Prognostic Value of Dynamic Changes in Pulmonary Congestion During Exercise Stress Echocardiography in Heart Failure With Preserved Ejection Fraction

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Short title: Exercise lung ultrasound in HFpEF

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Background: Patients with heart failure (HF) with preserved ejection fraction (HFpEF) typically develop dyspnoea and pulmonary congestion upon exercise. Lung ultrasound (LUS) is a simple diagnostic tool, providing semi-quantitative assessment of extravascular lung water through B-lines. It has been shown that HFpEF patients develop B-lines upon submaximal exercise stress echocardiography; however, whether exercise-induced pulmonary congestion carries prognostic implications is unknown. This study aimed at evaluating the prognostic value of B-line assessment during exercise in HFpEF patients.

Methods: Sixty-one I–II NYHA class HFpEF patients underwent standard echocardiography, LUS (28-scanning point method), and B-type natriuretic peptide (BNP) assessment during supine exercise echocardiography (baseline and peak exercise). The primary endpoint was a composite of cardiovascular death or HF hospitalization at one-year.

Results: B-lines, E/e', and BNP significantly increased during exercise (P <0.001 for all). By multivariable analysis, both peak (HR 1.50, 95% CI 1.21–1.85, P <0.001), and change (HR 1.34, 95% CI 1.12–1.62, P =0.002) B-lines were retained as independent predictors of outcome (HRs per 1 B-line increment), along with BNP and E/e' ratio. Importantly, adding peak B-line on top of a clinical model significantly improved prognostic accuracy (C-index increase=0.157, 0.056 to 0.258, P =0.002), and net reclassification (continuous NRI =0.51, 0.09 to 0.74, P =0.016), with similar results for B-line change.

Conclusions: Detection of exercise-induced pulmonary congestion by LUS is an independent predictor of outcome in HFpEF patients; its use may help refining the routine risk-stratification of these patients on top of well-established clinical variables.

Keywords Heart failure with preserved ejection faction; Pulmonary congestion; B-lines; Lung ultrasound, E/e', BNP.

Abbreviations

HF, heart failure

HFpEF, Heart failure with preserved ejection fraction.

PCWP, pulmonary capillary wedge pressure.

LV, left ventricular

TRV, tricuspid regurgitation velocity

PASP, pulmonary artery systolic pressure

LUS, lung ultrasonography

EVLW, extravascular lung water

BNP, B-type natriuretic peptide

BP, blood pressure

TAPSE, Tricuspid annular peak systolic excursion

SD, standard deviation

IQR, interquartile range

CAD, coronary artery disease

NRI, net reclassification improvement

HFrEF, Heart failure with reduced ejection fraction

Introduction

Heart failure (HF) with preserved ejection fraction (HFpEF) patients tend to develop dyspnoea, hemodynamic derangements, and pulmonary congestion upon exercise (1). Findings from invasive stress test studies demonstrated that increase in pulmonary capillary wedge pressure (PCWP) during exercise is correlated to dyspnoea symptoms (2), and associated with long-term mortality (3) in HFpEF patients. Exercise stress echocardiography allows assessment of symptoms, and estimation of left ventricular (LV) filling pressures during exercise (4). Recommended diastolic parameters during exercise echocardiography are early mitral inflow E-wave velocity and early tissue Doppler-derived mitral annular velocity (e'), and their ratio (E/e′, to estimate LV filling pressures), as well as tricuspid regurgitation velocity (TRV), a surrogate of pulmonary artery systolic pressure (PASP) (4).

In HFpEF, dynamic changes in estimated LV filling pressures during exercise test can be investigated by E/e'. However, E/e' changes show only moderate correlation with invasively and simultaneously-measured PCWP (5), and conflicting results have been obtained in HFpEF patients regarding the value of exercise E/e' (6, 7). An approach based mainly on E/e' and TRV evaluation is also hampered by accuracy issues (5, 8).

Lung ultrasonography (LUS) provides a simple and reproducible semi-quantitative evaluation of extravascular lung water (EVLW) in HF patients (9), providing a new opportunity to explore pulmonary congestion during exercise (10, 11). At rest, B-line count as measured by LUS, showed a higher correlation with invasively measured LV filling pressures if compared to echocardiographic indices in a cohort of patients with significant dyspnea (i.e. NYHA class≥2), and significantly increased the diagnostic accuracy in predicting haemodynamic congestion on top of clinical variables (12). Recently, it was observed that the development of lung congestion during submaximal exercise, as assessed by LUS, was significantly associated with elevations in PCWP and right atrial pressures, as well as with derangements in right ventricular- to pulmonary circulation coupling (13). Additionally, we have recently demonstrated for the first time that exercise may result in the rapid

appearance, or increase, of B-lines (14) in HFpEF patients; those changes were mirrored by significant variations in B-type natriuretic peptide (BNP) concentrations, TRV, and E/e' and were of greater magnitude if compared to hypertensive control subgroup (14). Yet, the prognostic value of exercise LUS in HFpEF patients remains unexplored.

This study aims at evaluating the prognostic value of changes in pulmonary congestion during exercise stress echocardiography among HFpEF patients, and correlating these variations with changes in other congestion markers (i.e., BNP, E/e', and PASP).

Methods

Participants

The data that support the findings of this study are available from the corresponding author on reasonable request.

Consecutive HFpEF outpatients referred to the Cardiology Clinic of the Niska Banja Institut from June 1 2016 to February 1 2018 were prospectively enrolled in this cohort, which expanded findings coming from a previously reported study, which included 31 HFpEF patients (14). Participants, diagnosed according to the European HF guidelines (15), were haemodynamically stable, and exercise stress echocardiography was performed after >8 weeks of any previous HF hospitalization. Given the well-known limitations of natriuretic peptide diagnostic thresholds in diagnosing HFpEF (16), patients having BNP concentrations lower than the cut-off proposed by European HF guidelines (i.e. 35 pg/ml) (15) were included if they had had a previous HF hospitalization. Inclusion criteria were: (i) ability to perform bicycle exercise stress echocardiography; (ii) sinus rhythm; (iii) good echocardiographic window; (iv) no pulmonary fibrosis, or other diseases potentially hampering image acquisition (pleural effusion, severe emphysema, previous pneumectomy or lobectomy, pulmonary cancer or metastases). The study was approved by the local ethics committee and managed in accordance with Good Clinical Practice and

the Declaration of Helsinki (17). All patients provided written informed consent. The clinical outcome of interest, a composite of cardiovascular death or HF hospitalization, was ascertained by chart review, institution's electronic medical records, and telephone contact at one year. All endpoints, defined according to previously established definitions (18), were adjudicated by three cardiologists (M. D., E.C., G.A.) with extensive experience in endpoint adjudication, who were blinded to LUS data. Particularly, cardiovascular death included deaths resulting from a myocardial infarction, sudden death, HF, stroke, other cardiovascular causes. There were no missing data with respect to the study endpoint.

