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**Exercise-induced B-lines for the Diagnosis of Heart Failure with Preserved Ejection Fraction:
a two-centre study**

Short title: exercise B-lines for HFpEF diagnosis

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Background: Diagnosis of heart failure with preserved ejection fraction (HFpEF) remains challenging despite the use of scores/algorithms. This study intended to assess the diagnostic value of exercise lung ultrasound (LUS) for HFpEF diagnosis.

Methods: We studied two independent case-control studies of HFpEF patients and control subjects undergoing different exercise protocols: i) submaximal exercise stress echocardiography (ESE) with LUS performed by expert cardiologists (N=116, HFpEF=65.5%), and ii) maximal cycle ergometer test (CET) (N=54, HFpEF=50%) with LUS performed by unexperienced physicians shortly trained for the study. B-line kinetics (i.e. peak values and their changes from rest) were assessed

Results: In the ESE cohort, the C-index (95% CI) of peak B-lines for HFpEF diagnosis was 0.985 (0.968-1.000), whereas the C-index of rest and exercise HFA-PEFF scores (i.e. including stress echo findings) were <0.90 (CI 0.823-0.949), and that of H2FPEF scores was <0.70 (CI 0.558-0.764). The C-index increase of peak B-lines on top of the above-mentioned scores was significant (C-index increase >0.090 and P-value <0.001 for all). Similar results were observed for change B-lines. Peak B-line >5 (sensitivity=93.4%, specificity=97.5%) and change B-line >3 (sensitivity=94.7%, specificity=87.5%) were the best cut-offs for HFpEF diagnosis. Adding peak or change B-lines on top of HFpEF scores and BNP significantly improved diagnostic accuracy. Peak B-lines showed a good diagnostic accuracy in the LUS beginner-led CET cohort (C-index=0.713, 0.588-0.838).

Conclusions: exercise LUS showed excellent diagnostic value for HFpEF diagnosis regardless of different exercise protocols/level of expertise, with additive diagnostic accuracy on top of available scores and natriuretic peptides.

Keywords Heart failure with preserved ejection fraction; Pulmonary congestion; B-lines; Exercise Test; Diagnostic accuracy.

Introduction

Heart failure (HF) with preserved ejection fraction (HFpEF) is defined by a left ventricular (LV) ejection fraction (LVEF) $\geq 50\%$, structural and/or functional cardiac abnormalities (including raised natriuretic peptides), in the presence of signs and symptoms of congestion, according to latest European HF guidelines¹. However, HFpEF is a complex heterogeneous clinical syndrome, encompassing a broad spectrum of cardiovascular risk factors and extra cardiac comorbidities^{1,2}. Recently, two important diagnostic tools have been proposed, the H2FPEF score³ and the HFA-PEFF algorithm⁴ with the aim of resolving these diagnostic uncertainties. However, a non-negligible proportion of patients is identified as having HFpEF with intermediate probability by both scores, for whom additional testing is recommended^{1,3,4}.

Patients with HFpEF often complain of exertional dyspnoea⁵, and an increase in extravascular lung water (EVLW) during exercise contributes to symptom manifestation⁶. Lung ultrasonography (LUS) provides a reliable semi-quantitative evaluation of EVLW in HF patients in different settings⁷⁻¹⁴. Coupled with submaximal exercise stress echocardiography (ESE), LUS enables to monitor pulmonary congestion development in HFpEF (through the quantification of B-lines), which occurs together with exercise-induced worsening of diastolic function and increase of natriuretic peptides^{15,16}. Our group demonstrated that exercise B-line is an independent predictor of outcomes in patients with HFpEF, even on top of well-established prognosticators¹⁷. Yet, the diagnostic value of exercise B-lines in identifying HFpEF has been scarcely studied.

We aimed at assessing the diagnostic value of exercise LUS for HFpEF diagnosis by using different exercise protocols and performed by professionals with different expertise; we also compared diagnostic capability of exercise B-lines with that of currently recommended diagnostic scores (H2FPEF score and HFA-PEFF scores).

Methods

Participants

A detailed description of clinical parameters, eligibility criteria, exercise stress protocols, echocardiography/lung ultrasound, calculation of HFpEF scores, and statistical methods is available in *Supplementary Material*.

In the ESE cohort, 116 consecutive outpatients referred to the Cardiology Clinic of the Niska Banja Institut were prospectively enrolled between June 2016 and February 2018 (N=76 with HFpEF, and N=40 controls). Controls were hypertensive subjects (with other cardiovascular risk factors such as dyslipidaemia, diabetes, or smoking habit), free of history of HF or significant coronary or valvular heart disease. Inclusion criteria for both cases and controls were: (i) ability to perform bicycle ESE; (ii) sinus rhythm; (iii) good echocardiographic window; (iv) no pulmonary fibrosis, or other pulmonary diseases potentially hampering image acquisition (pleural effusion, severe emphysema, previous pneumectomy or lobectomy, pulmonary cancer or metastases). The final subgroup of HFpEF patients (N=76) was extracted from an initial population which was made up of 92 HFpEF patients. Of these, 16 patients did not satisfy the inclusion criteria, 7 patients had atrial fibrillation, and 9 patients had severe pulmonary diseases, other conditions limiting image acquisition, or poor echocardiographic window. In the control subgroup, all patients fulfilled the inclusion criteria. Patients were enrolled in an outpatient setting in order to assess the diagnostic and prognostic value of lung ultrasound coupled with submaximal ESE in HFpEF, and further details have been published elsewhere^{15,17,18}; overall, their baseline data were used for the analysis reported herein.

In the cycle ergometer test (CET) cohort, 54 consecutive outpatients (N=27 with HFpEF, and N=27 controls) referred to the Department of Cardiology-CHRU Nancy were prospectively enrolled between January 2021 and December 2021. Both cases and controls (age ≥ 65 years, no history of HF, non-dyspnoeic) were scheduled with an indication for an exercise test. Patients were enrolled as part of the Effort-Lung Ultra Sound (E-LUS) study (ClinicalTrials.gov Identifier:

NCT04644692), a prospective, non-randomised, monocentric case-control study. The main objective was to evaluate the association of exercise LUS data with the diagnosis of HFpEF in a cohort of patients referred to a tertiary care centre with an indication for CET. All controls underwent CET for risk stratification; for HFpEF patients, a CET was performed to assess exercise tolerance and to investigate potential factors contributing to worsening HF [ischemia, arrhythmia, blood pressure (BP) profile). The study protocol envisaged the following main exclusion criteria: i) pulmonary or pleural pathology preventing reliable acquisition of LUS images (e.g. pulmonary fibrosis, diffuse interstitial lung disease, lung cancer or history of thoracic surgery), ii) pregnancy, iii) inability to express consent, and iv) recent (i.e. <15 days) discharge from hospital for a worsening heart failure hospitalization. The final cohort of (N=54, 27 cases and 27 controls) was extracted from an initial population made up of 56 patients (28 cases and 28 controls). Of these, two patients had exclusion criteria. All patients provided written informed consent.

