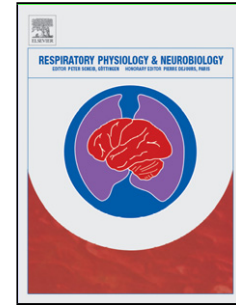


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**Surfactant proteins changes after acute hemodynamic improvement in patients
with advanced chronic heart failure treated with Levosimendan**

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HIGHLIGHTS

Levosimendan improves cardiopulmonary hemodynamics and reduces lung fluids in HF.

BNP levels and cardiopulmonary exercise test parameters improved after infusion.

Levosimendan did not change DLCO, reduced SPs except for mature SP-B which increased.

SPs are fast responders to alveolar-capillary membrane condition changes.

ABSTRACT

Alveolar-capillary membrane evaluated by carbon monoxide diffusion (DLCO) plays an important role in heart failure (HF). Surfactant Proteins (SPs) have also been suggested as a worthwhile marker. In HF, Levosimendan improves pulmonary hemodynamics and reduces lung fluids but associated SPs and DLCO changes are unknown.

Sixty-five advanced HF patients underwent spirometry, cardiopulmonary exercise test (CPET) and SPs determination before and after Levosimendan.

Levosimendan caused natriuretic peptide-B (BNP) reduction, peak VO_2 increase and VE/VCO₂ slope reduction. Spirometry improved but DLCO did not. SP-A, SP-D and immature SP-B reduced (73.7 ± 25.3 vs. 66.3 ± 22.7 ng/mL*, 247 ± 121 vs. 223 ± 110 ng/mL*, 39.4 ± 18.7 vs. 34.4 ± 17.9 AU*, respectively); while mature SP-B increased (424 ± 218 vs. 461 ± 243 ng/mL, *=p<0.001).

Spirometry, BNP and CPET changes suggest hemodynamic improvement and lung fluid reduction. SP-A, SP-D and immature SP-B reduction indicates a reduction of inflammatory stress; conversely mature SP-B increase suggests alveolar cell function restoration.

In conclusion, acute lung fluid reduction is associated with SPs but not DLCO changes. SPs are fast responders to alveolar-capillary membrane condition changes.

KEYWORDS: Alveolar capillary membrane; gas diffusion; surfactant proteins

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INTRODUCTION

Lung function abnormalities play an important role in chronic and acute heart failure (HF) being both lung mechanics and gas exchange impaired (Agostoni et al., 2006; Chua and Coats, 1995; Hosenpud et al., 1990; Naum et al., 1992; Puri et al., 1995). Clinically we know that dyspnea, one of the pivotal symptom of HF, arise from the lung and that lung dysfunction severely impedes cardiac performance via cardio-pulmonary interaction. In particular, alveolar-capillary membrane abnormalities, which include increase in interstitial fluids, reduction in the number of the alveolar-capillary units, interstitial fibrosis, local thrombosis and an increase in cellularity, have a role in HF syndrome and significantly influence its clinical course (Guazzi et al., 2002). Alveolar capillary membrane dysfunction is most frequently analyzed in terms of functional abnormalities using carbon monoxide or nitric oxide as markers of lung diffusion (Graham et al., 2017; Zavorsky et al., 2017), but recently several surfactant proteins (SPs), both in the blood and alveolar fluid, have been proposed as biological indicators of alveolar capillary membrane damage including SP type A, B and D. (Banfi and Agostoni, 2016; Gargiulo et al., 2014; Swenson et al., 2002; Whitsett and Weaver, 2002). Specifically SP-A has been suggested as a predictor of lung damage produced by smoking and high altitude (Kobayashi et al., 2008; Swenson et al., 2002), SP-D as a predictor of cardiovascular morbidity and mortality over classical risk factors as well as a prognostic marker of chronic kidney and lung disease, while SP-B, both in its immature and mature forms, has been proposed as a biomarker of an alveolar capillary barrier damage in HF (De Pasquale et al., 2004; De Pasquale et al., 2003; Magri et al., 2009). Specifically, an increased SP-B immature form plasma level has been reported in chronic HF with a strong correlation with alveolar capillary membrane gas diffusion (Magri et al., 2009). Moreover, SP-B, which differently from others SPs is produced only in alveolar cells, has a definite prognostic role in chronic HF (De Pasquale et al., 2004; Magri et al., 2015). SP-B has also been reported to be elevated in acute pulmonary edema and to