Submaximal exercise stress echocardiography protocol

After clinical examination (see Data Supplement for detailed description), resting echocardiography and LUS were performed (see details below); patients underwent submaximal exercise stress echocardiography (supine, lying slightly tilted on their left side) on a tilting table using a cycle ergometer, as proposed by Cardiff-MEDIA protocol (slightly modified in order to provide a suitable acquisition period to perform both echocardiography and LUS) (19). Submaximal exercise stress echocardiography has a good feasibility, allowing image acquisition throughout exercise (4, 20). Briefly, exercise started at an initial 15W workload, with 5 W increments every minute and maintaining a pedalling rate of 55–65 r.p.m. Once heart rate of >100 b.p.m. was reached, workload was kept constant for ~5-6 min while echocardiography, LUS imaging (Figure I and Figure II in the Data Supplement), and blood sampling for natriuretic peptides assessment were performed; once these measurements were carried out, a 10 minute recovery phase started. Echocardiography and lung ultrasonography were performed: 1) at rest; 2) during exercise phase, i.e. at the heart rate of >100 b.p.m, or whenever symptoms develop (whichever first); 3) during the last 5 min of recovery, ECG and blood pressure (BP) were continuously monitored, and recorded every 2 minutes over the entire test. For echocardiographic images, at least three consecutive beats were

acquired. Exercise testing was interrupted in the event of typical chest pain, constraining breathlessness, dizziness, muscular exhaustion, >10 mmHg drop in systolic BP or severe hypertension (systolic BP ≥250 mmHg), development of significant ventricular arrhythmias, or ST depression.

Two-dimensional-tissue Doppler echocardiography and lung ultrasonography

All patients underwent detailed echocardiographic examination and lung ultrasonography at rest and at the maximal workload sustained during exercise (see Data Supplement for expanded methods). All measurements were averaged over three beats. Early filling (E) and atrial (A) peak velocities, as well as deceleration time of early filling were measured from trans-mitral flow. Septal and lateral peak mitral annular early diastolic velocity (e′) were acquired, and averaged, by using real-time pulse-wave tissue Doppler method (21). LV filling pressures were then estimated by E/e′ ratio (21). Tricuspid annular peak systolic excursion (TAPSE) was assessed using M-mode echocardiography. Estimation of PASP was performed using the Bernoulli formula according to TRV (21). Left ventricular end-systolic elastance at rest and during exercise was derived as previously reported (20).

Lung ultrasound examinations were performed with the method of the 28-scanning points, as previously described (22), immediately after echocardiogram. Secondary analyses were performed according to 8- and 4-scanning point methods by reviewing patients' data sets as previously described (23). B-lines were defined as discrete, laser-like vertical hyperechoic reverberation artefacts arising from the pleural line, extending to the bottom of the screen without fading, and moving synchronously with lung sliding (22). Usually, when a scanning site displays a few B-lines, they are easily discernible and easy to count. If B-lines were greater in number and confluent (i.e. not enough distinguishable to be counted), the percentage of the screen below the pleural line occupied by B-lines was considered, and then divided by 10. The sum of B-lines recorded in each of the 28 scanning points yields a score (ranging from 0 to 280) denoting the extent of extravascular fluid of

the lung. Scanning sites with missing B-line data (e.g. due to minimal pleural effusions or difficulty in detecting pleural sliding) were not taken into account and were consequently judged as 'zero B-lines'. As previously reported by our group(24), LUS reliability is excellent, with both intra- and inter-observer correlation coefficients ≥0.85.

All echocardiographic images, performed using a 1–4 MHz phased-array probe, were recorded and analysed off-line; the number of B-lines was assessed in real time. All exams were performed by a single operator (D.S.), blinded to patients' data and who did not take part in their clinical management. Typically, 3 minutes, or less, were necessary to perform echocardiographic acquisitions at peak exercise (focused on cardiac volumes, PASP, E/e' ratio) or LUS. The present study fulfilled all the applicable requirements reported in the Expert Consensus Document on LUS in HF studies (25).

Laboratory examinations

Immediately before exercise stress echocardiography, peripheral venous blood samples were obtained to determine blood count, sodium, potassium, creatinine (and estimated glomerular filtration rate by modification of diet in renal disease), and BNP; an additional blood sample for BNP was obtained at peak exercise (during echocardiographic image acquisition), before the beginning of the recovery period (26). BNP concentrations were assessed by ALERE ^{TM} Triage® BNP MeterPro Assay (Alere San Diego Inc., San Diego, California)

Statistical methods

Continuous variables are expressed as mean ± standard deviation (SD), or median and interquartile range (IQR), as appropriate; categorical variables are presented as counts and percentages. Distribution of variables was visually checked. Differences in baseline characteristics according to exercise B-lines (peak B-lines >10 as reference, which also approximately corresponds

to median and average peak value in the total cohort) were performed using unpaired t-test or Mann-Whitney test for continuous variables, and $\chi 2$ test or Fisher's exact test for categorical variables, as appropriate. Baseline characteristic between patients who experienced the outcome, or not, were also compared. Univariable analyses by Cox proportional hazards models were performed to assess the association between each variable (listed in Table 1 and Table 2) and outcome. All variables with P <0.10 by univariable analysis (listed in Table 3) were considered as candidate variables and included in the multivariable model. Multivariable analyses were performed using a backward selection procedure; namely, at each step, the least significant stepwise variable was removed from the model until all of the remaining ones showed a statistically significant contribution to the model (the threshold P-value was set at 0.10). To account for the inherent correlation between variables, three separate multivariable analyses were performed by introducing B-lines at rest (rest B-lines), B-lines at peak exercise (peak B-lines), or B-line change (from rest to peak) along with other significant predictors of the outcome. Multicollinearity diagnostics was performed for each model. We also performed multivariable analyses with associations with the outcome for continuous variables expressed per each 1-SD increase.

The Kaplan–Meier method was used to estimate survival probabilities; differences between survival curves were evaluated using the log-rank test. According to previously proposed B-line scoring (27), we tested univariable associations with the outcome for three different cut-offs: 1) B-lines >5, 2) B-lines >15, 3) B-lines >10.

Correlations between congestion parameters (i.e. B-lines, E/e', PASP, and BNP) at rest, at peak exercise, and their change were tested by Spearman test.