In the ESE cohort, all HFpEF patients were diagnosed according to 2016 European HF guidelines¹⁹ and the vast majority (about 90%) had a previous HF hospitalization. Given the limitations of natriuretic peptide diagnostic thresholds in diagnosing HFpEF²⁰, patients having B-type natriuretic peptide (BNP) concentrations lower than the 35 pg/ml cut-off proposed by European HF guidelines¹⁹ were included if they had had a previous HF hospitalization.

In the CET cohort, all HFpEF patients had a previous HF hospitalization; detailed information on natriuretic peptides and echocardiographic characteristics were not collected as per study protocol.

Exercise stress echocardiography and cycle stress test protocol

In the ESE cohort, after clinical examination, resting echocardiography and LUS were performed (see below); patients underwent submaximal ESE (supine, slightly tilted on their left side) on a tilting table using a cycle-ergometer, in order to provide a suitable acquisition period to

perform both echocardiography and LUS²¹. Exercise started at an initial 15 watt workload, with 5 watt increments every minute. Once heart rate >100 b.p.m. was reached, workload was kept constant for ~5-6 min while echocardiography and LUS imaging were performed; subsequently, a 10-minute recovery phase started. Echocardiography and lung ultrasonography were performed: 1) at rest; 2) during exercise, i.e. at the heart rate of >100 b.p.m, or whenever symptoms developed (whichever first); 3) during the last 5 min of recovery.

In the CET cohort, all patients underwent maximal exercise test according to the protocol recommended by current guidelines²². Namely, a maximal graded cycling test, using increments of 25 watts/2 min, was performed starting at an initial workload of 25 watts. Lung ultrasound scanning was performed in the upright position at rest (before exercise) and soon after peak exercise (within the first two minutes of the recovery phase). In both exercise protocols, 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 \geq 250 mmHg), development of significant ventricular arrhythmias, or ST depression.

Two-dimensional-tissue Doppler echocardiography and lung ultrasonography

In the ESE cohort, all patients underwent detailed baseline echocardiographic examination (Esaote-MyLab Alpha eHD Crystalline series 7400; see *Supplementary Material* for details). Echocardiographic images, acquired using a 1–4 MHz phased-array probe, were recorded and analysed off-line. A single operator, (D.S.), a cardiologist with extensive experience in LUS assessment who was blinded to the patients' data and who did not take part in their clinical management, performed all exams.

LV structure and function were evaluated from standard apical four-chamber, two-chamber, and parasternal long- and short-axis views. LVEF was calculated using the biplane method of disks (modified Simpson's rule)²³. Peak velocities of early (E) and late (A) waves of transmitral flow and the E-wave deceleration time (DT), were measured from the pulsed-wave

Doppler obtained between the tips of the mitral leaflets. Septal and lateral mitral annular velocities (for measurement of e' and s') were obtained by pulsed tissue Doppler imaging (TDI). Average E/e' ratio was calculated using the peak E-wave velocity and the average of septal and lateral e' ²⁴. Right ventricular function was estimated by measuring tricuspid annular plane peak systolic excursion (TAPSE, in mm), and lateral tricuspid annulus peak systolic velocity by TDI (s' , cm/s). Estimation of pulmonary artery systolic pressure (PASP) was performed using the Bernoulli formula according to tricuspid regurgitation velocity (TRV)²⁴. LUS examinations were performed by the 28-scanning point method¹² immediately after echocardiogram.

In the CET cohort, an echocardiographic examination was not planned for all the patients by study protocol; LUS examinations were performed with the 8-zone protocol, as previously described²⁵, using a pocket-sized, hand-held ultrasound system (Vscan with phased-array transducer for the first half, and Vscan Extend with linear transducer for the second half of patients; GE Healthcare). All exams were performed by four physicians (two cardiologists specialized in exercise testing and two residents in nuclear medicine), with no experience with LUS (“LUS beginners”), unaware of group assignment, and who took no part in the clinical management. Two experienced cardiologists (N.G., M.E.) trained those operators on B-line assessment with a very short training (30 minutes of theory and practice on two patients).

In both cohorts, scanning sites with missing B-line data (e.g. due to minimal pleural effusions or difficulty in detecting pleural sliding) were judged as ‘zero B-lines’. Echocardiographic images were recorded and analysed off-line. The number of B-lines was assessed in real time; additionally, B-line clips were recorded in order to allow off-line analysis.

Laboratory examinations

In the ESE cohort, peripheral venous baseline blood samples were obtained to measure blood count, sodium, potassium, creatinine, and BNP. BNP concentrations were assessed by ALERETM Triage® BNP MeterPro Assay (Alere San Diego Inc., San Diego, California).

Calculation of H2FPEF and HFA-PEFF scores

The H2FPEF score was calculated as previously described³. Briefly, the H2FPEF score (ranging from 0 to 9) comprises the following dichotomous variables: body mass index >30 Kg/m², treatment with ≥ 2 antihypertensive medications, atrial fibrillation, PASP >35 mm Hg, age >60 years, and average E/e' ratio >9 ; each clinical variable scores 1 point, except obesity (2 points), and atrial fibrillation (3 points). A score of 6 or more can diagnose HFpEF with 90% probability³. The HFA-PEFF score has previously been described in detail as part of the HFA-PEFF diagnostic algorithm for suspected HFpEF⁴. The HFA-PEFF score includes the three different domains (functional, morphological and biomarker), individually scoring a maximum of 2 points. The highest HFA-PEFF score is 6, and an HFA-PEFF score of ≥ 5 is diagnostic of HFpEF. For the calculation of the HFA-PEFF score, we used resting parameters of laboratory and echocardiography data in the ESE cohort, and all parameters needed were available for all the patients (cases and controls). We also calculated an HFA-PEFF score incorporating echocardiographic indices of diastolic dysfunction from ESE as per Consensus Recommendations (“HFA-PEFF exercise”, 2 points for exercise average E/e' ≥ 15 , 3 points for exercise average E/e' ≥ 15 and TRV >3.4 m/s)⁴.

