Simultaneous measurement of lung diffusing capacity and pulmonary hemodynamics reveals exertional alveolar-capillary dysfunction in heart failure with preserved ejection fraction. J Am Heart Assoc. 2021;10(16). doi:10.1161/JAHA.120.019950.
7. Guazzi M, Pontone G, Brambilla R, Agostoni P, Rèina G. Alveolar--capillary membrane gas conductance: a novel prognostic indicator in chronic heart failure. Eur Heart J. 2002 Mar;23(6):467-76. doi: 10.1053/euhj.2001.2803. PMID: 11863349.
8. Weaver LJ, Carrico CJ. Congestive heart failure and edema. In: Staub NC, Taylor AE. Edema. New York, NY: Raven Press; 1984.
9. Jambrik Z, Monti S, Coppola V, et al. Usefulness of ultrasound lung comets as a nonradiologic sign of extravascular lung water. *American Journal of Cardiology*. 2004; 93(10):1265-1270. doi:10.1016/j.amjcard.2004.02.012.

   Agricola E, Picano E, Oppizzi M, et al. Assessment of Stress-induced Pulmonary Interstitial Edema by Chest Ultrasound During Exercise Echocardiography and its Correlation with Left Ventricular Function. *Journal of the American Society of Echocardiography*. 2006; 19(4): 457-463. doi:10.1016/j.echo.2005.11.013.

10. Scali MC, Cortigiani L, Simionuc A, Gregori D, Marzilli M, Picano E. Exercise-induced B-lines identify worse functional and prognostic stage in heart failure patients with depressed left ventricular ejection fraction. Eur J Heart Fail. 2017; 19(11): 1468-1478. doi: 10.1002/ejhf.776.
11. Roughton FJ and Forster RE. Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lung, with special reference to true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. J Appl Physiol 1957; 11: 290–302. doi: 10.1152/jappl.1957.11.2.290.
12. Guazzi M, Bandera F, Ozemek C, Systrom D, Arena R. Cardiopulmonary exercise testing: what is its value? J Am Coll Cardiol 2017; **70**:1618–36.
13. Guazzi M, Arena R, Halle M, Piepoli MF, Myers J, Lavie CJ. 2016 Focused Update: Clinical Recommendations for Cardiopulmonary Exercise Testing Data Assessment in Specific Patient Populations. Circulation 2016;133:e694-711. doi: 10.1161/CIR.0000000000000406.
14. Scali MC, Zagatina A, Simova I, Zhuravskaya N, Ciampi Q, Paterni M et al. B-lines with lung ultrasound: the optimal scan technique at rest and during stress. Ultrasound Med Biol. 2017; 43: 2558–66.
15. Merli E, Ciampi Q, Scali MC, Zagatina A, Merlo PM, Arbucci R et al. Pulmonary congestion during exercise stress echocardiography in ischemic and heart failure patients. Circ Cardiovasc Imaging 2022; 15(5): e013558. doi: 10.1161/CIRCIMAGING.121.013558.
16. Austin PC. An Introduction to Propensity Score Methods for Reducing the Effects of Confounding in Observational Studies. Multivariate Behav Res 2011. PMID: 21818162.
17. Van Iterson EH, Johnson BD, Borlaug BA, Olson TP. Physiological dead space and arterial carbon dioxide contributions to exercise ventilatory inefficiency in patients with reduced or preserved ejection fraction heart failure. *Eur J Heart Fail*. 2017;19:1675–1685. doi: 10.1002/ejhf.913.
18. Obokata M, Olson TP, Reddy YN, Melenovsky V, Kane GC, Borlaug BA. Haemodynamics, dyspnoea, and pulmonary reserve in heart failure with preserved ejection fraction. *Eur Hear J*. 2018; 39:2810–2821. doi: 10.1093/eurheartj/ehy268.
19. Reddy YNV, Obokata M, Wiley B, Koepp KE, Jorgenson CC, Egbe A, Melenovsky V, Carter RE, Borlaug BA. The haemodynamic basis of lung congestion during exercise in heart failure with preserved ejection fraction. *Eur Heart J*. 2019; 40:3721–3730. doi: 10.1093/eurheartjehz713.
20. Fayyaz AU, Edwards WD, Maleszewski JJ, Konik EA, DuBrock HM, Borlaug BA, Frantz RP, Jenkins SM, Redfield MM. Global pulmonary vascular remodelling in pulmonary

hypertension associated with heart failure and preserved or reduced ejection fraction. *Circulation*. 2018; 137:1796–1810. doi: 10.1161/CIRCULATIONAHA.117.031608.