We used C-statistics for time-to-event data to quantify the ability of each parameter to identify patients who had an outcome, as well as their increase in diagnostic accuracy on top of clinical model [i.e. age, male sex, and coronary artery disease (CAD)] by using the Harrell's method (28). In addition, continuous net reclassification improvement (NRI) was performed to assess the

additive prognostic value of each baseline parameter on top of the above-mentioned model (29). A P-value <0.05 was considered significant. Statistical analyses were performed using the SPSS package version 25.0 (Chicago, L, USA) and the R software (R Foundation for Statistical Computing).

Results

Patient characteristics

The main characteristics of the 61 patients enrolled are showed in Table 1 and Table 2. In the whole population, 44% of patients were male, mean age was 64.7 ± 8.9 years, and median LV ejection fraction (LVEF) was 56.0% [IQR 52.5-63.0]. About one-quarter of patients were in NYHA class >1, and about 80% of patients had had a previous HF hospitalization. According to exercise LUS findings, patients with peak B-lines >10 were more likely to have CAD; interestingly, there were no significant differences with respect to medications at baseline, including diuretics. No significant differences were observed with respect to physical examination findings. Additionally, patients with peak B-lines >10 had higher BNP levels at any stage (Table 1), LV mass index, wall thicknesses, peak and change average E/e', TRV change, and rest B-lines (Table 2).

At rest, no B-lines were detectable in 12 patients (19%), and overall median rest B-lines were 2 [IQR 1-3]; according to B-line grading (27) pulmonary congestion was absent at rest (i.e. B-lines ≤5) in all participants. B-line reading was interpretable in all patients.(i.e. none of the patients had missing B-lines in some of the zones). Overall, exercise test lasted 6.4±1.8 min, mean achieved workload was 42.0 ± 9.0 W, peak heart rate averaged 104.9 ±5.1 b.p.m, with no significant difference according to the outcome. As expected, changes in B-lines, E/e' ratio, TRV, and BNP were significant upon exercise (P <0.001 for all, Table I in the Data Supplement); additionally, B-lines, E/e' ratio, TRV tended to decrease toward baseline upon recovery, although showing higher values if compared to the rest phase (P <0.001 for all, Table I in the Data Supplement). Rest, peak and changes in B-lines, BNP, and E/e' ratio were significantly higher among patients who

experienced the outcome; in contrast, no significant differences were observed for TRV (Table II in the Data Supplement). Patients experiencing the outcome were more likely to have CAD, to receive diuretics or anti-aldosterone agents; additionally, they had higher LV mass or wall thicknesses (Table II in the Data Supplement). With regard to 8- and 4-scanning point methods, differences in B-line scores were overall significantly higher among patients who experienced the outcome (Table III in the Data Supplement).

Correlations between B-lines and other markers of congestion at rest ad upon exercise

Overall, as compared to BNP, B-line count correlations were higher than that observed for E/e' ratio and TRV (Table IV in the Data Supplement). Particularly, peak B-lines showed the closest correlations with BNP (r=0.61 with rest BNP, r=0.73 with peak BNP, and r=0.63 with change BNP; all P <0.001). Change B-line was also highly correlated with peak and change E/e' ratio (r=0.75, and r=0.69, respectively) (Table IV in the Data Supplement*).*

Prognostic value of lung ultrasound

At one-year follow-up, 21 patients experienced a total of 25 events (20 HF hospitalizations and 5 cardiovascular deaths). Exercise-induced B-lines were significantly associated with outcome by univariable analysis, both for peak (HR 1.41, 95% CI 1.23–1.62, P <0.001) and change (HR 1.39, 95% CI 1.18–1.64, P <0.001) values; significant associations were also observed between BNP and outcome, especially for rest, and peak values (both P values <0.001, HR >1.10 for a 10-unit increase) (Table 3).

We then constructed three different multivariable models by introducing separately each B-line variable (i.e. rest, peak, and change). Peak values of BNP and E/e' were not entered along with rest BNP and E/e' because of multicollinearity issues. By multivariable analysis, peak (HR 1.50, 95% CI 1.21–1.85, P <0.001), and change (HR 1.34, 95% CI 1.12–1.62, P =0.002) B-lines were retained as

significant predictors for the combined endpoint, along with resting values of BNP, and E/e' ratio (Table 3). Peak B-lines was retained as a significant predictor also when introducing peak values of both BNP and E/e' (in place of rest BNP and E/e') as candidate variable in the multivariable model (HR 1.39, 95% CI 1.06–1.82, P =0.018). Associations with the outcome expressed per 1-SD increase are shown in Table V in the Data Supplement.

Among B-line scores obtained with 8- and 4-scanning point methods, peak B-lines with 8-scanning point method was retained as a significant predictor (HR 1.59, 95% CI 1.03–2.42, P =0.037), also by forcing peak values for BNP and E/e' ratio (HR 1.58, 95% CI 1.03–2.42, P =0.038).

We then tested different cut-offs (i.e. >5, >10, and >15) for peak, and B-line change by univariable analysis; B-lines >10, both peak (HR 7.81, 95% CI 2.62–23.33, P <0.001) and change (HR 4.97, 95% CI 2.08–11.90, P <0.001) values, showed the most significant associations (Table VI in the Data Supplement). One-year event-free survival was 25±11% in patients with B-lines change >10 (N=16) and 80±6% in those with B-lines change ≤10 (N=45) (P <0.001) (Figure 1A). The same pattern was observed for peak B-lines, with an event-free survival of 35±9% in patients with >10 B-lines (N=26) and 89±5% in those with ≤10 B-lines (N=35) (P <0.001) (Figure 1B).

Prognostic accuracy and improvement in reclassification associated with lung ultrasonography, echocardiographic variables, and natriuretic peptides

As showed in Table 4, peak B-lines [C-Index increase of 0.157 (0.056 to 0.258), P =0.002] , B-line change [C-Index increase of 0.130 (0.017 to 0.243), P =0.024], peak B-lines >10 [C-Index increase of 0.115 (0.006 to 0.223), P =0.039], peak B-lines with 8-scanning point method [C-Index increase of 0.123 (0.025 to 0.221), P =0.014], were the only variables, along with peak BNP, significantly associated with an increase in prognostic accuracy as assessed by C-index on top of clinical model (age, male gender, and CAD history). Peak [continuous NRI 0.51, 0.09 to 0.74, P =0.016] and change [continuous NRI 0.57, 0.01 to 0.78, P =0.049] B-lines were also significantly

associated with an improvement in reclassification, along with B-line increase >10 on top of clinical model whereas peak BNP was not (P =0.14). Noteworthy, LV mass (which was not retained as indipendent predictor by multivariable analysis), had no additive prognostic value on top of clinical model; similar results were observed for relative wall thickness (Table 4).

Discussion

The main finding of the present study is that the development of pulmonary congestion during exercise, as easily assessed by LUS, is an independent predictor of adverse outcome in patients with HFpEF. In addition, peak stress B-lines and their increase relative to baseline provide additive prognostic information (as assessed with NRI) on top of other established prognostic predictors in our cohort, whereas BNP did not. These findings suggest that exercise LUS could be useful to further refine risk-stratification of HFpEF patients.

