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Impact of anaemia on lung function and exercise capacity in patients with stable severe chronic obstructive pulmonary disease

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【Abstract】

Objective: This study intended to search for potential correlations between anaemia in patients with severe COPD (GOLD stage 3) and pulmonary function at rest, exercise capacity, as well as ventilatory efficiency, utilizing pulmonary function test (PFT) and cardiopulmonary exercise testing (CPET).

Setting: Study was undertaken at Shanghai pulmonary hospital, a tertiary level centre affiliated to Tongji University. It caters to a large population base within shanghai and referrals from centers in other cities as well.

Participants: 155 Chinese patients with stable severe COPD were divided into two groups: anemia group [hemoglobin (Hb) <12.0 g/dl for males, and <11 g/dL for females (n=46)] and non-anemia group (n=109).

Primary and secondary outcome measures: Arterial blood gas (ABG), PFT and CPET were tested in all patients.

Results: (1) Diffusing capacity for carbon monoxide (DL_{CO}) corrected by Hb was significantly lower in anemia group [(15.3±1.9) ml/min/mmHg] than in non-anemia group [(17.1±2.1) ml/min/mmHg] (P<0.05). Significant difference did not exist in the level of forced expiratory volume in 1 second (FEV₁), FEV₁%pred, FEV₁/ forced vital capacity (FVC), inspiratory capacity (IC), residual volume (RV), total lung capacity (TLC) and RV/TLC (P>0.05). (3) Peak Load, Peak oxygen uptake ($\dot{V} O_2$), Peak $\dot{V} O_2$ %pred, Peak $\dot{V} O_2$ /Kg, Peak $\dot{V} O_2$ pulse and the ratio of $\dot{V} O_2$ increase to WR increase ($\Delta\dot{V} O_2/\Delta WR$) were significantly lower in anemia group (P<0.05), however, Peak minute ventilation (VE), Lowest $\dot{V} E$ / carbon dioxide output ($\dot{V} CO_2$), Peak dead space/tidal volume ratio (VD/VT) were similar between the two groups (P>0.05). (4) A strong positive correlation was found between Hb concentration and Peak $\dot{V} O_2$ in anemic patients. (r=0.702, P<0.01).

Conclusion: Anemia has a negative impact on gas exchange and exercise tolerance during exercise in severe COPD patients. The decrease amplitude of Hb levels is related to the quantity of oxygen uptake.

【Key words】 anemia; chronic obstructive pulmonary disease; cardiopulmonary exercise test; hemoglobin

Article summary

Heading: Impact of anaemia on lung function and exercise capacity in patients with stable severe chronic obstructive pulmonary disease

This study searched for potential correlations between anaemia in patients with severe COPD (GOLD stage 3) and pulmonary function at rest, exercise capacity, as well as ventilatory efficiency. Our results show that anemia has a negative impact on gas exchange and exercise capacity in severe COPD patients. The decrease amplitude of Hb levels is related to the quantity of oxygen uptake. Although cardiopulmonary exercise testing (CPET) parameters have been widely used to grade the severity of exercise limitation and provide a quantitative assessment on patients' ventilator response and cardiovascular response during exercise, all of which might be impaired due to anemia. It has seldom been utilized for functional assessment of COPD patients with anemia. The limitations of the study is that the present data were derived from patients with severe (GOLD stage III) COPD and thus cannot be applied to the overall population of COPD patients.

Introduction

Chronic obstructive pulmonary disease (COPD) affects various functional and structural domains of the lungs and is recognized as a systemic disease, frequently present in addition to other diseases.¹ Polycythemia, traditionally thought to be highly prevalent in COPD, is less frequent nowadays with more rigorous correction of hypoxaemia.² Reversely, recent research show that anaemia is highly prevalent in patients with COPD.^{3,4}

Anemia, which is a well recognized comorbidity in many chronic illnesses occurs in 10-30% of COPD patients.³⁻¹² Anemia has been related with increased mortality and morbidity including increased health care costs and hospitalization.³⁻¹² Although cardiopulmonary exercise testing (CPET) parameters have been widely used to grade the severity of exercise limitation and provide a quantitative assessment on patients' ventilator response and cardiovascular response during exercise,¹³ all of which might be impaired due to anemia. It has seldom been utilized for functional assessment of COPD patients with anemia.

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Based on the aforementioned situation, the aims of our study were to (1) investigate the prevalence of anemia in stable severe COPD patients and (2) evaluate the impact of anemia on pulmonary function at rest, ventilatory efficiency and exercise capacity in COPD patients, utilizing PFT and CPET.

MATERIALS AND METHODS

Study subjects

The relevant population consisted of consecutive, clinically stable patients with a diagnosis of severe COPD (GOLD stage III)¹⁴, who visited the Respiratory Unit of Shanghai Pulmonary Hospital as outpatients between December 2011 and November 2013. Patients were diagnosed as clinically stable if they had had no hospital admission, exacerbation, respiratory infection or change in medication three months prior to entering the study. Exclusion criteria included: (1) a history of bronchiectasis, asthma or other concomitant respiratory diseases; (2) a diagnosis of malignancy; (3) inability to complete CPET according to the American Thoracic Society/American College of Chest Physicians guidelines,¹⁵ such as unstable angina, acute myocardial infarction, uncontrolled arrhythmias causing symptoms and orthopedic impairment; and (4) any disease that could affect haemoglobin levels (thyroid disease, liver disease, chronic renal failure, chronic heart failure, previous history of gastrointestinal bleeding and chronic inflammatory rheumatic disease). Anaemia was defined as the presence of haemoglobin levels <12 g/dL in males and <11 g/dL in females according to China-specific criteria¹⁶. The study was approved by the hospital Ethics Committee. Informed consent was obtained from all patients before undergoing any study procedure.

Study Procedures

During the initial visit, all the patients with a potential or known diagnosis of COPD underwent physical examination, arterial blood gas analysis (ABG) and lung function testing. All stable severe COPD patients (if $30\% \leq FEV1 \leq 50\%$ predicted) underwent peripheral venous blood sampling to determine full blood count, liver transaminase, total protein (albumin and globulin), thyroid hormone, serum levels of

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3 urea and creatinine. Patients who were subsequently presented with abnormal
4 thyroid hormone values (either high or low), glomerular filtration rate (GFR) below
5 60mL/min¹⁷ and increased liver enzymes twice the upper limit of normal were
6 excluded. The included patients in the study visited the outpatient clinic within two
7 weeks from the initial visit and performed CPET.
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10 11 **Arterial blood gas analysis and resting pulmonary function measurements**

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13 ABG measurements such as PH, partial pressure of oxygen (PaO₂), partial
14 pressure of carbon dioxide (PaCO₂) and arterial oxygen saturation (SaO₂) were taken
15 just after 10 minutes of rest. Each subject underwent resting PFT of forced vital
16 capacity (FVC), forced expiratory volume in 1second (FEV₁), inspiratory capacity
17 (IC), diffusing capacity for carbon monoxide (DL_{co}) corrected by Hb, residual
18 volume (RV) and total lung capacity (TLC) using standard methodology¹² and
19 equipment (Jaeger Corp., Hoechberg, Germany). All resting lung function values were
20 reported in absolute terms and normalized to percent of predicted (% pred). Predicted
21 spirometry values were calculated using accepted equations for Chinese adults.¹⁸
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32 **Cardiopulmonary exercise test measurements**

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34 All the patients and controls performed on a cycle ergometer using a
35 breath-by-breath system according to the American Thoracic Society/American
36 College of Chest Physicians Statement on CPET.¹⁵ The equipment was calibrated in
37 accordance with manufacturer's specifications using reference and calibration gases
38 before each test. Standard 12 lead electrocardiograms (ECGs) and pulse oximetry
39 were continuously monitored. Arterial blood pressure (BP) was measured every two
40 minutes with an automatic cuff. The protocol was comprised of three minutes of rest,
41 three minutes of unloaded cycling at 55-65 revolutions per minute (rpm), followed by
42 a progressively increasing work rate of 5 to 15 watts (W)/min for PH patients, 20 to
43 25 W/min for the normal subjects to the maximum tolerance, and four minutes of
44 recovery.¹⁸
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55 Most CPET values were reported in absolute terms and normalized to percentage
56 of predicted (% pred). Predicted values were calculated using accepted equations.¹⁹
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CPET variables including oxygen uptake ($\dot{V}O_2$), minute ventilation ($\dot{V}E$), carbon dioxide output ($\dot{V}CO_2$), workload (WR) and dead space/tidal volume ratio (VD/VT) were calculated at peak exercise. Peak $\dot{V}O_2$ was defined as the highest 30-second average of oxygen uptake in the last minute of exercise and other peak parameters were calculated at the same time. Lowest $\dot{V}E/\dot{V}CO_2$ was determined by averaging the lowest consecutive 90 sec data points.²⁰

Statistical analysis

Statistical analysis was performed using SPSS (version 16, SPSS, Chicago). Parameters were expressed as mean \pm SD. Most PFT and CPET values are expressed in absolute terms and % pred. Unpaired Student t test was used to identify differences between groups, whereas χ^2 test was used to assess differences in categorical variables between groups. Correlations between CPET parameters and Hb levels were determined by Pearson's correlation test. P value of < 0.05 was considered significant.

Results

Baseline clinical and demographic characteristics

One hundred and fifty-five patients diagnosed with severe COPD (GOLD stage III) in outpatients were included. The vast majority (93%) of patients in the dataset were males. The mean age was 61 years. The characteristics of the anemic and non anemic COPD patients are presented in Table 1. Forty-eight (31%) patients from this population fulfilled both laboratory and clinical criteria for anemia, therefore, the prevalence of anemia among stable COPD (GOLD stage III) outpatients in our study was 31%. The Hb level for anemic patients was 10.6 ± 0.8 g/dl. No difference was noted in BMI, ages, pack-years of smoking and arterial blood gas analysis parameters (PH , PaO_2 , $PaCO_2$ or SaO_2) between anemic and non anemic patients.

