INTRODUCTION
Heart failure (HF) is a chronic disease whose prognosis remains poor. Vitamin D (Vit D) is classically involved in bone homeostasis, but many studies strongly suggest extra skeletal functions including pleiotropic effects on cardiovascular health. Evidences indicate that low serum 25-hydroxivitamin D (25OHD, a biomarker of Vit D status) levels are associated with increased risk of cardiovascular disease (CVD), impaired exercise performance in HF patients and Left Ventricle dysfunction. A major limit of all studies in literature is the measurement of serum 25OHD: since traditional immunoassay are often inaccurate and have a remarkable intra and inter-assay variability. High Performances Liquid Chromatography (HPLC-MS-MS) has shown high accuracy and specificity and can resolve this problem.

AIMS
1. To define 25 OH-D levels in the HF population by a RETROSPECTIVE analysis. To reveal the condition of vitamin-D deficiency or insufficiency in the HF population, since vitamin D status is often a neglected data in these patients.
2. To correlate 25 OH-D levels and HF outcome markers (biochemical and instrumental evaluation) by RETROSPECTIVE analysis.

MATERIALS AND METHODS
1. Patients: N = 261
CLINICAL DATABASE at Fondazione Toscana Gabriele Monasterio (FTGM), Unit of Cardiology (Pisa): data were collected from interviews, physical examinations and biochemical and instrumental test. All patients with heart failure (HF) in different NYHA classes consecutively seen in FTGM ambulatories: 1) Anthropometric data (age, sex, weight, height, BMI); 2) Anamnestic records and drugs

Biochemical evaluation: 1) GENERAL: hemoglobin, serum electrolytes, 2) NEUROHORMONES: PRA, aldosterone, catecolamine, BNP levels, 3) KIDNEY FUNCTION: creatinine and glomerular filtration rate as MDRD 4) BONE METABOLISM: albumin adjusted serum calcium, PTH levels

2. 25-OH-VITAMIN D QUANTIFICATION
We used the blood samples (serum) collected at the baseline evaluation and stored at -80 C. Fast isotope dilution Mass Spectrometry coupled to High Performances Liquid Chromatography (HPLC-MS-MS) method was developed for the accurate measurement of 25-OH-vitamin D status. HPLC-MS-MS offers a good quantification accuracy and the contribution of interfering compounds to the final results is limited (as previously described)

3. INSTRUMENTAL EVALUATION
1) Ejection fraction (echocardiography)
2) Cardiopulmonary exercise test (CPET) parameters: peak oxygen consumption (VO2), VE/VCO2 slope and Watt peak have utility in prognostic stratification for patients with heart failure. 3) Calculation of Mecki score index (Figure 1)

RESULTS
Patients were 47 females and 214 males (ratio M:F=4:1), with a mean age of 65±12 years and mean BMI of 28±4. They had stable HF disease in prevalent NYHA 2 class (Figure 2) and prevalent HF causes were 1) dilative non ischemic cardiomyopathy, 2) ischemic cardiomyopathy, 3) cardiomyopathy secondary to valvular disease. Mean EF (ejection fraction) was 33±8%; patients had mild kidney failure (creatinine 1.12±0.3 mg/dl) and they were normocalcemic and normo-PTH. Levels of 25OHvitaminD ranged 2-45 ng/ml, with mean of 17±9 ng/ml. Twenty-five% (n=65) patients had vitamin D deficiency (<10ng/ml), 62% (n=161) had vitamin insufficiency (between 10 and 30 ng/ml) and 13% (n=35) had vitaminD>30 ng/ml, without any supplementation. The linear regression analysis showed that 25OHvitaminD levels were positively correlated with CPET paramethers and negatively with mortality Mecki score (Figure 3-5) and this relation was even stronger in patient with Vit D insufficiency (Figure 6).

CONCLUSIONS
This pilot study confirms the condition of vitamin D deficiency or insufficiency in the HF population
This study confirms that HPLC-MS-MS is a valuable alternative to Immunoassay and offers a good quantification accuracy
This study correlates 25 OHD levels and HF outcome in terms of CPET parameters, particularly in HF patients with hypovitaminosis D
Vitamin D represents an emerging factor in the development and progression of cardiovascular disease and is a potential "modifiable factor"
Further studies needed: prospective study

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Calcium & Vitamin D metabolism
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