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Global longitudinal strain assessment of cardiac function and extravascular lung water formation after diving using semi-closed circuit rebreather

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Abstract:	AbstractPurpose: The aim of the present investigation is to study the relationship of ventricular Global Longitudinal Strain (GLS) and ultrasound lung comets (ULC) formation to establish a link between extravascular pulmonary water formation and cardiac contractile dysfunction. Methods: This is a prospective observational study including 14 active military divers. The subjects performed two sea dives of 120 minutes each with a semi-closed SCUBA circuit at 10 meters depth. Divers were examined at baseline, 15 minutes (D1) and 60 minutes (D2) after diving. The

	evaluation included pulmonary and cardiac echography (including speckle tracking techniques). Blood samples were drawn at baseline and after diving, assessing hs-TnT and Endothelin-1.Results: ULC were detected in 9 (64,2 %) and 8 (57,1%) of the subjects after D1 and D2 respectively. No differences were found in right and left ventricular GLS after both immersions (RV: Baseline: -17.9 ± 4.9 vs. D1: -17.2 ± 6.5 and D2: -16.7 ± 5.8 s-1; p=0.757 and p=0.529; LV: Baseline: -17.0 ± 2.3 vs. D1: -17.4 ± 2.1 and D2: -16.9 ± 2.2 s-1; p=0.546 and p=0.783). However, a decrease in atrial longitudinal strain parameters have been detected after diving (RA: Baseline: 35.5 ± 9.2 vs. D1: 30.3 ± 12.8 and D2: 30.7 ± 13.0 s-1; p=0.088 and p=0.063; LA: Baseline: 39.0 ± 10.0 vs. D1: 31.6 ± 6.1 and D2: 32.4 ± 10.6 s-1; p=0.019 and p=0.054).Conclusion: In the present study, no ventricular contractile dysfunction was observed. However, increase pulmonary vasoconstriction markers were present after diving.
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Dear editor,

Please find enclosed our manuscript entitled "**Global longitudinal strain assessment of cardiac function and extravascular lung water formation after diving using semi-closed circuit rebreather"**, which we would like the Editorial Board to consider for publication as Original Article in *European Journal of Applied Physiology.*

Since the description of immersion pulmonary edema (IPE) by Wilmshurst in 1989, the cardiovascular and pulmonary changes proposed are not fully understood. Particularly, the role of ventricle function is still uncertain. Authors have suggested a primary role of the right ventricle increasing cardiac preload thus increasing the pulmonary capillary pressure. Other authors have associated IPE to stress myocardiopathy (Tako-Tsubo syndrome) as an underlying cause of ventricular dysfunction. Although IPE incidence is low, extravascular lung water detection is a common phenomenon. Both entities have almost the same predisposing risk factors such as strenuous exercise, cold water, age > 50 years old, female sex, and the use of rebreathers. More importantly, there is an individual predisposition described for both entities. Since cardiac temporal dysfunction has been proposed, the rapid and accurate detection of this pathological phenomenon may be important to identify those individuals at higher risk for increased extravascular water formation and might developing IPE.

We present a prospective observational study with healthy active Navy divers using a semi-closed rebreather. In order to assess changes in cardiac function, speckle tracking technologies (longitudinal strain), which are more sensitive to any abnormality, have been incorporated.

Our results suggest that there is no ventricular contractile dysfunction, as no marker of impartment was observed after diving, including hs-TnT and global longitudinal strain of both ventricles. However, a decrease in right atrial longitudinal strain and an increase in Endothelin-1 levels have been observed after diving. All these data may suggest a primary involvement of the pulmonary capillary pressure in extravascular water formation after diving, which may explain the individual predisposition to this phenomenon.

We believe that the present study is novel and of current interest. We confirm that this work is original and none of the article contents are under consideration for publication in any other journal or have been published in any journal. No portion of the text has been copied from other material in the literature (unless in quotation marks, with citation). All authors have participated in the work and have reviewed and agree with the content of the article. I am aware that it is the authors' responsibility to obtain permission for any figures or tables reproduced from any prior publications, and to cover fully any costs involved. Such permission must be obtained before final acceptance. We hope that the Editor will find this manuscript suitable for publication in European Journal of Applied Physiology.

Thank you for your consideration.

Kind regards,

Antonio Tello-Montoliu, MD, PhD

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Global longitudinal strain assessment of cardiac function and extravascular lung water formation after diving using semi-closed circuit rebreather

Running Head: Global longitudinal strain after diving

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Authors' contribution

MMV conceived the study, collected data, performed the data analysis, interpreted the results and drafted the manuscript; ATM conceived the study, collected data, performed the data analysis, interpreted the results and drafted the manuscript; AO conceived the study, interpreted the results and drafted the manuscript; AP conceived the study, interpreted the results and drafted the manuscript DS performed the

data analysis, interpreted the results and drafted the manuscript; SM collected data, and drafted the manuscript; NV collected data, and drafted the manuscript; ACM collected data, and drafted the manuscript; NRP collected data, and drafted the manuscript; NV collected data, and drafted the manuscript; MMH collected data and performed the data analysis; IRM collected data, performed the data analysis and drafted the manuscript; JAV collected data, performed the data analysis and drafted the manuscript; MGN performed the data analysis; GDM collected data, performed the data analysis; DP drafted the manuscript.

Abbreviation List

- BP: blood pressure
- GLS: global longitudinal strain
- HR: heart rate
- Hs-TnT: high sensitivity troponin T
- ICV: inferior cava vein
- IPE: immersion induced pulmonary edema
- LA: left atrium
- LV: left ventricle
- LVEDV: left ventricle end-diastolic volume
- LVESV: left ventricle end-systolic volume
- Nm: nautical mile
- O2sat: Basal oxygen saturation
- RA: right atrium
- RVSF: right ventricle shortening fraction
- SCUBA: self-contained underwater breathing apparatus
- ULC: ultrasound lung comets

Declarations

Funding: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Conflicts of interest/Competing interests: The authors declare that they have no competing interests.

Availability of data and material (data transparency): The data and the study materials will not be made available to other researchers because they are used for other unpublished projects and analyses.

Code availability (software application or custom code): N/A

Ethics approval: The study complied with the Declaration of Helsinki and was approved by the Ethics Committee of the Hospital Central de la Defensa Gómez Ulla in Madrid.

Consent to participate: All subjects provided written informed consent.

Consent for publication (include appropriate statements) All authors have provided their consent

Abstract

Purpose: The aim of the present investigation is to study the relationship of ventricular Global Longitudinal Strain (GLS) and ultrasound lung comets (ULC) formation to establish a link between extravascular pulmonary water formation and cardiac contractile dysfunction.