Resting pulmonary function measurements

The PFT parameters of the anemic and non anemic patients are presented in Tables 2. There was no statistical significance in FEV_1 , $FEV_1\%$ pred, FEV_1/FVC , IC, RV, TLC, RV/TLC between the two groups, indicating resting pulmonary ventilation

function was similar to each other. However, DL_{CO} corrected by Hb was significantly lower among anemic patients compared to nonanemic patients.

Cardiopulmonary exercise testing

All individuals completed their CPET studies without incident. Nearly all patients stopped exercise because of leg fatigue and/or acute shortness of breath. All subjects achieved a respiratory exchange ratio (RER) >1.10, indicating sufficient metabolic stress. Because the anaerobic threshold (AT) may not be determined by V-slope method²¹ or the interpreter may feel that the AT is unreliable after reviewing an exercise test in a substantial number of severe COPD patients, CPET variables including $\dot{V}O_2$, $\dot{V}E$, $\dot{V}CO_2$, workload, and VD/VT were calculated at peak exercise without AT. Exercise responses of all the COPD patients at peak are presented in Table 3. Overall, there was a negative impact of anemia on exercise capacity. PeakLoad, Peak $\dot{V}O_2$, Peak $\dot{V}O_2$ /Kg, Peak $\dot{V}O_2$ pulse, and the ratio of $\dot{V}O_2$ increase to WR increase ($\Delta\dot{V}O_2 / \Delta WR$ slope) were significantly lower among anemic patients compared with non anemic patients. None of the exercise parameters indicative of respiratory limitation and ventilatory efficiency (Peak $\dot{V}E$, Lowest $\dot{V}E / \dot{V}CO_2$, Peak VD/VT) differed between the two groups.

Correlations

Peak $\dot{V}O_2$ is frequently used as the most reliable measure of overall exercise capacity, so we choose Peak $\dot{V}O_2$ as the typical CPET parameters to investigate for potential associations between Hb levels and exercise capacity. The correlations between Hb concentration and Peak $\dot{V}O_2$ for the anemic and non anemic COPD patients are shown in Figure 1. A strong positive correlation was found between Hb concentration and Peak $\dot{V}O_2$ in anemic patients ($r=0.702$, $P<0.01$) (Figure1A) , but no statistical significant correlation was found with non anemic patients ($r=0.055$, $P>0.05$) (figure1B).

Discussions

There is increasing evidence regarding the impact of systemic inflammation on COPD. In theory, it has been postulated that the pathophysiology of COPD-associated anaemia might be an inflammatory and immunological response, mediated by IL-1, IL-6, TNF- α and interferon gamma, which could make variations in iron metabolism, reducing iron absorption thereby altering normal haematopoiesis.^{22,23} These mediators increase in patients with COPD and could be regarded as factors related to the onset of anaemia.²⁴ One previous study has reported that anaemia in COPD is at least in part, on account of inflammation and resistance to elevated levels of serum erythropoietin.²⁵

In the current study, anaemia was present in 31% of severe COPD patients. The result confirms that anaemia is a common phenomenon in patients with severe COPD. The main aim of the present study was to search for potential correlations between anaemic severe COPD patients and resting pulmonary function, exercise capacity, as well as ventilatory efficiency based on PFT and CPET.

The usual PFT parameters like FEV₁, FEV₁% pred, FEV₁/FVC, IC, RV, TLC and RV/TLC of the two groups were similar to each other except for DLco corrected by Hb. A severe obstructive pattern characterized the population studied. The finding that patients in the anaemia group had significant lower DLco values (corrected by Hb) (P<0.05) indicates that the decreased level of haemoglobin may affect the rate of oxygen uptake across the alveolar-capillary bed and reduce the diffusing capacity of the lungs, without having obvious influence on the pulmonary ventilation function.

Although anaemia has been associated with dyspnoea and reduced exercise tolerance in patients with chronic kidney disease, heart failure and cancer,²⁶⁻²⁸ few studies have been published concerning the effect of anaemia on exercise capacity in COPD patients. Cote et al studied patients with stable COPD, observed that the presence of anaemia affects the general feeling of dyspnoea measured in the MRC scale and concluded that anaemia decreases exercise tolerance measured in 6MWT.²⁹ However, the present study is the first to investigate the impact of anaemia on exercise capacity and ventilatory efficiency of severe COPD patients utilizing CPET.

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On the one hand, the present study showed that anemic patients with severe COPD had lower PeakLoad, Peak $\dot{V} O_2$ (%pred), Peak $\dot{V} O_2$ /Kg, $\Delta O_2/\Delta WR$ slope and Peak oxygen pulse (O_2 pulse) compared with the non anemic ones, demonstrating the negative effect of anemia on exercise capacity. Peak $\dot{V} O_2$ is frequently used as the most reliable measure of overall exercise capacity. By Fick's principle^{20,30-32}, $\dot{V} O_2 =$ cardiac output*arteriovenous O_2 difference, the arteriovenous O_2 difference is dependent on the availability of hemoglobin, blood oxygenation in the lung, and extraction of oxygen in the periphery. For a given increase in cardiac output, the anemic patient, who has decreased level of haemoglobin and arterial O_2 concentration, will have a lower Peak $\dot{V} O_2$. O_2 pulse is the quotient of the $\dot{V} O_2$ and heart rate. $\Delta \dot{V} O_2/\Delta WR$ slope is an important measurement to evaluate exercise capacity in patients with heart disease. The decreased $\Delta VO_2/\Delta WR$ slope is a marker of decreased aerobic exercise capacity caused by decreased oxygen transport.³³ In our study, we found that COPD patients with anemia had a lower $\Delta VO_2/\Delta WR$ slope than those without anemia. This result further confirmed that anemic patients may have a more serious limitation in exercise capacity than non anemic patients.

On the other hand, there were no differences in CPET parameters as Peak $\dot{V} E$, Lowest $\dot{V} E/\dot{V} CO_2$, Peak VD/VT (%) between the two groups. Both Peak VD/VT and Lowest $\dot{V} E/\dot{V} CO_2$ indicate the ventilatory efficiency of oxygen uptake and carbon dioxide elimination in patients, mainly due to the limitation of blood flow perfusion, i.e. Q/VA mismatch. Therefore, anaemia may have a negative effect on exercise capacity of patients with severe COPD, while its influence on ventilatory efficiency is little.

Apart from that, a strong positive correlation was found between Hb concentration and Peak $\dot{V} O_2$ in anemic patients ($r=0.702$, $P<0.01$), but no statistical significant correlation was found with non anemic patients. All these results strongly indicate that oxygen carrying capacity may depend upon the level of haemoglobin for

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the anemic patients and oxygen delivery is crucial for the maintenance of oxidative metabolism. Previous study showed that in normal individuals, 15 g/dl of Hb carry approximately 21 ml of oxygen per 100 ml of blood and a 3g/dl decrease in Hb levels would result in a reduction of the total oxygen-carrying capacity by 4/100 ml.³⁴ Moreover, anaemia may result in limited oxygen supply with the consequent increase in ventilatory drive. Among patients with COPD whose ventilatory reserve were decreased, the accompanying increased ventilatory demand may result in dyspnoea.

Our study is not free of limitations. The proportion of female patients was small. The significantly lower prevalence of COPD in females is the possible explanations for this phenomenon. Moreover, the present data were derived from patients with severe (GOLD stage III) COPD and thus cannot be applied to the overall population of COPD patients. The reason why we choose severe (GOLD stage III) COPD patients as our object of study is that patients with anaemia had a tendency to have more severe COPD^{35,36}, while very severe COPD patients may not manage to complete CPET. Efforts should be made to further assess the prevalence of anaemia in other stages of COPD.

Conclusions:

Our study demonstrated that COPD can be accompanied by anemia and implied the potential correlations between anaemia in patients with COPD and respiratory physiology utilizing PFT and CPET. The results indicated that both the function of gas exchange and exercise capacity decreased in the severe COPD patients with anaemia compared with the patients without anaemia, while the influence of anaemia on pulmonary ventilation function and ventilatory efficiency was little. Future studies are needed in order to evaluate the possible therapeutic approaches in COPD patients with anaemia.

contributorship statement

Conceived and designed the experiments: JML XGS JG CZ.

Performed the experiments: JG CZ QX WLY.

Analyzed the data: JML JG CZ QX GGS QHZ.

Contributed reagents/materials/analysis tools: JML JG LW JH XS.

Wrote the paper: JML JG CZ QX .

competing interests

All of the authors have declared that no competing interests exist.

funding

There is no specific funding for our work.

data sharing statement

All the primary data were obtained at Shanghai pulmonary hospital. They are both secure and available anytime through the medical records section of the hospital, both in print and digital formats. We the authors are aware about sensitivity of data security. And with regards to data used from other sources due permission has been obtained and cited accordingly. We are ready to answer to any queries that may arise in the future.

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Figure captions

Figure 1. Figure 1A and 1B show the correlation between Peak $\dot{V}O_2$ and Hb concentration in COPD patients with and without anaemia respectively.

Significant positive correlation was found between Hb concentration and Peak $\dot{V}O_2$ in anemic patients ($r=0.702$, $P<0.01$) (Figure1A), but no statistical significant correlation was found with nonanemic patients ($r=0.055$, $P>0.05$) (Figure1B).

Table 1. Demographic and clinical characteristics of patients included in the study

Variable	Patients with low haemoglobin Concentration (%)	Patients with normal haemoglobin concentration (%)
Number of patients (n = 207) (%)	48 (31%)	109(69%)
Sex	Men (M) 45(94%)	Men (M)99 (91%)
Age (years)	61.32 ± 6.03	60.82 ± 7.13
BMI (kg/m ²)	21.72 ± 2.93	22.18 ± 2.15
Haemoglobin concentration (g/dL)	10.6 ± 0.8*	12.9 ± 1.1*
Smoking index (Pack-years)	43.58 ± 7.70	45.56 ± 4.63
PH	7.39 ± 0.017	7.38 ± 0.023
PaCO ₂ (mm Hg)	44.67 ± 1.32	44.31 ± 1.28
PaO ₂ (mm Hg)	68.11 ± 3.01	67.33. ± 3.62
SaO ₂ (%)	94.44 ± 0.94	94.77 ± 1.13

PaO₂= oxygen partial pressure; PaCO₂ = carbon dioxide partial pressure;
values represent numbers of patients or means+SD, as appropriate.

