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# Exercise performance in patients with heart failure and impaired iron transport: a single-centre retrospective study

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## ABSTRACT

**Objectives** Iron deficiency (ID) is frequent in heart failure (HF). Among patients with HF and ID, those with impaired iron transport (IIT) (transferrin saturation (TSAT) <20%) have the worst ID phenotype. In HF, exercise limitation is mainly related to abnormality in oxygen delivery ($VO_2$) and utilisation and/or to ventilation inefficiency. We evaluated whether it is possible to identify the leading cause of exercise limitation in patients with HF and IIT.

**Design** Observational study.

**Setting** Retrospective study.

**Participants** We analysed 1043 consecutive hospitalised patients with HF (66±14 years, 49.8% females) who underwent cardiopulmonary exercise test (CPET).

**Outcome measures** Associations between CPET parameters and TSAT were explored using general linear models adjusted for potential confounders (haemoglobin, left ventricle ejection fraction, age, gender, C reactive protein, serum creatinine).

**Results** We observed that: (a) 413 patients with HF and IIT had worse functional capacity compared with non-IIT cases: peak $VO_2$ (15±3 vs 16±6 mL/min/kg, p<0.0001) and ventilation/carbon dioxide relationship ($VE/VCO_2$) slope (38±9 vs 33±8, p<0.0001); (b) $VE/VCO_2$ values remained significantly different between IIT and non-IIT cases after adjusting for confounding variables including peak $VO_2$; differently, peak $VO_2$ after adjusting also for $VE/VCO_2$ slope, resulted not different in IIT compared with non-IIT HF cases; (c) patients with both low peak $VO_2$ (<14 mL/min/kg) and high $VE/VCO_2$ (≥34) had a higher B-type natriuretic peptide (BNP), lower TSAT and higher MECKI (Metabolic Exercise combined with Cardiac and Kidney Index) score compared with patients with high peak $VO_2$ and low $VE/VCO_2$ (BNP 951±1041 vs 413±623 pg/mL (p<0.0001); TSAT 19.5%±10.5% vs 26.9%±10.2% (p<0.0001); MECKI score 14.8%±1.4% vs 1.2%±2.3% (p<0.0001).

**Conclusions** High $VE/VCO_2$ slope is directly associated with IIT, independent of peak $VO_2$, suggesting a pivotal role for ventilation inefficiency in exercise impairment in patients with IIT and HF. In patients with HF, the worst exercise performance is associated with low TSAT, high BNP and the highest MECKI score.

## STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Largest single-centre study in well-characterised patients with heart failure grouped according to transferrin saturation.
⇒ Cardiopulmonary exercise test (symptoms limited ramp exercise protocol) in all patients.
⇒ Retrospective nature of the study.

## INTRODUCTION

Iron deficiency (ID) is frequently observed in patients with heart failure (HF) even in the absence of anaemia.[1–3] In HF, ID is associated with reduced exercise performance.[4 5] Exercise limitation in HF has multiple causes, primarily related to abnormalities in oxygen delivery and utilisation and/or to ventilatory inefficiency.[6–10] Indeed, both peak oxygen intake ($VO_2$) and ventilation/carbon dioxide relationship ($VE/VCO_2$ slope) are pivotal yet independent indicators of HF severity and prognosis.[11–14] Of note, ID in HF affects both peak $VO_2$ and $VE/VCO_2$ slope—reducing the former and increasing the latter—regardless of the presence of anaemia.[15–17] An elevated $VE/VCO_2$ slope may be related to increased dead space ventilation and/or to heightened reflex-mediated ventilation.[18 19] In this regard, it is known that untreated ID increases central $CO_2$ reflex activity, and that this hyperactivated reflex is reduced after treatment with ferric carboxymaltose.[20] In contrast, a reduction in peak $VO_2$ in patients with HF and ID may be related to several mechanisms, including impaired cardiac performance due to the dysregulation of myocardial iron-related genes,[21] or to reduced mitochondrial activity in peripheral muscles independent of oxygen transport.[22 23] The present study was conceived to evaluate whether the leading cause of exercise limitation in patients with HF and ID is due to oxygen delivery abnormalities, ventilation

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inefficiency or a combination of both. Elucidating this will improve our understanding of the pathophysiology of exercise limitation in patients with HF and ID. To this end, we retrospectively analysed a sizeable population of patients hospitalised for HF worsening who underwent cardiopulmonary exercise test (CPET) after clinical stabilisation.