Statistical methods

The main analysis was performed in the ESE cohort, for which a complete echocardiographic, LUS, and laboratory dataset was available. In the CET cohort, the analysis was focused on B-lines. Continuous variables are expressed as mean \pm 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. In both cohorts (i.e. ESE and

CET cohorts), differences between HFpEF and controls in terms of demographic, clinical, echocardiographic, and lung ultrasound characteristics were assessed by unpaired t-test or Mann-Whitney test for continuous variables, and χ^2 test or Fisher's exact test for categorical variables, as appropriate. In both cohorts, we calculated sensitivity, specificity, negative predictive value, and positive predictive value of different cut-offs for exercise B-lines (peak and change B-lines); in the ESE cohort, we also tested relevant cut-offs for average E/e', BNP, and HFpEF scores⁴. In the ESE cohort, receiver operating characteristic (ROC) curves were created to assess the ability of exercise B-lines and of HFA-PEFF and H2FPEF scores in HFpEF diagnosis. Optimal cut-offs by Youden's index or exercise B-line were also assessed; we then tested those cut-offs in the CET cohort.

C-index was calculated to assess the individual performance in diagnosing HFpEF for the following variables: average E/e' (rest and exercise), BNP, H2FPEF score, HFA-PEFF scores (i.e. rest, and exercise), and B-lines (rest, peak and change B-lines). In the CET cohort, taking into account a possible learning curve effect, we also assessed C-index for exercise B-lines in the first half and the second half of consecutive patients. In addition, the increase in C-index was calculated to assess the additional diagnostic value of each variable on top of the three scores for HFpEF diagnosis. We also tested the increase in C-index of B-lines on top different models, including HFpEF score and BNP. C-index was overall assessed for all covariates, both as continuous and dichotomous (optimal and relevant cut-offs) variables. Statistical analyses were performed using SPSS package version 25.0 (Chicago, L, USA) and R version 3.6.1 (the R foundation for Statistical Computing).

Results

Baseline characteristics

Main clinical, echocardiographic and LUS findings in the two cohorts are showed in *Table 1*. In the submaximal ESE cohort (N=116, 76 with HFpEF; mean achieved workload=42±8

watts, mean maximum heart rate= 105 ± 5 b.p.m.), 41.4% of patients were male, and mean age was 63.2 ± 9.9 . Compared with controls, HFpEF patients were older, and were more likely to have diabetes, dyslipidemia, coronary artery disease, or a previous episode of atrial fibrillation or HF hospitalization (all P values <0.02). In addition, patients with HFpEF had higher BP upon exercise, BNP concentrations, LV mass indexed by body surface area, LVEF, average E/e' upon exercise and resting or exercise B-lines as compared with controls (all P values <0.02); they also had lower E/A ratio, and resting average e' (all P values <0.0001).

In the maximal CET cohort, (N=54, 27 with HFpEF; mean achieved workload= 98 ± 58 watts, mean percentage of theoretical maximum heart rate= $86 \pm 18\%$), 61.1% of patients were male, and mean age was 73.9 ± 7.0 years. Differences between HFpEF and controls in demographic, medical history, therapy and vital signs were overall comparable to those seen in the ESE cohort (all P values <0.05). Both resting and exercise B-lines were higher in HFpEF as compared with controls (all P values <0.02).

Diagnostic performances of lung ultrasound, HFpEF score and other variables

In the ESE cohort, exercise B-lines showed an excellent diagnostic value (C-index for peak B-lines=0.985, 95% CI 0.968 - 1.000; C-index for change B-lines=0.975, 95% CI 0.953 - 0.997), which was better than all considered HFpEF score (C-index for H2FPEF=0.661, 95% CI 0.558 - 0.764; C-index for resting HFA-PEFF=0.884, 95% CI 0.823 - 0.944; C-index for exercise HFA-PEFF=0.894, 95% CI 0.839 - 0.949) (Table 2 and Figure 1). Among other parameters, exercise average E/e' had good diagnostic value (C-index=0.877, 95% CI 0.811- 0.944). Peak B-lines >5 (sensitivity=93.4%, specificity 97.5%) and change B-lines >3 (sensitivity=94.7%, specificity 87.5%) were the optimal cut-offs for exercise B-lines (Table S1).

In the LUS beginner-led CET cohort, the diagnostic value of exercise B-lines was good but lower than that observed in the ESE cohort (C-index for peak B-lines= 0.713, 95% CI 0.588 - 0.838; C-index for change B-lines= 0.665, 95% CI 0.538 - 0.793) (Figure 2); we also observed a

certain degree of learning curve effect, with improvement of C-index from the first to second half of patients (C-index 1st half= 0.692, 95% CI 0.495 - 0.890; C-index 2nd half= 0.737, 95% CI 0.576 - 0.898). When applying optimal cut-off values of ESE cohort in the CET cohort, specificity remained excellent (>95% for both peak and change B-lines), but sensitivities were considerably lower, equal to about 30% (*Table S1 in Supplementary Material and Figure 2*). Overall, specificities of other tested cut-off for exercise average E/e', BNP, H2FPEF and HFA-PEFF scores were excellent (98-100%), but sensitivities were poor, ranging from 11 to 47% (*Table S1 in Supplemental Material*).

Incremental diagnostic performance of lung ultrasound on top of HFpEF scores and natriuretic peptides

A significant increase in C-index over H2FPEF score (C-index increase= 0.325, 95% CI 0.222 - 0.428), resting HFA-PEFF score (C-index increase= 0.103, 95% CI 0.045 - 0.161), and exercise HFA-PEFF score (C-index increase=0.093, 95% CI 0.040 - 0.145) was identified for peak B-lines (all P values <0.001) (*Table 2*); similar results were observed for change B-lines (all P values <0.001), peak B-line >5 and change B-line >3 (all P values <0.01). Significant improvement in diagnostic performance was observed for BNP (all P values <0.04). Rest and exercise average E/e' showed significant increase in C-index over HFA-PEFF and H2FPEF score, respectively (*Table 2*).

