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The role of impaired iron transport on exercise performance and prognosis in patients with chronic heart failure

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ABSTRACT

Background: Iron deficiency (ID) is frequent in chronic heart failure (HF). Among HF-ID patients those with impaired iron transport (IIT) (Transferrin saturation (TSAT) < 20 %) have the worst prognosis. In HF survival is strictly related to exercise limitation but the link between IIT, exercise limitation and survival is at present undefined.

Methods: We evaluated in 999 consecutive patients hospitalized for worsening HF whether IIT affects prognosis through cardiopulmonary exercise test (CPET), i.e. peak oxygen uptake (VO_2) and ventilation vs. carbon dioxide (VE/VCO_2) slope. In all patients at stabilization iron metabolism and maximal CPET were performed. Survival was assessed as all cause death, urgent LVAD and heart transplant were considered death equivalents. The causal relationship between survival and IIT, peakVO_2 and VE/VCO_2slope was assessed applying path analysis.

Results: PeakVO_2, VE/VCO_2slope and TSAT were 68 ± 44 %pred, 35 ± 9 and 24.4 ± 12.9, respectively. PeakVO_2 and, VE/VCO_2slope were 61 ± 18 vs. 72 ± 53 %pred and 38 ± 10 vs. 33 ± 8, in IIT vs. non IIT patients ($p < 0.0001$ in both). At univariable and multivariable analysis a correlation between survival and VO_2, VE/VCO_2 slope and TSAT was observed; at Kaplan-Myer lower peakVO_2, higher VE/VCO_2slope and lower TSAT showed worst survival; at path analysis IIT showed both an important effect on survival independent from peakVO_2 and VE/VCO_2slope (48 %) and an effect on survival independently mediated by VE/VCO_2slope and peakVO_2 (52 %), contributing to the IIT negative effect on survival.

Conclusions: The adverse impacts of low TSAT on prognosis are in part direct and in part mediated by mechanisms related to reduced peakVO_2 and increased @VE/VCO_2slope

1. Introduction

In chronic heart failure (HF) iron deficiency (ID) are frequently observed in HF with an independent and additive negative role on prognosis and exercise performance [1]. Specifically, ID is associated with recurrent HF hospitalizations, increased cardiovascular (CV) and all-cause mortality rates irrespective of anemia [2,3]. Of note, in the context of HF, ID exceeds anemia in prevalence [4].

In the current guidelines from the European Society of Cardiology (ESC) and American College of Cardiology/American Heart Association/HF Society of America (ACC/AHA/HFSA), the prevailing definition of ID is ferritin level < 100 μg/L or ferritin level ranging between 100 and 300 μg/L coupled with a transferrin saturation (TSAT) < 20 % [5,6]. Accordingly, ID comprehend reduced iron deposit defined by low ferritin levels and impaired iron transport (IIT) defined as a TSAT level < 20 % irrespective of ferritin levels. IIT is commonly observed in chronic HF patients hospitalized for worsening HF and directly impacts their outcome which is worst compared to other forms of ID [7]. Actually, HF patients with IIT have a more severe clinical condition and poorer prognosis compared to all other ID or non-ID HF patients.

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As regards exercise performance the gold standard method is cardiopulmonary exercise testing (CPET) [8] being peak VO_2 and VE/VCO_2 slope the key CPET obtained prognostic indicators in HF assessment [5,9]. Recent studies considering the relationship between IIT in HF patients and CPET findings, reveal that individuals with IIT exhibit lower peak VO_2 and higher VE/VCO_2 slope compared to HF ID patients with low iron reserve (serum ferritin < 100 μg/L) [10]. Moreover, HF patients with IIT have a worst clinical outcome compared to non IIT cases.

Given the prognostic significance of IIT and CPET in HF patients, along with their interrelation, this study aimed to investigate whether or not IIT affects prognosis of HF patients through the two main CPET parameters: VO_2 and VE/VCO_2 slope. To do so we applied an advanced statistical method (path analysis) able to clarify the putative causal path between variables of interest [11–13].