21. Dupuis J, Guazzi M. Pathophysiology and clinical relevance of pulmonary remodelling in pulmonary hypertension due to left heart diseases. Can J Cardiol. 2005;31(4):416-29. doi: 10.1016/j.cjca.2014.10.012.
22. YS Lee. Electron microscopic studies on the alveolar-capillary barrier in the patients of chronic pulmonary edema. Jpn Circ J. 1979; 43(10): 945-54. doi: 10.1253/jcj.43.945.
23. Pezzuto B, Contini M, Berna G, et al. Dynamic trend of lung fluid movement during exercise in heart failure: From lung imaging to alveolar-capillary membrane function. *Int J Cardiol*. 2024; 407. doi:10.1016/j.ijcard.2024.132041.
24. Cattadori G, Wasserman K, Meloni C, Mustaq S, Contini M, Apostolo A, Andreini D, Magrì D, Sciomer S, Veglia F, Berna G, Introcaso G, Palermo P, Fiorentini C, Agostoni P. Alveolar membrane conductance decreases as BNP increases during exercise in heart failure. Rationale for BNP in the evaluation of dyspnea. J Card Fail. 2009 Mar;15(2):136-44. doi: 10.1016/j.cardfail.2008.10.002. Epub 2008 Nov 28. PMID: 19254673.
25. Scali MC, Zagatina A, Ciampi Q et al. Lung Ultrasound and Pulmonary Congestion During Stress Echocardiography. JACC Imaging. 2020; 13:2085-2095. https://doi.org/10.1016/j.jcmg.2020.04.020.

Table 1. Patient Characteristics and medications

	Controls n = 26	HFpEF n = 40	p-value
Patient characteristics			
Age (yrs)	61±13	75±8	<0,001
Sex (% female)	65	57	0,5
Body Mass Index (kg/m^2)	25±4	27,1±4,3	0,047
BSA (m^2)	1,76±0,19	1,8±0,2	0,4
NYHA functional class I/II/III	26/0/0	0/30/10	<0,001
Hypertension (%)	69	80	0,06
Smoking history (%)	15	20	0,1
Diabetes mellitus (%)	0	32	<0,001
NT-proBNP (pg/ml) at rest	52,5 (20-296)	362 (30-2940)	<0,001
Hemoglobin (g/dl)	13,4±0,92	13,5±1,9	0,1
Creatinine (mg/dl)	1±0,19	1,2±0,6	0,1
eGFR (ml/min per m^2)	78±12,6	52±16,6	0,02
Atrial Fibrillation (%)	0	25%	<0,001
Medications			
Loop diuretic agent (%)	0	50	<0,001
ACE-I (%)	15	32	0,4
ARB (%)	35	35	0,6
MRA (%)	0	32	0,02
B-Blockers (%)	38	56	0,23
Calcium Channel Blockers (%)	27	37	0,32
SGLT2-i (%)	0	27	0,01

Abbreviations: BSA, Body Surface Area; NYHA, New York Heart Association; NT-ProBNP, N-Terminal Pro B-type Natriuretic Peptide; ACE-I, Angiotensin-Converting Enzyme Inhibitors; ARB, Angiotensin Receptor Blockers; MRA, Mineralocorticoid Receptor Antagonist; SGLT2-i, Sodium-Glucose Transporter 2-Inhibitors.

Table 2. Resting cardiopulmonary function, lung diffusion, B-lines and echocardiographic data

	Controls n = 26	HFpEF n = 40	p-value
Resting cardiopulmonary function			
VO2 (ml/kg/min)	6,2±2,3	5,7±1,7	0,3
Rest respiratory exchange ratio	0,76±0,06	0,74±0,06	0,4
V_E (l/min)	8,1±1,5	9,2±2,2	0,2
PetCO2 (mmHg)	32,1±4,1	29,3±3,5	0,004
Lung diffusion and congestion			
DLCO (ml/mm Hg/min)	19,7±5,5	16,7±5,7	0,04
Dm (ml/mmHg/min)	23,1±7,9	19,3±7,6	0,048
VA (l)	4,79±1,31	4,42±1,51	0,32
Vcap (ml)	112,7±32	136,7±41,4	0,42
B-Lines (n)	0,77±0,8	2,7±2,7	<0,001
Cardiac hemodynamic and Echocardiography			
Heart rate (beats/min)	73±13	70±13	0,39
Systolic BP (mm Hg)	135±19	133±16	0,73
Ejection Fraction (%)	59,4±6,9	58,7±6,4	0,68
LV mass index (mg/m2)	71,1±21	89,9±24,7	0,04
LA volume index (ml/m2)	19,9±7,34	41,7±12,3	<0,001
E/e'_{avg} ratio	7,9±2,5	11±4,7	<0,001
Cardiac Output (l/min)	4,2±0,9	3,9±1	0,35
SV (ml/beat)	58±11,3	57,4±13,9	0,83
PASP (mmHg)	25,2±4,4	31,7±6,8	<0,001
mPAP (mmHg)	17,4±2,7	21±4,16	<0,01