Methods: This is a prospective observational study including 14 active military divers. The subjects performed two sea dives of 120 minutes each with a semi-closed SCUBA circuit at 10 meters depth. Divers were examined at baseline, 15 minutes (D1) and 60 minutes (D2) after diving. The evaluation included pulmonary and cardiac echography (including speckle tracking techniques). Blood samples were drawn at baseline and after diving, assessing hs-TnT and Endothelin-1.

Results: ULC were detected in 9 (64,2 %) and 8 (57,1%) of the subjects after D1 and D2 respectively. No differences were found in right and left ventricular GLS after both immersions (RV: Baseline: -17.9 ± 4.9 vs. D1: -17.2 ± 6.5 and D2: -16.7 ± 5.8 s^{-1}; p=0.757 and p=0.529; LV: Baseline: -17.0 ± 2.3 vs. D1: -17.4 ± 2.1 and D2: -16.9 ± 2.2 s^{-1}; p=0.546 and p=0.783). However, a decrease in atrial longitudinal strain parameters have been detected after diving (RA: Baseline: 35.5 ± 9.2 vs. D1: 30.3 ± 12.8 and D2: 30.7 ± 13.0 s^{-1}; p=0.088 and p=0.063; LA: Baseline: 39.0 ± 10.0 vs. D1: 31.6 ± 6.1 and D2: 32.4 ± 10.6 s^{-1}; p=0.019 and p=0.054).

Conclusion: In the present study, no ventricular contractile dysfunction was observed. However, increase pulmonary vasoconstriction markers were present after diving.

Keywords: longitudinal strain, ultrasound lung comets, Endothelin-1, diving medicine.

Introduction

Immersion pulmonary edema (IPE) is a relatively new pathology related to various underwater activities such as swimming, breath-hold diving, and SCUBA diving (Adir et al. 2004; Andersson et al. 2004; Arborelius et al. 1972; Wilmshurst 1989; Wilmshurst et al. 2019). IPE is clinically similar to other forms of pulmonary edema, but usually has a good prognosis and a rapid resolution after surfacing. However, the correct recognition of IPE is crucial since an individual predisposition is suggested (Carter et al. 2014) with frequently recurrences, and the second IPE episode may be fatal (Boussuges et al. 2017; Cochard et al. 2005).

The pathophysiology if IPE is still unclear. Authors have suggested a primary role of the right ventricle, having a right-left ventricle mismatch, increasing cardiac preload thus increasing the pulmonary capillary pressure (Löllgen et al. 1981). Others authors have reported cases of stress myocardiopathy (Tako-Tsubo syndrome) as underlying cause of ventricular dysfunction (Demoulin et al 2020; Gempp et al. 2013). In fact, a rise of natriuretic peptides (such as BNP or NT-proBNP) and troponin were significantly increased among divers admitted with IPE in comparison with those with decompressive sickness, suggesting a primary involvement of ventricles (Louge et al. 2016).

Nowadays, speckle tracking technology has added important diagnostic, and prognostic information about systolic dysfunction beyond classical ejection fraction (Potter and Marwick 2018). The volume-based measurement of left ventricular ejection fraction (LVEF) is fundamentally different from direct measurement of myocardial motion by tissue Doppler imaging and myocardial deformation, being more sensitive to any abnormality (Potter and Marwick 2018). In fact, global longitudinal strain (GLS) has been proposed as the test of choice in guidelines for monitoring of asymptomatic cardiotoxicity related to chemotherapy (Thavendiranathan et al. 2014).

Although IPE incidence is low, extravascular lung water detection is a common phenomenon. Some authors have reported up to 75% of divers showing extravascular lung water detected as ultrasound lung comets (ULC) (Castagna et al. 2017). The rapid and accurate detection of these pathological phenomena may be important to identify those individuals at higher risk for increased ULC formation and might developing IPE (Bates et al. 2011; Boussuges et al. 2006; Marabotti et al. 2013; Peacher et al. 2015). Thus, the aim of the present investigation is to examine the relationship of GLS of both ventricles and ULC

formation assessment after diving. Moreover, the role of biomarkers related with high pulmonary vasoreactivity were also explored after diving.

Methods

The current study was designed as a prospective observational study with healthy volunteers. Our model is based on the use of a semi-closed circuit rebreather since it has been proposed as predisposing risk factor for IPE and extravascular lung water formation (Castagna et al. 2018). Spanish Navy has routinely used semi-closed circuit rebreathers in special operations for more than 30 years. The rebreather system currently used, is a mechanically controlled device made with nonmagnetic materials.

All divers were selected from the navy explosive ordnance disposal (EOD) team of the Spanish Navy 1st Mine Countermeasures Squadron, based in Cartagena-Spain. It is a highly experienced team in military diving (more than 10 years), with a mean of 3-years experience using the present breathing apparatus. All of them were healthy, non-smokers, and had no history or evidence of arterial hypertension cardiovascular, pulmonary, or another invalidating disease. No diving incidents were recorded among the team using any breathing apparatus. Moreover, no IPE cases have ever been reported by any of the team members. Baseline examinations at rest were performed on all subjects.

All recruits were enrolled to complete two sea dives in different days using semi-closed circuit breathing apparatus (Crabe Aqua Lung, Carros, France), with Nitrox mixture 60% as breathing gas. They performed a sea dive at 10 meters depth, during 120 ±10 min to cover 0,75 nm (~1400 m), wearing 5 mm neoprene wet suits. At the deep planned to perform the dive, the equipment delivered an O_2 concentration between 18.95% and 26.75%). Thus, every diver is exposed to PO_2 0,38 – 0,53 ATA. Before diving, all subjects were asked for any symptom related. The present diving exercise was established to perform 2 dives on consecutive days. Both repetitive dives have the same diving profile. However, after surfacing subjects underwent echocardiographic assessment within 15 minutes on first dive (D1) and within 60 minutes on second one (D2) in order to explore possible time-induced changes. The rest of examination protocol was performed within 30 minutes after surfacing after both dives.

In each examination the subjects underwent cardiopulmonary symptom screening, physical examination with blood pressure (BP), heart rate (HR) and basal oxygen saturation (O2sat) measurement. The O2sat was assessed using a fingertip plethysmography device. After this, al subjects underwent a pulmonary ultrasound and echocardiographic examination. Finally, Blood sampling was performed before diving and 30 minutes after diving. The study complied with the Declaration of Helsinki and was approved by the Ethics Committee of the Hospital Central de la Defensa Gómez Ulla in Madrid. All subjects provided written informed consent.