2. Material and methods

2.1. Study population

We retrospectively analyzed data of consecutive chronic HF patients hospitalized for HF worsening between January 2016 and June 2023 at the Heart Failure Unit of Centro Cardiologico Monzino (Milan, Italy). The study inclusion criteria were hospitalization for HF worsening, availability of iron status, cardiac ultrasound, and a CPET. Of note iron status and cardiac ultrasound are measured in all patients hospitalized in our HF unit while CPET is performed whenever patients' clinical conditions allow it. We excluded from the present analysis the following patients: subjects admitted for acute myocardial infarction, acute pulmonary embolism, acute myocarditis or pericarditis, severe aortic stenosis, cardiogenic shock, and endocarditis. Patients with a concomitant non-cardiovascular disease affecting exercise performance independently from HF were excluded as COPD patients with more than mild COPD (GOLD 1) Moreover, patients receiving O_2 supplementation, blood transfusion, i.v. treatment except diuretics in the 24 h preceding CPET or any sort of iron supplementation between iron status evaluation and CPET were also excluded. Furthermore, pregnant or breast-feeding women were excluded. Moreover, we excluded from the present analysis exercise tests performed with a wearable ergospirometer or performed applying a protocol different from a personalized progressively increasing workload exercise performed on a *cyclo*-ergometer. Of note the left ventricular ejection fraction (LVEF) was not among the exclusion criteria. A total of 999 patients fulfilled the study inclusion/exclusion criteria and were considered in the present analysis. In case of multiple hospitalizations only the first was considered. In the present analysis we considered only IIT HF patients defined as TSAT level < 20 %, irrespectively from ferritin value.

2.2. Study measurements

CPET, cardiac ultrasound, and laboratory data evaluated in the present analysis were performed after clinical stabilization before hospital discharge. As part of the routine analysis, we performed hemoglobin, hematocrit, red blood cell count, iron status (serum iron, ferritin, transferrin saturation (TSAT).

All CPETs were performed by means of a stationary ergospirometer using an electronically braked cycle ergometer (Quark PFT Cosmed, Roma, Italy). CPETs were performed and analyzed by experts using a standard approach [14]. In brief, we applied a progressively increasing workload exercise protocol (ramp) aimed at achieving peak exercise in ~ 10 min. In the absence of clinical events, CPET was self-interrupted by the subjects when they stated that had reached maximal effort. No other causes of exercise interruption, but clinical events, were considered. Breath by breath recording of expiratory gases and ventilation was performed, while exercise data at peak and ventilatory anaerobic threshold (AT) are reported as 20-s average. Peak exercise was defined as the highest VO_2 value recorded. The AT was measured by the V-slope method plotting VCO_2 and VO_2 on an equal scale graph. VE/VCO_2 slope was calculated as the slope of the linear relationship between VE and VCO_2 from 1 min after the beginning of the loaded exercise to the end of the isocapnic buffering period. The oxygen pulse was measured as VO_2/HR. The respiratory exchange ratio was measured as VCO_2/VO_2. In the present study among CPET parameters we evaluated peak VO_2 and VCO_2, VO_2 at AT, VE/VCO_2 slope, workload (Watt), heart rate (HR) both at the peak and AT and derived parameters.

Cardiac ultrasounds were recorded (Affiniti CVx, Philips Healthcare, Cambridge, Massachusetts, USA) and analyzed as standard. Left ventricle ejection fraction (LVEF) measured applying the Simpson's biplane method of disks, involving the segmentation of the left ventricle into a series of disks from apical four-chamber and two-chamber views, and the volumes calculated from these measurements to determine ejection fraction.

2.3. Patients follow-up and study endpoint

The follow up was conducted by analyzing patients' follow-up data set based on a individualize schedule defined by the referring HF unit physician in charge of the patient. In case of lack of follow up evaluation patients, or they relatives, were contacted by phone calls. Data were confirmed, when needed, by analyzing the Lombardia patients' data registry (fascicolo sanitario elettronico).

The study endpoint was death irrespectively of its cause. Urgent LVAD and heart transplant were considered as death equivalent.

2.4. Ethics committee

The study complied with Declaration of Helsinki, was approved by the local ethics committee on human research, and all patients signed an informed consent form at the time of enrolment (IRB protocol number R116/14-CCM127).

2.5. Statistical analysis

Continuous variables are presented as mean ± standard deviation or as median and interquartile ranges, in normally and non-normally distributed variables, respectively. Categorical data were represented as frequencies and percentages.

The possible associations between the peak VO_2, VE/VCO_2 slope and TSAT and outcome (composite of cardiovascular death, urgent heart transplant or LVAD implantation) were investigated by Kaplan-Meier considering clinically recognized cut off values (Peak $VO_2 < 50$ % pred, $VE/VCO_2 \geq 34$ and < 20 % for TSAT). The survival analysis was also assessed considering comorbidities. Moreover, for these 3 variables (peak VO_2, VE/VCO_2 slope and TSAT) Hazard Ratio (HR) vs. the study endpoint both with uni and multivariable Cox model analysis was calculated. Follow-up analysis was arbitrarily truncated at 5 years.