Overall, the incremental diagnostic value of exercise B-lines (i.e. peak or change B-lines) persisted as significant also over models including HFpEF scores and BNP (*Tables S2 and S3 in Supplementary Material*). Namely, a significant increase in C-index over HFpEF score + BNP was observed for peak B-lines as continuous variable (all P values <0.005) (*Table S2 in Supplementary Material*); similar results were found for B-line changes. Additionally, the use of peak or change B-lines yielded a significant increase over models including each HFpEF score as dichotomous variable (i.e. by using diagnostic cut-offs for both scores) and BNP (all P values

<0.001) (*Table S2 in Supplementary Material*). Significant results were also observed for peak B-lines >5 (all P values <0.02), and change B-lines >3 (all P values <0.05) (*Table S3 in Supplementary Material*).

Discussion

Our results show that exercise pulmonary congestion, as assessed by B-line quantification with LUS, has excellent diagnostic value for HFpEF diagnosis in expert hands, and good diagnostic accuracy when performed by trained LUS beginners with a simple protocol and “low-tech” equipment. Additionally, in expert hands, exercise B-lines has an incremental diagnostic value on top of available HFpEF scores (i.e. H2FPEF and HFA-PEFF scores), or over models including score and natriuretic peptides. Our findings suggest that a semi quantitative assessment of pulmonary congestion with exercise B-lines could represent a new promising tool in the diagnostic of HFpEF, also being largely scalable given its simplicity.

LUS and exercise LUS is an excellent prognostic tool for predicting adverse outcome in both HF with reduced ejection fraction (HFrEF)^{12,26} and HFpEF^{17,27}. Additionally, in an acute setting, LUS has demonstrated excellent value in diagnosing decompensate HF, which is superior to physical examination and chest X-ray^{14,28}. However, regarding a chronic HF setting, we currently have no evidence on the diagnostic value of LUS. According to the latest HF guidelines^{1,2}, HFrEF diagnosis only requires the presence of symptoms and/or signs of HF and a reduced LVEF (i.e. $\leq 40\%$). On the other hand, HFpEF diagnosis can represent a complex challenge for the clinician due to the frequent presence of comorbidities that can mimic this pathology, and to the common absence of symptoms or functional alterations at rest in a non-negligible part of these patients¹⁷. The present study showed that exercise LUS is a promising and reliable tool for HFpEF diagnosis, even in non-expert hands.

Kagami et al recently assessed the diagnostic value of exercise LUS in a cohort of 255 patients (134 with HFpEF) undergoing maximal ESE²⁹. LUS was performed 1 min after the

initiation of each stage by using a 4-scanning point method (right third and fourth spaces along the mid-axillary and mid-clavicular lines). Prevalence of exercise EVLW (defined as B-line ≥ 1 only during exercise or B-line change ≥ 1 from rest) increased during exercise to a greater extent in patients with HFpEF than that in controls; namely, the most prominent difference was observed during the recovery phase. B-lines change from rest to the recovery period showed good accuracy in diagnosing HFpEF (AUC= 0.74, $P < 0.0001$), and improved model performance when added to H2FPEF score and left atrial strain (χ^2 by nested models 54.2 vs. 45.6, $P = 0.003$). These findings are overall in line with our results. First, the recovery phase represent the optimal timeframe to assess exercise B-lines. Additionally, the study by Kagami et al confirmed that exercise B-lines has incremental diagnostic capability over H2FPEF score. Diagnostic performance of B-line change in that study (AUC=0.74) was comparable to that observed in our CET cohort (C-index=0.67), but lower than our ESE cohort (C-index=0.98). However, taking into account differences in terms of LUS scanning methods, exercise protocols, and level of operator expertise, a direct comparison with our study cannot be made.

Two diagnostic scores have recently been proposed to support the clinician in HFpEF diagnosis, the H2FPEF score³ and the HFA-PEFF algorithm⁴. The H2FPEF score, (derived from an American cohort of 414 patients undergoing invasive hemodynamic study during exercise for dyspnea of unknown origin), provided a good diagnostic accuracy (AUC 0.841, 95% CI 0.802 – 0.881, $P < 0.0001$). It is also significantly superior to expert opinion definitions (delta AUC=0.169 vs 2016 HF guidelines, $p < 0.0001$). A high score (i.e. ≥ 6) has $\geq 90\%$ probability of diagnosing HFpEF³. The HFA-PEFF score has been developed according to previous findings and expert opinions⁴. It was externally validated in two independent cohorts³⁰: the diagnostic accuracy of the score was excellent (AUC 0.90, 95% CI 0.84–0.96), and, similarly to H2FPEF score, a high-likelihood category (i.e. 5–6 points) can rule-in HFpEF with high specificity (93%). However, conflicting results were subsequently reported by validation analyses in external cohorts for both scores³¹⁻³⁴. In a cohort of 156 patients with chronic dyspnea and LVEF $\geq 50\%$ (HFpEF diagnosis

based on invasive hemodynamic definition), the diagnostic accuracies of both scores appeared to be lower (AUC=0.73 for HFA-PEFF and = 0.74 for H2FpEF score)³⁴. The H2FPEF score showed lower diagnostic accuracy (AUC=0.720) as compared to the seminal study also in another cohort of 621 patients from the Alberta HEART study, mainly driven by a reduced performance in the subgroups of patients presenting with dyspnea (AUC=0.580, specificity for high score =69.1%)³²; conversely, in a retrospective case-control study (with invasively-proven HFpEF diagnosis) including 736 patients with unexplained dyspnea, H2FPEF score had superior diagnostic performance compared with the HFA-PEFF score (AUC 0.845 and 0.710, respectively; delta AUC=-0.134, p <0.001)³³. In a cohort of 363 consecutive patients with suspected HFpEF, the diagnostic performance of both score was good (AUC=0.77 for H2FPEF and =0.88 for HFA-PEFF); however, 41% of patients were classified into different likelihood categories depending on the score used³¹.

Overall, both scores have moderate to good diagnostic capability for HFpEF diagnosis. The main limitation of both scores is the relevant proportion of patients who are classified as having HFpEF with intermediate probability, for whom an advanced work-up is needed (i.e. an ESE with non-invasive estimation of filling pressure or invasive hemodynamic measurements at rest or upon exercise)^{3,4}. That proportions of patients ranged from ≈ 37 to 58% for HFA-PEFF^{30,31,33,34} and from ≈ 44 to 61% H2FPEF^{3,31-34}, which roughly corresponds to half of patients with a suspected diagnosis of HFpEF; additionally, physicians may not have access to the additional suggested testing for advanced-work-up. Our study shows how exercise LUS can provide additional information to available scores, and can integrate those validated tools for HFpEF diagnosis.