## MATERIAL AND METHODS

### Patients selection

We retrospectively collected data from consecutive chronic patients with HF, regardless of left ventricle ejection fraction (LVEF) or New York Heart Association (NYHA) class, who were hospitalised for HF worsening at the Heart Failure Unit of Centro Cardiologico Monzino (Milan, Italy) between January 2016 and June 2023. The study inclusion criteria were as follows: hospitalisation for HF worsening, availability of iron status measurements, cardiac ultrasound data and CPET results. Iron status and cardiac ultrasound are routinely measured in all patients hospitalised in our HF unit, while CPET is performed whenever a patients' clinical condition allows it. Accordingly, although patients were intensively treated, some residual congestion may still have been present, which could affect CPET results. Exclusion criteria were as follows: admission for acute myocardial infarction, acute pulmonary embolism, acute myocarditis or pericarditis, severe aortic stenosis, cardiogenic shock or endocarditis. We also excluded patients with concomitant non-cardiovascular diseases that could independently affect exercise performance, those receiving oxygen supplementation, iron treatment or blood transfusion. Moreover, patients with worsening HF associated with a COVID-19 infection, a recent history of COVID-19 infection (<6 months) or long COVID-19 syndrome were excluded from the present study. To avoid pharmacological interference with CPET results, patients who received intravenous treatments (except for diuretics) in the 24 hours preceding the CPET were excluded. Pregnant or breastfeeding women were also excluded. Furthermore, to ensure comparability between CPETs, we did not include in the present analysis exercise tests performed with a wearable ergospirometer or those that did not follow a standardised protocol of personalised, progressively increasing workload on a cycle ergometer.[24] In case of multiple hospitalisations, only the first was considered. A total of 1043 patients fulfilled the study inclusion/exclusion criteria.

CPET, cardiac ultrasound and laboratory data evaluated in the present analysis were performed after clinical stabilisation shortly before hospital discharge. Transthoracic echocardiography was conducted using a Philips Epiq CVx ultrasound machine (Philips Medical Systems, Andover, Massachusetts) equipped with an X5-1 probe. A comprehensive standard 2D analysis was performed, with left chamber volumes and LVEF measured from four-chamber and two-chamber views using the biplane Simpson's method.[25]

As part of the routine analysis, we performed the following measurements: haemoglobin (Hb), haematocrit, red cell count (via the Sysmex XN method); iron status, including serum iron (Ferrozine method), ferritin (Chemiluminescent Immunoassay), transferrin saturation (TSAT) and total iron binding capacity (immunoturbidimetry); B-type natriuretic peptide (BNP–chemiluminescent immunoassay) and soluble suppression of tumourigenicity 2 protein (sST2–immunoturbidimetry), which is a member of the interleukin 1 receptor family. Blood samples were drawn after ensuring that intense physical effort was avoided in the previous 3 hours and following a 5-minute rest period in a sitting position. Regarding the CPET, we evaluated $VO_2$, $VCO_2$, workload (Watt), heart rate (HR)—both at peak exercise and at the anaerobic threshold (AT)—as well as the $VE/VCO_2$ slope and other derived parameters.

Among patients with ID, only those with impaired iron transport (IIT), identified as a TSAT level <20% regardless of ferritin value, were analysed. Consequently, patients who fulfilled the established ID definition (ferritin <100 µg/L) but had a TSAT >20% were excluded from the present analysis. All CPETs were performed using a stationary ergospirometer with an electronically braked cycle ergometer (Quark PFT Cosmed, Roma, Italy). CPETs were conducted and analysed by experts according to a standard approach.[24] Briefly, a progressively increasing workload exercise protocol (ramp) was used, aiming for peak exercise to be achieved in approximately 10 min. In the absence of clinical events, CPET was terminated by the subjects themselves on reporting maximal effort. Expiratory gases and ventilation were recorded breath by breath. Data for both peak exercise and AT are reported as 20 s averages. Peak exercise was defined as the highest recorded $VO_2$ value. The AT was identified using the V-slope method by plotting $VCO_2$ and $VO_2$ on an equal-scale graph. The $VE/VCO_2$ slope was calculated as the slope of the linear relationship between ventilation and $VCO_2$ from 1 min after the beginning of the loaded exercise until the end of the isocapnic buffering period. The oxygen pulse was calculated as $VO_2/HR$, and the respiratory exchange ratio was measured as $VCO_2/VO_2$.

### Patient and public involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

### Statistical analysis

Continuous variables are presented as mean±SD and were compared using an independent samples t-test. Non-normally distributed variables are presented as median and IQRs, and were compared using the Wilcoxon rank sum test. Categorical data were compared using the $\chi^2$ test or Fisher's exact test, as appropriate. Non-normally distributed variables were transformed into logarithms for statistical analysis. The assessment of normality was

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performed using the Shapiro-Wilk test, along with visual inspection of histograms and Q–Q plots. For grouping purposes, clinically established cut-off values were applied: TSAT (≥20% vs <20%), peak $VO_2$ (≥14 mL/min/kg vs <14 mL/min/kg) and $VE/VCO_2$ slope (≥34 vs <34).