To investigate the possible direct and indirect effects of TSAT on outcome, using CPET variables as mediators, Path Analysis was implemented [11–13]. Indeed, Path analysis is a method used in social sciences to examine and understand the relationships between variables within a hypothesized model. It allows to identify the direct and indirect causal linkages among variables, as well as break down correlations into causal and noncausal components. Of note Path analysis is not frequently used in medical studies but helps to disentangle complex interrelationships.. All analysis were conducted using the SAS Proc CALIS procedure (SAS Institute Inc., Cary, NC, USA) based on structural equation modeling. Results are presented as standardized Beta [15]. All tests were 2-tailed, and a $P < 0.05$ was required for statistical significance. All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC).

3. Results

3.1. Patients population

We analyzed data from 999 consecutive patients (age of 66 ± 13 years) hospitalized for HF worsening, encompassing 724 males and 275 females (72.5 and 27.5 %, respectively), with an average age of 66 ± 13 years. Patients characteristics are reported in Table 1 (demographic and laboratory data) and Table 2 (comorbidities and treatment data), as the hole population and grouped by TSAT values, <20 % and ≥ 20 %, respectively. Of the entire study population 537 patients had ferritin < 100 ng/ml, 372 patients had ferritin between 100 and 300 ng/mL and 90 > 300 ng/ml.

HF treatment and follow up was according to guidelines. HF phenotypes according to LVEF were: reduced LVEF (<40 %) 39 %, middle range (40–50 %) 16 % and preserved ejection fraction (>50 %) 45 %. Grouping patients by LVEF phenotypes IIT was observed in 39 % of the overall population, 46, 30 and 35 % of HF patients with LVEF < 40 %, between 40 and 50 % and > 50 %, respectively (p < 0.001).

3.2. Iron metabolism

Average serum iron, ferritin, and TSAT were 78.4 ± 37.4 (μg/dL), 137 (42 – 177.3, μg/L), 24.4 ± 12.9 (%), respectively. Three-hundred-ninety-three out of 999 HF patients (39.3 %) had the diagnosis of IIT (TSAT < 20 %). Of those 295 patients had ferritin < 100 ng/ml, 88 patients had ferritin between 100 and 300 ng/mL and 10 > 300 ng/ml.

Table 1
Patient Population Characteristics: demographic and laboratory data.

Variable	All (n = 999)	TSAT < 20 % (n = 393)	TSAT ≥ 20 % (n = 606)	P-value
Age (years)	66 ± 13	68 ± 12	65 ± 13	0,018
Male gender	724 (72.4 %)	267 (67.9 %)	457 (75.4 %)	0.078
Hb (g/dL)	13.5 ± 1.9	12.6 ± 1.9	14.1 ± 1.7	<0.0001
HCT (%)	41 ± 5	39 ± 5	42 ± 5	<0.0001
MCV (fL)	88.3 ± 7.1	86.2 ± 6.9	89.7 ± 7	<0.0001
RDW (%)	14.7 ± 2.3	15.9 ± 2.6	14 ± 1.8	<0.0001
INR	1.5 ± 0.8	1.7 ± 0.9	1.3 ± 0.6	<0.0001
Iron blood level (μg/dL)	78.4 ± 37.4	47.7 ± 14.5	98.2 ± 34.2	<0.0001
Transferrin (mg/dL)	266 ± 51	289.1 ± 57	251.1 ± 40.1	<0.0001
TSAT (%)	24.4 ± 12.9	13.2 ± 4.2	31.6 ± 11.5	
TIBC (μg/dL)	334 ± 65	365 ± 72	314 ± 50	<0.0001
Creatinine (mg/dL)	1.3 ± 0.5	1.4 ± 0.6	1.2 ± 0.5	<0.0001
eGFR (mL/min/1.73 m^2)	64.9 ± 24	58.8 ± 24.7	69 ± 22.7	<0.0001
Na (mmol/L)	140.8 ± 2.8	140.6 ± 3	140.9 ± 2.6	0.203
CRP (mg/L)	6.83 (0.9 – 5.4)	10.9 (1.5 – 8.3)	4.2 (0.6 – 3.6)	<0.0001
Ferritin (μg/L)	137 (42 – 177.3)	78 (23.4 – 101.2)	174.6 (66.9 – 216.7)	<0.0001
BNP (pg/mL)	512 (61–617)	809 (145–1096)	319 (43–331)	<0.0001
ST2 (ng/mL)	33.4 (21.4–36.8)	39.9 (24.1–42.8)	30.2 (20.7 – 33.9)	<0.0001
LVEF (%)	43 ± 16	41 ± 16	45 ± 15	<0.0001
Weight (kg)	76.9 ± 15.9	74.7 ± 15.5	78.5 ± 15.9	<0.0001
Height (m)	1.7 ± 0.1	1.69 ± 0.1	1.71 ± 0.1	<0.0001
BMI (kg/m^2)	26 ± 4.5	26 ± 4.7	26.5 ± 4.3	0.087

Data are expressed as mean ± standard deviation or median and interquartile range or number of cases and percentage.
BMI, body mass index;
BMI, body mass index; BNP, brain natriuretic peptide; CRP, C reactive protein; eGFR, esteemed glomerular filtration rate;
Hb, hemoglobin; HCT, hematocrit; INR, international normalized ratio; MCV, mean corpuscular volume; RDW, red distribution width; TIBC, total iron binding capacity; TSAT, transferrin saturation.