Transthoracic (cardiac and pulmonary) echography was performed by experienced sonographers, using a commercially available echocardiograph (HD15 Philips Healthcare; Best, Netherlands) with a 2 to 4 MHz echocardiographic probe. The image analysis was performed offline by experts in Doppler echocardiography, using commercially available software (Phillips QLAB version 10.7. Koninklijke Philips Electronics N.V. 2016).

For pulmonary assessment, subjects were at rest in supine position. Ultrasound lung images were obtained scanning the anterior and lateral chest on the right and left hemithorax, from the second to the fourth (on the right side to the fifth) intercostal spaces, from the axillary line to the parasternal line at right hemithorax and from the parasternal line to the axillary line at left hemithorax. The presence of extravascular lung water was assessed using lung ultrasound by counting the number of B-lines or ULCs (Jambrick et al. 2014, Lichtenstein et al. 1997, Picano et al. 2006).

Echocardiography was performed with subjects at rest in the left lateral decubitus position. We recorded a four second duration loop long axis view, 3 short axis views (apical, mitral valve and base), apical four-chamber view, apical two and three-chamber view and subcostal long axis view. A pulsed-wave Doppler tracing of transmitral and transtricuspidal blood flow and a pulsed-wave at left outflow tract were also recorded. Left and right chambers size and function parameters were quantified according to current guidelines (Lang et al. 2015). Diastolic properties were assessed according to guidelines (Paulus et al. 2007) by determining transmitral early (E -wave) and late (A -wave) flow velocities on pulsed wave Doppler and by tissue Doppler peak diastolic velocities of the septal and lateral mitral annulus (E′). The E /E′ ratio was calculated.

Strain analysis was performed using Phillips QLAB versión 10.7. Koninklijke Philips Electronics N.V. 2016. The observed traced the endocardial border wall offline to include the myocardial wall. Then, the software automatically tracked wall motion over the entire cardiac cycle. The observer was allowed to manually readjust some but not all aspects of the tracking procedure. Global LS was assessed from the left ventricle (LV), right ventricle (RV), left atrium (LA), and right atrium (RA). For speckle tracking analysis the two-dimensional apical four-chamber view was recorded with a frame rate ranging between 60 and 80 frames/s.

Two blood samples were drawn for every subject: baseline and post-dive. Post-dive blood samples were obtained 30 minutes after surfacing of the first dive. All samples were drawn by antecubital puncture using a 21-gauge needle. Samples were placed into vacutainer blood collecting tubes containing 3.8% trisodium citrate, and serum tubes without stasis and were centrifuged at 3,800 rpm for 15 min. Plasma and serum samples were collected into aliquots and stored at -80 ºC until batch analysis.

The biomarkers analyzed were high sensitivity troponin T (hs-TnT) as a marker of myocardial damage, and Endothelin-1 as a marker related to pulmonary vasoconstriction (Bärtsch et al. 1991). Serum hs-TnT was assayed by an Elecsys Cobas e 601 analyzer (Roche Diagnostics, Mannhein, Germany). Values below the detection limit are indicated as <3 ng/L. Serum concentration of Endothelin-1, was measured using a commercially available ELISA kit (Invitrogen. EIAET1. Endothelin-1 ELISA kit).

Continuous variables were tested for normal distribution using the Kolmogorov–Smirnov test. Continuous variables are presented as mean (± standard deviation, SD), and categorical variables as percentage. Correlations between two continuous variables were performed using the Pearson correlation coefficient, or Spearman rank correlation if the variables were not normally distributed. For analysis proposes, the population was divided into two groups depending on the ULC formation after diving: No ULC formers and ULC–forming divers (No ULC vs ULC-forming). Differences between 2 independent variables were compared using t-Student or U Mann Whitney; and for 2-dependent variables using t-Student for paired samples or Wilcoxon test in parametric or non-parametric distribution. $P < 0.05$ was accepted as statistically significant. Statistical analysis was performed using SPSS v20.0 software (SPSS, Inc., Chicago, IL, USA).

Results

Fourteen Spanish Navy active military divers were recruited. The mean age of subjects was 39.2 ± 5.6 years and the mean body mass index 26.1 ± 2.3 (weight 84.6 ± 11.6 Kg, height 178.6 ± 9.2 cm). All subjects showed normal BP, HR and basal O2sat. Dives were carried out in the morning hours with a constant sea temperature of 22°C and without any stream. Divers performed the immersions without any symptoms of pulmonary edema or other disturbances. Although there were statistical differences between baseline and after dives BP, HR, and O2sat, the difference in those were not enough to reach pathological meaning. All data is depicted in Table 1.

No ULCs were detected at baseline and cardiac parameters were normal (Table 2). ULCs were detected in 9 (64,2%) of the divers after D1 and in 8 (57,1%) subjects after D2. Interestingly, all subjects with ULC after the first dive showed ULC after the second dive as well, except for one subject.

Echocardiographic parameters before and after dives are shown in Table 2. Reduction in left ventricle end-diastolic volume (LVEDV) after D1 was observed (baseline: 113.3 ± 19 mL vs 99.4 ± 10.8 mL; p=0.011), showing a less decrease after D2 (113.3 ± 19 mL vs 102.9 ± 19.6 mL; p=0.105). Left Ventricle end-systolic volume (LVESV) showed a similar pattern after diving (Baseline: 45.1 ± 10.3 mL vs. D1: 36.5 ± 8.9 mL and D2: 35.8 ± 8.7 mL; p= 0.006 and p=0.024 respectively). On the other hand, right ventricle parameters were not altered in this study except for a decrease of right ventricle shortening fraction (RVSF) after D2 (baseline: 45.3 ± 8.65 vs. D1: 43.5 ± 13.1 and D2: 39.4 ± 10.6 % p= 0.035). No differences were found between the ratio RV/LV after diving. Also, no changes in inferior cava vein (ICV) or both atrial dimensions were recorded (data shown in Table 2).

Some differences at LV filling parameters were observed: the mitral E peak decreased significantly after the D1 and D2 (Baseline: 0.80 ± 0.11 m/s vs. D1: 0.63 ± 0.10 m/s and D2: 0.63 ± 0.08; p<0.001 and 0.002 respectively), however, there was no difference in A peak after diving. Thus, the E/A ratio also decreased (Baseline: 1.4 ± 0.21 vs. D1: 1.07 ± 0.22 and D2: 1.10 ± 2.11; p< 0.001 and p=0.005, respectively). However, there was no statistical differences among tissue Doppler filling parameter, only numerically decrease in lateral E/e' index (Baseline: 5.6 ± 1.3 vs. D1: 5.3 ± 0.8 and D2: 5.1 ± 1.02; p=0.423 and p=0.072).

