**METHODS**

**Study Population**. We prospectively enrolled consecutive ambulatory patients with cardiovascular risk factors, signs and symptoms of HF, from January 2018 to January 2020. All patients were referred to our dedicated excellence HF centre to establish if they have HFpEF, according to guidelines. In order to evaluate the prediction power of each echocardiographic parameter for HFpEF diagnosis, patients with HFpEF were compared to a group of patients with similar risk factor profile and DD, but without HF signs and symptoms, and normal NTproBNP level (preHF group), also prospectively enrolled on a 2:1 ratio model (HFpEF:preHF). The study protocol conforms to the ethical guidelines as reflected in a priori approval by the Institution's Human Research Committee of the University of Medicine and Pharmacy Carol Davila. Informed consent was obtained in all subjects prior to enrolment.

Inclusion criteria for HFpEF group were: age > 18 years, sinus rhythm, stable patient with clinical, biological, and echocardiographic criteria suggestive for HFpEF, according to 2016 guidelines (4), informed consent signed. Exclusion criteria were recent hospitalization for acute HF (< 4weeks), sustained atrial/ventricular arrhythmia, significant valvular heart disease, hypertrophic cardiomyopathy, pericardial disease, previous history of myocarditis, any systemic inflammatory disease or vasculitis*,* active cancer in the last year*,* renal failure with haemodialysis, pulmonary causes of dyspnoea, moderate to severe anaemia, inappropriate quality of echocardiographic images for STE analysis. preHF group was selected from the asymptomatic patients with risk factors, without clinical and biological criteria for HFpEF. All patients had clinical examination, 12-lead electrocardiogram, screening laboratory tests, NTproBNP, and a comprehensive 2DE.

Demographic and clinical data were collected before echo protocol (age, gender, BMI, heart rate, systolic and diastolic blood pressure, HF symptoms and NYHA class, if any, HF aetiology, risk factors (hypertension, diabetes mellitus, smoker status, pulmonary diseases, sleep apnea, obesity, dyslipidaemia, ischemic disease documented by ECG, coronarography or other imaging technique). Treatment with all potentially cardiovascular active drugs was recorded on enrolment. After complete evaluation of the patients, the HFA-PEFF score was calculated, according to the current consensus.

**Echocardiographic protocol**

**Conventional echocardiography** was performed using a Vivid E9 echocardiographic ultrasound system (GE Healthcare, Horten, Norway) with a 3.5 MHz transducer. The electrocardiographic tracing was adjusted to show a well-defined P wave. Standard images were acquired and digitally stored for offline analysis using a vendor specific software Echo PAC PC, version BT12. Echocardiographic protocol included three apical views (four-chamber, two-chamber, and long-axis) optimized for LV, followed by dedicated apical four-chamber and two-chamber views for LA, avoiding foreshortening of the LV and LA, during acquisition. For each view, three consecutive heart cycles were recorded with a frame rate ranging between 50 and 80 frames/sec. A good quality electrocardiogram (ECG) trace with well visible P was recorded. All images were digitally stored and exported to a separate workstation for offline analysis. The operators performing the echocardiographic analysis were blinded to the patient’s clinical details. The quantification of the cardiac chamber size and function was performed in agreement with the current guidelines. LV mass indexed (LVMI) was calculated using the linear method from the parasternal long axis view (Cube formula) and indexed to the body surface area (BSA).

LAVi max was calculated using the biplane disk summation technique and indexed to BSA, as a mean from the apical 4- and 2-chamber views. Transmitral pulsed-wave Doppler velocities (E, A) and tissue Doppler velocities of the septal and lateral mitral annulus were recorded and mean of both velocities was calculated (E’). Tricuspid regurgitant jet velocity and inferior vena cava diameter were measured for the estimation of the sPAP. DD was assessed in a step-by-step algorithm, based on the 2016 American Society of Echocardiography recommendations.

**LV global longitudinal strain (GS)** was measured from apical 2D views (four-, two-, and three-chamber views), by manually tracing the endocardial border of the LV at the end of systole, at the smallest LV chamber size. GS was calculated as the average strain values of all 18 LV segments, according to the guidelines.