*p < 0.05, COPD patients with anaemia versus patients without anaemia using unpaired t test

Table 2. The comparison of pulmonary function indices in COPD patients with and without anaemia

Variable	Patients with low haemoglobin concentration(n = 48)	Patients with normal haemoglobin concentration(n = 109)
FEV ₁ (L)	1.33 ± 0.27	1.41 ± 0.13
FEV ₁ (% predicted)	42.15 ± 7.53	43.28 ± 5.72
FEV ₁ /FVC (%)	41.32 ± 2.19	43.36 ± 1.38
RV (L)	3.98 ± 1.55	4.11 ± 1.24
TLC (L)	7.82 ± 0.93	7.11 ± 1.78
RV/TLC (%)	60.12 ± 6.78	53.27 ± 6.79
IC (L)	1.52 ± 0.47	1.61 ± 0.43
DLco (L)	15.31 ± 1.94*	17.10 ± 2.05*

FEV₁, forced expiratory volume in one second; FVC, forced vital capacity; TLC, total lung capacity; RV, residual volume; IC, inspiratory capacity; DLco, carbon monoxide diffusion capacity corrected for Hb concentration

values represent numbers of patients or means+SD, as appropriate

*p < 0.05, COPD patients with anaemia versus patients without anaemia using unpaired t test

Table 3. The comparison of cardiopulmonary exercise testing parameters in COPD patients with and without anaemia

Variable	Patients with low haemoglobin concentration	Patients with normal haemoglobin concentration
Peak Load (W)	66.36 ± 16.62*	69.46 ± 28.18*
Peak $\dot{V}O_2$ (ml/min)	866.28 ± 274.32**	1180.42 ± 319.18**
Peak $\dot{V}O_2$ (%pred)	56.21 ± 14.79**	69.11 ± 18.72**
Peak $\dot{V}O_2$ /Kg (mL/min/kg)	14.88 ± 3.92*	18.72 ± 3.86*
Peak $\dot{V}E$ (L/min)	37.89 ± 6.33	36.71 ± 5.42
$\Delta O_2/\Delta WR$ slope (ml/min/watt)	8.02 ± 1.03*	9.20 ± 1.42*
Peak O_2 pulse (mL/beat)	7.88 ± 2.63*	11.3 ± 2.19*
Lowest $\dot{V}E/\dot{V}CO_2$	32.15 ± 1.28	31.28 ± 2.90
Peak VD/VT (%)	33.2 ± 7.2	34.9 ± 6.1

%pred = percent of predicted; $\dot{V}O_2$ = peak oxygen uptake; $\dot{V}E$ = minute ventilation; $\dot{V}CO_2$ =

carbon dioxide output; $\Delta O_2/\Delta WR$ slope = the ratio of $\dot{V}O_2$ increase to WR increase;

VD/VT = dead space/tidal volume ratio

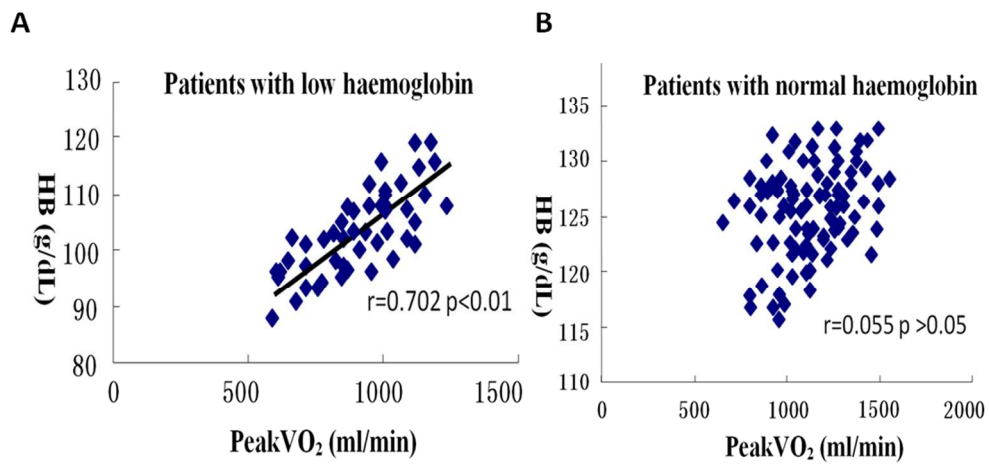
values represent numbers of patients or means ± SD, as appropriate.

*P < 0.05, **P < 0.01, COPD patients with anaemia versus patients without anaemia using unpaired

t test

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Figure 1



Peer review only

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Impact of anaemia on lung function and exercise capacity in patients with stable severe chronic obstructive pulmonary disease

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Manuscripts

1 **Impact of anaemia on lung function and exercise capacity in patients**
2 **with stable severe chronic obstructive pulmonary disease**

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19 * This three authors contributed equally to this work

20 **【Abstract】**

21 **Objective:** This study intended to search for potential correlations between anaemia
22 in patients with severe COPD (GOLD stage 3) and pulmonary function at rest,
23 exercise capacity, as well as ventilatory efficiency, utilizing pulmonary function test
24 (PFT) and cardiopulmonary exercise testing (CPET).

25 **Setting:** Study was undertaken at Shanghai pulmonary hospital, a tertiary level centre
26 affiliated to Tongji University. It caters to a large population base within shanghai and
27 referrals from centers in other cities as well.

28 **Participants:** 157 Chinese patients with stable severe COPD were divided into two
29 groups: anemia group [hemoglobin (Hb) <12.0 g/dl for males, and <11 g/dL for
30 females (n=48)] and non-anemia group (n=109).

31 **Primary and secondary outcome measures:** Arterial blood gas (ABG), PFT and
32 CPET were tested in all patients.

33 **Results:** (1) Diffusing capacity for carbon monoxide (DL_{CO}) corrected by Hb was
34 significantly lower in anemia group [(15.3±1.9) ml/min/mmHg] than in non-anemia
35 group [(17.1±2.1) ml/min/mmHg] (P<0.05). Significant difference did not exist in the
36 level of forced expiratory volume in 1 second (FEV₁), FEV₁%pred, FEV₁/ forced vital
37 capacity (FVC), inspiratory capacity (IC), residual volume (RV), total lung capacity
38 (TLC) and RV/TLC (P>0.05). (2) Peak Load, Peak oxygen uptake ($\dot{V} O_2$), Peak \dot{V}
39 O₂%pred, Peak $\dot{V} O_2$ /Kg, Peak O₂ pulse and the ratio of $\dot{V} O_2$ increase to WR increase
40 ($\Delta\dot{V} O_2/\Delta WR$) were significantly lower in anemia group(P<0.05), however, Peak
41 minute ventilation (VE), Lowest $\dot{V} E$ / carbon dioxide output ($\dot{V} CO_2$), Peak dead
42 space/tidal volume ratio (VD/VT) were similar between the two groups (P>0.05). (3)
43 A strong positive correlation was found between Hb concentration and Peak $\dot{V} O_2$ in
44 anemic patients. (r=0.702, P<0.01).

45 **Conclusion:** Anemia has a negative impact on gas exchange and exercise tolerance
46 during exercise in severe COPD patients. The decrease amplitude of Hb levels is
47 related to the quantity of oxygen uptake.

48 **【Key words】** anemia; chronic obstructive pulmonary disease; cardiopulmonary
49 exercise test; hemoglobin

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51 **Article summary**

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4 52 Heading: Impact of anaemia on lung function and exercise capacity in patients with
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6 53 stable severe chronic obstructive pulmonary disease
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9 54 This study searched for potential correlations between anaemia in patients with
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11 55 severe COPD (GOLD stage 3) and pulmonary function at rest, exercise capacity, as
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13 56 well as ventilatory efficiency. Our results show that anemia has a negative impact on
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15 57 gas exchange and exercise capacity in severe COPD patients. The decrease amplitude
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17 58 of Hb levels is related to the quantity of oxygen uptake. Although cardiopulmonary
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19 59 exercise testing (CPET) parameters have been widely used to grade the severity of
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21 60 exercise limitation and provide a quantitative assessment on patients' ventilator
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23 61 response and cardiovascular response during exercise, all of which might be impaired
24
25 62 due to anemia. It has seldom been utilized for functional assessment of COPD patients
26
27 63 with anemia. The limitations of the study is that the present data were derived from
28
29 64 patients with severe (GOLD stage III) COPD and thus cannot be applied to the overall
30
31 65 population of COPD patients.

66 **Introduction**

32
33 67 Chronic obstructive pulmonary disease (COPD) affects various functional and
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35 68 structural domains of the lungs and is recognized as a systemic disease, frequently
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37 69 present in addition to other diseases.¹ Polycythemia, traditionally thought to be highly
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39 70 prevalent in COPD, is less frequent nowadays with more rigorous correction of
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41 71 hypoxaemia.² Reversely, recent research show that anaemia is highly prevalent in
42
43 72 patients with COPD.^{3,4}

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45 73 Anemia, which is a well recognized comorbidity in many chronic illnesses
46
47 74 occurs in 10-30% of COPD patients.³⁻¹² Anemia has been related with increased
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49 75 mortality and morbidity including increased health care costs and hospitalization.³⁻¹²
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51 76 Although cardiopulmonary exercise testing (CPET) parameters have been widely
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53 77 used to grade the severity of exercise limitation and provide a quantitative assessment
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55 78 on patients' ventilator response and cardiovascular response during exercise,¹³ all of
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57 79 which might be impaired due to anemia. It has seldom been utilized for functional
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59 80 assessment of COPD patients with anemia.
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81 Based on the aforementioned situation, the aims of our study were to (1)
82 investigate the prevalence of anemia in stable severe COPD patients and (2) evaluate
83 the impact of anemia on pulmonary function at rest, ventilatory efficiency and
84 exercise capacity in COPD patients, utilizing PFT and CPET.