Interestingly, we demonstrated that exercise LUS during a CET performed by LUS beginners had good diagnostic value (AUC=0.713), which was comparable to diagnostic accuracy of H2FPEF in our study, and to both scores in other cohorts³²⁻³⁴. Performing LUS during a CET is a scalable approach; on the other hand, ESE needs specific training and experienced operators, and

it may be difficult to implement in some clinical arenas. Our study suggests that operators with limited or no experience with LUS, after completing a non-intensive training program, can easily perform exercise LUS, achieving a good diagnostic value for HFpEF diagnosis. This also indicates that this technique should be not restricted to experienced physicians or healthcare professionals, as already demonstrated in other settings³⁵, and it could be therefore widely applied in most centers. It should be considered that the observed good performance of exercise LUS in the CET cohort was obtained in optimal conditions in terms of ease of implementation, but suboptimal conditions to unveil B-lines. In addition to the use of a pocket-sized ultrasound system, sensibility in B-line assessment may have been decreased by orthostatic position³⁶. This can also partially explain the observed difference in terms of diagnostic accuracy between the two cohorts in our study.

We also showed that exercise LUS is effective in diagnosing HFpEF with different LUS protocols (i.e. 28-scanning point and 8-zone protocols) or exercise workloads. At this purpose, as expected, in the maximal CET cohort, HFpEF patients attained lower workload as compared with controls (57 ± 33 vs. 140 ± 47 watts, $P < 0.0001$; data not shown); in the submaximal ESE cohort, there were no differences in terms of achieved workload intensity. Although those differences, HFpEF showed significantly higher exercise B-lines than controls in both cohorts. These results point out the notions that an increase in B-lines can occur in patients with HFpEF even in those with low exercise capacity during either CET or ESE^{15,37}

These results represent a promising avenue for future research; indeed, this seminal data on the usefulness of exercise LUS for HFpEF diagnosis suggests another possible application for LUS in a chronic HF setting. We acknowledge these results need to be replicated in further larger studies or trials, also including a wider phenotypic spectrum. In the absence of these validating studies, our work should be considered as hypothesis generating. In principle, detection of B-lines does not necessarily imply their cardiogenic origin¹⁷. However, a sudden increase during effort is nearly certain related to a cardiogenic origin of EVLW. Additionally, case and controls were not

matched on age and sex; namely, patients with HFpEF were significantly older than controls in both cohorts (*Table 1*), and some effects on B-line count cannot be excluded³⁸. With regard to LUS protocols, best cut-offs (28-scanning point protocol) were peak B-line >5 and change B-line >3 in the ESE cohort. It could be expected that similar cut-offs adapted to the 8-zone protocol (which roughly corresponds to peak B-line >2 and change B-line >1) would performed better in the CET cohort; however, the best cut-off were the same. This difference in thresholds may partially be attributed to a certain degree of heterogeneity between the two population cohorts (mainly age, and differences in terms of risk factor prevalence) and the exercise/imaging protocols (steeper resistance in the CET cohort when compared to the ESE cohort). Finally, in both cohorts, control subgroups exclusively included non-dyspneic patients, and this may have partially influenced exercise LUS diagnostic accuracy³³.

In summary, exercise LUS can represent a new diagnostic tool HFpEF diagnosis with different exercise protocols and levels of expertise, by showing incremental diagnostic accuracy on top of available score/algorithms for HFpEF diagnosis and natriuretic peptides.

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Statements and Declarations

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Table 1 Baseline demographic and clinical characteristics (HFpEF versus controls) in different settings (exercise stress echocardiography and cycle ergometer test cohorts)