A decrease in atrial LS parameters (LA LS: Baseline: 39.0 ± 10.0 vs. D1: 31.6 ± 6.1 and D2: 32.4 ± 10.6 s^{-1}; p=0.019 and p=0.054; RA LS: Baseline: 35.5 ± 9.2 vs. D1: 30.3 ± 12.8 and D2: 30.7 ± 13.0 s^{-1}; p=0.088 and p=0.063) was observed. However, no differences were found in right ventricle GLS (Baseline: -17.9 ± 4.9 vs. D1: -17.2 ± 6.5 and D2: -16.7 ± 5.8 s^{-1}; p=0.757 and p=0.529), and left ventricle GLS (Baseline: -17.0 ± 2.3 vs. D1: -17.4 ± 2.1 and D2: -16.9 ± 2.2 s^{-1}; p=0.546 and p=0.783) after diving (Figure 1).

All subjects were studied in two groups according to the ULC formation (Table 3). No differences were found between groups except a significant reduction in LVESV in ULC-forming group in comparison with no-ULC group in D1 (ULC-forming: 33.27± 6.26 vs. no-ULC: 46.15 ± 9.18 mL; p=0.019). ULC-forming group showed a significant decrease in RA LS in D1 (ULC-forming: 25.27± 11.22 vs. no-ULC: 41.68 ± 8.36 s^{-1}; p=0.025), with no differences in D2 (ULC-forming :29.12 ± 14.08 vs. no-ULC: 33.06 ± 12.62 s^{-1}; p=0.629) (Figure 2).

No differences were found in hs-TnT levels between baseline and post-dive (baseline: 7.30 ± 3.42 vs. post-dive: 7.01 ± 1.95 ng/L; p=0.734). Endothelin-1 levels showed a non-significant increase after diving (baseline: 14.10 ± 5.4 vs. post-dive: 16.82 ± 6.6 pg/mL; p=0.154)

There were no differences in hs-TnT and Endothelin-1 levels between ULC-forming and No ULC subjects at baseline before diving. After diving, no differences in hs-TnT levels were found (7.00 ± 2.43 vs. 6.72 ± 1.62 ng/L, p=0.822). However, significantly higher levels of Endothelin-1 were observed in ULC-forming than in no-ULC subjects (13.44 ± 6.10 vs. 20.00 ± 5.41 pg/mL, p=0.021) (Figure 3A).

Number of ULC showed a negative moderate correlation with RA LS (r:-0.816, p=0.001). Also, number of ULC correlated positively with Endothelin-1 levels (r:0.606, p=0.028). No other clinically and significantly correlations were observed in this study (Figure 3B).

Discussion

There are several factors associated with asymptomatic changes in cardiovascular and pulmonary physiology, therefore linked to the development of extravascular lung water (Marinovic et al. 2010). Some authors have related this phenomenon to a mismatch between right and left cardiac function, determined as increased systolic arterial pulmonary pressure and increase in right-side pressures measured by echocardiography (Castagna et al. 2017; Löllgen et al.1981; Mahon et al. 2002). Changes showing a LV rapid early filling related to higher pulmonary artery pressures and secondary to an increased work done by the RV has been suggested by those reports (Ware et al. 2005; Bates et al. 2011; Mahon et al. 2002). Besides, similar to stress myocardiopathy, a reduction in left ventricular contractile function has been suggested with common features as the reversible impairment of the LVEF (Demoulin et al. 2020; Gempp et al. 2013; Marinovic et al. 2010). In fact, increased levels of NT terminal part of the brain natriuretic peptide (NT-proBNP) and TnT has been reported as part of the contractile impairment (Louge et al. 2016).

Our study showed different results. We observed no changes in right-ventricle dimensions but a late (D2) decrease in RVSF, which may represent a small increase in right-ventricle post-load. On the other hand, a decrease in E wave peak (although not a clear diastolic dysfunction pattern) was observed after D1, with changes in E/e' lateral index. These findings may represent an increase in left-ventricle pre-load. No reduction in LVEF was observed in this population. Importantly, no impairment of GLS was observed in both ventricles. Although some authors reported reductions in ventricles contractile function, these reductions were within the normal range limits of "preserved" ejection fraction (Marabotti et al. 2013). GLS is a technique from speckle tracking analysis which has been developed as a useful tool identifying populations at risk of heart failure, being the only sign of ventricular dysfunction in those patients (Potter and Marwick 2018). Moreover, there is no rise in the myocardial damage markers assessed as normal hs-TnT levels obtained after diving in all subjects. Our group has also analyzed NT-proBNP levels after diving and no significant increased have been found (data published elsewhere). Finally, no altered RV/LV ratio was demonstrated after dives in this population.

A decrease in atrial LS was observed after diving. Atrial LS is also a speckle tracking related technique that has been promoted as early marker of pulmonary hypertension and is associated with prognosis in

hypertensive patients (Alenezi et al. 2018; Bärtsch et al. 1991; Rai et al.2015). It is important to highlight that all echocardiographic changes were attenuated during time after surfacing (changes observed in D1 but almost disappeared in D2). This observation translates the transitory evolution of this phenomenon after leaving the immersion conditions.

We found an increase in Endothelin-1 levels after diving although it has not reached statistical significance. Endothelin-1 is an endothelium-derived peptide, which acts as a potent and long-lasting pulmonary vasoconstrictor (Giaid et al. 1993; Loeffler et al.1994). Endothelin-1 plays an important role in the regulation of pulmonary vascular tone, and its concentrations are closely associated to the severity of pulmonary hypertension in humans (Carter et al. 2014; Alenezi et al. 2018). This finding suggests a pulmonary vasoconstriction after diving due to the release of this potent agent. The relationship between endothelin-1 levels and high pulmonary pressure has been de described in animal models (45,46), patients with pulmonary hypertension (Giaid et al. 1993), and subjects prone to develop high-altitude pulmonary edema (HAPE) (Sartori et al. 1999; Sartori et al. 2007). HAPE is a form of non-cardiogenic pulmonary edema characterized by exaggerated pulmonary hypertension (Bärtsch et al. 1991; Sartori et al. 2007).

The increase of pulmonary pressure during underwater exercise has been previously demonstrated (Peacher et al. 1985; Wester et al. 2009). In the present investigation, we have corroborated the pulmonary artery increase at shallow immersion (the equivalent to 2 ATA) using a novel technique which perform a direct, non-invasive estimation of the pulmonary capillary pressure.

Interestingly, both atrial LS and Endothelin-1 levels correlated with the ULC formation suggesting the role of pulmonary vasoconstriction in the pathophysiology of extravascular lung water formation.