significantly increase whenever the lung is acutely stressed as during positive pressure ventilation (Agostoni et al., 2011a; De Pasquale et al., 2003). At present, the correlation between SPs plasma levels and acute hemodynamic changes in severe HF is unknown, but there is a need to better understand the alveolar cell response to an hemodynamic improvement and its correlation to alveolar capillary membrane gas diffusion. To do so we used the inodilator Levosimendan which combines positive inotropic, vasodilator and cardioprotective effects, without increase of myocardial oxygen consumption, and it has been shown to rapidly improve symptoms and hemodynamics in patients with HF (Nieminen et al., 2013). Furthermore, it sustains the normalization of neurohormones levels (Nieminen et al., 2013). Recently we demonstrated that, on top of standard medical treatment, Levosimendan treatment in severe HF patients significantly promotes oxygen uptake at peak exercise (peak VO_2) and reduces ventilation efficiency (VE/VCO_2 slope) and brain natriuretic peptide (BNP) compared to placebo, (Mushtaq et al., 2015).

The aim of this study was therefore to evaluate whether the positive effects of Levosimendan are associated with a change of SPs production and whether this translates in an alveolar capillary membrane function improvement.

METHODS

Patients selection

Patients who according to the European Society of Cardiology definitions (Ponikowski et al., 2016) had advanced (AD) HF were evaluated for the present study. Specifically, to be eligible patients had to have severe HF symptoms [New York Heart Association (NYHA) classes III to IV], multiple episodes of fluid retention and/or peripheral hypoperfusion, and objective evidence of severe cardiac dysfunction. Moreover, patients had also severe impairment of functional capacity, history of >1 HF hospitalizations in the past 6 months, and the presence of all the previous features despite

optimal medical therapy. All patients belonged to a cohort of chronic HF patients regularly followed at our institution and had experience with cardiopulmonary exercise test (CPET) in our laboratory.

Patients were hospitalized because of acute hemodynamic instabilization, but, at study enrollment, patients were returned to a stable clinical condition (NYHA III/IV). Patients were free from both inotrope support and other i.v. therapies for at least 48 h prior to study inclusion, except for i.v. diuretics therapy. Other study inclusion criteria were: left ventricular ejection fraction at echocardiography $\leq 35\%$, age ≥ 18 years, peak $\text{VO}_2 \leq 12$ mL/min/kg, peak respiratory quotient ≥ 1.05 , and standard HF therapy.

Exclusion criteria were: ongoing mechanical ventilation, recent or acute coronary and respiratory syndromes, recent sustained ventricular tachycardia/ventricular fibrillation/cardiopulmonary resuscitation maneuvers, severe aortic, pulmonary or mitral valve disease (or known malfunctioning artificial heart valve), hypertrophic cardiomyopathy, uncorrected thyroid disease, presence of any comorbidities which might *per se* influence exercise performance. Patients with left ventricle assist devices as well as patients with a pacemaker-guided heart rate at rest or during exercise and patients in which Levosimendan was not indicated were also excluded from the present study.

Patients' eligibility was assessed after 48 to 72 h of clinical stabilization including medical history, physical examination and standard echocardiography. Afterwards a blood sample was taken for general chemistry including BNP, hemoglobin, creatinine levels, and blood urea nitrogen, and for SPs determination. Thereafter complete spirometry and CPET were performed. Levosimendan infusion started approximately 3 h after tests had been completed. Levosimendan (12.5 mg in 500 mL in 5% glucose solution) was infused starting at 0.05 $\mu\text{g}/\text{kg}/\text{min}$, and progressively increased up to 0.2 $\mu\text{g}/\text{kg}/\text{min}$, based on patient clinical status and blood pressure, until the entire infusion had been administered. Notably no Levosimendan bolus was administered. Within 24 h after infusion completion blood samples, complete spirometry and CPET were repeated.