Development of pulmonary congestion during stress in HFpEF

Pathophysiology of HFpEF is still not entirely understood. Exercise intolerance and exertional dyspnoea are pivotal symptoms of HFpEF. Several alternative mechanisms beyond diastolic dysfunction have been proposed, such as subtle systolic impairment (30), impaired myocardial energetics and microvascular inflammation (31). In healthy subjects, adequate filling of the ventricles occurs without abnormal elevation of intracavitary pressures, both at rest and during exercise. As compared to healthy controls, both patients with clinically suspected (32) and invasively-proven HFpEF (2) were associated with greater increase in PCWP and PASP, along with a blunted increase in heart rate, also at submaximal exercise; particularly, those haemodynamic derangements were observed at a workload intensity which was lower than that achieved in our

cohort. With respect to heart rate, chronotropic incompetence represents another relevant feature among HFpEF, described in approximately one-third of patients (33).

A rise in PCWP leads to increased extravasation of fluid from the vascular bed to the pulmonary interstitial space, exceeding the attractive force of the oncotic pressure or overwhelming other physiological adaptations (i.e. enhanced alveolar fluid clearance, changes in basal membrane permeability, increased lymphatic drainage) (34), and thus resulting in a net increase of EVLW (35). Recently, in a cohort of 61 HFpEF patients, Reddy et al (13) demonstrated that B-line appearance or increase was related to elevations of both PCWP and central venous pressures during submaximal exercise, which in turn were secondary and well correlated to abnormalities in right ventricular-to-pulmonary circulation coupling (as assessed by the ratios of different echocardiographic indices of right ventricular function and invasive mean pulmonary arterial pressure); the authors also speculated that, in the setting of elevated central venous pressures, lymphatic drainage of EVLW is hampered, further increasing lung congestion. In our cohort we observed a non-significant reduction in the ratio between TAPSE and TRV according to B-line increase >10 (data not shown); however, taken into account that both estimated right atrial and systolic pulmonary pressures were not assessed in our cohort, a direct comparison cannot be made with the study of Reddy et al (13).

In our cohort, at peak exercise, we observed good correlations of B-lines with BNP and E/' ratio, whereas, the correlation between exercise B-lines and PASP (as assessed by TRV) was negligible (Table IV in the Data Supplement). This is in line with previous reports including HF patients with mixed LVEF, showing low but significant correlations of B-lines with PASP only in patients with reduced LVEF (36). Whether these differences according to HF phenotypes are due to feasibility and accuracy problems -as recently suggested (5)-, different timings of PASP rise during exercise, or intrinsic limitations of this parameter (i.e. TRV is an indirect estimate of LV filling pressures), is not completely understood. Additionally, substantial right atrial pressure elevation

during exercise in HF patients occurs (13, 37), and this is not taken into account for PASP estimation by TRV, thus resulting in underestimation of non-invasive PASP measurement.

Prognostic value of B-lines during exercise in HFpEF patients

Detection of pulmonary congestion at rest by LUS has demonstrated diagnostic and prognostic value in different HF settings and subsets (38); indeed, it can be intended as an extension of clinical examination in HF patients (39), also being able to overcome limitations of Doppler-derived indices (21). Additionally, this technique allows real-time monitoring of the consequences on pulmonary congestion elicited by exercise, showing a prompt increase in B-line count (10). Agricola et al (36) first described B-line development upon exercise (with LUS performed in the recovery phase, i.e., >6 minutes after the end of the exercise phase) in a mixed cohort of 72 patients referred for exercise echocardiography (about 75% with a LVEF <40%); B-line score increased significantly with exercise (5.9 ±14.9 vs 11.0 ± 20.7, P = 0.0001), and its change was correlated to variations of estimated PCWP and PASP (36). With respect to HFpEF, we recently demonstrated that submaximal exercise stress echocardiography coupled with LUS unveils development of pulmonary congestion, which occurs together with dynamic changes of echocardiographic markers of congestion (i.e. E/e', and TRV), and of natriuretic peptides, as compared to hypertensive controls (14). However, evidence on the prognostic value of exercise LUS is scarce, and derived exclusively from HF with reduced ejection fraction (HFrEF) cohorts (35, 40). Scali et al first (35) investigated this issue in a cohort of 103 HFrEF patients by performing LUS at rest and at peak exercise of maximal exercise stress echocardiography. The degree of pulmonary congestion in that previous report was slightly higher than in our cohort in terms of median B-lines, both at rest (5 versus 2) and at peak exercise (12 versus 9). HFrEF and HFpEF patients tend to show similar ranges of pulmonary congestion as assessed with LUS (41); however these differences may be attributed to more advanced HF stages in the report of Scali et al., as indicated by higher NYHA and BNP concentrations in those patients

compared to ours. However, regardless of absolute values, similar to us, Scali et al. also showed a significant association of peak B-lines with the combined outcome of death or HF hospitalization by univariable analysis (HR per 1 B-line increase=1.04, 95% CI 1.03–1.06, P <0.0001); those findings were recently confirmed in a cohort of 105 HFrEF patients, reporting similar associations with the composite endpoint of cardiovascular death or HF hospitalization for each B-line increase (40). Recently, B-line change (Δstress-rest B-lines, assessed by the 4-scanning point method) was found to be independently associated with adverse outcome in a mixed cohort of 3,410 patients with known or suspected CAD and/or HF (12% with HFpEF) undergoing stress echocardiography with different stressors(42); however, taking into account differences in terms of aims, patient characteristics, stress protocol, and LUS methodology, the study of Ciampi et al. cannot be directly compared with our study, which instead is specifically focused on the HFpEF patients undergoing exercise echocardiography. From a methodological point of view, findings by Ciampi et al(42) validated a previous preliminary study which aimed at comparing various scanning protocols (28-, 16-, 8-, and 4-site scan) in order to identify the one that best balances feasibility and accuracy(23); correlation coefficients of 4- (R^2= 0.91) and 8- (R^2= 0.95) with the 28-scanning point method were excellent. As such, in a context of stress imaging for clinical purposes, both the 8- and (to a greater extent) the 4-scanning point methods may be regarded as valuable alternatives to the more comprehensive 28-site scan method, allowing a considerable reduction of imaging and analysis time with no substantial loss of information.