TAPSE/PASP (mm/mmHg)	0,88±0,21	0,62±0,17	<0,001

Abbreviations: VO2, Oxygen consumption; V_E, minute Ventilation; PetCO2, End-tidal CO2 pressure; DLCO, Diffusing capacity of the Lungs for Carbon Monoxide; Dm, membrane conductance; VA, Alveolar Volume; Vcap, capillary Volume; BP, Blood Pressure; LV, Left Ventricle; LA, Left Atrium; SV, Stroke Volume; PASP, Pulmonary Artery Systolic Pressure; mPAP mean Pulmonary Systolic Pressure; TAPSE, Tricuspid Annular Plane Systolic Excursion.

Table 3. Cardiopulmonary function, lung diffusion and congestion, cardiac hemodynamic and echocardiography during exercise

	Controls n = 26	HFpEF n = 40	p-value
Cardiopulmonary function			
Peak workload (W)	103,6±47,3	53±25,7	<0,001
Peak VO2 (ml/kg/min)	21,8±5,9	14,2±3,0	<0,001
Peak respiratory exchange ratio	1,05±0,9	0,96±0,98	0,002
VO2 at VAT (ml/kg/min)	16,7±2,9	11,2±4,2	<0,001
VE/VCO2 slope	30,8±5,4	38,9±7,8	<0,001
PetCO2 (mmHg)	35,3±5,6	31,9±5	0,02
VO2/HR (ml/min/kg/bpm)	11,3±3,8	10±2,7	0,16
V_E (l/min)	58,2±15,4	40,3±11,7	<0,001
Lung congestion and natriuretic peptide			
B-Lines (n)	1,1±1	5,4±3,3	<0,001
NT-proBNP (pg/ml) at peak	60,5 (20-328)	386 (31-3130)	<0,001
Cardiac hemodynamic and Echocardiography			
HR (beats/min)	130,7±18,6	107,4±21,1	<0,001
Systolic BP (mm Hg)	175,8±26,4	157,8±22,5	0,007
LA volume index (ml/m2)	20,7±8,3	40,7±10,8	<0,001
E/E'_{avg} ratio	8,8±2,8	14,5±5,5	<0,001
Cardiac Output (l/min)	8,75±1,77	6,76±1,71	<0,001
SV (ml/beat)	68,8±11,7	64,4±14,9	0,186
PASP (mmHg)	41,5±7,8	50,5±7,1	<0,001
mPAP (mmHg)	27,3±4,78	32,7±4,26	<0,001
TAPSE/PASP (mm/mmHg)	0,66±0,22	0,41±0,11	<0,01

mPAP/CO slope (mmHg/L/min)	2,1±0,3	4,24±0,9	<0,001

Abbreviations: VO2, Oxygen consumption; VAT, Ventilatory Anaerobic Threshold; VE/VCO2 slope, minute Ventilation/Carbon Dioxide production; PetCO2, end-tidal CO2 Pressure; HR, Heart Rate; V_E, minute Ventilation; NT-ProBNP, N-Terminal Pro B-type Natriuretic Peptide; BP, Blood Pressure; LA, Left Atrial; SV, Stroke Volume; PASP, Pulmonary Artery Systolic Pressure; mPAP, mean Pulmonary Systolic Pressure; TAPSE, Tricuspid Annular Plane Systolic Excursion; CO, Cardiac Output.

Highlights

- HFpEF patients exhibit lung congestion at peak exercise compared to controls
- Impaired pulmonary diffusing capacity at rest is relevant in HFpEF pathophysiology
- Dm and DLCO at rest are correlate with exercise B lines
- A reduction in pulmonary gas exchange seems predictive of B-lines extent during exercise in HFpEF