HPLC-MS-MS 25OHvitaminD levels are associated with prognosis markers of Heart Failure

Federica Saponaro, S. Frascarelli, A. Saba, A. Clerico, T. Prontera, F. Cetani, R. Zucchi, C. Passino, C. Marcocci

UNIVERSITY OF PISA, ITALY

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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 extraskeletric 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

RETROSPECTIVE STUDY

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.

To correlate 25 OH-D levels and HF outcome markers (biochemical and instrumental evaluation) by RETROSPECTIVE analysis.
Hypothesis: vitamin D represents an emerging factor in the development and progression of HF and a potential "modifiable factor risk

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, catecolammine, BNP levels, 3) KIDNEY FUNCTION: creatinine and glomerular filtration rate as MDRD 4) BONE METABOLISM: albumin adjusted serum calcium, PTH

MATERIALS AND METHODS 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)

MATERIALS AND METHODS 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±14. 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).***

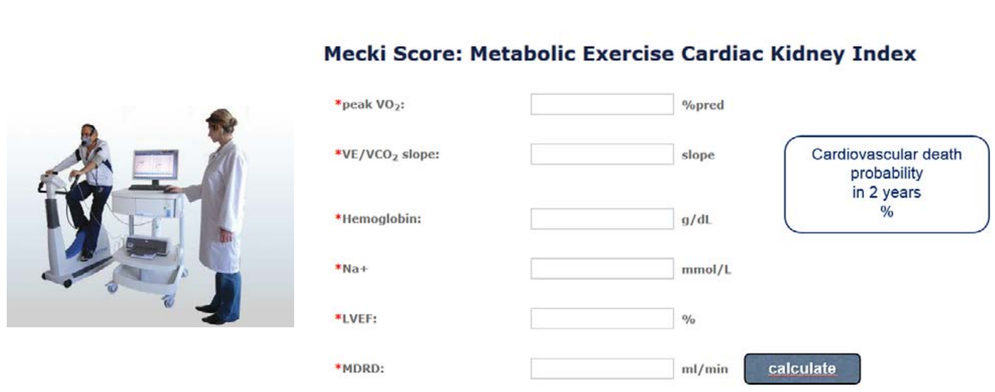

Figure 1: Cardiopulmonary test execution and Mecki score calculation

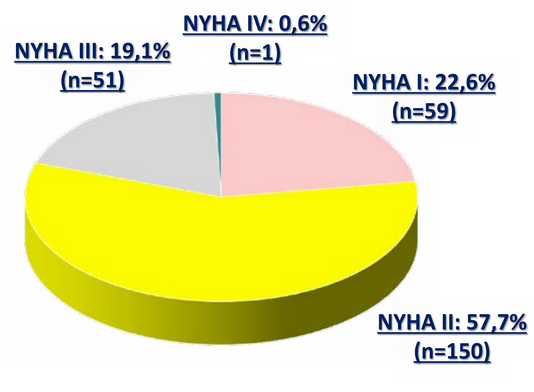

Figure 2: Patients NYHA classes distribution

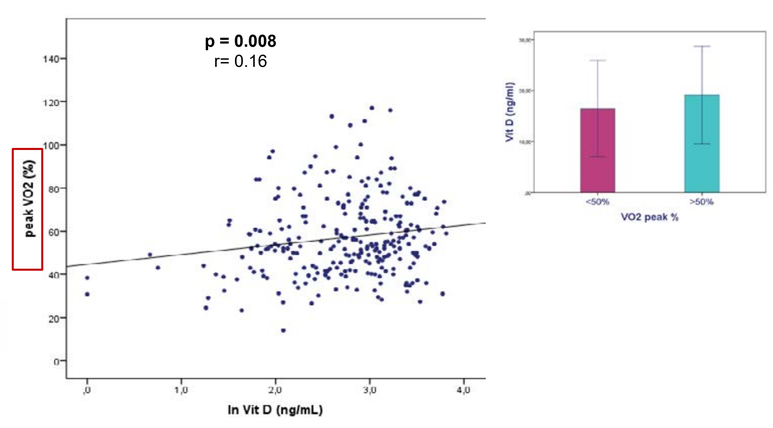

Figure 3: *A)* **Positive correlation between 25OHD and VO2 peak (p<0.1)** in a multivariate model (Age, BMI, Hb, MDRD, NT-proBNP); B) 25-OHD levels were lower in patients with VO2 peak% <50% and severe HF (p = 0.025)