	Exercise stress echocardiography cohort (N=116)	HFpEF (N=76, 65.5%)	Controls (N=40, 44.5%)	P value	Cycle ergometer test cohort (N=54)	HFpEF (N=27, 50%)	Controls (N=27, 50%)	P value
Demographics								
Male, n (%)	48 (41.4%)	34 (44.7%)	14 (35.0%)	0.312	33 (61.1%)	13 (48.1%)	20 (74.1%)	0.051
Age (years), mean (±SD)	63.2 ± 9.9	65.2 ± 8.3	59.6 ± 11.7	0.009	73.9 ± 7.0	78.7 ± 6.8	69.4 ± 3.3	<0.0001
BMI (Kg/m ²), mean (±SD)	28.8 ± 4.1	29.4 ± 3.6	27.6 ± 4.6	0.043	28.8 ± 4.1	28.9 ± 5.8	27.2 ± 4.6	0.162
Medical history								
Hypertension, n (%)	116 (100%)	76 (100%)	40 (100%)	NA	42 (77.8%)	25 (92.6%)	17 (63.0%)	0.009
Diabetes, n (%)	33 (28.4%)	28 (36.8%)	5 (12.5%)	0.006	14 (25.9%)	12 (44.4%)	2 (7.4%)	0.002
Dyslipidemia, n (%)	97 (83.6%)	74 (97.4%)	23 (57.5%)	<0.0001				
Coronary artery disease, n (%)	28 (24.1%)	24 (31.6%)	4 (10.0%)	0.01	17 (31.5%)	9 (33.3%)	8 (29.6%)	0.770
Current smoking, n (%)	27 (23.3%)	20 (26.3%)	7 (17.5%)	0.286	4 (7.4%)	1 (3.7%)	3 (11.1%)	0.299
Previous HHF, n (%)	65 (56.0%)	65 (85.5%)	0 (0.0%)	<0.0001	27 (50.0%)	27 (100%)	0 (0.0%)	<0.0001
Previous AF episode, n (%)	13 (11.2%)	13 (17.1%)	0 (0.0%)	0.006	17 (31.5%)	17 (63%)	0 (0.0%)	<0.0001
Therapy								
ACE-i or ARB, n (%)	109 (94.0%)	72 (94.7%)	37 (92.5%)	0.631	37 (68.5%)	19 (70.4%)	18 (66.7%)	0.770
Beta-blocker, n (%)	115 (99.1%)	76 (100.0%)	35 (97.5%)	0.166	27 (50.0%)	18 (66.7%)	9 (33.3%)	0.014
Statin, n (%)	97 (83.6%)	72 (94.7%)	25 (62.5%)	<0.0001	17 (31.5%)	5 (18.5%)	12 (44.4%)	0.04
Diuretics, n (%)	91 (78.4%)	65 (85.5%)	26 (65.0%)	0.011	35 (64.8%)	27 (100%)	8 (29.6%)	<0.0001
Nitrates, n (%)	17 (14.7%)	15 (19.7%)	2 (5.0%)	0.033				
Aldosterone antagonist, n (%)	11 (9.5%)	11 (14.5%)	0 (0.0%)	0.011	27 (50.0%)	18 (66.7%)	9 (33.3%)	0.014
Vital signs								
Systolic BP, rest (mmHg), mean	124.4 ± 8.6	124.9 ± 8.4	124.4 ± 9.0	0.365	124.4 ± 8.6	128.1 ± 21.2	135.6 ± 20.3	0.155
Systolic BP, peak (mmHg), mean	143.5 ± 10.7	147.5 ± 10.0	136.0 ± 7.3	<0.0001	143.5 ± 10.7	156.4 ± 28.3	178.7 ± 18.4	0.001
Diastolic BP, rest (mmHg), mean	75.3 ± 7.0	75.0 ± 7.1	75.9 ± 6.9	0.519	75.3 ± 7.0	70.1 ± 12.0	78.0 ± 8.5	0.004
Diastolic BP, peak (mmHg), mean	78.8 ± 5.8	79.8 ± 7.8	76.9 ± 5.5	0.010	78.8 ± 5.8	75.7 ± 13.8	84.7 ± 14.0	0.022
Laboratory								
Haemoglobin (gr/dl), mean (±SD)	13.5 ± 1.2	13.4 ± 1.4	13.6 ± 0.9	0.348				
Creatinine (mg/dl), mean (±SD)	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.3	0.601				
Clearance creatinine (ml/min/1.73),	75.3 ± 20.6	79.5 ± 20.7	67.3 ± 18.2	0.002				
BNP (pg/ml), mean (±SD)	24.6 (11.1-41.5)	31.1 (19.5-59.2)	10.8 (6.3-20.2)	<0.0001				
Echocardiography-LUS								
LV mass index (gr/m ²), mean (±SD)	120.8 ± 27.8	129.4 ± 25.6	104.3 ± 24.4	<0.0001				
LV ejection fraction (%), median	58.6 (54.0-64.0)	56.0 (52.2-63.0)	60 (58.0-64.0)	0.001				
Left atrial volume index (ml/m ²),	27.7 ± 6.9	28.5 ± 7.5	26.1 ± 5.4	0.050				
E/A, mean (±SD)	0.68 ± 0.4	0.69 ± 0.4	1.0 ± 0.3	<0.0001				
Average e', median (IQR)	8.5 ± 2.3	7.8 ± 2.1	10.0 ± 1.8	<0.0001				
Average E/e', median (IQR)	7.9 ± 2.1	7.8 ± 2.3	7.9 ± 1.7	0.804				
Peak exercise average E/e', median	11.3 ± 3.8	12.9 ± 3.6	8.2 ± 1.5	<0.0001				
TAPSE (mm), mean (±SD)	22.1 ± 2.6	22.0 ± 2.6	22.3 ± 2.6	0.572				
s' (cm/s), median (IQR)	13.7 ± 2.3	14.1 ± 2.6	12.9 ± 1.8	0.003				
TR max velocity- (m/s), median	1.7 ± 0.5	1.7 ± 0.5	1.6 ± 0.6	0.209				
B-lines median (IQR)	1 (0-3)	2 (0-3)	0 (0-1)	<0.0001	0 (0-2)	1 (0-4)	0 (0-0)	0.012
Peak exercise B-line, median (IQR)	8 (3-11.8)	10 (8-13)	2 (1-3)	<0.0001	0 (0-5)	4 (0-9)	0 (0-1)	0.003
Change B-lines, median (IQR)	6 (2.3-9)	8 (6-11)	2 (1-3)	<0.0001	0 (0-1.3)	0 (0-3)	0 (0-0)	0.017

HFpEF, heart failure with preserved ejection fraction; BMI, body mass index; HHF, hospitalization for heart failure; AF, atrial fibrillation; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin I receptor blocker; BP, blood pressure; BNP, B-type natriuretic peptide; LV, left ventricular, SD, standard deviation, IQR, interquartile range; TAPSE, tricuspid annular plane systolic excursion; TR, tricuspid regurgitation.

Table 2. Diagnostic performance and additive diagnostic accuracy of B-line and other echocardiographic or bio-humoral tools on top of different HFpEF score in the diagnosis of HFpEF (Exercise stress echocardiography cohort)