85 **MATERIALS AND METHODS**

86 **Study subjects**

87
88 The relevant population consisted of consecutive, clinically stable patients with a
89 diagnosis of severe COPD (GOLD stage III)¹⁴, who visited the Respiratory Unit of
90 Shanghai Pulmonary Hospital as outpatients between December 2011 and November
91 2013. Patients were diagnosed as clinically stable if they had had no hospital
92 admission, exacerbation, respiratory infection or change in medication three months
93 prior to entering the study. Exclusion criteria included: (1) a history of bronchiectasis,
94 asthma or other concomitant respiratory diseases; (2) a diagnosis of malignancy; 3)
95 inability to complete CPET according to the American Thoracic Society/American
96 College of Chest Physicians guidelines,¹⁵ such as unstable angina, acute myocardial
97 infarction, uncontrolled arrhythmias causing symptoms and orthopedic impairment;
98 and 4) any disease that could affect haemoglobin levels (thyroid disease, liver disease,
99 chronic renal failure, chronic heart failure, previous history of gastrointestinal
100 bleeding and chronic inflammatory rheumatic disease). Anaemia was defined as the
101 presence of haemoglobin levels <12 g/dL in males and <11 g/dL in females according
102 to China-specific criteria¹⁶. The study was approved by the hospital Ethics Committee.
103 Informed consent was obtained from all patients before undergoing any study
104 procedure.

105 **Study Procedures**

106 During the initial visit, all the patients with a potential or known diagnosis of
107 COPD underwent physical examination, arterial blood gas analysis (ABG) and lung
108 function testing. All stable severe COPD patients (if $30\% \leq FEV1 \leq 50\%$ predicted)
109 underwent peripheral venous blood sampling to determine full blood count, liver
110 transaminase, total protein (albumin and globulin), thyroid hormone, serum levels of

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4 111 urea and creatinine. Patients who were subsequently presented with abnormal
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6 112 thyroid hormone values (either high or low), glomerular filtration rate (GFR) below
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8 113 60mL/min¹⁷ and increased liver enzymes twice the upper limit of normal were
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10 114 excluded. The included patients in the study visited the outpatient clinic within two
11
12 115 weeks from the initial visit and performed CPET.

116 **Arterial blood gas analysis and resting pulmonary function measurements**

117 ABG measurements such as PH, partial pressure of oxygen (PaO₂), partial
118 pressure of carbon dioxide (PaCO₂) and arterial oxygen saturation (SaO₂) were taken
119 just after 10 minutes of rest. Each subject underwent resting PFT of forced vital
120 capacity (FVC), forced expiratory volume in 1second (FEV₁), inspiratory capacity
121 (IC), diffusing capacity for carbon monoxide (DL_{co}) corrected by Hb, residual
122 volume (RV) and total lung capacity (TLC) using standard methodology¹² and
123 equipment (Jaeger Corp., Hoechberg, Germany). All resting lung function values were
124 reported in absolute terms and normalized to percent of predicted (% pred). Predicted
125 spirometry values were calculated using accepted equations for Chinese adults.¹⁸

126 **Cardiopulmonary exercise test measurements**

127 All the patients performed on a cycle ergometer using a breath-by-breath system
128 according to the American Thoracic Society/American College of Chest Physicians
129 Statement on CPET.¹⁵ The equipment was calibrated in accordance with
130 manufacturer's specifications using reference and calibration gases before each test.
131 Standard 12 lead electrocardiograms (ECGs) and pulse oximetry were continuously
132 monitored. Arterial blood pressure (BP) was measured every two minutes with an
133 automatic cuff. The protocol was comprised of three minutes of rest, three minutes of
134 unloaded cycling at 55-65 revolutions per minute (rpm), followed by a progressively
135 increasing work rate of 5 to 15 watts (W)/min for the COPD patients, and four
136 minutes of recovery.¹⁸

137 Most CPET values were reported in absolute terms and normalized to percentage
138 of predicted (% pred). Predicted values were calculated using accepted equations.¹⁹

139 CPET variables including oxygen uptake ($\dot{V}O_2$), minute ventilation ($\dot{V}E$), carbon

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4 140 dioxide output ($\dot{V}CO_2$), workload (WR) and dead space/tidal volume ratio (VD/VT)
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6 141 were calculated at peak exercise. Peak $\dot{V}O_2$ was defined as the highest 30-second
7
8 142 average of oxygen uptake in the last minute of exercise and other peak parameters
9
10 143 were calculated at the same time. Lowest $\dot{V}E/\dot{V}CO_2$ was determined by averaging the
11
12 144 lowest consecutive 90 sec data points.²⁰

145 **Statistical analysis**

146 Statistical analysis was performed using SPSS (version 16, SPSS, Chicago).
147 Parameters were expressed as mean \pm SD. Most PFT and CPET values are expressed
148 in absolute terms and % pred. Unpaired Student t test was used to identify differences
149 between groups, whereas χ^2 test was used to assess differences in categorical
150 variables between groups. Correlations between CPET parameters and Hb levels were
151 determined by Pearson's correlation test. P value of < 0.05 was considered significant.

152 **Results**

153 **Baseline clinical and demographic characteristics**

154 One hundred and fifty-seven patients diagnosed with severe COPD (GOLD stage
155 III) in outpatients were included. The vast majority (93%) of patients in the dataset
156 were males. The mean age was 61 years. The characteristics of the anemic and non
157 anemic COPD patients are presented in Table 1. Forty-eight (31%) patients from this
158 population fulfilled both laboratory and clinical criteria for anemia, therefore, the
159 prevalence of anemia among stable COPD (GOLD stage III) outpatients in our study
160 was 31%. The Hb level for anemic patients was 10.6 ± 0.8 g/dl. No difference was
161 noted in BMI, ages, pack-years of smoking and arterial blood gas analysis parameters
162 (PH, PaO₂, PaCO₂ or SaO₂) between anemic and non anemic patients.

163 **Resting pulmonary function measurements**

164 The PFT parameters of the anemic and non anemic patients are presented in
165 Tables 2. There was no statistical significance in FEV₁, FEV₁%pred, FEV₁/FVC, IC,
166 RV, TLC, RV/TLC between the two groups, indicating resting pulmonary ventilation
167 function was similar to each other. However, DLco corrected by Hb was significantly
168 lower among anemic patients compared to nonanemic patients.

169 **Cardiopulmonary exercise testing**

170 All individuals completed their CPET studies without incident. Nearly all
171 patients stopped exercise because of leg fatigue and/or acute shortness of breath. All
172 subjects achieved a respiratory exchange ratio (RER) >1.10, indicating sufficient
173 metabolic stress. Because the anaerobic threshold (AT) may not be determined by
174 V-slope method²¹ or the interpreter may feel that the AT is unreliable after reviewing
175 an exercise test in a substantial number of severe COPD patients, CPET variables
176 including $\dot{V}O_2$, $\dot{V}E$, $\dot{V}CO_2$, workload, and VD/VT were calculated at peak exercise
177 without AT. Exercise responses of all the COPD patients at peak are presented in
178 Table 3. Overall, there was a negative impact of anemia on exercise capacity.
179 PeakLoad, Peak $\dot{V}O_2$, Peak $\dot{V}O_2/Kg$, Peak $\dot{V}O_2$ pulse, and the ratio of $\dot{V}O_2$ increase
180 to WR increase ($\Delta\dot{V}O_2 / \Delta WR$ slope) were significantly lower among anemic patients
181 compared with non anemic patients. None of the exercise parameters indicative of
182 respiratory limitation and ventilatory efficiency (Peak $\dot{V}E$, Lowest $\dot{V}E / \dot{V}CO_2$, Peak
183 VD/VT) differed between the two groups.

184 **Correlations**

185 Peak $\dot{V}O_2$ is frequently used as the most reliable measure of overall exercise
186 capacity, so we choose Peak $\dot{V}O_2$ as the typical CPET parameters to investigate for
187 potential associations between Hb levels and exercise capacity. The correlations
188 between Hb concentration and Peak $\dot{V}O_2$ for the anemic and non anemic COPD
189 patients are shown in Figure 1. A strong positive correlation was found between Hb
190 concentration and Peak $\dot{V}O_2$ in anemic patients ($r=0.702$, $P<0.01$) (Figure1A), but
191 no statistical significant correlation was found with non anemic patients ($r=0.055$,
192 $P>0.05$) (figure1B).

194 **Discussions**

195 In the current study, anaemia was present in 31% of severe COPD patients. The

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4 196 result confirms that anaemia is a common phenomenon in patients with severe COPD.
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6 197 The main aim of the present study was to search for potential correlations between
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8 198 anaemic severe COPD patients and resting pulmonary function, exercise capacity, as
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10 199 well as ventilatory efficiency based on PFT and CPET.

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12 200 The usual PFT parameters like FEV₁, FEV₁% pred, FEV₁/FVC, IC, RV, TLC and
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14 201 RV/TLC of the two groups were similar to each other except for DLco corrected by
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16 202 Hb. A severe obstructive pattern characterized the population studied. The finding that
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18 203 patients in the anaemia group had significant lower DLco values (corrected by Hb)
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20 204 (P<0.05) indicates that the decreased level of haemoglobin may affect the rate of
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22 205 oxygen uptake across the alveolar-capillary bed and reduce the diffusing capacity of
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24 206 the lungs, without having obvious influence on the pulmonary ventilation function.

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26 207 Although anaemia has been associated with dyspnoea and reduced exercise
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28 208 tolerance in patients with chronic kidney disease, heart failure and cancer,²²⁻²⁴ few
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30 209 studies have been published concerning the effect of anaemia on exercise capacity in
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32 210 COPD patients. Cote et al studied patients with stable COPD, observed that the
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34 211 presence of anaemia affects the general feeling of dyspnoea measured in the MRC
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36 212 scale and concluded that anaemia decreases exercise tolerance measured in 6MWT.²⁵
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38 213 However, the present study is the first to investigate the impact of anaemia on
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40 214 exercise capacity and ventilatory efficiency of severe COPD patients utilizing CPET
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42 215 in Chinese population.