Table 2
Patient population characteristics: comorbidities and drugs.

Variable	Overall	TSAT < 20 % (n = 393)	TSAT ≥ 20 % (n = 606)	P-value
CAD (n)	454 (45 %)	198 (50.38 %)	256 (42.24 %)	0.014
DM II (n)	226 (22.6 %)	113 (28.75 %)	113 (18.65 %)	<0.0001
COPD (n)	156 (15.6 %)	80 (20.36 %)	76 (12.54 %)	0.0012
Arterial Hypertension (n)	517 (51.7 %)	196 (49.87 %)	321 (52.97 %)	0.372
Smokers (n)	213 (21.3 %)	73 (18.58 %)	140 (23.1 %)	0.104
ICD (n)	239 (24 %)	125 (31.81 %)	114 (18.81 %)	<0.0001
CRT (n)	116 (11.6 %)	54 (13.74 %)	62 (10.23 %)	0.112
PM (n)	95 (9.5 %)	54 (13.74 %)	41 (6.77 %)	<0.0001
AF/AFL (n)	328 (32.8 %)	170 (43.26 %)	158 (26.07 %)	<0.0001
OSAS (n)	77 (7.7 %)	31 (7.89 %)	46 (7.59 %)	0.96
Anti-vit. K antagonists (n)	97 (9.7 %)	50 (12.72 %)	47 (7.76 %)	0.013
DOAC (n)	249 (24.9 %)	122 (31.04 %)	127 (20.96 %)	<0.0001
Antiplatelet (n)	232 (23.3 %)	87 (22.14 %)	145 (23.93 %)	0.563
Beta-blockers	782 (78.2 %)	316 (80.41 %)	466 (76.90 %)	0.217
ACEi-ARB /ARNI (n)	730 (73 %)	263 (67.3 %)	467 (77.5 %)	0.0015
MRA (n)	520 (52 %)	226 (57.51 %)	294 (48.51 %)	0.0066

ACE-I, angiotensin-converting enzyme inhibitors; AF/AFL, atrial fibrillation/atrial flutter; ARBs, angiotensin II receptor blockers; ARNI, angiotensin receptor-neprilysin inhibitor; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; CRT, cardiac resynchronization therapy; DM II, diabetes mellitus type II; ICD, implantable cardioverter defibrillator; MRA, mineralocorticoid receptor antagonist; DOAC, direct oral anticoagulants; OSAS, obstructive sleep apnea syndrome; PM, pace-maker.

3.3. Grouping patients by TSAT

CPET, LVEF, comorbidities, and specifically diabetes, COPD, anemia, coronary artery disease, atrial fibrillation and renal insufficiency and HF biomarkers, NT-proBNP and ST2, showed that on the average TSAT < 20 % patients had, at hospital discharge, moderate to severe HF with a severity greater compared to HF patients with TSAT ≥ 20 %. Moreover, the MECKI score, which is among the HF prognostic score recommended by ESC guidelines [6] was 10.2 ± 13.2 and 4.3 ± 8.0 (p < 0.001) in HF patients with and without IIT, respectively. As regards CPET (Table 3) IIT patients had a more compromised exercise performance has shown by VO_2 at peak and AT, peak workload as well as by the VE/VCO_2 relationship slope. According to RER value all patients performed a maximal or near maximal exercise.

3.4. Patients follow-up and prognosis

The mean follow-up was 2.68 years ± 1.86 years. At 5 years the study endpoint, death regardless of the death cause was reached by 215 (21 %) subjects which included 8 subjects who underwent heart transplant or LVAD implant. We evaluated three main variables to identify their influence on the prognosis: VO_2 %pred, VE/VCO_2 slope and TSAT. HR showed at univariable analysis an association between each of them and prognosis: The same association was found at multivariable analysis (Table 4).

We further assessed the prognostic role of peak VO_2 (%pred), VE/VCO_2 slope and TSAT by Kaplan-Meier survival analysis..For each of the

Table 3
Cardiopulmonary exercise test parameters.