Considered Parameter	C-index Considered Parameter	C-index increase of Considered Parameter on top of H2FPEF score	P-value	C-index increase of Considered Parameter on top of HFA PEFF score rest	P-value	C-index increase of Considered Parameter on top of HFA-PEFF score exercise	P-value
Echocardiography							
Average E/e' (rest)	0.510 (0.403 - 0.617)	0.008 (-0.019 - 0.036)	0.55	0.034 (0.003 - 0.064)	0.029	0.033 (0.004 - 0.063)	0.028
Average E/e' (rest) \geq 9	0.551 (0.470 - 0.633)	0.002 (-0.012 - 0.017)	0.76	0.014 (0.001 - 0.026)	0.032	0.009 (0.001 - 0.017)	0.031
Average E/e' (exercise)	0.877 (0.811 - 0.944)	0.222 (0.117 - 0.326)	<0.0001	0.026 (-0.006 - 0.058)	0.11	0.016 (-0.011 - 0.043)	0.25
Average E/e' (exercise) \geq 15	0.651 (0.599 - 0.703)	0.093 (0.041 - 0.144)	0.0004	0.010 (-0.001 - 0.021)	0.064	0.000 (0.000 - 0.000)	1.00
Natriuretic peptides							
BNP	0.849 (0.781 - 0.917)	0.191 (0.094 - 0.288)	0.0001	0.029 (0.002 - 0.056)	0.037	0.033 (0.005 - 0.062)	0.023
BNP \geq 35 pg/ml	0.718 (0.656 - 0.779)	0.122 (0.051 - 0.193)	0.0007	0.018 (-0.006 - 0.042)	0.14	0.019 (-0.004 - 0.042)	0.11
Lung ultrasound							
Rest B-line	0.841 (0.776 - 0.906)	0.199 (0.102 - 0.296)	<0.0001	0.051 (0.013 - 0.090)	0.008	0.049 (0.012 - 0.085)	0.010
Peak B-line	0.985 (0.968 - 1.000)	0.325 (0.222 - 0.428)	<0.0001	0.103 (0.045 - 0.161)	0.0005	0.093 (0.040 - 0.145)	0.0005
Peak B-lines > 1	0.637 (0.567 - 0.708)	0.071 (0.016 - 0.127)	0.011	0.026 (0.004 - 0.048)	0.018	0.026 (0.004 - 0.047)	0.018
Peak B-lines > 2	0.781 (0.702 - 0.860)	0.192 (0.101 - 0.284)	<0.0001	0.053 (0.013 - 0.093)	0.010	0.050 (0.012 - 0.087)	0.009
Peak B-lines > 3	0.881 (0.814 - 0.948)	0.234 (0.141 - 0.328)	<0.0001	0.067 (0.024 - 0.110)	0.002	0.064 (0.023 - 0.105)	0.002
Peak B-lines > 4	0.924 (0.870 - 0.977)	0.273 (0.177 - 0.370)	<0.0001	0.069 (0.024 - 0.114)	0.003	0.064 (0.022 - 0.105)	0.003
Peak B-lines > 5	0.955 (0.917 - 0.992)	0.303 (0.194 - 0.413)	<0.0001	0.083 (0.034 - 0.132)	0.001	0.075 (0.031 - 0.119)	0.001
Change B-lines	0.975 (0.953 - 0.997)	0.314 (0.214 - 0.414)	<0.0001	0.095 (0.040 - 0.150)	0.0007	0.085 (0.035 - 0.135)	0.0009
Change B-lines > 1	0.662 (0.589 - 0.736)	0.074 (0.019 - 0.130)	0.008	0.026 (0.005 - 0.048)	0.017	0.026 (0.005 - 0.048)	0.017
Change B-lines > 2	0.824 (0.749 - 0.900)	0.202 (0.111 - 0.294)	<0.0001	0.053 (0.012 - 0.094)	0.011	0.050 (0.012 - 0.089)	0.010
Change B-lines > 3	0.911 (0.853 - 0.969)	0.262 (0.166 - 0.357)	<0.0001	0.068 (0.023 - 0.114)	0.003	0.063 (0.021 - 0.105)	0.003

BNP, B-type natriuretic peptide.

Figure legends

Figure 1: Diagnostic accuracy of exercise B-lines (B-line peak) and different score in the diagnosis of HFpEF (Exercise stress echocardiography cohort)

Diagnostic accuracy of exercise B-lines and different scores in the diagnosis of HFpEF

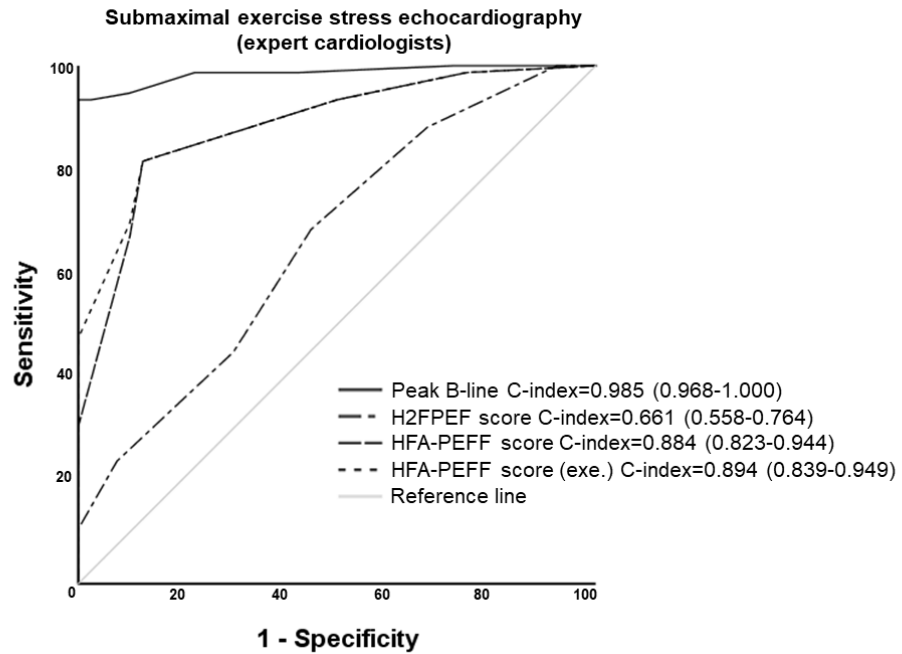


Figure 2: Diagnostic accuracy of exercise B-lines (B-line peak) in diagnosing HFpEF with different protocols and operator experience