Associations between CPET parameters and TSAT were investigated using a general linear model, adjusting for potential confounders (Hb, LVEF, age, gender, C reactive protein (CRP) and serum creatinine).

Spearman correlation coefficients were calculated, and general linear models used to identify possible predictors of BNP levels.

All tests were two-tailed and a p<0.05 considered statistically significant. All analyses were performed using SAS V.9.4 (SAS Institute).

Based on group sizes (413 patients with TSAT<20% and 630 patients with TSAT ≥20%) and an estimated effect size of approximately 0.68 for the difference in the various CPET parameters, the present study has an excellent statistical power (>99%) to detect the observed difference in functional capacity between the groups.

As the percentage of missing data for all variables was below 5%, imputation was not necessary.

## RESULTS

We analysed data from 1043 hospitalised patients with HF, comprising 524 males and 519 females, with a mean age of 66±14 years. The cohort was categorised based on TSAT. Patients characteristics are reported in table 1 (demographic, clinical and laboratory data) and table 2 (comorbidities and treatments). 413 individuals (210 males and 203 females) exhibited IIT (TSAT levels<20%), while 630 patients (314 males and 316 females) had TSAT levels ≥20%. Patients with IIT presented with more severe HF, as evidenced by their clinical status, elevated HF biomarkers and poorer kidney function. Furthermore,

**Table 1** Patient population characteristics: laboratory data and NYHA class

| Variable | All (n=1043) | TSAT <20% (n=413) | TSAT ≥20% (n=630) | P value |
|---|---|---|---|---|
| Age (years) | 66±13 | 67±12 | 65±13 | 0.0189 |
| Hb (g/L) | 135±19 | 126±19 | 141±17 | <0.0001 |
| HCT (%) | 40±5.2 | 39±5.2 | 42±4.8 | <0.0001 |
| MCV (fL) | 88.3±7.2 | 86.2±6.9 | 89.7±7 | <0.0001 |
| RDW (%) | 14.8±2.3 | 15.9±2.5 | 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±37 | 48±14 | 98±34 | <0.0001 |
| Transferrin (mg/dL) | 266±51 | 288±57 | 251±40 | <0.0001 |
| TIBC (µg/dL) | 334±64 | 363±72 | 315±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 $m^2$) | 64.7±24.1 | 58.6±24.5 | 68.8±22.9 | <0.0001 |
| $Na^+$ (mmol/L) | 140.8±2.7 | 140.6±3 | 140.1±2.6 | 0.0649 |
| CRP (mg/L) | 2 (0.9–5.4) | 3.7 (1.5–8.3) | 1.4 (0.6–3.6) | <0.0001 |
| Ferritin (µg/L) | 91.2 (42.5–176) | 47 (23.8–102) | 117.5 (66–214.8) | <0.0001 |
| BNP (pg/mL) | 217 (63–649) | 441 (151–1072) | 134 (45–358) | <0.0001 |
| sST2 (ng/mL) | 27.8 (21.4–36.8) | 33.1 (24–42.8) | 26 (20.7–34) | <0.0001 |
| Weight (kg) | 77.0±15.8 | 74.7±15.5 | 78.4±15.9 | 0.0002 |
| Height (m) | 1.71±0.1 | 1.68±0.1 | 1.71±0.1 | 0.2799 |
| BMI (kg/$m^2$) | 26.3±4.5 | 26±4 | 26.5±4.3 | 0.0895 |
| Male (%) | 71 | 67 | 74 | 0.0345 |
| NYHA (%) | | | | |
| Class I (%) | 21.9 | 15.5 | 25.6 | <0.0001 |
| Class II (%) | 45.1 | 37.6 | 49.4 | |
| Class III (%) | 30.9 | 43.1 | 23.8 | |
| Class IV (%) | 2.1 | 3.8 | 1.2 | |

Data are expressed as mean ±SD or median and IQR or percentage.
BMI, body mass index; BNP, brain natriuretic peptide; CRP, C reactive protein; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; HCT, haematocrit; INR, international normalised ratio; MCV, mean corpuscular volume; $Na^+$, sodium; NYHA, New York Heart Association; RDW, red cell distribution width; sST2, soluble suppression of tumourigenicity 2 protein; TIBC, total iron binding capacity; TSAT, transferrin saturation.