All patients provided written informed consent before entering the study. The protocol was approved by the Institutional Ethics Board (Cardiology Center Ethical Committee, N° S199/312).

Study Procedures

Standard spirometry and lung diffusion for carbon monoxide (DLCO) measurements were always performed before CPET by expert medical personnel. Forced expiratory volume in 1 s (FEV₁) and vital capacity (FVC) were measured in triplicate and calculated according to the American Thoracic Society criteria (Graham et al., 2017; Miller et al., 2005) using a mass flow sensor (Sensor Medics 2200, Sensor Medics Co., Yorba Linda, CA, USA). DLCO and its subcomponents membrane diffusion (DM) and capillary volume (VCap) were measured by the single-breath method (Sensor Medics 229D, Sensor Medics Yorba Linda, CA) by breathing gas mixtures containing three different O₂ concentrations (21%, 40% and 60%) and 0.3% CO and 0.3% methane (CH₄) as originally described by Roughton and Forster (Roughton and Forster, 1957).

DLCO was corrected for hemoglobin (Graham et al., 2017; Roughton and Forster, 1957). Prediction equations were derived for FEV₁ and FVC from Miller et al. (Miller et al., 2005) and for DLCO from Graham et al. (Graham et al., 2017) and Huang et al. (Huang et al., 1994).

CPET (Sensor Medics Vmax 29D, Sensor Medics, Yorba Linda, CA) was performed on a cycloergometer (Sensor Medics Ergo 800S, Sensor Medics, Yorba Linda, CA) using a personalized ramp protocol, aimed at achieving peak exercise in 10 min (Agostoni et al., 2005a), calculated on clinical status and previous CPET. If the exercise duration was < 7 min, the CPET was repeated the following day; in these cases, the blood chemistry measurements were also repeated. Expiratory O₂, CO₂, and ventilation (VE) were measured breath by breath. Peak VO₂ was considered as the highest VO₂ achieved during the exercise (mean of 20 s). Percentage of predicted peak VO₂ was derived from Hansen and Wasserman regression equation (Wasserman et al., 2012). The VO₂ vs. work-rate relationship was calculated throughout the entire exercise. The VE/VCO₂ relationship was calculated as the slope of the linear relationship between VE and VCO₂ from the beginning of the

loaded exercise to the end of the isocapnic buffering period. Oxygen pulse was calculated as $VO_2/\text{heart rate}$. Exercise-induced periodic breathing was defined as a cyclic fluctuation of VE during exercise (Agostoni et al., 2017). Twelve-lead electrocardiograms were also continuously recorded (Case 800, Marquette, Milwaukee, WI, USA). Blood pressure was measured during CPET every 2 min, by sphygmomanometer.

Complete spirometry and CPET were repeated after Levosimendan infusion using the same protocol. Medical and laboratory personnel performing and interpreting pulmonary function and CPET were unaware of the research protocol. CPET readings were performed *a posteriori* by blinded experts.

Specimen handling and assay

SP-B determination was performed as previously described. Briefly, fresh blood (5 mL) was drawn into Vacutainer tubes containing citrate 0.129 mol/L as an anticoagulant. Plasma was immediately prepared by means of centrifugation at $1,500\times g$ for 10 minutes at 4°C , divided into aliquots and frozen at -80°C until assayed. Plasma levels of mature SP-B were performed by an ELISA purchased from Uscn Life Science Inc. (Wuhan, China), with inter-assay and intra-assay coefficient of variation being $11.6\pm 2.1\%$ and $7.9\pm 1.5\%$, respectively. Immature form of SP-B was performed by Western blotting on plasma samples, as previously described (Gargiulo et al., 2014).