Our study substantially expands previous findings, by investigating for the first time HFpEF patients. Along with E/e' ratio and BNP, B-line upon exercise (both peak value and its increase from baseline) was an independent predictor of adverse outcome; specifically, each B-line increase during exercise conferred a 40% higher risk adverse outcome (Table 3). B-line development upon exercise may represent a marker of higher susceptibility to subsequent adverse events in HFpEF events, as in HFrEF patients. In our cohort, out of 61 patients, 21 (34%) experienced the combined endpoint, with

20 HF hospitalizations and 5 cardiovascular deaths; the risk was substantial if considering the relatively small sample size and the length follow-up. This may have been influenced by the fact that a large proportion (50/61, 82%) had a previous HF hospitalization, and, although all patients were judged as clinically stable and enrolled at least >2 months after the HF admission, a certain degree of residual congestion or clinical instability may have partially driven the events. In this regard, the event-risk was comparable to that of the HFrEF cohort of Scali et al (i.e. 35.9% at a median follow-up of 8 months) (35).

With regard to the optimal cut-off, B-lines >10 (both as change from rest and as peak value) appeared as the best risk-stratifier in our cohort. Importantly, we also demonstrated the incremental prognostic value of peak and increase in B-lines, which were the only two variables in our dataset to be associated with both a significant increase in C-index and with improved net reclassification on top of usual risk stratification variables.

Limitations

This is a single-centre cohort study, and this may limits the generalizability of these results. There may be unknown or unmeasured confounding variables which were not adjusted for, and which could have affected some or all of the observed relationships; additionally, we observed a non-significant trend in higher previous HF hospitalization and reduced renal function among patients who experienced the outcome, and this might have partially influenced the results. Additionally, estimation of associations is limited by the number of patients, particularly for B-line cut-offs, which show wide HR confidence intervals. Nonetheless, the lower border of the confidence interval of >10 B-lines in the univariable analysis is 2.62 for peak B-lines, and 2.08 for change B-lines, suggesting a fair prognostic value even in the worst-case scenario. Additionally, given the relatively low number of events per variable (EPV) (i.e. 7), results from multivariable Cox analyses should be cautiously interpreted; however, some authors showed that rules of thumb for multivariable analyses (such as

10 or more EPV) may be too conservative, whereas 5 to 9 EPV may be satisfactory(43). We acknowledge that further larger studies are warranted to confirm associations observed in our study. In the absence of these validating studies, our work should be considered as hypothesis-generating. Patients with atrial fibrillation were excluded because of well-known limitations in the assessment of Doppler diastolic function(21); however, given the not negligible prevalence of atrial fibrillation among HFpEF patients(44), this might limit the generalizability of our results.

Pulse oximetry monitoring was not included in our protocol; in this respect, as recently reported in a cohort of 61 HFpEF patients undergoing submaximal exercise, patients with and without exercise B-lines showed similar levels of arterial saturation, both at rest and upon exercise(13). Thus, pulse oximetry seems to be insufficiently sensitive to capture functional changes during exercise (in contrast with LUS data) in HFpEF patients.

In principle, detection of B-lines does not necessarily imply their cardiogenic origin, as they are a very sensitive but non-specific sign, and although we excluded patients with pulmonary fibrosis (a disease associated with "dry" B-lines), we cannot rule out the possibility that some of the patients included in this cohort had B-lines of non-cardiac origin. However, this phenomenon can hardly explain exercise-induced B-lines, and a sudden increase during effort is almost certainly related to a cardiogenic origin of EVLW. Additionally, real-time analysis by a single operator may represent a potential source of bias, although it represents the closest approach to the routine clinical practice.

Conclusions

Exercise-induced pulmonary congestion, as assessed by LUS, is an independent predictor of adverse outcome in NYHA I/II HFpEF patients. This simple and inexpensive test might allow to further refine the risk-stratification of these patients on top of well-established clinical variables. These findings should be further replicated in larger HFpEF cohorts to prompt the implementation of LUS as a risk-stratifier in clinical practice.

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Conflict of interest: N.G. reports personal fees from Novartis, personal fees from Boehringer, outside the submitted work. G.A. reports personal fees from Angelini, personal fees from Behring, personal fees from Menarini, outside the submitted work. All the other authors have nothing to declare.

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Figure legends

Fig.1: Kaplan–Meier survival curves for best B-line cut-off

A: Twelve-month event-free survival for the primary endpoint (cardiovascular death or HF hospitalization) is 25±11% in patients with >10 B-line change upon effort and 80±6% in those with ≤10 B-lines ($P < 0.001$).

B: Twelve-month event-free survival for the primary endpoint (cardiovascular death or HF hospitalization) is 35±9% in patients with >10 B-line peak upon effort and 89±5% in those with ≤10 B-lines ($P < 0.001$).

Supplementary material

Fig. 1: illustrative figure showing how to count B-lines at rest and at peak exercise.

Fig. 2: illustrative figure showing left ventricular volumes and ejection fraction measurements at rest and at peak exercise.

Table 1: Baseline demographic and clinical characteristics (whole cohort and according to B-line peak >10 or ≤10)

	Total cohort (N=61)	B-line peak ≤10 (N=35)	B-line peak >10 (N=26)	P value
Demographics-anthropometry				
Male gender, n (%)	27 (44%)	16 (46%)	11 (42.3%)	0.79
Age (years), mean (±SD)	64.7 ± 8.9	63.4 ± 9.0	66.4 ± 8.5	0.21
BMI (Kg/m2), mean (±SD)	29.3 ± 3.8	29.4 ± 3.9	29.2 ± 3.6	0.87
Medical history				
Hypertension, n (%)	61 (100%)	35 (100%)	26 (100%)	NA
Diabetes, n (%)	21 (34%)	15 (43%)	6 (23%)	0.11
Dyslipidemia, n (%)	60 (98%)	35 (100%)	25 (96%)	0.24
Coronary artery disease, n (%)	18 (30%)	5 (14%)	13 (50%)	0.002
Current smoking, n (%)	17 (28%)	9 (26%)	8 (31%)	0.66
COPD, n (%)	3 (5%)	2 (6%)	1 (4%)	0.74
Previous HHF, n (%)	50 (82%)	28 (80%)	22 (85%)	0.64
Previous AF episode, n (%)	10 (16%)	4 (11%)	6 (23%)	0.22
Therapy				
ACE-i or ARB, n (%)	58 (95%)	34 (97%)	24 (92%)	0.39
Beta-blocker, n (%)	61 (100%)	35 (100%)	26 (100%)	NA
Statin, n (%)	57 (93%)	31 (89%)	26 (100%)	0.08
Diuretics, n (%)	51 (84%)	29 (83%)	22 (85%)	0.85
Nitrates, n (%)	11 (18%)	5 (14%)	6 (23%)	0.38
Anti-aldosterone agents, n (%)	6 (10%)	2 (6%)	4 (15%)	0.21
Physical examination				
Systolic BP (mmHg), mean (±SD)	123.9 ± 8.4	122.8 ± 7.6	125.5 ± 9.2	0.21
Diastolic BP (mmHg), mean (±SD)	74.8 ± 7.2	74.6 ± 6.6	75.1 ± 8.2	0.80
Heart rate (bpm), mean (±SD)	66.1 ± 7.9	65.5 ± 7.5	66.9 ± 8.5	0.50
NYHA class >1	15 (25%)	10 (29%)	5 (19%)	0.40
Jugular venous distension, n (%)	0 (0%)	0 (0%)	0 (0%)	NA
Ankle edema, n (%)	21 (35%)	11 (31%)	10 (39%)	0.57
Pulmonary rales, n (%)	2 (3%)	1 (31%)	1 (4%)	0.83
Laboratory				
Haemoglobin (gr/dl), mean (±SD)	13.6 ± 0.1	13.7 ± 1.1	13.4 ± 1.5	0.32
Sodium (mEq/L), mean (±SD)	143.8 ± 3.1	144.0± 2.8	143.7 ± 3.6	0.79
Creatinine (mg/dl), mean (±SD)	1.04 ± 0.3	1.1 ± 0.2	1.7 ± 0.3	0.39
eGFR (ml/min), mean (±SD)	82.0 ± 21.0	84.3 ± 21.0	78.9 ± 21.1	0.32
BNP-rest (pg/ml), median (IQR)	31.4 (16.9-56.6)	24.8 (12.8-40.2)	55.0 (30.3-118.2)	<0.001
BNP-peak (pg/ml), median (IQR)	57.1 (38.2-94.7)	42.6 (32.4-59.0)	106.5 (67.0-175.0)	<0.001
BNP-change, (pg/ml), median (IQR)	24.5 (13.0-51.9)	17.3 (7.5-26.6)	51.9 (24.8-67.7)	<0.001