Diagnostic accuracy of exercise B-lines (Peak B-lines) in the diagnosis of HFpEF

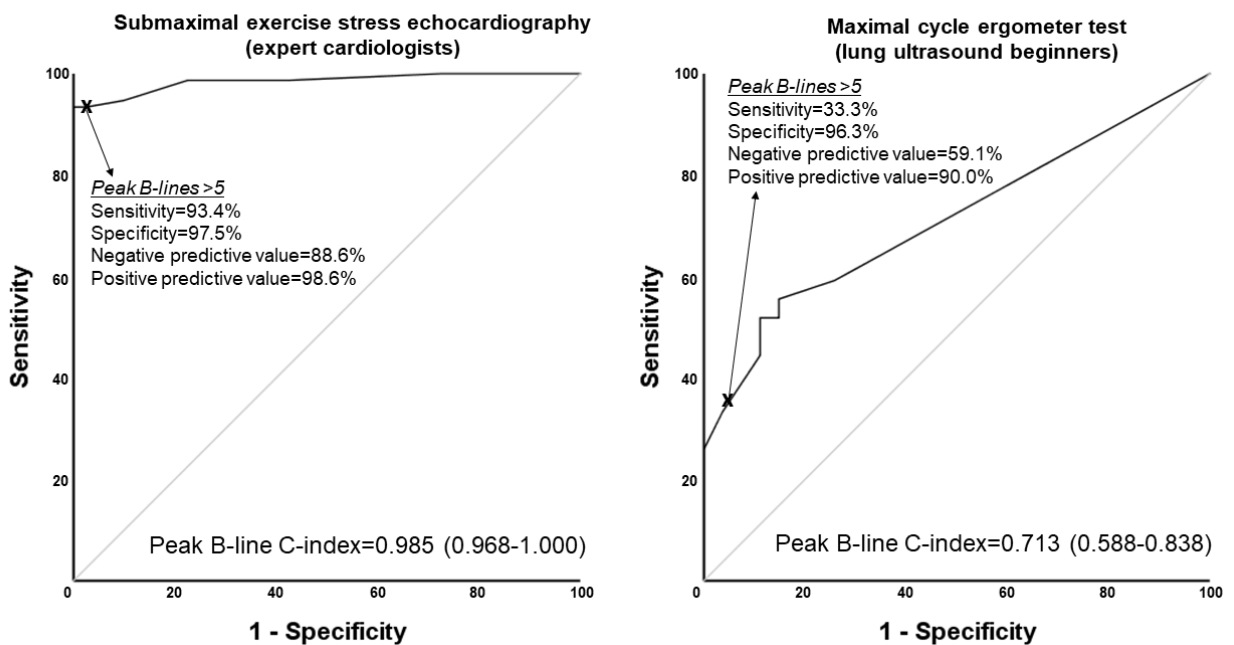
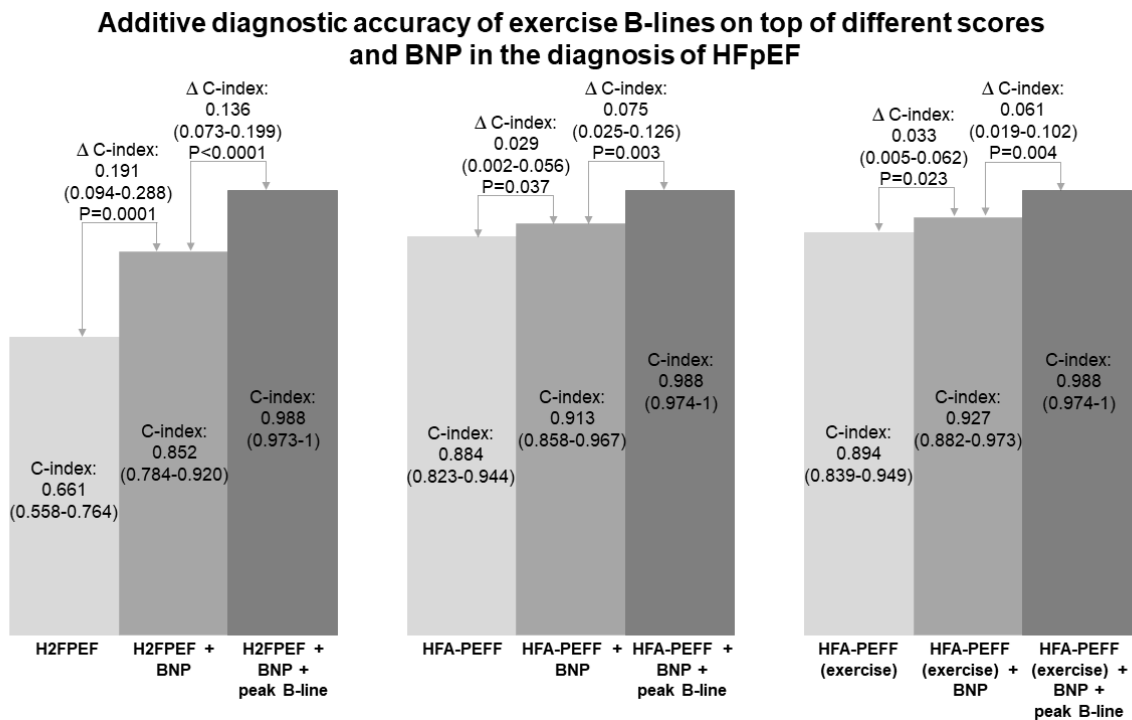


Figure 3: Additive diagnostic accuracy of exercise B-lines on top of different scores and BNP in the diagnosis of HFpEF (Exercise stress echocardiography cohort)



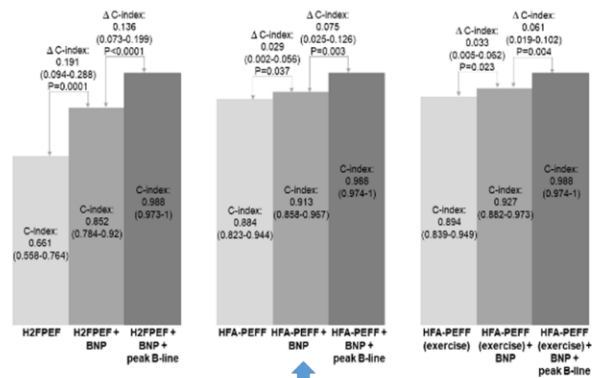
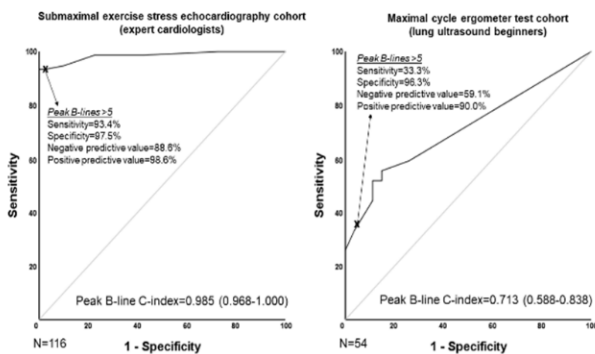
Graphical Abstract

Exercise-induced B-lines for the Diagnosis of Heart Failure with Preserved Ejection Fraction

Aim: assess the diagnostic value of exercise lung ultrasound (LUS) for HFpEF diagnosis and compare its diagnostic capability with that of currently recommended diagnostic scores (H2FPEF score and HFA-PEFF scores)

Methods

- Two independent case-control studies:
 - Study 1) submaximal exercise stress echocardiography (ESE) with LUS performed by expert cardiologists.
 - Study 2) maximal cycle ergometer test with LUS performed by beginners.
- Assessment of B-line kinetics (i.e. peak values and their changes from rest).



LUS demonstrated additive diagnostic accuracy on top of available scores and natriuretic peptides (BNP) in the ESE cohort.

Exercise LUS showed good/excellent diagnostic value for HFpEF diagnosis regardless of different exercise protocols/level of expertise.