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Table 2 Patient population characteristics: comorbidities and drugs

| Variable | All (n=1043) | TSAT <20% (n=413) | TSAT ≥20% (n=630) | P value |
|---|---|---|---|---|
| CAD (n) | 480 (45.9%) | 211 (51.2%) | 269 (42.5%) | 0.0057 |
| DM II (n) | 211 (21%) | 112 (28.5%) | 113 (18.6%) | 0.0005 |
| COPD (n) | 156 (15.5%) | 80 (20.3%) | 76 (12.5%) | 0.0008 |
| HBP (n) | 519 (49.6%) | 197 (47.7%) | 322 (50.9%) | 0.3163 |
| Smokers (n) | 216 (20.7%) | 74 (17.9%) | 142 (22.5%) | 0.0778 |
| ICD (n) | 239 (24.0%) | 125 (32.1%) | 114 (18.7%) | <0.0001 |
| CRT (n) | 116 (11.6%) | 54 (13.8%) | 62 (10.2%) | 0.0809 |
| PM (n) | 95 (9.6%) | 54 (13.9%) | 41 (6.8%) | 0.0002 |
| AF/AFL (n) | 348 (33.3%) | 182 (44.1%) | 166 (26.2%) | <0.0001 |
| VKA (n) | 98 (9.8%) | 51 (12.9%) | 47 (7.7%) | 0.0063 |
| DOAC (n) | 240 (23.9%) | 122 (31%) | 118 (19.3%) | 0.0001 |
| Antiplatelet (n) | 177 (17.6%) | 72 (18.3%) | 105 (17.2%) | 0.4744 |
| Beta-blockers | 824 (79.2%) | 335 (81.7%) | 489 (77.6%) | 0.1122 |
| ACEi-ARB (n) | 559 (53.8%) | 204 (49.8%) | 355 (56.3%) | 0.0372 |
| ARNI (n) | 218 (21%) | 77 (18.8%) | 141 (22.4%) | 0.1633 |
| MRA (n) | 554 (35.3%) | 239 (58.3%) | 315 (50.1%) | 0.0095 |

ACEi, ACE inhibitors; AF/AFL, atrial fibrillation/atrial flutter; ARB, angiotensin II receptor blockers; ARNI, angiotensin receptor-neprilysin inhibitor; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; CRT, cardiac resynchronisation therapy; DM II, diabetes mellitus type II; DOAC, direct oral anticoagulants; HBP, hypertensive blood pressure; ICD, implantable cardioverter defibrillator; MRA, mineralocorticoid receptor antagonist; PM, pace-maker; TSAT, transferrin saturation; VKA, vitamin K antagonist.

IIT was associated with a higher prevalence of anaemia and atrial fibrillation.

CPET (table 3) reveals a worse functional capacity in patients with HF and IIT compared with non-IIT cases. Notably, individuals with TSAT<20% displayed a reduced peak exercise $VO_2$ (967±377 mL/min) compared with those with TSAT ≥20% (1269±529 mL/min, p<0.0001). Additionally, a higher $VE/VCO_2$ slope (38±9) was observed in patients with TSAT <20% compared with those with TSAT ≥20% (33±8, p<0.0001) (table 3).

As an average the present HF population showed an impaired exercise performance. Indeed, considering the most widely used severity prognostic values for peak $VO_2$ and $VE/VCO_2$ slope, <14 mL/min/kg and ≥34, respectively, 519 (50%) had peak $VO_2$<14 mL/min/kg and 524 (50%) ≥14 mL/min /kg while 501 (48%) cases had a $VE/VCO_2$ slope ≥34 and 542 (52%) <34. Importantly, peak $VO_2$ and $VE/VCO_2$ slope retained statistical significance even after adjusting for all the variables that resulted different in tables 1 and 2 including the most frequently recognised potential confounding variables, such as Hb, LVEF, age, gender, CRP and serum creatinine (table 3). Finally, $VE/VCO_2$ slope values retained significant differences between TSAT<and ≥20% patients even further adjusting for peak $VO_2$; in contrast, peak $VO_2$, after adjustment for the confounding variables listed above plus $VE/VCO_2$ slope, was not significantly correlated to IIT, demonstrating that $VE/VCO_2$ slope is associated with IIT independently from peak $VO_2$ but not vice versa (figure 1 Upper and lower panel, respectively).