BMI, body mass index; COPD, chronic obstructive pulmonary disease; HHF=heart failure hospitalization; AF, atrial fibrillation; ACE-i= angiotensin converting enzyme inhibitor; ARB= angiotensin II receptor blocker; BP, blood pressure; NYHA=New York Heart Association; eGFR, estimated glomerular filtration rate; IQR, interquartile range; BNP, B-type natriuretic peptide; SD, standard deviation; IQR, interquartile range; NA, not applicable. **on**

Table 2: Echocardiographic and lung ultrasound characteristics (whole cohort and according to B-line peak >10 or ≤10)

	Total cohort (N=61)	B-line peak ≤10 (N=35)	B-line peak >10 (N=26)	P value
Echocardiography and lung ultrasound				
LV ejection fraction (%),mean (±SD)	56.0 (52.5-63.0)	55.0 (52.0-62.0)	56.5 (53.8-63.3)	0.58
Septal wall thickness (mm), mean (±SD)	13.6 ± 1.5	13.0 ± 1.1	14.3 ± 1.6	0.001
Posterior wall thickness (mm), mean (±SD)	11.4 ± 1.2	11.0 ± 1.1	12.1 ± 1.1	<0.001
Relative wall thickness, mean (±SD)	0.45 ± 0.1	0.44 ± 0.1	0.48 ± 0.1	0.006
Diastolic LV internal diameter (mm), mean (±SD)	50.9 ± 3.9	50.6 ± 4.2	51.2 ± 3.7	0.56
LVEDV (ml/m2), mean (±SD)	44.8 ± 9.5	44.6 ± 9.6	45.0 ± 9.5	0.90
LV mass index (gr/m2), mean (±SD)	130.2 ± 25.8	121.0 ± 19.8	142.7 ± 28.1	0.001
Left atrial volume index (ml/m2), mean (±SD)	28.5 ± 7.5	26.9 ± 7.8	30.5 ± 6.6	0.06
ELVI rest ($mmHg \cdot mL^{-1} \cdot m^{-2}$), mean (±SD)	6.2 ± 7.5	6.1 ± 1.6	6.3 ± 1.4	0.61
ELVI peak ($mmHg \cdot mL^{-1} \cdot m^{-2}$), mean (±SD)	8.1 ± 2.1	8.1 ± 2.4	8.0 ± 1.8	0.81
E/A, mean (±SD)	0.7 ± 0.4	0.7 ± 0.2	0.7 ± 0.6	0.73
E/e' average-rest, median (IQR)	7.6 (6.3-9.2)	7.5 (5.3-9.3)	7.7 (6.4-9.2)	0.33
E/e' average-peak, median (IQR)	13.1 (10.0-14.8)	12.0 (8.9-13.7)	14.8 (13.3-16.1)	<0.001
E/e' average-change, median (IQR)	5.0 (2.4-6.9)	3.8 (1.3-5.6)	6.7 (4.6-8.2)	<0.001
TAPSE (mm), mean (±SD)	21.9 ± 2.5	22.2 ± 2.5	21.6 ± 2.9	0.45
TR max velocity-rest (m/s), median (IQR)	1.7 (1.3-2.2)	1.8 (1.3-2.2)	1.7 (1.3-2.2)	0.79
TR max velocity-peak (m/s), median (IQR)	2.7 (2.1-3.0)	2.6 (1.9-2.9)	2.8 (2.4-3.0)	0.07
TR max velocity-change (m/s), median (IQR)	0.9 (0.4-1.2)	0.7 (0.3-1.1)	1.1 (0.6-1.4)	0.007
B-lines-rest, median (IQR)	2 (1-3)	2 (0-3)	3 (2-4)	0.004
B-lines-peak, median (IQR)	9 (8-13)	8 (7-9)	13 (12-14)	<0.001
B-line-change, median (IQR)	7 (6-11)	6 (5-7)	11 (9-12)	<0.001

LV, left ventricular; LVEDV, left ventricular end-diastolic volume; ELVI: left ventricular end-systolic elastance; TAPSE=tricuspid annular plane systolic excursion; TR, tricuspid regurgitation; SD, standard deviation; IQR, interquartile range.