Study endpoints

The primary endpoint was to determine the different SP-isoforms changes after Levosimendan infusion. The secondary endpoints were changes in BNP, DLCO, VO_2 and VE/VCO_2 slope after the treatment administrations.

Statistical analysis

Statistical analysis was performed using SPSS 23.0 software (SPSS Inc, Chicago, IL, USA). Continuous variables were expressed as means \pm standard deviation or median and interquartile

range as appropriate, while discrete variables as absolute numbers and percentages. Comparisons between variables pre- and post- treatment were performed using paired t-tests for normally distributed variables, and Wilcoxon signed rank test for non-normally distributed variables. $P < 0.05$ was considered statistically significant.

RESULTS

Sixty-five patients (57 males, age 70 ± 9 years) fulfilled the study inclusion criteria, participated and completed the study without relevant unwanted side effects. HF etiology was ischemic heart disease in 40% of cases and cardiomyopathy in the remaining 60%. Standard echocardiography showed: left ventricle ejection fraction $25 \pm 7\%$, left ventricle end-diastolic volume 213 ± 74 mL and systolic volume 162 ± 66 mL and pulmonary systolic pressure 44 ± 13 mmHg. Before Levosimendan infusion blood sample showed some degree of renal dysfunction and high BNP levels (Table 1). Standard spirometry showed below normal reduced FEV₁ and FVC, 77 ± 16 L and 80 ± 18 L, respectively. DLCO was 70 ± 17 %pred due to a low membrane diffusion partially compensated by an increase in VCap (Table 1). CPET was performed in all cases but in 3 cases exercise performance was extremely limited and CPET judged not evaluable. Baseline peak VO₂ was severely reduced as well as there was a decrease in peak workload, oxygen pulse and the VO₂/work relationship. Conversely VE/VCO₂ slope was high (Table 1). Periodic breathing was observed in 32 cases (54% of cases) with an average on the total population of loaded exercise time with periodic breathing equal to $38\% \pm 0$. Levosimendan infusion was associated with relevant BNP reduction and unchanged kidney function (Table 1). Furthermore a significant increase in peak VO₂ was observed together with reduction in VE/VCO₂ slope. Post Levosimendan infusion FEV₁, FVC and alveolar volume increased while DLCO and all its components were unchanged (Table 1). In parallel all the measured CPET parameters improved (Table 1) and periodic breathing disappeared in 9 out of 32

cases. In patients with periodic breathing at baseline after infusion its lengths was reduced from 71 ± 25 to 38 ± 33 of loaded exercise. None of the patients developed periodic breathing after infusion. All analyzed SPs showed significant but diverging changes after Levosimendan infusion. Specifically, SP-A, SP-D and the immature form of SP-B reduced while the mature form of SP-B increased (Table 2).

DISCUSSION

This study shows that Levosimendan infusion in patients with ADHF significantly improves exercise parameters and reduces BNP levels. This is associated with a reduction of SP-A, D and immature form of SP-B but with an increase of mature SP-B. Regardless DLCO was unchanged.

The population we studied is a classical ADHF population with a very recent hemodynamic instabilization. In a similar population we recently showed, in a double blind placebo controlled study, that Levosimendan infusion is associated with relevant exercise performance improvement and BNP reduction (Mushtaq et al., 2015). Therefore, we conceived the present study to assess the effects of these predicted hemodynamic improvement on DLCO and SPs. As in our previous report (Mushtaq et al., 2015), we observed with acute Levosimendan infusion a $>50\%$ reduction of BNP in parallel to a reduction of VE/VCO₂ slope and an increase of peak VO₂, oxygen pulse and VO₂ work slope. Similarly standard spirometry measurements, as expected with a lung fluid reduction, improved (Agostoni et al., 2000; Guazzi et al., 1999; Puri et al., 1999). Also acute VE/VCO₂ changes are directly linked to lung fluid changes, so that a rapid rise in lung fluids is associated with a VE/VCO₂ increase (Paolillo et al., 2013; Pellegrino et al., 2003; Robertson et al., 2004). Accordingly observed standard spirometry, BNP and CPET parameters changes all-together suggest an hemodynamic improvement and reduction of previously increased lung fluid. However, no