**LA volumetric and functional assessment by 2DE.** LA analysis was performed by two experienced operators, experts in STE analysis (RCR and SMB). LA volumetric assessment was done from 4C and 2C dedicated views, and reported as a mean value and indexed by BSA, as follows:

* LA maximal volume (LAVi max) - volume at the LV end-systole, before the mitral valve opening.
* LA pre-A volume (LAVi pre-A) - volume before the onset of the P-wave on the ECG tracing.
* LA minimal volume (LAVi min) - volume at the LV end-diastole, after mitral valve closure.

**LA phasic functions** were generated by using published formulas, based on LA volumes [28]:

* **Reservoir function**:

LA total emptying fraction (EF) = (LAV max – LAV min)/LAV max x 100

LA expansion index= (LAV max – LAV min) /LAV min x100

* **Conduit function**: LA passive EF= (LAV max - LAV preA)/LAV max x 100
* **Pump function**: LA active EF= (LAV preA – LAV min)/LAV preA x100

**LA longitudinal deformation analysis by STE**. A detailed LA longitudinal deformation evaluation by 2DSTE was published previously by our echocardiography lab. In summary, the LA strain curves were generated by manually tracing the endocardial border in the apical four- and two-chamber views (P-P gating). The average deformation values from the apical four- and two-chamber views were used for analysis. LA phasic functions were defined as follows:

* **LA reservoir function**: LA reservoir strain (S\_R) deformation between MVC to MVO (calculated as the sum between the peak negative longitudinal strain (absolute value), and LA reservoir strain rate (SR\_R) during systole, defined as peak positive strain rate from de strain rate curve.
* **LA conduit function**: LA conduit strain (S\_CD) as peak positive strain (Figure 1A), and LA conduit strain rate (SR\_CD) as peak negative strain rate during early LV diastole.
* **LA booster pump function**: LA contractile strain (S\_CT) as peak negative strain (**Figure 1A**) and strain rate during late diastole, corresponding to atrial contraction, defined as and strain rate (SR\_CT).
* **LA overload indices.** We defined two non-invasive indices as markers of LA overload:
	+ **stiffness index (SI)**, estimated by the ratio between E/E’ ratio and S\_R, as a marker of pressure overload, representing the amount of pressure required to induce a change in LA deformation during the reservoir phase.

**distensibility index (DI)**, estimated by the ratio between S\_R and LAVi max, as a marker of volume overload, representing the amount of deformation for any volume change of LA, knowing that the excursion is lower for an already dilated LA.We also evaluated the time added for measurement of all LA parameters, in order to estimate how much time adds a functional assessment of the LA at the conventional echocardiographic evaluation proposed by the guidelines.

**Reproducibility**. Intra- and inter-observer variability from our echo lab for all conventional echocardiographic and TDI parameters, and also for STE parameters were already reported elsewhere. We also assessed intra- and inter-observer variability for all LA strain and strain rate parameters, on 10 patients that were randomly selected, using an online random selection generator. The intra-observer assessment was performed at 2 weeks apart (RRC). For inter-observer variability assessment, the same patients were analysed by a second blinded observer (SMB).

**Statistical Analysis.** All continuous variables were assessed for the normal distribution by Kolmogorov–Smirnov test. Normally distributed continuous variables were reported as mean ± SD and compared for statistical significance with Independent Samples T Test. Non-normally distributed continuous variables were presented as the median and interquartile range (IQR) and compared using the Mann Whitney *U* Test. A p value of <0.05 was considered significant. Categorical variables were expressed as percentages and compared with Chi-square test. Correlation between continuous variables was performed using Pearson’s or Spearman’s correlation coefficient as appropriate. Multivariate logistic regression was used to identify predictors of HFpEF and to calculate the corresponding odds ratios. The receiver operating characteristic (ROC) curve was used to identify prediction of HFpEF diagnosis for each echocardiographic parameter and to determine cut-off values. Sensitivity (Se) and specificity (Sp) were calculated. ICCs and their 95% confident intervals were calculated usingSPSS statistical package, based on an absolute-agreement, 2-way mixed-effects model. Statistical analysis was performed using SPSS version 21 (SPSS Inc., Chicago, IL, USA).