Table 3 Cardiopulmonary exercise parameters

| Variables | Transferrin saturation <20% | Transferrin saturation ≥20% | P value for trend | P value adjusted for age, sex, LVEF, CRP, creatinine and Hb | P value adjusted for all* |
|---|---|---|---|---|---|
| Peak $VO_2$/kg (mL/min/kg) | 15.1±3.7 | 16.2±5.8 | <0.0001 | <0.0002 | 0.0431 |
| Peak $VO_2$ (mL/min) | 968±377 | 1269±529 | <0.0001 | 0.0046 | 0.0203 |
| $VO_2$ AT (ml/min) | 689±290 | 881±359 | <0.0001 | 0.0678 | 0.3059 |
| $VE/VCO_2$ slope | 38±9 | 33±8 | <0.0001 | <0.0001 | <0.0001 |

Data are expressed as mean±SD.
*All includes all variables reported in tables 1 and 2 which resulted different between groups.
AT, anaerobic threshold; CRP, C reactive protein; Hb, haemoglobin; LVEF, left ventricle ejection fraction; $VE/VCO_2$, ventilation/carbon dioxide relationship; $VO_2$, oxygen consumption.



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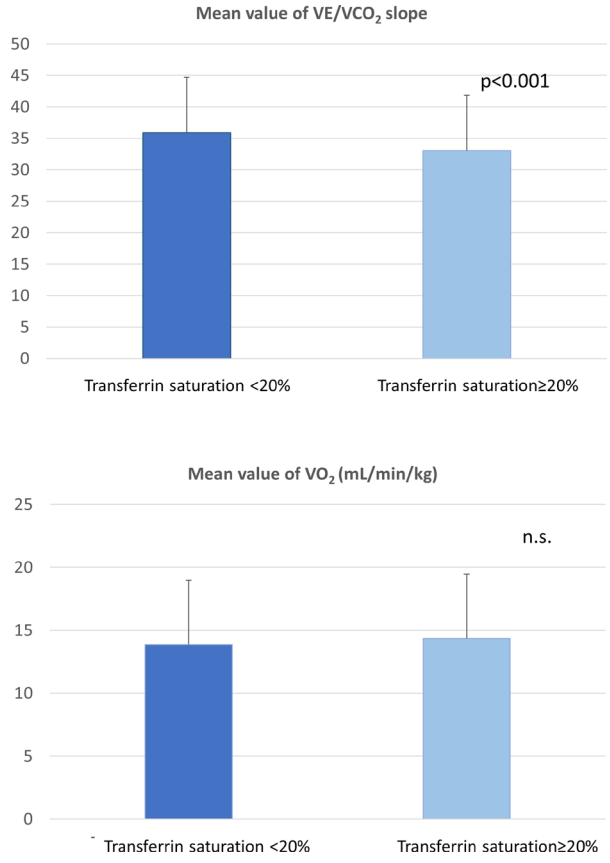


**Figure 1** Upper panel: Mean value of $VE/VCO_2$ slope adjusted for age, gender, C reactive protein, left ventricle ejection fraction, haemoglobin and peak $VO_2$. Lower panel: Mean value of peak $VO_2$ adjusted for age, gender, C reactive protein, left ventricle ejection fraction, haemoglobin and $VE/VCO_2$ slope. $VE/VCO_2$, ventilation/carbon dioxide relationship; $VO_2$, oxygen consumption.

BNP value was significantly associated with peak $VO_2$ (r=−0.5623, p<0001) and $VE/VCO_2$ slope (r=0.4935, p<0.0001) (table 4). At multiple variable analysis BNP remained significantly associated with TSAT, age, LVEF, serum creatinine, CRP, peak $VO_2$ and $VE/VCO_2$ slope.

Considering peak $VO_2$ and $VE/VCO_2$ slope HF severity cut-off values, 341 patients had both low peak $VO_2$ and high $VE/VCO_2$ slope (group 1), 337 patients had either low peak $VO_2$ and $VE/VCO_2$ slope or high peak $VO_2$ and VE/VCO2 slope (group 2) and 365 patients had high peak $VO_2$ and low $VE/VCO_2$ slope (group 3) (table 5). Specifically, among IIT cases (n=198) 48% of patients had peak $VO_2$<14 mL/min/kg and $VE/VCO_2$ slope ≥34, 30% (n=124) of cases had peak $VO_2$<14 but $VE/VCO_2$ slope <34 or peak $VO_2$≥14 but $VE/VCO_2$ slope ≥34 and 22% (n=91) of cases had peak $VO_2$≥14 and $VE/VCO_2$ slope<34. Finally, in the entire study population, patients with both low peak $VO_2$ and high $VE/VCO_2$ slope had a higher BNP and low TSAT compared with patients with high peak $VO_2$ and low $VE/VCO_2$ slope (figure 2). An intermediate position on the TSAT versus BNP graph was observed for those patients with low peak $VO_2$ and $VE/VCO_2$ slope or high peak $VO_2$ and $VE/VCO_2$ slope.

## DISCUSSION

The present study confirms that IIT, defined as a TSAT <20%, is a frequent comorbidity in patients with chronic HF hospitalised for acute worsening and exerts a direct influence on the patients' exercise performance.