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44 216 On the one hand, the present study showed that anemic patients with severe
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46 217 COPD had lower PeakLoad, Peak $\dot{V} O_2$ (%pred), Peak $\dot{V} O_2$ /Kg, $\Delta O_2/\Delta WR$ slope and
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48 218 Peak oxygen pulse (O₂ pulse) compared with the non anemic ones, demonstrating the
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50 219 negative effect of anemia on exercise capacity. Peak $\dot{V} O_2$ is frequently used as the
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52 220 most reliable measure of overall exercise capacity. By Fick's principle^{20,26-28}, $\dot{V} O_2 =$
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54 221 cardiac output*arteriovenous O₂ difference, the arteriovenous O₂ difference is
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56 222 dependent on the availability of hemoglobin, blood oxygenation in the lung, and
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58 223 extraction of oxygen in the periphery. For a given increase in cardiac output, the
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60 224 anemic patient, who has decreased level of haemoglobin and arterial O₂ concentration,

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4 225 will have a lower Peak $\dot{V} O_2$. O_2 pulse is the quotient of the $\dot{V} O_2$ and heart rate. $\Delta\dot{V}$
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6 226 $O_2/\Delta WR$ slope is an important measurement to evaluate exercise capacity in patients
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8 227 with heart disease. The decreased $\Delta VO_2/\Delta WR$ slope is a marker of decreased aerobic
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10 228 exercise capacity caused by decreased oxygen transport.²⁹ In our study, we found that
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12 229 COPD patients with anemia had a lower $\Delta VO_2/\Delta WR$ slope than those without anemia.
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14 230 This result further confirmed that anemic patients may have a more serious limitation
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16 231 in exercise capacity than non anemic patients, which corroborate with the study by
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18 232 Boutou et al.³⁰ However, we have just included subjects with GOLD III rather than
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20 233 theirs which included other groups as well. This study also differs in the fact that we
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22 234 have a larger sample size and the anemia cut off values were pertinent to the Chinese
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24 235 population rather than WHO defined general cut off values for anemia. The study by
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26 236 Rutten et al showed the 6MWD and the BODE score were not different among the
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28 237 patients with and without anemia which contradicts our finding.³¹ However, the
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30 238 patient profiles in our study is fundamentally different from that of Rutten et al as we
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32 239 have taken a lower Hb cut off value to define anemia. Nevertheless the patients in our
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34 240 study have much lower Hb levels on average indicating the degree of anemia was
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36 241 more severe.

37 242 On the other hand, there were no differences in CPET parameters as Peak $\dot{V} E$,
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39 243 Lowest $\dot{V} E/\dot{V} CO_2$, Peak VD/VT (%) between the two groups. Both Peak VD/VT
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41 244 and Lowest $\dot{V} E/\dot{V} CO_2$ indicate the ventilatory efficiency of oxygen uptake and
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43 245 carbon dioxide elimination in patients, mainly due to the limitation of blood flow
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45 246 perfusion, i.e. Q/VA mismatch. Therefore, anaemia may have a negative effect on
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47 247 exercise capacity of patients with severe COPD, while its influence on ventilatory
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49 248 efficiency is little.

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51 249 Apart from that, a strong positive correlation was found between Hb
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53 250 concentration and Peak $\dot{V} O_2$ in anemic patients ($r=0.702$, $P<0.01$), but no statistical
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55 251 significant correlation was found with non anemic patients. All these results strongly
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57 252 indicate that oxygen carrying capacity may depend upon the level of haemoglobin for
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4 253 the anemic patients and oxygen delivery is crucial for the maintenance of oxidative
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6 254 metabolism. Previous study showed that in normal individuals, 15 g/dl of Hb carry
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8 255 approximately 21 ml of oxygen per 100 ml of blood and a 3g/dl decrease in Hb levels
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10 256 would result in a reduction of the total oxygen-carrying capacity by 4/100 ml.³²
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12 257 Moreover, anaemia may result in limited oxygen supply with the consequent increase
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14 258 in ventilatory drive. Among patients with COPD whose ventilatory reserve were
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16 259 decreased, the accompanying increased ventilatory demand may result in dyspnoea.

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18 260 There is increasing evidence regarding the impact of systemic inflammation on
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20 261 COPD. In theory, it has been postulated that the pathophysiology of COPD-associated
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22 262 anaemia might be an inflammatory and immunological response, mediated by IL-1,
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24 263 IL-6, TNF- α and interferon gamma, which could make variations in iron metabolism,
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26 264 reducing iron absorption thereby altering normal haematopoiesis.^{33,34} These mediators
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28 265 increase in patients with COPD and could be regarded as factors related to the onset
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30 266 of anaemia.³⁵ One previous study has reported that anaemia in COPD is at least in part,
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32 267 on account of inflammation and resistance to elevated levels of serum
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34 268 erythropoietin.³⁶

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36 269 Our study is not free of limitations. The proportion of female patients was small.
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38 270 The significantly lower prevalence of COPD in females is the possible explanations
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40 271 for this phenomenon. Moreover, the present data were derived from patients with
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42 272 severe (GOLD stage III) COPD and thus cannot be applied to the overall population
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44 273 of COPD patients. The reason why we choose severe (GOLD stage III) COPD
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46 274 patients as our object of study is that patients with anaemia had a tendency to have
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48 275 more severe COPD^{30,37}, while very severe COPD patients may not manage to
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50 276 complete CPET. Efforts should be made to further assess the prevalence of anaemia in
51
52 277 other stages of COPD.

53 278 **Conclusions:**

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55 279 Our study demonstrated that COPD can be accompanied by anemia and implied
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57 280 the potential correlations between anaemia in patients with COPD and respiratory
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59 281 physiology utilizing PFT and CPET. The results indicated that both the function of gas
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282 exchange and exercise capacity decreased in the severe COPD patients with anaemia

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3 283 compared with the patients without anaemia, while the influence of anaemia on
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5 284 pulmonary ventilation function and ventilatory efficiency was little. Future studies are
6
7 285 needed in order to evaluate the possible therapeutic approaches in COPD patients with
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9 286 anaemia.

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13 288 **contributorship statement**

14 289 Conceived and designed the experiments: JML XGS JG CZ.

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16 290 Performed the experiments: JG CZ QX WLY.17
18 291 Analyzed the data: JML JG CZ QX GGS QHZ.19
20 292 Contributed reagents/materials/analysis tools: JML JG LW JH XS.21
22 293 Wrote the paper: JML JG CZ QX .23
24 29425
26 295 **competing interests**

27 296 All of the authors have declared that no competing interests exist.

28
29 297 **funding**

30 298 There is no specific funding for our work.

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32
33 299 **data sharing statement**34 300 All the primary data were obtained at Shanghai pulmonary hospital. They are both
35
36 301 secure and available anytime through the medical records section of the hospital, both
37
38 302 in print and digital formats. We the authors are aware about sensitivity of data security.
39
40 303 And with regards to data used from other sources due permission has been obtained
41
42 304 and cited accordingly. We are ready to answer to any queries that may arise in the
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44 305 future.45
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Figure captions

Figure 1. Figure 1A and 1B show the correlation between Peak $\dot{V} O_2$ and Hb concentration in COPD patients with and without anaemia respectively.

Significant positive correlation was found between Hb concentration and Peak $\dot{V} O_2$ in anemic patients ($r=0.702$, $P<0.01$) (Figure1A), but no statistical significant correlation was found with nonanemic patients ($r=0.055$, $P>0.05$) (Figure1B).

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Table 1. Demographic and clinical characteristics of patients included in the study

Variable	Patients with low haemoglobin	Patients with normal haemoglobin
	Concentration (%)	concentration (%)
Number of patients (n = 157) (%)	48 (31%)	109(69%)
Sex	Men (M) 45(94%)	Men (M)99 (91%)
Age (years)	61.32 ± 6.03	60.82 ± 7.13
BMI (kg/m ²)	21.72 ± 2.93	22.18 ± 2.15
Haemoglobin concentration (g/dL)	10.6 ± 0.8*	12.9 ± 1.1*
MCV(fl)	85.9 ± 9.1	87.1 ± 10.2
Smoking index (Pack-years)	43.58 ± 7.70	45.56 ± 4.63
PH	7.39 ± 0.017	7.38 ± 0.023
PaCO ₂ (mm Hg)	44.67 ± 1.32	44.31 ± 1.28
PaO ₂ (mm Hg)	68.11 ± 3.01	67.33 ± 3.62
SaO ₂ (%)	94.44 ± 0.94	94.77 ± 1.13

30 442 PaO₂= oxygen partial pressure; PaCO₂ = carbon dioxide partial pressure; MCV = Mean
31 443 corpuscular (erythrocyte) volume;
32 444 values represent numbers of patients or means+SD, as appropriate.