Some authors have reported an individual predisposition not only to develop IPE but also ULCs. In fact, other dysbaric pathologies as HAPE have been related to an individual predisposition (Carter et al. 2014). The wide variation of pulmonary artery pressure under immersion has been suggested as plausible explanation for this individual predisposition (Peacher et al. 1985). In the present investigation, no differences between ULC-forming and no ULC former subjects were found except for RA LS and endothelin-1 levels (Sartori et al. 1999) have described an exaggerated Endothelin-1 release or reduced clearance in subjects forming to develop HAPE. HAPE and IPE have some similarities, since both are

pathologies due to dysbaric environment exposition. Moreover, both have related an individual predisposition. Finally, endothelin-1 levels have been reported to be similar in HAPE-forming subjects who did and who did not develop pulmonary edema (Sartori et al. 1999). This observation has been interpreted as the exaggerated stimulation of endothelin-1 release is a primary phenomenon and does not occur secondary to edema. This conclusion may be in line with the higher levels in ULC-forming subjects.

It is necessary to highlight that the link between the ULC and the clinically patent IPE is still unsolved. One crucial question is whether ULC-forming subjects would develop an IPE, and what would be the necessary trigger for it. Studies analyzing biomarkers in IPE showed increased (above the cut-off limit) levels mainly of hs-TnT and NT-proBNP (Louge et al. 2016). Thus, these markers might be related with the clinical consequences and not with the preclinical state or the possible trigger. In other words, the rise in the pulmonary pressure should be enough to develop a clinical IPE, and a ventricular overload as consequence (Picano et al. 2006). But what are the possible triggers to reach the pulmonary edema are the next questions to solve. More studies are guaranteed for identifying those individuals.

Finally, we should acknowledge some limitations related with the present study. First, the inherent limitations related with a pilot investigation with arbitrary sample size. Although the risk for bias is present, other authors have chosen similar sizes, mainly because of the difficulty to find enough number of individuals with expertise in diving with those SCUBA systems. Second, the assessment after the dive does not provide the acute effects of the dysbaric environment on echocardiographic assessment. Thus, there might be changes that have been not or partially observed. Another limitation to acknowledge is the relative shallow deep of the immersion in the present study, in comparison with previous reports. The different pressure exposition may explain the different changes found in the echocardiographic examination, particularly in chamber volumes (Castagna et al. 2018, Bates et al. 2011; Boussuges et al. 2006). However, the development of ULC and the changes in pulmonary pressure are present at any deep, suggesting a lack between pressure exposition and extravascular lung water formation (Castagna et al. 2017).

Conclusion

SCUBA diving using a semi-closed circuit rebreather is associated with ULC development. No marker of ventricular contractile dysfunction was observed after diving, including hs-TnT and GLS of both ventricles. However, a decrease in RA LS and an increase in Endothelin-1 levels have been observed after diving, with a strong difference between ULC-forming and no ULC subjects.

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Figure legends

Figure 1. Changes in both atria and ventricles GLS after diving. Results represent baseline value and D1 and D2 values. Lines show standard deviation (SD).

Figure 1 Changes in echocardiographic parameters in No-ULC and ULC-forming subjects. A) Differences in left ventricle volumes after diving. B) Differences in RA LS after diving. Lines denote SD.

Figure 3. A) Differences between no-ULC and ULC-forming subjects in hs-TnT and Endothelin-1 serum levels at baseline and post-diving. Bars represent serum levels, and lines SD. B) Linear correlation between RA LS and Endothelin-1 levels and number of ULC after diving.

Figure 1

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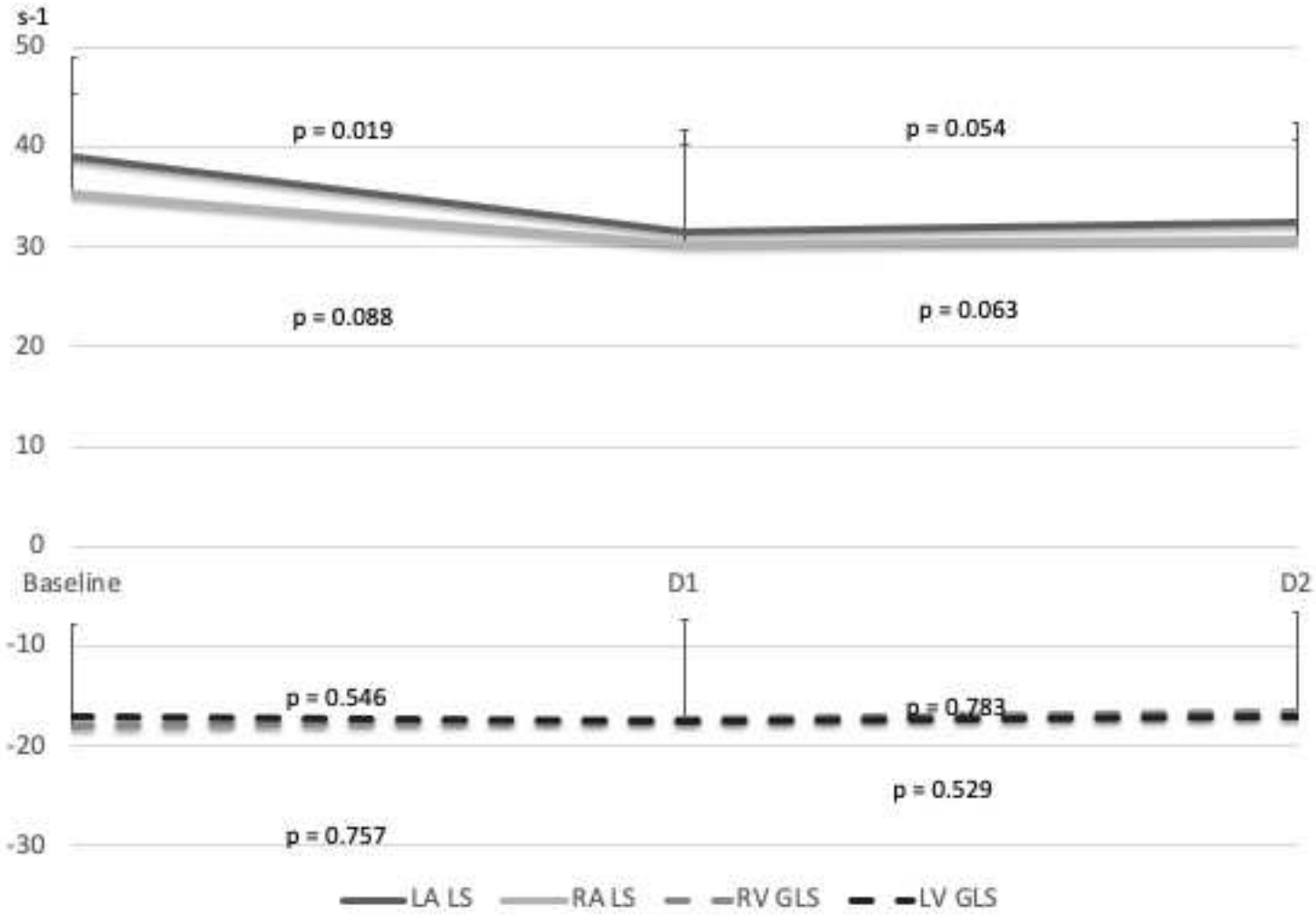