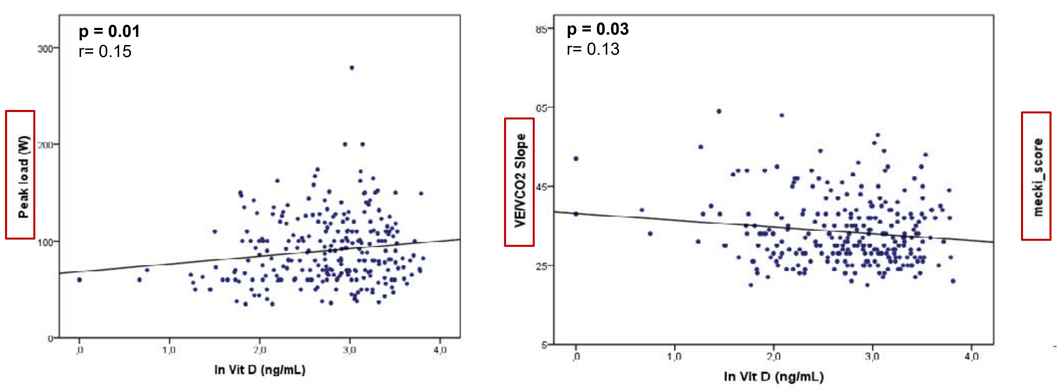

Figure 4: **Positive correlation between 25OHD and peak load** (CPET) and **negative correlation between 25OHD and VE/VCO2 slope** (CPET) in a multivariate model (Age, BMI, Hb, MDRD, NT-proBNP) p<0.1

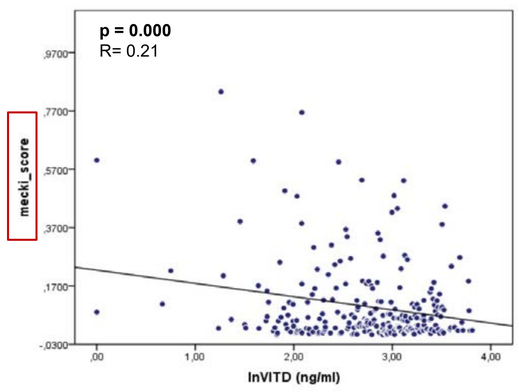

Figure 5: **INVERSE CORRELATION between 25OHD and MECKI score** of mortality in a multivariate model (Age, BMI, Hb, MDRD, NT-proBNP) p<0.0001

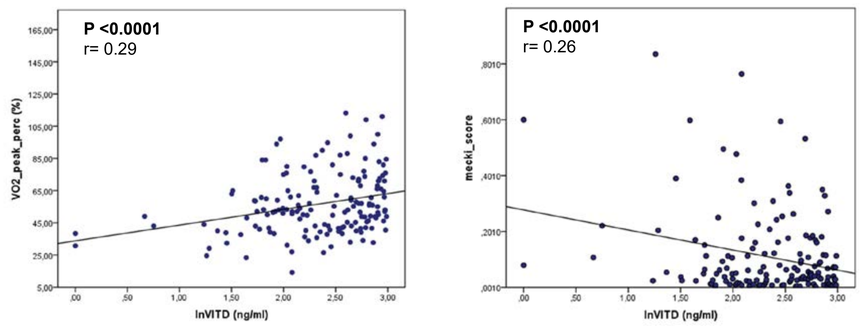

Figure 6: **Positive correlation between 25OHD and VO2 peak** and **negative correlation between 25OHD and Mecki score** in a multivariate model (Age, BMI, Hb, MDRD, NT-proBNP) p<0.0001 in **in patients with hypovitaminosis D (<20 ng/ml – n=158 - 60%)**

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 OH-D levels and HF outcome in terms of CPET paramethers, particularly in HF patients with hypovitaminosis D
- Vitamin D represents an emerging factor in the development and progression of cardiovascular disease and a potential "modifiable factor risk"
- Further studies needed: prospective study

federica.saponaro@gmail.com