Consistent with previous literature, both peak $VO_2$ and $VE/VCO_2$ slope were adversely affected in patients with HF and IIT, being significantly lower and higher, respectively, compared with non-IIT patients. However, our analysis indicates that the predominant exercise abnormalities in IIT are those related to ventilation inefficiency assessed as the slope of the relationship between ventilation and $VCO_2$. Indeed, the significant correlation between TSAT and $VE/VCO_2$ slope persisted after adjustment for several relevant confounding factors, including peak $VO_2$ (table 4). Furthermore, we demonstrated that TSAT was independently associated with BNP levels. Finally, the worst exercise performance—characterised

**Table 4** Correlation matrix

| | $VE/VCO_2$ slope | Peak $VO_2$ | Peak $VO_2$ %pred | CRP | TSAT | BNP |
|---|---|---|---|---|---|---|
| $VE/VCO_2$ slope | 1 | −0.50569 | −0.41655 | 0.20846 | −0.24816 | 0.49347 |
| | | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Peak $VO_2$ | | 1 | 0.59039 | −0.28079 | 0.31978 | −0.56234 |
| | | | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Peak $VO_2$ %pred | | | 1 | −0.27312 | 0.2113 | −0.51666 |
| | | | | <0.0001 | <0.0001 | <0.0001 |
| CRP | | | | 1 | −0.31485 | 0.35683 |
| | | | | | <0.0001 | <0.0001 |
| TSAT | | | | | 1 | −0.31925 |
| | | | | | | <0.0001 |
| BNP | | | | | | 1 |

BNP, brain natriuretic peptide; CRP, C reactive protein (expressed as ln); TSAT, transferrin saturation; $VE/VCO_2$, ventilation/carbon dioxide relationship; $VO_2$, oxygen consumption.

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Table 5 Patient population characteristics

| Variable | Group 1 (n=365) | Group 2 (n=337) | Group 3 (n=341) | P value for trend |
|---|---|---|---|---|
| Age (years) | 72±9 | 68±12 | 60±14 | <0.0001 |
| Hb (g/L) | 128±20 | 135±18 | 143±16 | <0.0001 |
| HCT (%) | 39±6 | 41±5 | 43±4 | <0.0001 |
| MCV (fL) | 88.1±7.7 | 88.9±7.8 | 88±5.9 | 0.2071 |
| RDW (%) | 15.8±2.6 | 14.7±2.3 | 13.8±1.6 | <0.0001 |
| INR | 1.7±0.9 | 1.4±0.7 | 1.3±0.6 | <0.0001 |
| Iron blood level (µg/dL) | 67±37 | 79±33 | 88±39 | <0.0001 |
| Transferrin (mg/dL) | 273±58 | 265±48 | 261±46 | 0.035 |
| TIBC (µg/dL) | 342±75 | 333±61 | 328±56 | 0.0125 |
| Creatinine (mg/dL) | 1.6±0.7 | 1.2±0.44 | 1±0.3 | <0.0001 |
| eGFR (mL/min/1.73 $m^2$) | 51±21 | 65±22 | 65±22 | <0.0001 |
| Na (mmol/L) | 140±3 | 141±2 | 141±2 | <0.0001 |
| CRP (mg/L) | 11±30 | 6±11 | 6±11 | <0.0001 |
| Ferritin (µg/L) | 145±281 | 130±129 | 130±129 | 0.0954 |
| BNP (pg/mL) | 951±1041 | 413±623 | 413±623 | <0.0001 |
| sST2 (ng/mL) | 45±33 | 31.7±17 | 25±9 | <0.0001 |
| Weight (kg) | 75±15 | 77±12 | 78±15 | 0.5533 |
| BMI (kg/$m^2$) | 26±5 | 26.7±4 | 28±4 | 0.1882 |
| Height (m) | 1.7±0.1 | 1.7±0.1 | 1.7±0.1 | 0.5533 |
| MECKI score | 14.8±14 | 4±5.7 | 1.2±2.3 | <0.0001 |

Data are expressed as mean±SD.
Group 1: low peak$VO_2$ (14 mL/min/kg) and high $VE/VCO_2$ slope ≥34; Group 2: either low peak $VO_2$ and $VE/VCO_2$ slope or high peak $VO_2$ and $VE/VCO_2$ slope; Group 3: high peak $VO_2$ and low $VE/VCO_2$ slope.
BMI, body mass index; BNP, brain natriuretic peptide; CRP, C reactive protein; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; HCT, haematocrit; INR, international normalised ratio; MCV, mean corpuscular volume; MECKI, Metabolic Exercise test data combined with Cardiac and Kidney Indexes; Na, sodium; RDW, red cell distribution width; sST2, soluble suppression of tumourigenicity 2 protein; TIBC, total iron binding capacity.

by low peak $VO_2$ and high $VE/VCO_2$—was associated with the lowest TSAT and highest BNP levels. Conversely, the best exercise phenotype—with high peak $VO_2$ and low $VE/VCO_2$—was associated with the highest TSAT and lowest BNP.