DLCO changes were recorded albeit alveolar volume increased; the latter a datum again supportive of lung fluid reduction. The lack of DLCO improvement should not be considered as an unexpected event. As a matter of fact in a similar population we observed that ultrafiltration, a technique which produces a rapid lung fluid reduction, does not affect DLCO which remains unchanged despite pulmonary mechanics and hemodynamic amelioration both at rest and during exercise (Agostoni et al., 2000; Agostoni et al., 1993; Agostoni et al., 1995; Costanzo et al., 2017). Indeed in chronic HF the alveolar capillary membrane dysfunction is associated with interstitial fibrosis, local thrombosis and an increased in cellularity on top of lung fluid increase. Moreover long term treatment with drugs which marginally affect pulmonary hemodynamic is associated with improvement (ACE-inhibitors and mineralcorticoid receptor antagonists) or worsening (unselective β -blockers) of DLCO (Agostoni et al., 2005b; Contini et al., 2013; Guazzi et al., 1997). The suggested mechanisms are bradikinin increase, antifibrotic action and alveolar β -2 receptor blockage for ACE-inhibitors, mineralocorticoid receptor antagonists blockers and unselective β -blockers, respectively (Agostoni et al., 2005b; Contini et al., 2013; Guazzi et al., 1997).

SPs showed significant and apparently discordant changes after Levosimendan infusion. Reduction of SP-A and D, having both a widespread multiorgan production, may be linked with the well documented antiinflammatory effect of Levosimendan (Adamopoulos et al., 2006; Yilmaz and Mebazaa, 2011). The reduction of the immature form of SP-B likely indicates a reduction of the hemodynamic stress on the alveolar capillary membrane. Indeed an acute hemodynamic and/or respiratory stress on the alveolar capillary membrane increases SP-B plasma level (Agostoni et al., 2011a; Agostoni et al., 2011b; Banfi and Agostoni, 2016). The immature form of SP-B is most unlikely found in the blood stream. When it is found, it is a clear indicator of alveolar cell stress, dysfunction, if not even death. Conversely the finding of the mature form of SP-B in the blood may suggest a restoration of alveolar cell function with an overproduction of SP-B which reaches the blood stream when its intracellular catabolic process is terminated (Banfi and Agostoni, 2016). Clearly the present interpretation of our data is highly speculative but has relevant functional

meaning. Regardless it is totally unknown if a prolonged increase of SP-B mature form leads to DLCO improvement but, in any cases, SPs are fast responder markers of the alveolar capillary membrane function.

This study has several limitations. The most important being that our interpretation is highly speculative with almost no experimental evidence. Regardless it seems to us physiologically convincing. Secondly, we have short term but not long term results of effects of Levosimendan or of prolonged hemodynamic improvement by any cause. Indeed, multiple blood samples for SPs determination would have been desirable for assessing changes of SPs and DLCO with time in case of prolonged HF improvement. Thirdly, we do not know what the effects of possible confounders such as concomitant HF treatment or HF comorbidities are. Fourthly, we do not know what happens on the other side of the alveolar capillary membrane with the likelihood of SPs being elevated in the alveolar fluid of HF patients. However analyzing multiple samples of alveolar fluids was considered inconvenient if not unethical. Fifthly, we do not know what happens with regards to DLCO and SPs changes in case of acute HF without preexisting alveolar capillary damage. Finally, it must be recognized that Levosimendan infusion was not placebo controlled but CPET readings were performed a posteriori by experts in a blind fashion.