Variables	All (n = 999)	TSAT < 20 % (n = 393)	TSAT ≥ 20 % (n = 606)	P-value
Peak VO_2 (mL/min)	1163 ± 488	984 ± 370	1280 ± 520	<0.0001
Peak VO_2/kg (mL/min/kg)	15 ± 5	13 ± 4	16 ± 6	<0.0001
Peak VO_2 (% of predicted)	68 ± 44	61 ± 18	72 ± 53	<0.0001
VO_2 AT (mL/min)	811 ± 345	691 ± 288	878 ± 356	<0.0001
VE/VCO_2 slope	35 ± 9	38 ± 10	33 ± 8	<0.0001
VO_2/WR (mL/min/W)	9.3 ± 1.9	8.9 ± 1.7	9.6 ± 1.9	<0.0001
Peak VE (L/min)	51.8 ± 18.7	47.5 ± 16.7	54.5 ± 19.5	<0.0001
Peak workload (W)	86 ± 47	69 ± 35	96 ± 50	<0.0001
Peak RER	1.14 ± 0.99	1.11 ± 0.12	1.16 ± 1.27	0.295
Peak VO_2/HR (mL/b)	11.1 ± 7.6	9.9 ± 5.3	11.8 ± 8.6	<0.0001
Peak HR (b.p.m.)	111 ± 27	105 ± 26	115 ± 27	<0.0001

Data are expressed as mean ± standard deviation.
AT, anaerobic threshold; HR, heart rate; RER, respiratory exchange ratio; VCO_2, carbon dioxide production; VE, ventilation; VO_2, oxygen consumption; WR, work.

Table 4
Univariable and Multivariable Analysis of Prognostic Factors: Peak VO_2/kg, VO_2 % pred, VE/VCO_2 slope, and TSAT.

UNIVARIABLE ANALYSIS				
Variable	**Beta**	**HR**	**95 % Confidence Limits**	**P-value**
VO_2 % pred	−1.24384	0.288	0.216 – 0.386	0.0001
VE/VCO_2 slope	1.0504	2.859	2.105 – 3.883	0.0001
TSAT (%)	−0.68508	0.504	0.376 – 0.675	0.0001
MULTIVARIABLE ANALYSIS				
Variable	**Beta**	**HR**	**95 % Confidence Limits**	**P-value**
VO_2 % pred	−0.96155	0.382	0.281 – 0.520	<0.0001
VE/VCO_2 slope	0.70838	2.031	1.468 – 2.808	<0.0001
TSAT (%)	−0.3758	0.687	0.508 – 0.928	0.0144

HR, hazard ratio; TSAT, transferrin saturation; VCO_2, carbon dioxide production; VE, ventilation; VO_2, oxygen consumption. Data were analyzed as categorical variables.

three variables used, we arbitrarily identified two groups: peak VO_2 < 50 % pred value vs. ≥ 50 %, VE/VCO_2 slope ≤ 34 vs. > 34, and TSAT < 20 % vs. ≥ 20 %. Kaplan-Meier curves indicate a noticeable divergence in survival distribution between the two groups of each variable. Considering VO_2 % pred (Fig. 1A) and VE/VCO_2 slope (Fig. 2B) patients with higher VO_2 or lower VE/VCO2 slope consistently showed a higher survival rate ($p < 0.0001$ in both cases) with the curves diverging shortly after the beginning of follow-up in both cases. Finally, also TSAT (Fig. 1C) showed a clear difference in survival ($p < 0.0001$) between HF patients with TSAT < 20 % vs. TSAT ≥ 20 % and in parallel with peak VO_2 and VE/VCO_2 slope with an early separation of the 2 curves. Of note, adjusting for HF comorbidities including COPD, presence of coronary artery disease, diabetes and atrial fibrillation, does not influence our findings and the above reported association remained statistically significant. Furthermore, we also analyzed the association between IIT and outcome adjusting for HF subtype based on LVEF and therapy and found that that association remained statistically significant ($p = 0.028$).

The path analysis (Fig. 2) approach was used to investigate whether the prognostic role of TSAT was mediated or not from peak VO_2 and VE/VCO_2 slope. TSAT showed both a negative direct and an indirect effect on the outcome the latter mediated through VE/VCO_2 slope and peak VO_2 % pred. TSAT was linked to the study outcome directly at 48 % and through peak VO_2 and VE/VCO_2 at 52 %, respectively. The latter was 48 % by peak VO_2 and 52 % by VE/VCO_2.

