Supplementary material

Trial design

The FACE ('Ferinject Assessment in patients with COPD and iron deficiency to improve Exercise tolerance') study was an unicentric, single-blind, parallel group, placebo controlled clinical trial. Patients were included from January 2018 to January 2020 and were subsequently randomised with a 2:1 sequence to receive a single dose of ferric carboxymaltose or placebo. The study followed the CONSORT guidelines[1]. The study was approved by the local Ethics Committee of the hospital (CEIC Parc de Salut Mar, registration nº2016/6730). The trial was registered in EudraCT with the number 2016-001238-89.

Participants

Eligibility criteria, recruitment, and follow-up

Patients must show clinical stability of chronic obstructive pulmonary disease (COPD) for at least 8 weeks prior to the inclusion in the study. Clinical stability was considered when there were no changes either in bronchodilators or other chronic treatments and no need of antibiotics or systemic corticosteroids for COPD exacerbation. Iron deficiency (ID) was defined as a ferritin level < 100 ng/mL or a ferritin level between 100 and 299 ng/mL with a transferrin saturation (TSAT) < 20 %[2,3]. Mild anaemia was defined using the World Health Organization guidelines as an haemoglobin (Hb) between 12-13 g/dL in men, and 11-12 g/dL in women[4].

The study inclusion and exclusion criteria were the following:

Table 1. Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Patients of both genres aged between 45 and 80 years.	All conditions preventing the performance of the cardiopulmonary exercise test, including cardiovascular (heart failure with left ventricle fraction ejection below 60%), neurological, kidney, musculoskeletal alterations, or uncontrolled psychiatric disorders.
Clinical stability for at least 8 weeks prior to inclusion in the study.	Other respiratory obstructive diseases such as asthma, bronchiectasis or following long-term oxygen therapy.
No change for the last 8 weeks in COPD and comorbidities pharmacotherapy treatment since last antibiotics intake and/ or systemic steroids for COPD exacerbation.	Obesity (Body Mass Index >30 Kg/m^2).
Patients who have signed the informed consent indicating that they have been informed of all pertinent aspects of the trial.	Loss of blood by any other clinical reason, pregnancy or breast-feeding, chronic liver disease, active oncologic disease.
	Allergy, or hypersensitivity to parenteral iron or any of the excipients.
	Hb ≤ 12 g/dL in men and ≤ 11 g/dL in women.
	Treatment in the previous month with either erythropoietin, iron (oral or intravenous) or any transfusions.
	Patients must have not participated in a clinical drug research trial in the 3 months before drug administration/study entry.

COPD, Chronic obstructive pulmonary disease; Hb, haemoglobin.

Throughout the study we recorded the following variables: Charlson Comorbidity Index[5], physical examination, chest x-ray, arterial blood gases and blood sample test, electrocardiography, bio impedance test (Quantum X, RJL Systems, Clinton Township, MI, USA), 6-minute walk test (6MWT)[6], incremental exercise test (IET) and constant work-rate exercise test (CWRET)[6–8].

Both IET and CWRET were conducted on a cycle ergometer (Ergoline Medgraphics, St. Paul, MN, USA). IET was performed with a ramp of 10 watts/minute. Typically, CWRET work-rate are selected to be 75% of IET work-rate peak[9]. Patients were not told that we targeted endurance time during constant high work-rate exercise 3'-8' ($t_{LIM3'-8'}$). One patient did not achieve the baseline ($t_{LIM3'-8'}$), so the test was repeated at -5 W after 60 min; conversely, 4 cases of $t_{LIM>8'}$ at CWRET75% prompted another test at 90% peak (CWRET90%).

Patients rated the intensity of dyspnoea and leg discomfort using the modified Borg score at rest and at the end of exercise[10]. Daily physical activity (DPA) was assessed using an accelerometer SenseWear® Pro2 Armband (SWA; Body media, Pittsburgh, PA). The questionnaires used included Mediterranean diet[11] and COPD Assessment Test (CAT)[12]. Blood sample test included: Hb (g/dL), haematocrit (%), serum iron (µg/dL), transferrin (g/dL), transferrin saturation (%TSAT, defined as serum iron divided by serum transferrin multiplied by 100%), serum ferritin (ng/mL) and soluble transferrin receptor (mg/L; their levels appear to be unaffected by inflammation and may more accurately reflect iron status in chronic lung disease[13]).

Study therapy and blinding

Patients received 500 mg (10 mL) or 1000 mg (20 mL) of ferric carboxymaltose diluted in 250 mL of normal saline (0.9 % NaCl) and administered over 15 minutes. The iron dose was calculated by the principal investigator according to weight and haemoglobin as stated in the manufacturer product label of the Spanish Agency of Drugs[14].

Table 2. Calculated dose according to the manufacturer product label.

Haemoglobin (g/dL)	Patient weight		
	< 35 kg	35-70 kg	≥ 70 kg
< 10	*500 mg*	*1,500 mg*	*2,000 mg*
10-14	*500 mg*	*1,000 mg*	*1,500 mg*
≥ 14	*500 mg*	*500 mg*	*500 mg*

Placebo consisted of 250 mL of normal saline that was administered for 15 minutes.

Due to the dark brown appearance of ferric carboxymaltose solution, the blinding was performed by the clinical trial pharmacist as infusion equipment and the injection site were concealed by covering the infusion bag, the infusion equipment and the injection site using opaque drapes.

Participants were therefore blinded to the study drug. The physiotherapist and the pulmonologist of the laboratory of clinical exercise physiology were unaware

of treatment allocation and performed. Only the principal investigator was aware of the group assignments.

All patients received a single dose. Patients' blood pressure was monitored every 15 minutes for up to one hour right after the end of the administration of the treatment. The administration of the treatment was performed 15 minutes after the CWRET in the respiratory day-care hospital by the nurse in charge of the clinical trial within the premises of the Respiratory Medicine Department.

Outcomes

Severity was evaluated according to Common Terminology Criteria for Adverse Events (CTCAE, version 5.0)[15]. All potential adverse events were assessed in each visit after treatment infusion and up to the end of the study protocol. Attributions were performed according to Naranjo scale[16] by blinded study investigators.

Statistical analysis

Continuous variables were expressed with median (Q1-Q3). The primary endpoint was therefore analysed using Fisher's exact test and was presented in percentages. Risk ratio and absolute risk difference were calculated and described including the 95 % confidence interval. Secondary endpoints were assessed by Mann-Whitney U test and described as median (Q1-Q3). Paired analysis was conducted when outcomes from the same treatment arm were compared through the Wilcoxon signed-rank test.

In the post-hoc analysis, we tried to assess the impact of a ferritin level of less than 30 ng/mL. This this cut-off value has been identified with the higher sensitivity (92 %) and similar specificity (98 %) and is more widely use to reflect

total body iron stores[17]. Patients were divided into those with baseline values > 30 ng/mL and < 30 ng/mL and thereafter the achievement of the primary endpoint was compared using the Fisher's exact test, given the small number of patients. The endurance time, CAT questionnaire and DPA were assessed through Mann-Whitney U test and were expressed as median (Q1-Q3).

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