Figure 2

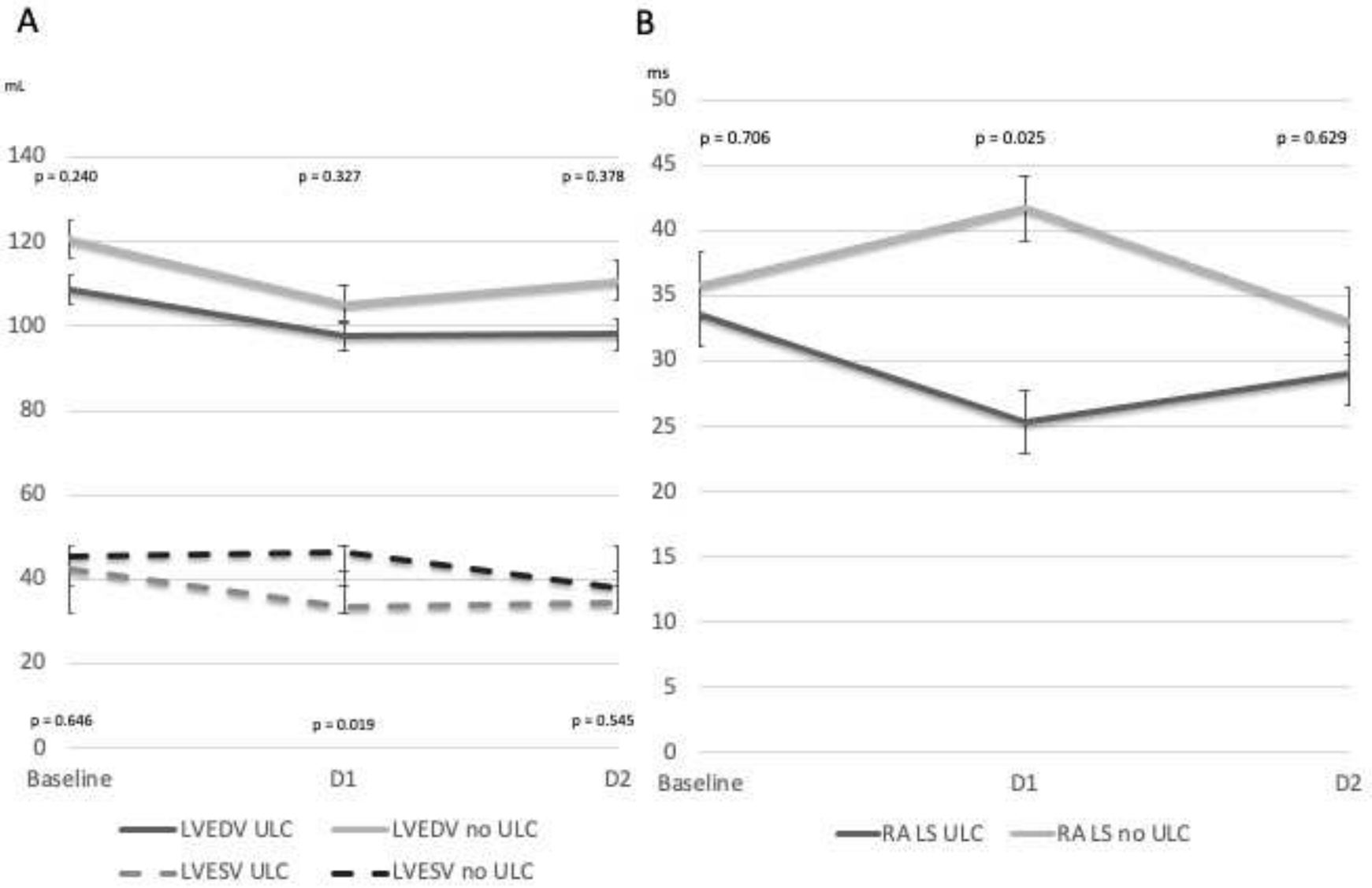

Figure 3

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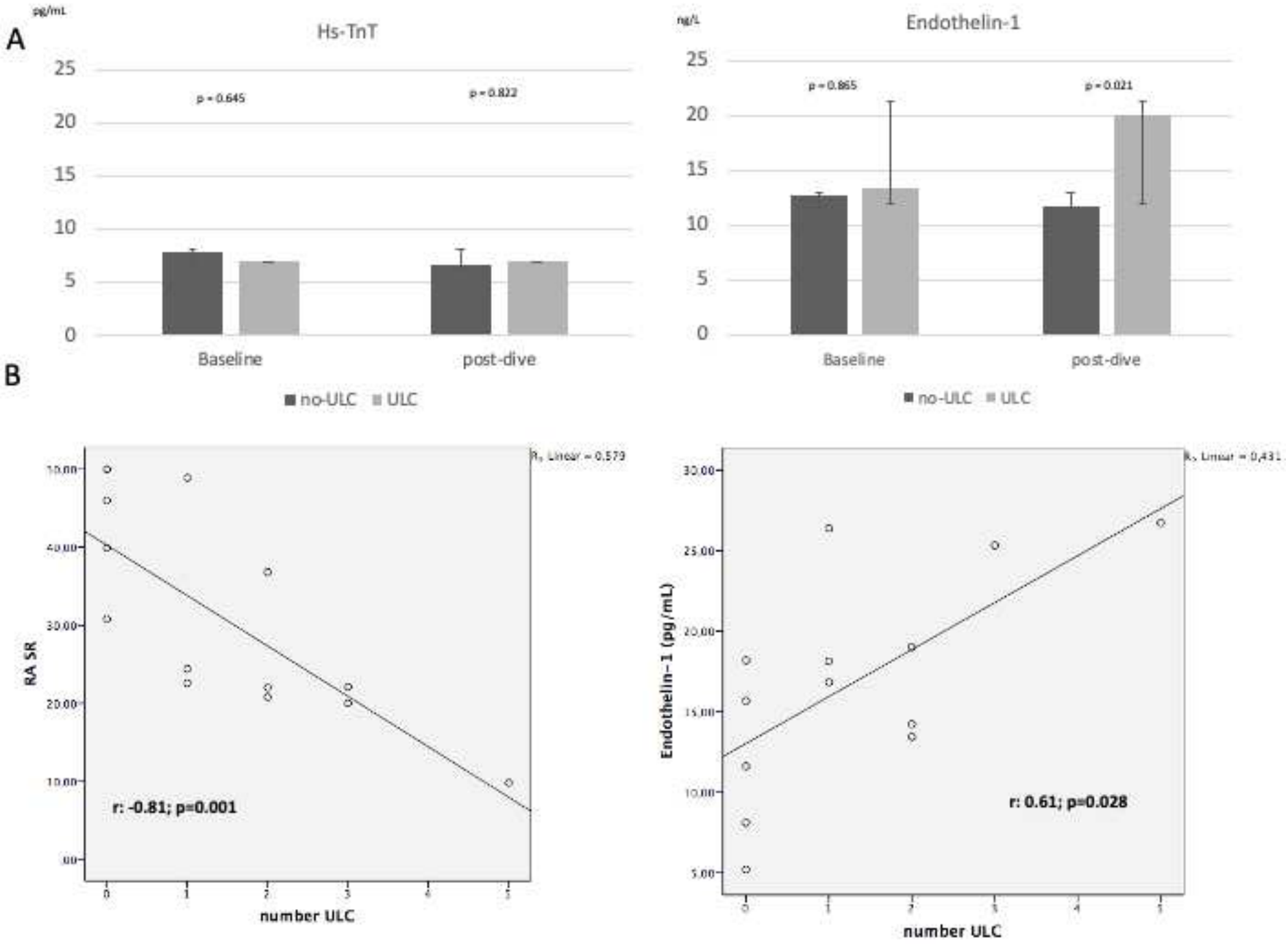

Table 1. Physical examination data (baseline and after dives).

	Baseline	D1	D2	p value*
HR (bpm)	61.6 ± 7.0	77.1 ± 16.7	72.2 ± 10.5	<0.001/0.001
SBP (mmHg)	126.1 ± 8.6	140.8 ± 16.7	136.1 ± 15.4	0.002/0.088
DBP (mmHg)	76.8 ± 7.2	86.4 ± 12.3	83.5 ± 5.1	0.012/0.009
O2sat (%)	98.3 ± 1.3	97.3 ± 1.1	96.8 ± 1.5	0.006/0.003

BMI, body mass index; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; O2sat, oxigen saturation.

* D1 vs basal/ D2 vs basal

Table 2

Table 2. Pulmonary ultrasound and echocardiography data.

	Basal	**D1**	**D2**	**p value***
ULC-forming subjects (%)	0	9 (64.2)	8 (57.1)	
LVEDV (ml)	113.3 ± 19	99.4 ± 10.8	102.9 ± 19.6	0.011/0.105
LVESV (ml)	45.1 ± 10.3	36.5 ± 8.9	35.8 ± 8.7	0.006/0.024
LVEDD (mm)	46.1 ± 3.6	45.3 ± 5.1	46.1 ± 3.2	0.651/0.655
LVESD (mm)	30.6 ± 3.1	30 ± 2.5	30 ± 4.1	0.676/0.241
LVEF (%)	60.3 ± 4.5	63.6 ± 6.1	65.2 ± 5.7	0.079/0.041
LAV (ml)	41.5 ± 16.2	44.9 ± 13.3	44.76 ± 18.2	0.394/0.973
Mitral E peak (m/s)	0.80 ± 0.11	0.63 ± 0.10	0.63 ± 0.08	<0.001/0.002
Mitral A peak (m/s)	0.57 ± 0.07	0.60 ± 0.08	0.59 ± 0.11	0.417/0.938
E/A ratio	1.4 ± 0.21	1.07 ± 0.22	1.10 ± 2.11	<0.001/0.005
E’ septal wall (m/s)	9.6 ± 1.9	7.7 ± 1.2	8.3 ± 1.5	0.006/0.042
E’lateral wall (m/s)	14.6 ± 2.5	12.2 ± 2.7	12.8 ± 2.6	0.027/0.057
Septal E/e’ index	8.6 ± 2.2	8.3 ± 1.4	7.8 ± 1.3	0.588/0.161