In conclusion, we showed several indirect evidences of lung fluid reduction after Levosimendan infusion in patients with ADHF. However, DLCO remains unchanged as the likely consequence of its multifactorial causes, but SP-B changes of both immature and mature forms, albeit opposite, are a signs compatible with alveolar cell functional improvement. The effects of these changes with time on membrane function remain unknown.

REFERENCES

Adamopoulos, S., Parissis, J.T., Iliodromitis, E.K., et al., 2006. Effects of levosimendan versus dobutamine on inflammatory and apoptotic pathways in acutely decompensated chronic heart failure. *The American journal of cardiology* 98, 102-106.

Agostoni, P., Banfi, C., Brioschi, M., et al., 2011a. Surfactant protein B and RAGE increases in the plasma during cardiopulmonary bypass: a pilot study. *The European respiratory journal* 37, 841-847.

Agostoni, P., Banfi, C., Magri, D., et al., 2011b. Kinetics of plasma SPB and RAGE during mechanical ventilation in patients undergoing major vascular surgery. *Respiratory physiology & neurobiology* 178, 256-260.

Agostoni, P., Bianchi, M., Moraschi, A., et al., 2005a. Work-rate affects cardiopulmonary exercise test results in heart failure. *European journal of heart failure* 7, 498-504.

Agostoni, P., Bussotti, M., Cattadori, G., et al., 2006. Gas diffusion and alveolar-capillary unit in chronic heart failure. *European heart journal* 27, 2538-2543.

Agostoni, P., Corra, U., Emdin, M., 2017. Periodic Breathing during Incremental Exercise. in press. *Annals of the American Thoracic Society*.

Agostoni, P., Magini, A., Andreini, D., et al., 2005b. Spironolactone improves lung diffusion in chronic heart failure. *European heart journal* 26, 159-164.

Agostoni, P.G., Guazzi, M., Bussotti, M., et al., 2000. Lack of improvement of lung diffusing capacity following fluid withdrawal by ultrafiltration in chronic heart failure. *Journal of the American College of Cardiology* 36, 1600-1604.

- Agostoni, P.G., Marenzi, G.C., Pepi, M., et al., 1993. Isolated ultrafiltration in moderate congestive heart failure. *Journal of the American College of Cardiology* 21, 424-431.
- Agostoni, P.G., Marenzi, G.C., Sganzerla, P., et al., 1995. Lung-heart interaction as a substrate for the improvement in exercise capacity after body fluid volume depletion in moderate congestive heart failure. *The American journal of cardiology* 76, 793-798.
- Banfi, C., Agostoni, P., 2016. Surfactant protein B: From biochemistry to its potential role as diagnostic and prognostic marker in heart failure. *International journal of cardiology* 221, 456-462.
- Chua, T.P., Coats, A.J., 1995. The lungs in chronic heart failure. *European heart journal* 16, 882-887.
- Contini, M., Apostolo, A., Cattadori, G., et al., 2013. Multiparametric comparison of CARvedilol, vs. NEbivolol, vs. BIsoprolol in moderate heart failure: the CARNEBI trial. *International journal of cardiology* 168, 2134-2140.
- Costanzo, M.R., Ronco, C., Abraham, W.T., et al., 2017. Extracorporeal Ultrafiltration for Fluid Overload in Heart Failure: Current Status and Prospects for Further Research. *Journal of the American College of Cardiology* 69, 2428-2445.
- De Pasquale, C.G., Arnolda, L.F., Doyle, I.R., et al., 2004. Plasma surfactant protein-B: a novel biomarker in chronic heart failure. *Circulation* 110, 1091-1096.
- De Pasquale, C.G., Arnolda, L.F., Doyle, I.R., et al., 2003. Prolonged alveolocapillary barrier damage after acute cardiogenic pulmonary edema. *Critical care medicine* 31, 1060-1067.
- Gargiulo, P., Banfi, C., Ghilardi, S., et al., 2014. Surfactant-derived proteins as markers of alveolar membrane damage in heart failure. *PloS one* 9, e115030.