Our study comprised a sizeable population of patients hospitalised for HF worsening between January 2016 and June 2023. Data of all patients who met the study inclusion/exclusion criteria were analysed. Accordingly, the studied population can be considered as representative of a worsening HF population. Patients underwent CPET, cardiac ultrasound and laboratory analysis after clinical stabilisation as confirmed by BNP values. Accordingly, patients were relatively homogenous and HF therapy was updated for the time when the data were collected. As a matter of fact, no patient was on sodium-glucose cotransporter-2 (SGLT2) inhibitors and angiotensin receptor-neprilysin inhibitor (ARNI) was around 20%.

The cut-off value we used for peak $VO_2$ (<14 mL/min/kg) and $VE/VCO_2$ slope (≥ 34) are those more commonly and historically used in HF to define prognosis.[26] Of note both these values were very close to the median value we observed for both peak $VO_2$ (14 (11.3–17.6)) and $VE/VCO_2$ slope (33.6 (29.0–40.0)). It is recognised that other cut-off values could be applied, including those calculated as a percentage of predicted[7 27 28] or those which vary, although for peak $VO_2$ only, according to the presence or absence of β-blocker treatment.[29]

It has been repeatedly reported that ID is associated with a worse HF prognosis and, among patients with ID, those with IIT have a higher BNP level, higher inflammatory status and worse prognosis.[30] In the present study, we confirmed in a large population of patients with HF stabilised after clinical worsening which needed hospitalisation that IIT patients had a higher BNP, sST2 and CRP values and more frequently HF comorbidities such as diabetes, chronic obstructive pulmonary disease (COPD), atrial fibrillation/flutter all of which are possible causes of impaired exercise performance but also are a clinical sign of more severe HF. Indeed, atrial fibrillation is frequently observed in severe HF although it is not directly linked to a worse prognosis[31] As regards exercise performance a worst physical performance was observed in patients with IIT considering either peak $VO_2$ or ventilation efficiency ($VE/VCO_2$ relationship slope). Of note with HF worsening peak $VO_2$ reduces and in parallel $VE/VCO_2$ slope increases. However, peak $VO_2$ and $VE/VCO_2$ slope are related to different causes being the former associated with a reduced $O_2$ delivery and utilisation by the working muscles and the latter to ventilation/perfusion mismatch and increased chemo and metabo reflex activity.[7–10 14 19] In the present study we showed that after adjusting for



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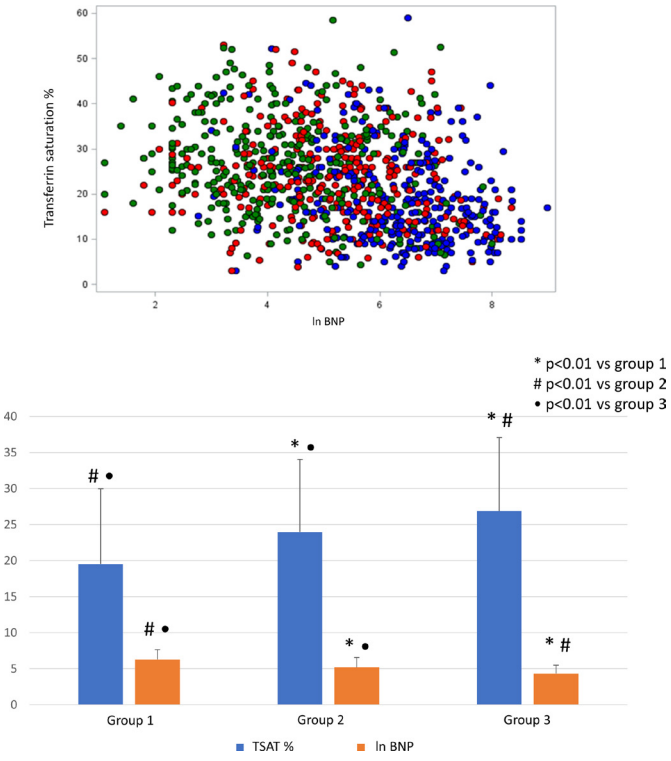