Lateral E/e’ index	5.6 ± 1.3	5.3 ± 0.8	5.1 ± 1.02	0.423/0.072
LA LS (s^{-1})	39.0 ± 10.0	31.6 ± 6.1	32.4 ± 10.6	0.019/0.054
LV GLS (s^{-1})	-17.0 ± 2.3	-17.4 ± 2.1	-16.9 ± 2.2	0.546/0.783
RVEDV (ml)	21.8 ± 4.3	21.1 ± 4.7	20.1 ± 7.3	0.634/0.485
RVESV (ml)	12.0 ± 2.6	11.4 ± 2.7	12.5 ± 3.6	0.602/0.690
RVSF (%)	45.3 ± 8.65	43.5 ± 13.1	39.4 ± 10.6	0.748/0.035
TAPSE (mm)	24.7 ± 4.7	23.5 ± 3.2	24.6 ± 3.5	0.537/0.902
RAV (ml)	48.9 ± 17.6	43.5 ± 13.1	39.4 ± 15.0	0.240/0.132

RV S-wave (m/s)	12.6 ± 2.8	12.6 ± 1.8	12.3 ± 2.2	0.966/0.621
RA LS (s^{-1})	35.5 ± 9.2	30.3 ± 12.8	30.7 ± 13.0	0.088/0.063
RV LS (s^{-1})	-17.9 ± 4.9	-17.2 ± 6.5	-16.7 ± 5.8	0.757/0.529
ICVD (mm)	14.1 ± 3.8	16.1 ± 0.4	15.3 ± 0.60	0.230/0.622
Ratio RV/LV	0.83 ± 0.19	0.85 ± 0.8	0.80 ± 0.17	0.690/0.759

ULC, ultrasound lung comets; LVEDV, left ventricle end-diastolic volume; LVESV, left ventricle end-systolic volume; LVEDD, left ventricle end-diastolic diameter; LVESD left ventricle end-systolic diameter; LVEF, left ventricle ejection fraction; LAV, left atrium volume; LA LS, left atrium longitudinal strain; LV GLS, left ventricle global longitudinal strain; RVEDV, right ventricle end-diastolic volume; RVESV, right ventricle end-systolic volume; RVSF, right ventricle shortening fraction; TAPSE, tricuspid annular plane systolic excursion; RAV, right atrium volume; RV S-wave, right ventricle S-wave; RA LS, right atrium longitudinal strain; RV GLS, right ventricle global longitudinal strain; ICVD, inferior cava vein diameter.