Graham, B.L., Brusasco, V., Burgos, F., et al., 2017. 2017 ERS/ATS standards for single-breath carbon monoxide uptake in the lung. *The European respiratory journal* 49.

Guazzi, M., Agostoni, P., Bussotti, M., et al., 1999. Impeded alveolar-capillary gas transfer with saline infusion in heart failure. *Hypertension* 34, 1202-1207.

Guazzi, M., Marenzi, G., Alimento, M., et al., 1997. Improvement of alveolar-capillary membrane diffusing capacity with enalapril in chronic heart failure and counteracting effect of aspirin. *Circulation* 95, 1930-1936.

Guazzi, M., Pontone, G., Brambilla, R., et al., 2002. Alveolar--capillary membrane gas conductance: a novel prognostic indicator in chronic heart failure. *European heart journal* 23, 467-476.

Hosenpud, J.D., Stibolt, T.A., Atwal, K., et al., 1990. Abnormal pulmonary function specifically related to congestive heart failure: comparison of patients before and after cardiac transplantation. *The American journal of medicine* 88, 493-496.

Huang, Y.C., Helms, M.J., MacIntyre, N.R., 1994. Normal values for single exhalation diffusing capacity and pulmonary capillary blood flow in sitting, supine positions, and during mild exercise. *Chest* 105, 501-508.

Kobayashi, H., Kanoh, S., Motoyoshi, K., 2008. Serum surfactant protein-A, but not surfactant protein-D or KL-6, can predict preclinical lung damage induced by smoking. *Biomarkers : biochemical indicators of exposure, response, and susceptibility to chemicals* 13, 385-392.

Magri, D., Banfi, C., Maruotti, A., et al., 2015. Plasma immature form of surfactant protein type B correlates with prognosis in patients with chronic heart failure. A pilot single-center prospective study. *International journal of cardiology* 201, 394-399.

- Magri, D., Brioschi, M., Banfi, C., et al., 2009. Circulating plasma surfactant protein type B as biological marker of alveolar-capillary barrier damage in chronic heart failure. *Circulation. Heart failure* 2, 175-180.
- Miller, M.R., Hankinson, J., Brusasco, V., et al., 2005. Standardisation of spirometry. *The European respiratory journal* 26, 319-338.
- Mushtaq, S., Andreini, D., Farina, S., et al., 2015. Levosimendan improves exercise performance in patients with advanced chronic heart failure. *ESC heart failure* 2, 133-141.
- Naum, C.C., Scirba, F.C., Rogers, R.M., 1992. Pulmonary function abnormalities in chronic severe cardiomyopathy preceding cardiac transplantation. *The American review of respiratory disease* 145, 1334-1338.
- Nieminen, M.S., Fruhwald, S., Heunks, L.M., et al., 2013. Levosimendan: current data, clinical use and future development. *Heart, lung and vessels* 5, 227-245.
- Paolillo, S., Pellegrino, R., Salvioni, E., et al., 2013. Role of alveolar beta2-adrenergic receptors on lung fluid clearance and exercise ventilation in healthy humans. *PloS one* 8, e61877.
- Pellegrino, R., Dellaca, R., Macklem, P.T., et al., 2003. Effects of rapid saline infusion on lung mechanics and airway responsiveness in humans. *J Appl Physiol* (1985) 95, 728-734.
- Ponikowski, P., Voors, A.A., Anker, S.D., et al., 2016. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *European heart journal* 37, 2129-2200.

Puri, S., Baker, B.L., Dutka, D.P., et al., 1995. Reduced alveolar-capillary membrane diffusing capacity in chronic heart failure. Its pathophysiological relevance and relationship to exercise performance. *Circulation* 91, 2769-2774.

Puri, S., Dutka, D.P., Baker, B.L., et al., 1999. Acute saline infusion reduces alveolar-capillary membrane conductance and increases airflow obstruction in patients with left ventricular dysfunction. *Circulation* 99, 1190-1196.