**Figure 2** Upper panel: Transferrin saturation (TSAT) and brain natriuretic peptide (BNP, log) in patients with low peak $VO_2$ (<14 mL/min/kg) and high $VE/VCO_2$ slope (≥34) (group 1, blue symbols), low peak $VO_2$ and $VE/VCO_2$ slope or high peak $VO_2$ and $VE/VCO_2$ slope (group 2, red symbols), and high peak $VO_2$ and low $VE/VCO_2$ slope (group 3, green symbols). Lower panel: Average TSAT and BNP in the three study groups. *p<0.01 vs group 1, #p<0.01 vs group 2, •p<0.01 vs group 3. $VE/VCO_2$, ventilation/carbon dioxide relationship; $VO_2$, oxygen consumption.

the most important epidemiological confounders $VE/VCO_2$ slope but not peak $VO_2$ remained associated with low TSAT (online supplemental material). Although the present study was not undertaken to dig into this matter it is possible to speculate that abnormal chemoreceptor response previously observed in patients with ID[20] plays a pivotal role. Indeed, a high $VE/VCO_2$ slope is associated with an increased chemoreflex activity in several diseases including HF and pulmonary hypertension.[32 33] On this regard Caravita *et al*[20] showed that in patients with chronic HF, intravenous iron treatment with ferric carboxymaltose reduces the enhanced chemoreflex response.

Natriuretic peptides are the biomarkers most frequently used in HF to assess HF severity and efficacy of HF treatment.[34] In the present study, we evaluated BNP and not N-terminal pro-BNP because the latter was not routinely performed at our HF unit when data were collected. Accordingly, for BNP analysis data of patients with ongoing ARNI treatment were not considered since ARNI may per se increase BNP value.[35] Of note, sST2 data, another HF biomarker, showed data comparable with those of BNP. At multivariable analysis $VE/VCO_2$ slope and peak $VO_2$ remained associated with BNP as remained associated other relevant variables including LVEF, Hb, CRP, serum creatinine and TSAT. The finding that patients with both low peak $VO_2$ and high $VE/VCO_2$ have the lowest TSAT and highest BNP suggest that TSAT and BNP can be used to predict exercise abnormalities in HF. It is possible that this translates in a worse prognosis as suggested by the higher value of MECKI (Metabolic Exercise test data combined with Cardiac and Kidney Indexes) score but this needs to be confirmed by dedicated studies.[14 36]

This study has several limitations that should be acknowledged. First of all, it is a retrospective study. Second, the potential influence of contemporary HF treatments, such as SGLT2 inhibitors (entirely absent in this cohort) and ARNI (present in only 20% of cases), on our findings remains unknown. Third, we cannot determine whether IIT treatments would ameliorate the observed CPET abnormalities. Fourth, as the study enrolled chronic patients hospitalised for HF worsening before hospital discharge, some degree of residual congestion was likely present. While this may have affected CPET results, it presumably did so uniformly across iron status groups. Consequently, our findings may not be generalisable to patients with different HF severity levels, such as those who are too sick to perform a CPET or those with clinically stable HF without recent decompensation. Similarly, since we studied only Caucasian subjects our results cannot be applied to non-Caucasian patients with HF. Fifth, when comparing peak $VO_2$ and $VE/VCO_2$ slope between patients with TSAT<20% vs those with TSAT ≥20% we considered only major confounders (Hb, LVEF, age, gender, CRP and serum creatinine) which may possibly directly affect exercise performance. However, we did not adjust for other clinical confounders as COPD, diabetes and atrial fibrillation as these are considered directly linked with low TSAT (atrial fibrillation and COPD) or renal insufficiency (diabetes). Finally, this analysis focused specifically on IIT cases and we do not know what happens in case of ID associated with low ferritin but TSAT>20%.

## Future directions

As underlined in the study limitation paragraph, several prospective and multicentre studies on IIT and CPET in patients with HF are needed. Most importantly, it must be investigated the independent prognostic role of TSAT, peak $VO_2$ and $VE/VCO_2$ slope.

In conclusion, our findings demonstrate that a high $VE/VCO_2$ slope is directly linked to IIT, suggesting a pivotal role of ventilation inefficiency in the exercise limitation observed in these patients. We also showed that patients exhibiting the worst exercise performance—characterised by concurrently low peak $VO_2$ and high $VE/VCO_2$ slope—presented with the lowest TSAT, highest BNP and the highest MECKI score, the latter suggesting a worse clinical prognosis.

**Contributors** All authors contributed to the research and have approved the final manuscript. PA is the guarantor.

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**Patient consent for publication** Not applicable.

**Ethics approval** The study complied with the Declaration of Helsinki, was approved by the local ethics committee for human research and all patients signed a general informed consent form for the use of their data (anonymised) at the time of enrolment. The study was approved by the IEO-Monzino Ethical Committee (IRB protocol number R116/14-CCM127).

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**Data availability statement** Data are available on reasonable request. Raw data will be available on request at www.zenodo.org.

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