33 445 *p < 0.05, COPD patients with anaemia versus patients without anaemia using unpaired t test
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40 450 **Table 2. The comparison of pulmonary function indices in COPD patients with and without**
41 451 **anaemia**

Variable	Patients with low haemoglobin	Patients with normal haemoglobin
	concentration(n = 48)	concentration(n = 109)
FEV ₁ (L)	1.33 ± 0.27	1.41 ± 0.13
FEV ₁ (% predicted)	42.15 ± 7.53	43.28 ± 5.72
FEV ₁ /FVC (%)	41.32 ± 2.19	43.36 ± 1.38
RV (L)	3.98 ± 1.55	4.11 ± 1.24
TLC (L)	7.82 ± 0.93	7.11 ± 1.78
RV/TLC (%)	60.12 ± 6.78	53.27 ± 6.79
IC (L)	1.52 ± 0.47	1.61 ± 0.43
DLco (L)	15.31 ± 1.94*	17.10 ± 2.05*

52 452 FEV₁, forced expiratory volume in one second; FVC, forced vital capacity; TLC, total lung capacity; RV,
53 453 residual volume; IC, inspiratory capacity; DLco, carbon monoxide diffusion capacity corrected for Hb
54 454 concentration

455 values represent numbers of patients or means+SD, as appropriate
 456 *p < 0.05, COPD patients with anaemia versus patients without anaemia using unpaired t test

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462 **Table 3. The comparison of cardiopulmonary exercise testing parameters in COPD patients**
 463 **with and without anaemia**

Variable	Patients with low haemoglobin concentration	Patients with normal haemoglobin concentration
PeakLoad (W)	66.36 ± 16.62*	69.46 ± 28.18*
Peak $\dot{V}O_2$ (ml/min)	866.28 ± 274.32**	1180.42 ± 319.18**
Peak $\dot{V}O_2$ (%pred)	56.21 ± 14.79**	69.11 ± 18.72**
Peak $\dot{V}O_2$ /Kg (mL/min/kg)	14.88 ± 3.92*	18.72 ± 3.86*
Peak $\dot{V}E$ (L/min)	37.89 ± 6.33	36.71 ± 5.42
$\Delta O_2/\Delta WR$ slope (ml/min/watt)	8.02 ± 1.03*	9.20 ± 1.42*
Peak O_2 pulse (mL/beat)	7.88 ± 2.63*	11.3 ± 2.19*
Lowest $\dot{V}E/\dot{V}CO_2$	32.15 ± 1.28	31.28 ± 2.90
Peak VD/VT (%)	33.2 ± 7.2	34.9 ± 6.1

464 %pred = percent of predicted; $\dot{V}O_2$ = peak oxygen uptake; $\dot{V}E$ = minute ventilation; $\dot{V}CO_2$ =

465 carbon dioxide output; $\Delta O_2/\Delta WR$ slope = the ratio of $\dot{V}O_2$ increase to WR increase;

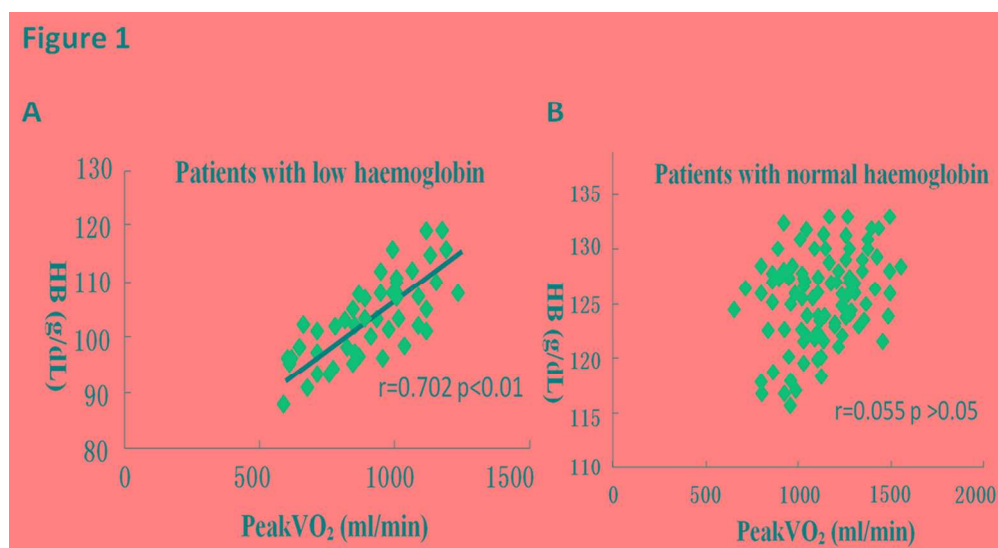
466 VD/VT = dead space/tidal volume ratio

467 values represent numbers of patients or means+SD, as appropriate.

468 *P<0.05, **P<0.01, COPD patients with anaemia versus patients without anaemia using unpaired

469 t test

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Figure 1A and 1B show the correlation between Peak V. O₂ and Hb concentration in COPD patients with and without anaemia respectively.

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Significant positive correlation was found between Hb concentration and Peak V. O₂ in anemic patients ($r=0.702$, $P<0.01$) (Figure1A), but no statistical significant correlation was found with nonanemic patients ($r=0.055$, $P >0.05$) (Figure1B).

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STROBE Statement

	Item No	Recommendation
Title and abstract	1	Impact of anaemia on lung function and exercise capacity in patients with stable severe COPD: an observational study
Introduction		
Background/rationale	2	We sought to explore the gas exchange impairment severity in severe COPD patients as such patients present with a lot of functional limitations and comorbidities. We found that decreased haemoglobin has a negative correlation with regards to gas exchange.
Objectives	3	To search for potential correlations between anaemia in patients with severe COPD (GOLD stage 3) and pulmonary function at rest, exercise capacity, as well as ventilatory efficiency, utilizing pulmonary function test (PFT) and cardiopulmonary exercise testing (CPET).
Methods		
Study design	4	Clinical Observational study
Setting	5	Study was undertaken at Shanghai pulmonary hospital, Tongji university. It caters to a large population base within shanghai and serves referrals from centres in other cities as well. Clinically stable outdoor patients of severe COPD (GOLD stage III) between December 2011- November 2013.
Participants	6	<i>Cohort study</i> — The relevant population consisted of consecutive, clinically stable patients with a diagnosis of severe COPD (GOLD stage III), who visited the Respiratory Unit of Shanghai Pulmonary Hospital as outpatients between December 2011 and November 2013. Patients were diagnosed as clinically stable if they had had no hospital admission, exacerbation, respiratory infection or change in medication three months prior to entering the study. They were followed up as outpatients.
Variables	7	During the initial visit, all the patients with a potential or known diagnosis of COPD underwent physical examination, arterial blood gas analysis (ABG) and lung function testing. All stable severe COPD patients (if $30\% \leq FEV1 \leq 50\%$ predicted) underwent peripheral venous blood sampling for a CBC. We used the levels of haemoglobin as per the Chinese criteria, anaemia [haemoglobin (Hb) <12.0 g/dl for males, and <11 g/dL for females.
Data sources/ measurement	8	We used the levels of haemoglobin as per the Chinese criteria, anaemia [haemoglobin (Hb) <12.0 g/dl for males, and <11 g/dL for females. Pulmonary function at rest, exercise capacity, as well as ventilatory efficiency was measured. We used the GOLD criteria to include just GOLD III patients. For the CPET we followed the ATS guidelines. The lab personnel and study authors were never in consultation during or after the selection of subjects.
Bias	9	To limit observer bias lab personnel were never consulted during or after the selection of consecutive subjects. Since we selected consecutive GOLD III stable COPD patients from the outpatient services selection bias was limited as other comorbidities in inpatients were excluded.
Study size	10	We followed selected patients for about two years. Those meeting the defined criteria's and not lost to follow up or died were automatically dropped those

remaining and willing to participate in further testing were included.

Quantitative variables	11	The subjects were divided as those with and those without anemia. We assessed the ABG, PFT, CBC and CPET parameters in both groups to reach our conclusion.
Statistical methods	12	Statistical analysis was performed using SPSS 16.0. Parameters were expressed as mean± SD. Unpaired Student t test was used to identify differences between groups, whereas χ^2 test was used to assess differences in categorical variables between groups. Correlations between CPET parameters and Hb levels were determined by Pearson's correlation test. P value of < 0.05 was considered significant. Since we already had a set number of subjects included into the study with complete relevant study reports we did not encounter missing data problems. Also the study population were selected over a period of over two years no single test parameter was used solely as a defining criteria to include them in the study. Almost all had those test on multiple occasions.

Results

Participants	13	157 Chinese patients with stable severe COPD, divided into two groups: anemia group [hemoglobin (Hb) <12.0 g/dl for males, and <11 g/dL for females (n=48)] and non-anemia group (n=109) were included and followed up.
Descriptive data	14	157 patients diagnosed with severe COPD (GOLD stage III) in outpatients were included. The demographic and clinical characteristics of patients are shown in the table:

Variable	Patients with low haemoglobin Concentration (%)	Patients with normal haemoglobin concentration (%)
Number of patients	48 (31%)	109(69%)
Sex	Men (M) 45(94%)	Men (M)99 (91%)
Age (years)	61.32±6.03	60.82±7.13
BMI (kg/m ²)	21.72±2.93	22.18±2.15
Haemoglobin concentration (g/dL)	10.6±0.8*	12.9±1.1*
MCV(fl)	85.9±9.1	87.1±10.2
Smoking index (Pack-years)	43.58±7.70	45.56±4.63

MCV = Mean corpuscular (erythrocyte) volume;

values represent numbers of patients or means±SD, as appropriate.

*p < 0.05, COPD patients with anaemia versus patients without anaemia using unpaired t test

Outcome data	15*	Forty-eight (31%) patients had anemia that had a negative impact on gas exchange and exercise tolerance during exercise in severe COPD patients.
Main results	16	(1) Diffusing capacity for carbon monoxide (DLCO) corrected by Hb was significantly lower in anemia group [(15.3±1.9) ml/min/mmHg] than in non-anemia group [(17.1±2.1) ml/min/mmHg] (P<0.05). Significant difference did not exist in the level of forced expiratory volume in 1 second (FEV1), FEV1%pred, FEV1/ forced vital capacity (FVC), inspiratory capacity (IC), residual volume (RV), total lung capacity (TLC) and RV/TLC (P>0.05). (2) Peak Load, Peak oxygen uptake (VO ₂), Peak VO ₂ %pred, Peak VO ₂ /Kg, Peak O ₂ pulse

and the ratio of $\dot{V}O_2$ increase to WR increase ($\Delta\dot{V}O_2/\Delta WR$) were significantly lower in anemia group ($P < 0.05$), however, Peak minute ventilation (VE), Lowest VE/ carbon dioxide output ($\dot{V}CO_2$), Peak dead space/tidal volume ratio (VD/VT) were similar between the two groups ($P > 0.05$).

(3) A strong positive correlation was found between Hb concentration and Peak $\dot{V}O_2$ in anemic patients. ($r = 0.702$, $P < 0.01$).