Robertson, H.T., Pellegrino, R., Pini, D., et al., 2004. Exercise response after rapid intravenous infusion of saline in healthy humans. *J Appl Physiol* (1985) 97, 697-703.

Roughton, F.J., Forster, R.E., 1957. 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. *Journal of applied physiology* 11, 290-302.

Swenson, E.R., Maggiorini, M., Mongovin, S., et al., 2002. Pathogenesis of high-altitude pulmonary edema: inflammation is not an etiologic factor. *Jama* 287, 2228-2235.

Wasserman, K., Hansen, J., Seu, D., et al., 2012. Principles of exercise testing and interpretation, fifth ed. Wolters Kluwer, Lippincott Williams & Wilkins.

Whitsett, J.A., Weaver, T.E., 2002. Hydrophobic surfactant proteins in lung function and disease. *The New England journal of medicine* 347, 2141-2148.

Yilmaz, M.B., Mebazaa, A., 2011. In vivo and in vitro evidence for pleiotropic effects of levosimendan in the intensive care setting. *Crit Care* 15, 182.

Zavorsky, G.S., Hsia, C.C., Hughes, J.M., et al., 2017. Standardisation and application of the single-breath determination of nitric oxide uptake in the lung. *The European respiratory journal* 49.

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Table 1: Laboratory, spirometry, DLCO and cardiopulmonary exercise test parameters before and after Levosimendan infusion

Parameter	Levosimendan		P-value
	Pre-infusion	Post-infusion	
Blood chemistry			
BNP (pg/mL)	954 (540-1736)	370 (165-874)	<0.001
BUN (mg/dL)	69 (56.0-94.5)	70 (49.0-98.0)	0.59
Creatinine (mg/dL)	1.46 (1.16-1.80)	1.40 (1.09-1.93)	0.12
Spirometry			
FEV ₁ (L)	2.09±0.56	2.26±0.63	<0.001
FVC (L)	2.87±0.74	3.09±0.83	<0.001
DLCO (mL/mmHg/min)	18.3±4.5	17.9±4.1	0.17
VCap (L)	114 (85-177)	120 (104-171)	0.79
DM (mL/min/mmHg)	24±9	22±6	0.07
VA (L)	4.67±1.18	4.84±1.17	0.01
Cardiopulmonary exercise test			
Peak VO ₂ (L/min)	0.74±0.23	0.84±0.22	<0.001
Peak VO ₂ (mL/Kg/min)	10.1±2.36	11.50±2.30	<0.001
Peak VO ₂ (% pred)	44±12.40	50±1.09	<0.001
Peak workload (watt)	42 (32-57)	49 (39-61)	<0.001
Peak O ₂ pulse (mL/beat)	8.2±2.64	8.80±2.65	0.002
VO ₂ /workload slope (mL/min/W)	9.0±1.9	9.6±1.5	0.001
VE/VCO ₂ slope	41±10	36±7	<0.001

BNP, brain natriuretic peptide; BUN, blood urea nitrogen; FEV₁, forced expiratory volume in one second; FVC, vital capacity; VCap, Capillary volume; DLCO, lung diffusion for carbon monoxide adjusted for hemoglobin; DM, membrane diffusion; VA, alveolar volume. VO₂, oxygen uptake; VE, ventilation; VCO₂, CO₂ productio.

Table 2: Pulmonary Surfactant proteins (SPs) levels before and after Levosimendan infusion

Parameter	Levosimendan		P- value
	Pre-infusion	Post-infusion	
SP-A (ng/mL)	73.7±25.3	66.3±22.7	<0.001
SP-D (ng/mL)	247±121	223±110	<0.001
SP-B immature (AU)	39.4±18.7	34.4±17.9	<0.001
SP-B mature (ng/mL)	424±218	461±243	<0.001

SP, Surfactant Protein; AU, arbitrary unit