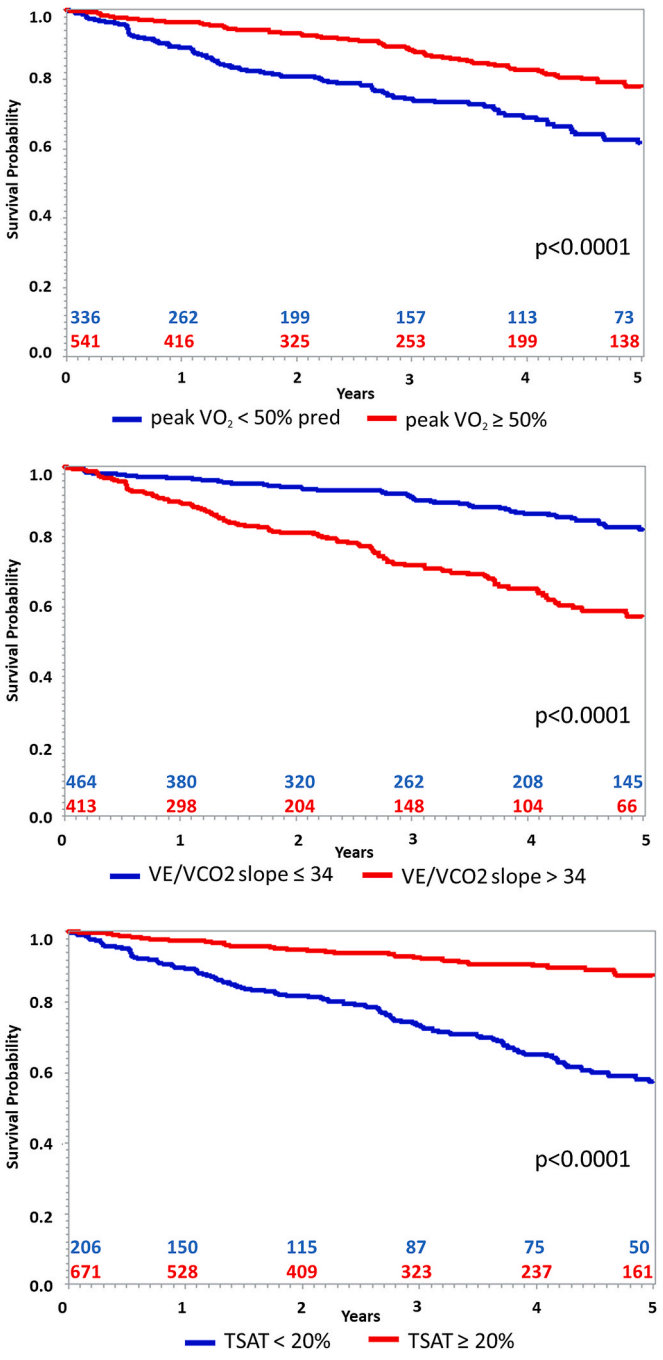

Fig. 1. Five years Kaplan-Meier survival curves by Predicted % VO_2, VE/VCO_2 slope and TSAT in HF patients. Survival is the composite of cardiovascular death, urgent heart transplant or LVAD implantation.

4. Discussion

The present study showed that IIT is frequently observed in HF patients, that it is associated to a reduced exercise performance and that it plays a significant negative prognostic role which is both in part independent and in part dependent from peak VO_2 and VE/VCO_2 slope.

On the average the population we studied had moderate HF as suggested by HF biomarkers, BNP and ST2, and CPET data. Of note both anemia and ID are frequently observed even in HF patients with moderate HF [16]. In the present population as in a few previously reported population studies IIT was frequently observed in HF patients (39.4 % of HF cases). IIT-HF patients had a more severe HF compared to non-IIT cases having lower Hb blood values and LVEF, higher BNP and ST2