Other analyses	17	N/A
Discussion		
Key results	18	Anemia had a negative impact on gas exchange and impaired exercise tolerance in severe COPD patients.
Limitations	19	The proportion of female patients was small. Moreover, the present data were derived from patients with severe (GOLD stage III) COPD and thus cannot be applied to the overall population of COPD patients.
Interpretation	20	<p>The results strongly indicate that oxygen carrying capacity may depend upon the level of haemoglobin for the anemic patients and oxygen delivery is crucial for the maintenance of oxidative metabolism. Previous study showed that in normal individuals, 15 g/dl of Hb carry approximately 21 ml of oxygen per 100 ml of blood and a 3g/dl decrease in Hb levels would result in a reduction of the total oxygen-carrying capacity by 4/100 ml. Moreover, anaemia may result in limited oxygen supply with the consequent increase in ventilatory drive. Among patients with COPD whose ventilatory reserve were decreased, the accompanying increased ventilatory demand may result in dyspnoea.</p> <p>The significantly lower prevalence of COPD in females is the possible explanations for the small proportion of female patients. The reason why we choose severe (GOLD stage III) COPD patients as our object of study is that patients with anaemia had a tendency to have more severe COPD, while very severe COPD patients may not manage to complete CPET. Our results corroborate with the study by Boutou et al, while contradicts the finding by Rutten et al. The reason is that the patient profiles in our study is fundamentally different form that of Rutten et al as we have taken a lower Hb cut off value to define anemia.</p>
Generalisability	21	The results indicated that both the function of gas exchange and exercise capacity decreased in the severe COPD patients with anaemia compared with the patients without anaemia, while the influence of anaemia on pulmonary ventilation function and ventilatory efficiency was little. Also applicability may be limited as we used a population specific criterion to define anemia. Nevertheless the gas exchange results from this study in such patient groups (stable severe anemic COPD, GOLD III) can serve as a reference for future investigators.
Other information		
Funding	22	No external funding was used for this study. Also all authors declare no financial or other conflicting interests.

BMJ Open

Impact of anaemia on lung function and exercise capacity in patients with stable severe chronic obstructive pulmonary disease: an observational study

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Primary Subject Heading:	Respiratory medicine
Secondary Subject Heading:	Sports and exercise medicine
Keywords:	anemia, chronic obstructive pulmonary disease, cardiopulmonary exercise test, hemoglobin

SCHOLARONE™
Manuscripts

1 **Impact of anaemia on lung function and exercise capacity in patients**
2 **with stable severe chronic obstructive pulmonary disease**

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19 * This three authors contributed equally to this work

20 **【Abstract】**

21 **Objective:** This study intended to search for potential correlations between anaemia
22 in patients with severe COPD (GOLD stage 3) and pulmonary function at rest,
23 exercise capacity, as well as ventilatory efficiency, utilizing pulmonary function test
24 (PFT) and cardiopulmonary exercise testing (CPET).

25 **Setting:** Study was undertaken at Shanghai pulmonary hospital, a tertiary level centre
26 affiliated to Tongji University. It caters to a large population base within shanghai and
27 referrals from centers in other cities as well.

28 **Participants:** 157 Chinese patients with stable severe COPD were divided into two
29 groups: anemia group [hemoglobin (Hb) <12.0 g/dl for males, and <11 g/dL for
30 females (n=48)] and non-anemia group (n=109).

31 **Primary and secondary outcome measures:** Arterial blood gas (ABG), PFT and
32 CPET were tested in all patients.

33 **Results:** (1) Diffusing capacity for carbon monoxide (DL_{CO}) corrected by Hb was
34 significantly lower in anemia group [(15.3±1.9) ml/min/mmHg] than in non-anemia
35 group [(17.1±2.1) ml/min/mmHg] (P<0.05). Significant difference did not exist in the
36 level of forced expiratory volume in 1 second (FEV₁), FEV₁%pred, FEV₁/ forced vital
37 capacity (FVC), inspiratory capacity (IC), residual volume (RV), total lung capacity
38 (TLC) and RV/TLC (P>0.05). (2) Peak Load, Peak oxygen uptake ($\dot{V} O_2$), Peak \dot{V}
39 O₂%pred, Peak $\dot{V} O_2$ /Kg, Peak O₂ pulse and the ratio of $\dot{V} O_2$ increase to WR increase
40 ($\Delta\dot{V} O_2/\Delta WR$) were significantly lower in anemia group(P<0.05), however, Peak
41 minute ventilation (VE), Lowest $\dot{V} E$ / carbon dioxide output ($\dot{V} CO_2$), Peak dead
42 space/tidal volume ratio (VD/VT) were similar between the two groups (P>0.05). (3)
43 A strong positive correlation was found between Hb concentration and Peak $\dot{V} O_2$ in
44 anemic patients. (r=0.702, P<0.01).

45 **Conclusion:** Anemia has a negative impact on gas exchange and exercise tolerance
46 during exercise in severe COPD patients. The decrease amplitude of Hb levels is
47 related to the quantity of oxygen uptake.

48 **【Key words】** anemia; chronic obstructive pulmonary disease; cardiopulmonary
49 exercise test; hemoglobin

50
51 **Article summary**

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4 52 Heading: Impact of anaemia on lung function and exercise capacity in patients with
5
6 53 stable severe chronic obstructive pulmonary disease
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8
9 54 This study searched for potential correlations between anaemia in patients with
10
11 55 severe COPD (GOLD stage 3) and pulmonary function at rest, exercise capacity, as
12
13 56 well as ventilatory efficiency. Our results show that anemia has a negative impact on
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15 57 gas exchange and exercise capacity in severe COPD patients. The decrease amplitude
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17 58 of Hb levels is related to the quantity of oxygen uptake. Although cardiopulmonary
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19 59 exercise testing (CPET) parameters have been widely used to grade the severity of
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21 60 exercise limitation and provide a quantitative assessment on patients' ventilator
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23 61 response and cardiovascular response during exercise, all of which might be impaired
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25 62 due to anemia. It has seldom been utilized for functional assessment of COPD patients
26
27 63 with anemia. The limitations of the study is that the present data were derived from
28
29 64 patients with severe (GOLD stage III) COPD and thus cannot be applied to the overall
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31 65 population of COPD patients.

66 **Introduction**

32
33 67 Chronic obstructive pulmonary disease (COPD) affects various functional and
34
35 68 structural domains of the lungs and is recognized as a systemic disease, frequently
36
37 69 present in addition to other diseases.¹ Polycythemia, traditionally thought to be highly
38
39 70 prevalent in COPD, is less frequent nowadays with more rigorous correction of
40
41 71 hypoxaemia.² Reversely, recent research show that anaemia is highly prevalent in
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43 72 patients with COPD.^{3,4}

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45 73 Anemia, which is a well recognized comorbidity in many chronic illnesses
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47 74 occurs in 10-30% of COPD patients.³⁻¹² Anemia has been related with increased
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49 75 mortality and morbidity including increased health care costs and hospitalization.³⁻¹²
50
51 76 Although cardiopulmonary exercise testing (CPET) parameters have been widely
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53 77 used to grade the severity of exercise limitation and provide a quantitative assessment
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55 78 on patients' ventilator response and cardiovascular response during exercise,¹³ all of
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57 79 which might be impaired due to anemia. It has seldom been utilized for functional
58
59 80 assessment of COPD patients with anemia. The present study is the first to
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4 81 investigate the impact of anaemia in COPD patients utilizing CPET in a Chinese
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6 82 population. The aims of our study were to (1) investigate the prevalence of anemia in
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8 83 stable severe COPD patients and (2) evaluate the impact of anemia on
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10 84 pulmonary function at rest, ventilatory efficiency and exercise capacity in COPD
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12 85 patients, utilizing PFT and CPET.
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86

87 **MATERIALS AND METHODS**

88 **Study subjects**

89 The relevant population consisted of consecutive, clinically stable patients with a
90 diagnosis of severe COPD (GOLD stage III)¹⁴, who visited the Respiratory Unit of
91 Shanghai Pulmonary Hospital as outpatients between December 2011 and November
92 2013. Patients were diagnosed as clinically stable if they had had no hospital
93 admission, exacerbation, respiratory infection or change in medication three months
94 prior to entering the study. Exclusion criteria included: (1) a history of bronchiectasis,
95 asthma or other concomitant respiratory diseases; (2) a diagnosis of malignancy; 3)
96 inability to complete CPET according to the American Thoracic Society/American
97 College of Chest Physicians guidelines,¹⁵ such as unstable angina, acute myocardial
98 infarction, uncontrolled arrhythmias causing symptoms and orthopedic impairment;
99 and 4) any disease that could affect haemoglobin levels (thyroid disease, liver disease,
100 chronic renal failure, chronic heart failure, previous history of gastrointestinal
101 bleeding and chronic inflammatory rheumatic disease). Anaemia was defined as the
102 presence of haemoglobin levels <12 g/dL in males and <11 g/dL in females according
103 to China-specific criteria¹⁶. The study was approved by the hospital Ethics Committee.
104 Informed consent was obtained from all patients before undergoing any study
105 procedure.

106 **Study Procedures**

107 During the initial visit, all the patients with a potential or known diagnosis of
108 COPD underwent physical examination, arterial blood gas analysis (ABG) and lung
109 function testing. All stable severe COPD patients (if $30\% \leq FEV1 \leq 50\%$ predicted)
110 underwent peripheral venous blood sampling to determine full blood count, liver

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4 111 transaminase, total protein (albumin and globulin), thyroid hormone, serum levels of
5
6 112 urea and creatinine. Patients who were subsequently presented with abnormal
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8 113 thyroid hormone values (either high or low), glomerular filtration rate (GFR) below
9
10 114 60mL/min¹⁷ and increased liver enzymes twice the upper limit of normal were
11
12 115 excluded. The included patients in the study visited the outpatient clinic within two
13
14 116 weeks from the initial visit and performed CPET.