Table 3 Univariable and multivariable analysis (backward conditional) for the combined endpoint (CV death or HF hospitalization)

	Univariable		Multivariable		Multivariable*		Multivariable	
		P-value	B-line rest	P-value	B-line peak	P-value	B-line change	P-value
Demographics								
Male gender	0.44 (0.17-1.13)	0.09						
Age (years) †	1.61 (0.93-2.79)	0.09						
Medical history								
Coronary artery disease	2.30 (0.97-5.41)	0.06						
Therapy								
Anti-aldosterone agents	3.94 (1.43-10.83)	0.008						
Laboratory								
BNP†	1.27 (1.15-1.40)	<0.001	1.29 (1.16-1.44)	<0.001	1.13 (1.01-1.28)	0.039	1.20 (1.08-1.34)	0.001
BNP peak†	1.11 (1.06-1.16)	<0.001						
BNP change†	1.12 (1.04-1.21)	0.004						
Echocardiography								
LV mass index (gr/m2) †	1.25 (1.07-1.47)	0.005						
E/e' average	1.20 (1.01-1.42)	0.036	1.44 (1.09-1.90)	0.01	1.35 (1.06-1.73)	0.017	1.30 (1.02-1.65)	0.037
E/e' average peak	1.27 (1.10-1.46)	0.001						
E/e' average-change	1.19 (1.02-1.39)	0.027	1.31 (1.10-1.57)	0.003				
TR max velocity-change	2.30 (0.91-5.80)	0.08						
B-lines rest	1.55 (1.14-2.10)	0.005						
B-lines peak	1.41 (1.23-1.62)	<0.001			1.50 (1.21-1.85)	<0.001		
B-line-change	1.39 (1.18-1.64)	<0.001					1.34 (1.12-1.62)	0.002

BNP, B-type natriuretic peptide; LV, left ventricular; TR, tricuspid regurgitation

3 separate multivariable analysis by introducing B-lines at rest, B-lines peak and B-lines change (from rest to peak).

* Peak B-lines was retained as a significant predictor also when introducing peak values of both BNP and E/e' in place of rest BNP and E/e'.

† =per 10 unit of increment.

BNP peak and E/e' peak were excluded from multivariable models as per collinearity diagnostics.

Table 4: Prognostic value of natriuretic peptides and echocardiographic variables on top of clinical model using Harrell's c-index and continuous net reclassification improvement at 1 year

Variable	Isolated prognostic value		Increase in prognostic value on top of clinical variables (age, sex, CAD)				
	Univariable c-index (CI 95%)	p-value	C-index (CI 95%) of model including clinical model + variable of interest	Δ c-index* (CI 95%)	P-value	cNRI at 1 year (CI 95%)	P-value
Age + Sex + CAD (clinical model)	0.689 (0.601 - 0.777)	<0.001	-	-	-	-	-
BNP rest	0.751 (0.640 - 0.863)	<0.001	0.792 (0.691 - 0.892)	0.103 (-0.006 to 0.212)	0.07	0.28 (-0.16 to 0.56)	0.16
BNP peak	0.733 (0.614 - 0.853)	0.001	0.787 (0.703 - 0.871)	0.098 (0.010 to 0.185)	0.029	0.38 (-0.15 to 0.61)	0.14
BNP-change	0.648 (0.514 - 0.782)	0.031	0.731 (0.647 - 0.815)	0.042 (-0.025 to 0.108)	0.22	0.12 (-0.20 to 0.43)	0.45
LV mass	0.646 (0.517 - 0.774)	0.026	0.752 (0.660 - 0.845)	0.063 (-0.021 to 0.147)	0.14	0.12 (-0.19 to 0.44)	0.31
RWT	0.574 (0.438 - 0.710)	0.28	0.697 (0.610 - 0.785)	0.008 (-0.027 to 0.043)	0.64	0.10 (-0.25 to 0.37)	0.51
LVEF	0.531 (0.403 - 0.659)	0.63	0.691 (0.605 - 0.778)	0.002 (-0.007 to 0.011)	0.66	0.05 (-0.25 to 0.44)	0.65
LVEDV	0.519 (0.408 - 0.629)	0.74	0.693 (0.607 - 0.779)	0.004 (-0.013 to 0.021)	0.66	-0.16 (-0.29 to 0.33)	1.16
LAVI	0.506 (0.380 - 0.633)	0.92	0.684 (0.595 - 0.774)	-0.005 (-0.019 to 0.009)	0.51	0.01 (-0.21 to 0.34)	0.59
E/A rest	0.493 (0.355 - 0.631)	0.92	0.706 (0.617 - 0.796)	0.017 (-0.010 to 0.044)	0.22	0.14 (-0.23 to 0.48)	0.43
E/e' rest	0.636 (0.520 - 0.753)	0.022	0.698 (0.607 - 0.789)	0.009 (-0.040 to 0.057)	0.73	0.23 (-0.25 to 0.49)	0.30
E/e' peak	0.715 (0.614 - 0.817)	<0.001	0.758 (0.668 - 0.848)	0.069 (-0.024 to 0.162)	0.15	0.53 (-0.10 to 0.71)	0.07
E/e' -change	0.626 (0.506 - 0.746)	0.040	0.731 (0.642 - 0.820)	0.042 (-0.037 to 0.120)	0.30	0.29 (-0.15 to 0.54)	0.14
TRV-rest	0.537 (0.404 - 0.671)	0.58	0.679 (0.584 - 0.773)	-0.011 (-0.054 to 0.032)	0.63	0.17 (-0.21 to 0.39)	0.40
TRV-peak	0.554 (0.433 - 0.675)	0.38	0.689 (0.601 - 0.777)	-0.000 (-0.006 to 0.005)	0.86	0.04 (-0.18 to 0.33)	0.59
TRV-change	0.634 (0.516 - 0.752)	0.026	0.692 (0.599 - 0.785)	0.002 (-0.034 to 0.039)	0.90	0.14 (-0.25 to 0.41)	0.37
B-lines rest	0.698 (0.596 - 0.800)	0.001	0.766 (0.678 - 0.855)	0.077 (-0.012 to 0.166)	0.09	0.27 (-0.15 to 0.52)	0.17
B-lines peak	0.826 (0.738 - 0.915)	<0.001	0.846 (0.769 - 0.923)	0.157 (0.056 to 0.258)	0.002	0.51 (0.09 to 0.74)	0.016
B-line-change	0.752 (0.649 - 0.855)	<0.001	0.819 (0.726 - 0.913)	0.130 (0.017 to 0.243)	0.024	0.57 (0.00 to 0.78)	0.049
B-lines change >5	0.605 (0.523 - 0.686)	0.012	0.699 (0.599 - 0.799)	0.010 (-0.067 to 0.087)	0.81	0.20 (-0.24 to 0.42)	0.31
B-lines change >10	0.678 (0.575 - 0.782)	0.007	0.784 (0.694 - 0.874)	0.094 (-0.011 to 0.200)	0.08	0.42 (0.00 to 0.65)	0.046
B-lines peak >10	0.732 (0.636 - 0.829)	<0.001	0.804 (0.708 - 0.900)	0.115 (0.006 to 0.223)	0.039	0.50 (-0.02 to 0.70)	0.06
B-lines peak >15	0.572 (0.493 - 0.651)	0.08	0.750 (0.662 - 0.838)	0.061 (-0.015 to 0.136)	0.12	0.14 (-0.35 to 0.41)	0.45
4-zone rest	0.650 (0.544 - 0.756)	0.006	0.731 (0.647 to 0.815)	0.042 (-0.033 to 0.117)	0.27	0.03 (-0.19 to 0.50)	0.30
4-zone peak	0.672 (0.558 - 0.786)	0.003	0.748 (0.666 to 0.829)	0.059 (-0.016 to 0.133)	0.12	0.31 (-0.09 to 0.57)	0.14
4-zone change	0.637 (0.524 - 0.750)	0.017	0.728 (0.639 to 0.817)	0.039 (-0.023 to 0.102)	0.22	0.26 (-0.13 to 0.51)	0.19
8-zone rest	0.667 (0.561 - 0.772)	0.002	0.742 (0.654 to 0.830)	0.053 (-0.019 to 0.124)	0.15	0.12 (-0.26 to 0.41)	0.32
8-zone peak	0.773 (0.677 - 0.869)	<0.001	0.812 (0.726 to 0.898)	0.123 (0.025 to 0.221)	0.014	0.28 (-0.06 to 0.60)	0.09
8-zone change	0.695 (0.587 - 0.804)	0.004	0.764 (0.676 to 0.853)	0.075 (-0.010 to 0.160)	0.08	0.23 (-0.21 to 0.52)	0.16
Log(BNP rest)	0.751 (0.640 - 0.863)	<0.001	0.777 (0.678 to 0.877)	0.088 (-0.019 to 0.195)	0.11	0.32 (-0.09 to 0.59)	0.12
Log(BNP peak)	0.733 (0.614 - 0.853)	0.001	0.765 (0.664 to 0.865)	0.076 (-0.037 to 0.188)	0.19	0.31 (-0.09 to 0.55)	0.13
Log(BNP change)	0.648 (0.514 - 0.782)	0.031	0.712 (0.618 to 0.807)	0.023 (-0.061 to 0.107)	0.59	0.09 (-0.21 to 0.38)	0.47