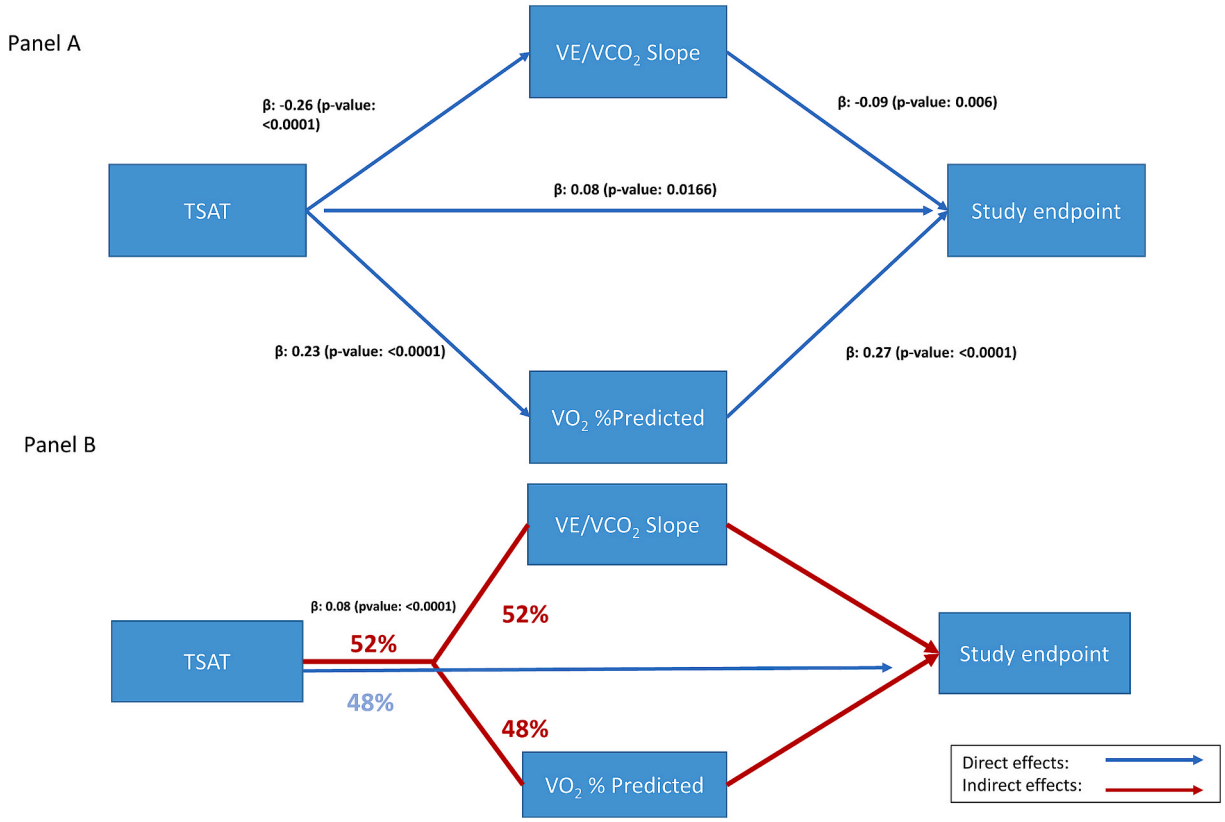

Fig. 2. Path analysis. Panel A with β; Panel B with percentages. The overall effect of TSAT on the study endpoint, composite of cardiovascular death, urgent heart transplant or LVAD was direct for 48% and mediated by peak VO_2 and VE/VCO_2 slope for 52%.

and more comorbidities including more frequent coronary artery disease, diabetes, renal insufficiency, and atrial fibrillation. Finally, IIT patients had a worst prognosis has predicted by MECKI score value and shown by survival analysis.

In IIT-HF patients RDW and CRP were higher compared to non-IIT-HF patients. A high RDW is associated with several chronic diseases including anemia, diabetes, metabolic syndrome as well as liver, kidney, and cardiovascular diseases. Overall, a high RDW is predictive of morbidity and mortality. Indeed, a high RDW indicates dysregulation of production/destruction of red blood cell. On the opposite a low RDW value has been reported in individuals with a high amount of physical activity who have a longer life expectancy [17,18] Recently, we and others reported that also in ID patients a high RDW is frequently observed so that RDW can be used to suspect the presence of ID when iron metabolism is not available. Moreover, RDW reduction has been shown in HF patients undergoing physical rehabilitation [19]. A few previous studies reported a correlation between inflammation and RDW [20]. For example, an increased removal of RBC from the blood is associated to an increased inflammatory state which involves macrophage phagocytosis and several inflammatory molecules. In the present study we observed in IIT-HF patients a high CRP value confirming a possible link between IIT and inflammation, high RDW and anemia leading all to a reduced exercise performance and worst prognosis.

We first estimated in IIT HF patient prognosis calculating the MECKI scores [21]. MECKI score is among the most powerful prognostic scores and it is recommended by most recent HF ESC guidelines [6]. MECKI score was higher in HF IIT patients compared to non IIT cases. Of note MECKI score has a holistic approach to HF combining 6 recognized parameters, LVEF, Hb, Na+, kidney function, peak VO_2 and VE/VCO_2 slope. In the present study prognosis was directly assessed according to TSAT, peak VO_2 and VE/VCO_2 slope values being these 3 parameters significantly interrelated between each other. Indeed, we and other previously reported that in HF a low TSAT is associated to poor prognosis and poor exercise performance and specifically low peak VO_2 and high VE/VCO_2 slope. Moreover, ID treatment is associated with improvement in exercise parameters [22–27]. We analyzed the capacity of predicting prognosis by TSAT, peak VO_2 and VE/VCO_2 slope using 3 different approaches.