* D1 vs basal/ D2 vs basal

Table 3

Table 3. Echocardiographic data divided by ULC-forming and No ULC subjects.

	Basal			**D1**			**D2**		
	No ULC	ULCF	P value	No ULC	ULCF	P value	No ULC	ULCF	P value
LVEDV (ml)	120.6±21.6	108.7±14.4	0.240	105.0±16.8	97.6±8.6	0.327	110.8±28.1	98.0±11.7	0.378
LVESV (ml)	45.2±16.1	42.4±6.9	0.646	46.2±9.2	33.3±6.3	0.019	37.8±11.3	34.6±7.2	0.545
LVEDD (mm)	4.7±0.4	4.6±0.3	0.635	4.7±0.7	4.3±0.3	0.144	4.2±0.7	4.8±0.18	0.202
LVESD (mm)	2.87±0.3	3.15±0.2	0.099	3.1±0.2	2.9±0.2	0.413	3.0±0.6	3.0±0.3	0.947
LVEF (%)	66.1±7.7	60.9±4.8	0.515	56.2±1.7	66.0±4.9	0.001	65.7±6.9	64.9±5.4	0.805
LAV (ml)	50.0±17.3	38.4±14.1	0.196	37.4±13.9	47.5±12.9	0.274	37.8±10.2	50.3±19.3	0.147
Mitral E	88.7±11.3	75.3±8.9	0.037	64.4±7.3	64.3±13.4	0.988	63.2±7.3	64.9±10.1	0.762

peak (m/s)									
Mitral A peak (m/s)	59.4±7.0	56.8±7.1	0.535	56.0±8.0	61.4±8.5	0.266	55.06±8.9	62.8±10.9	0.272
E/A ratio	1.5±0.3	1.3±0.1	0.131	1.2±0.3	1.1±0.3	0.415	1.2±0.3	1.1±0.2	0.398
E' septal wall (m/s)	9.5±1.8	9.7±2.0	0.840	7.6±1.2	7.8±1.2	0.759	8.8±2.0	7.9±1.0	0.279
E'lateral wall (m/s)	14.1±3.2	15.2±2.1	0.465	12.7±2.2	12.5±3.1	0.885	12.2±3.6	3.6±1.6	0.546
Septal E/e' index	9.7±2.7	8.0±1.6	0.178	9.5±1.8	9.7±2.0	0.840	8.7±2.0	8.2±1.2	0.571
Lateral E/e'	6.5±1.4	5.1±0.9	0.036	5.2±0.3	5.3±1.0	0.763	5.5±1.2	5.0±1.0	0.485

index									
LA LS (s^{-1})	37.3±6.9	40.2±11.1	0.602	29.1±8.6	32.7±4.8	0.480	33.3±9.9	31.9±11.7	0.832
LV LS (s^{-1})	-17.4±2.3	-16.8±2.4	0.664	-17.7±2.0	-17.3±2.9	0.752	-17.8±2.3	-16.3±2.0	0.239
RVEDV (ml)	22.3±3.7	21.5±4.9	0.744	20.6±5.7	21.7±5.2	0.699	16.7±4.0	21.4±7.7	0.288
RVESV (ml)	13.5±2.9	11.3±2.4	0.193	10.2±3.5	11.8±2.2	0.319	11.7±2.5	12.8±4.0	0.635
RVSF (%)	42.1±12.8	46.8±10.1	0.383	50.7±3.6	44.5±10.8	0.146	28.7±13.3	38.7±7.2	0.113
TAPSE (mm)	26.2±6.8	23.6±2.3	0.463	24.0±4.4	23.3±2.4	0.734	24.5±2.7	25.0±3.9	0.820
RAV (ml)	50.0±13.3	49.2±19.7	0.935	39.4±2.4	44.4±14.4	0.648	37.8±2.2	40.3±19.2	0.743
RV S-wave	13.3±4.0	12.0±1.4	0.463	12.7±2.4	12.5±1.4	0.823	12.3±1.7	12.0±2.3	0.772

(m/s)									
RA LS (s^{-1})	35.8±8.9	33.7±10.5	0.706	41.7±8.4	25.3±11.2	0.025	33.1v12.6	29.1±14.1	0.629
RV LS (s^{-1})	-18.7±5.7	-17.5±4.8	0.664	-15.7±9.2	-18.1±5.0	0.524	-17.2±6.3	-16.5±5.9	0.836
ICVD (mm)	1.2±0.5	1.5±0.3	0.120	1.6±0.7	1.6±0.2	0.998	1.4±0.7	1.6±0.5	0.702
Ratio RV/LV	0.8±0.1	0.8±0.2	0.697	0.7±0.1	0.9±0.2	0.156	0.8±0.1	0.8±0.2	0.929

ULCF, ultrasound lung comets forming group; LVEDV, left ventricle end-diastolic volume; LVESV, left ventricle end-systolic volume; LVEDD, left ventricle end-diastolic diameter; LVESD left ventricle end-systolic diameter; LVEF, left ventricle ejection fraction; LAV, left atrium volume; LA LS, left atrium longitudinal strain; LV GLS, left ventricle global longitudinal strain; RVEDV, right ventricle end-diastolic volume; RVESV, right ventricle end-systolic volume; RVSF, right ventricle shortening fraction; TAPSE, tricuspid annular plane systolic excursion; RAV, right atrium volume; RV S-wave, right ventricle S-wave, RA LS, right atrium longitudinal strain; RV GLS, right ventricle global longitudinal strain; ICVD, inferior cava vein diameter.

* D1 vs basal/ D2 vs basal

Authors' contribution statement

MMV conceived the study, collected data, performed the data analysis, interpreted the results and drafted the manuscript; ATM conceived the study, collected data, performed the data analysis, interpreted the results and drafted the manuscript; AO conceived the study, interpreted the results and drafted the manuscript; AP conceived the study, interpreted the results and drafted the manuscript DS performed the data analysis, interpreted the results and drafted the manuscript; SM collected data, and drafted the manuscript; NV collected data, and drafted the manuscript; ACM collected data, and drafted the manuscript; NRP collected data, and drafted the manuscript; NV collected data, and drafted the manuscript; MMH collected data and performed the data analysis; IRM collected data, performed the data analysis and drafted the manuscript; JAV collected data, performed the data analysis and drafted the manuscript; MGN performed the data analysis; GDM collected data, performed the data analysis; DP drafted the manuscript.