*Δ c-index is equal to the absolute difference between the c-index of model including the variable on interest (clinical model + variable of interest), and the c-index of clinical model (i.e. age, male gender, CAD).

cNRI: continuous Net Reclassification Improvement; CAD, coronary artery disease; BNP, B-type natriuretic peptide; LV, left ventricular; RWT, relative wall thickness; LVEF, left ventricular ejection fraction; LVEDV, left ventricular end-diastolic volume; LAVI, left atrial volume index; TRV, tricuspid regurgitation velocity.
Clinical model: age, male sex, CAD.

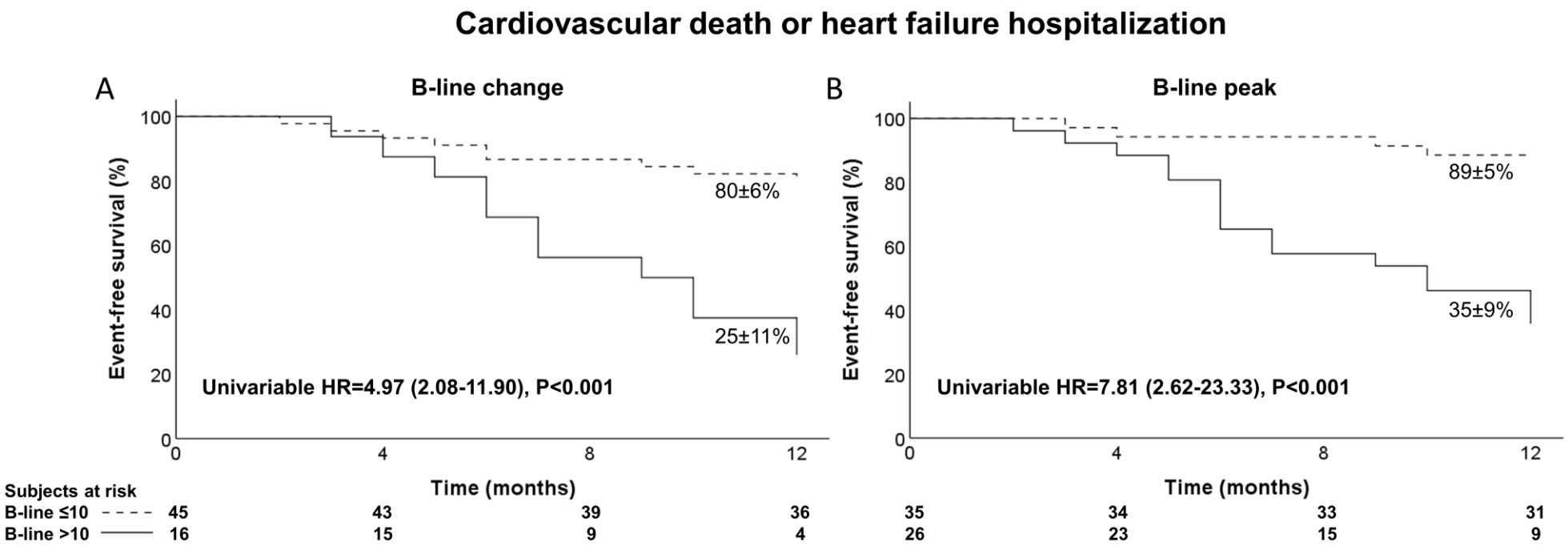
a. What is new?

- Heart failure (HF) with preserved ejection fraction (HFpEF) patients develop pulmonary congestion upon exercise in parallel with hemodynamic derangements, which is also responsible for symptoms. Lung ultrasonography (LUS) coupled with exercise stress echocardiography allows to easily assess increased extravascular lung water by B-line quantification.
- B-lines at peak exercise, as well as their change from baseline, independently predicted adverse outcome (i.e. cardiovascular death or HF hospitalization) at 12 months in HFpEF patients undergoing exercise echocardiography. Additionally, both peak and B-line change provided additive prognostic information on top of other established prognostic predictors.

b. What are the clinical implications?

- HFpEF, which accounts for about half of the cases of HF, represents a major burden on healthcare systems, being associated with significant morbidity and mortality. Reliable tools that provide clinicians with information on prognosis of this complex syndrome are much needed.
- This study demonstrates that exercise LUS could be useful to further refine risk-stratification of HFpEF patients, along with assessment of symptoms and estimation of left ventricular filling pressures during exercise.

Fig 1.


Cardiovascular death or heart failure hospitalization
A
B-line change
Event-free survival (%)
100
80
60
40
20
0
80±6%
25±11%
Univariable HR=4.97 (2.08-11.90), P<0.001
0
4
8
12
Time (months)
B
B-line peak
89±5%
35±9%
Univariable HR=7.81 (2.62-23.33), P<0.001
Subjects at risk
B-line ≤10 45 43 39 36 35 34 33 31
B-line >10 16 15 9 4 26 23 15 9