We used for survival death of all causes and considered urgent LVAD or heart transplant as death equivalent. We did so because of the holistic role of IIT. The Kaplan-Meier survival analysis clearly showed for TSAT, peak VO_2 and VE/VCO_2 slope a clear correlation to prognosis (Fig. 1A, B and C). The peak VO_2 and VE/VCO_2 slope cut off value we used, <50 pred and >34 for peak VO_2 and VE/VCO_2 slope, respectively, are likely the most frequently applied in clinical and research settings [9,28–33]. Similar to Kaplan-Meier analysis are the HR results (Table 4). Statistical significance was confirmed at a multivariable analysis considering VE/VCO_2 slope, peak VO_2 and TSAT. However, the present multivariable logistic model does not allow testing causal pathways. The use of path analysis overcame this limitation and allows showing that the impact of TSAT on survival is both direct and mediated by peak VO_2 reduction and VE/VCO_2 slope increase. The observation that TSAT effects on prognosis is mediated by peak VO_2 is somehow expected being low TSAT associated to anemia and low stroke volume, both relevant causes of peak VO_2 reduction in HF. Indeed, as regards stroke volume in HF several myocardial cells, including myocytes, fibroblast, and endothelial cells show that iron related genes are dysregulated reducing the amount of iron available for cardiac contraction and overall myocardial cells function [34,35].Consequently, iron abnormalities can be considered among the causes of a reduced inotropic function and stroke volume in chronic HF. The role of VE/VCO_2 abnormalities of TSAT effects on prognosis is, at a first glance, less clear. However, Caravita et al. [27] showed after ID treatment with carboxyferromaltose a reduction in VE/VCO_2 slope associated with an ameliorated chemo-reflex response.

Indeed, VE/VCO_2 slope in HF is highly dependent on chemo-reflex activity [36] as well as ventilation perfusion mismatch in the lung. While the former has been demonstrated for the latter it may be speculated that according to the holistic role of iron, in ID also pulmonary endothelial and alveolar cells are dysregulated [37].

Forty-eight % of the effects of low TSAT on prognosis are independent from peak VO_2 and VE/VCO_2 which means independent from exercise performance or from abnormalities affecting exercise performance. Several are the possible causes of poor survival unrelated to exercise performance including low adherence to treatment, the arrhythmic burden, obesity, or a progression of ischemic heart disease and/or diabetes with or without a new acute cardiac event. Except from adherence to treatment all the others are potentially affected by ID, at least in speculative terms.

5. Study limitations

The present study has several limitations which must be acknowledge. First, it is a retrospective study. Second, it is a single center study which limits the representative power of our finding to the general HF population but, on the other hand, standardize patients' evaluation and follow up. Third, patients were hospitalized for worsening HF and studied before hospital discharge. Accordingly, some residual congestion was likely present at least in a few cases. We do not know whether and how much this residual congestion influences directly exercise performance as well as patient's prognosis. Additionally, also the effects of left ventricle diastolic abnormalities as well as right ventricle function and degree of pulmonary hypertension on exercise performance and prognosis were not independently assessed. Moreover, albeit an undeniable solidity of our data in terms of size of the studied population and study procedures, the interpretation of our findings is merely speculative and needs to be reassessed by dedicated studies. Finally, path analysis based on structural equation modeling has the limit that underlying model does not consider, as a COX model, the time at which the event occurs.

6. Conclusions

In brief we showed that in chronic HF hospitalized for HF worsening IIT is associated to a reduced exercise performance and plays a significant negative prognostic role which is both in part independent and in part dependent from peak VO_2 and VE/VCO_2 slope.

7. Statement of authorship

Each author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation

CRediT authorship contribution statement

Jeness Campodonico: Writing – original draft, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Alice Bonomi:** Writing – original draft, Visualization, Validation, Formal analysis, Data curation. **Marina Alimento:** Writing – review & editing, Visualization, Validation, Investigation, Conceptualization. **Anna Apostolo:** Writing – review & editing, Visualization, Validation, Investigation. **Arianna Piotti:** Writing – review & editing, Visualization, Validation, Data curation. **Irene Mattavelli:** Writing – review & editing, Visualization, Validation, Data curation. **Elisabetta Salvioni:** Writing – review & editing, Visualization, Validation. **Massimo Mapelli:** Writing – review & editing, Visualization, Validation, Investigation. **Carlo Vignati:** Writing – review & editing, Visualization, Validation, Investigation. **Paola Gugliandolo:** Writing – review & editing, Visualization, Validation. **Beatrice Pezzuto:** Writing – review & editing, Visualization, Validation, Investigation. **Giulia Grilli:** Writing – review & editing, Visualization, Validation, Investigation. **Valentina Rusconi:** Writing – review & editing, Visualization, Validation, Investigation, Data curation. **Paolo Poggio:** Writing – original draft, Investigation, Data curation, Conceptualization. **Piergiuseppe Agostoni:** Writing – original draft, Visualization, Validation, Supervision, Project administration, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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