117 **Arterial blood gas analysis and resting pulmonary function measurements**

118 ABG measurements such as PH, partial pressure of oxygen (PaO₂), partial
119 pressure of carbon dioxide (PaCO₂) and arterial oxygen saturation (SaO₂) were taken
120 just after 10 minutes of rest. Each subject underwent resting PFT of forced vital
121 capacity (FVC), forced expiratory volume in 1second (FEV₁), inspiratory capacity
122 (IC), diffusing capacity for carbon monoxide (DL_{co}) corrected by Hb, residual
123 volume (RV) and total lung capacity (TLC) using standard methodology¹² and
124 equipment (Jaeger Corp., Hoechberg, Germany). All resting lung function values were
125 reported in absolute terms and normalized to percent of predicted (% pred). Predicted
126 spirometry values were calculated using accepted equations for Chinese adults.¹⁸

127 **Cardiopulmonary exercise test measurements**

128 All the patients performed on a cycle ergometer using a breath-by-breath system
129 according to the American Thoracic Society/American College of Chest Physicians
130 Statement on CPET.¹⁵ The equipment was calibrated in accordance with
131 manufacturer's specifications using reference and calibration gases before each test.
132 Standard 12 lead electrocardiograms (ECGs) and pulse oximetry were continuously
133 monitored. Arterial blood pressure (BP) was measured every two minutes with an
134 automatic cuff. The protocol was comprised of three minutes of rest, three minutes of
135 unloaded cycling at 55-65 revolutions per minute (rpm), followed by a progressively
136 increasing work rate of 5 to 15 watts (W)/min for the COPD patients, and four
137 minutes of recovery.¹⁸

138 Most CPET values were reported in absolute terms and normalized to percentage
139 of predicted (% pred). Predicted values were calculated using accepted equations.¹⁹

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4 140 CPET variables including oxygen uptake ($\dot{V}O_2$), minute ventilation ($\dot{V}E$), carbon
5
6 141 dioxide output ($\dot{V}CO_2$), workload (WR) and dead space/tidal volume ratio (VD/VT)
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8
9 142 were calculated at peak exercise. Peak $\dot{V}O_2$ was defined as the highest 30-second
10
11 143 average of oxygen uptake in the last minute of exercise and other peak parameters
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13 144 were calculated at the same time. Lowest $\dot{V}E/\dot{V}CO_2$ was determined by averaging the
14
15 145 lowest consecutive 90 sec data points.²⁰
16

17 146 **Statistical analysis**

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19 147 Statistical analysis was performed using SPSS (version 16, SPSS, Chicago).
20
21 148 Parameters were expressed as mean \pm SD. Most PFT and CPET values are expressed
22
23 149 in absolute terms and % pred. Unpaired Student t test was used to identify differences
24
25 150 between groups, whereas χ^2 test was used to assess differences in categorical
26
27 151 variables between groups. Correlations between CPET parameters and Hb levels were
28
29 152 determined by Pearson's correlation test. P value of < 0.05 was considered significant.
30

31 153 **Results**

32 154 **Baseline clinical and demographic characteristics**

33
34 155 One hundred and fifty-seven patients diagnosed with severe COPD (GOLD stage
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36 156 III) in outpatients were included. The vast majority (93%) of patients in the dataset
37
38 157 were males. The mean age was 61 years. The characteristics of the anemic and non
39
40 158 anemic COPD patients are presented in Table 1. Forty-eight (31%) patients from this
41
42 159 population fulfilled laboratory criteria for anemia, therefore, the prevalence of anemia
43
44 160 among stable COPD (GOLD stage III) outpatients in our study was 31%. The Hb
45
46 161 level for anemic patients was 10.6 ± 0.8 g/dl. No difference was noted in BMI, ages,
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48 162 pack-years of smoking and arterial blood gas analysis parameters (PH, PaO₂, PaCO₂
49
50 163 or SaO₂) between anemic and non anemic patients.

51 164 **Resting pulmonary function measurements**

52
53 165 The PFT parameters of the anemic and non anemic patients are presented in
54
55 166 Tables 2. There was no statistical significance in FEV₁, FEV₁%pred, FEV₁/FVC, IC,
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57 167 RV, TLC, RV/TLC between the two groups, indicating resting pulmonary ventilation
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4 168 function was similar to each other. However, DL_{CO} corrected by Hb was significantly
5 169 lower among anemic patients compared to nonanemic patients.
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7 **Cardiopulmonary exercise testing**

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9 171 All individuals completed their CPET studies without incident. Nearly all
10 172 patients stopped exercise because of leg fatigue and/or acute shortness of breath. All
11 173 subjects achieved a respiratory exchange ratio (RER) >1.10, indicating sufficient
12 174 metabolic stress. Because the anaerobic threshold (AT) may not be determined by
13 175 V-slope method²¹ or the interpreter may feel that the AT is unreliable after reviewing
14 176 an exercise test in a substantial number of severe COPD patients, CPET variables
15 177 including $\dot{V}O_2$, $\dot{V}E$, $\dot{V}CO_2$, workload, and VD/VT were calculated at peak exercise
16 178 without AT. Exercise responses of all the COPD patients at peak are presented in
17 179 Table 3. Overall, there was a negative impact of anemia on exercise capacity.
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27 180 PeakLoad, Peak $\dot{V}O_2$, Peak $\dot{V}O_2$ /Kg, Peak $\dot{V}O_2$ pulse, and the ratio of $\dot{V}O_2$ increase
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29 181 to WR increase ($\Delta\dot{V}O_2 / \Delta WR$ slope) were significantly lower among anemic patients
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31 182 compared with non anemic patients. None of the exercise parameters indicative of
32
33
34 183 respiratory limitation and ventilatory efficiency (Peak $\dot{V}E$, Lowest $\dot{V}E / \dot{V}CO_2$, Peak
35
36 184 VD/VT) differed between the two groups.
37

38 **Correlations**

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40 186 Peak $\dot{V}O_2$ is frequently used as the most reliable measure of overall exercise
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42 187 capacity, so we choose Peak $\dot{V}O_2$ as the typical CPET parameters to investigate for
43
44 188 potential associations between Hb levels and exercise capacity. The correlations
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46
47 189 between Hb concentration and Peak $\dot{V}O_2$ for the anemic and non anemic COPD
48
49 190 patients are shown in Figure 1. A strong positive correlation was found between Hb
50
51 191 concentration and Peak $\dot{V}O_2$ in anemic patients ($r=0.702$, $P<0.01$) (Figure1A) , but
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53 192 no statistical significant correlation was found with non anemic patients ($r=0.055$,
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55 193 $P>0.05$) (figure1B).
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195 **Discussions**

196 In the current study, anaemia was present in 31% of severe COPD patients. The
197 prevalence of anaemia was higher than in patients from previous studies³⁻¹², possibly
198 as the study population comprised of patients with more severe COPD. The result
199 confirms that anaemia is a common phenomenon in patients with severe COPD. The
200 main aim of the present study was to search for potential correlations between
201 anaemic severe COPD patients and resting pulmonary function, exercise capacity, as
202 well as ventilatory efficiency based on PFT and CPET.

203 The usual PFT parameters like FEV₁, FEV₁% pred, FEV₁/FVC, IC, RV, TLC and
204 RV/TLC of the two groups were similar to each other except for DLco corrected by
205 Hb. A severe obstructive pattern characterized the population studied. The finding that
206 patients in the anaemia group had significant lower DLco values (corrected by Hb)
207 (P<0.05) indicates that the decreased level of haemoglobin may affect the rate of
208 oxygen uptake across the alveolar-capillary bed and reduce the diffusing capacity of
209 the lungs, without having obvious influence on the pulmonary ventilation function.
210 Studies examining the effect of just anemia or anemia combined with disorder of the
211 alveolocapillary membrane consistently mention that anemia does affect the
212 diffusion capacity but the defect of the respiratory membrane on diffusing capacity
213 is greater.²²⁻²³ In the present study we have patients with anemia and additionally
214 defect with the respiratory membrane as is common in COPD, both contributing to the
215 reduced DLCO as noted. The initial poor correlation with blood gases in this study
216 we think is because the compensatory mechanisms are not yet fully dysfunctional and
217 the anemia not severe. Also all patients in the study were clinically stable and
218 under adequate medication.

219 Although anaemia has been associated with dyspnoea and reduced exercise
220 tolerance in patients with chronic kidney disease, heart failure and cancer,²⁴⁻²⁶ few
221 studies have been published concerning the effect of anaemia on exercise capacity in
222 COPD patients. Cote et al studied patients with stable COPD, observed that the
223 presence of anaemia affects the general feeling of dyspnoea measured in the MRC
224 scale and concluded that anaemia decreases exercise tolerance measured in 6MWT.²⁷

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3 225 However, the present study is the first to investigate the impact of anaemia on
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5 226 exercise capacity and ventilatory efficiency of severe COPD patients utilizing CPET
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7 227 in Chinese population.
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9 228 On the one hand, the present study showed that anemic patients with severe
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11 229 COPD had lower PeakLoad, Peak $\dot{V} O_2$ (%pred), Peak $\dot{V} O_2$ /Kg, $\Delta O_2/\Delta WR$ slope and
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13 230 Peak oxygen pulse (O_2 pulse) compared with the non anemic ones, demonstrating the
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15 231 negative effect of anemia on exercise capacity. Peak $\dot{V} O_2$ is frequently used as the
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17 232 most reliable measure of overall exercise capacity. By Fick's principle^{20,28-30}, $\dot{V} O_2 =$
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19 233 cardiac output*arteriovenous O_2 difference, the arteriovenous O_2 difference is
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21 234 dependent on the availability of hemoglobin, blood oxygenation in the lung, and
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23 235 extraction of oxygen in the periphery. For a given increase in cardiac output, the
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25 236 anemic patient, who has decreased level of haemoglobin and arterial O_2 concentration,
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27 237 will have a lower Peak $\dot{V} O_2$. O_2 pulse is the quotient of the $\dot{V} O_2$ and heart rate. $\Delta \dot{V}$
28
29 238 $O_2/\Delta WR$ slope is an important measurement to evaluate exercise capacity in patients
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31 239 with heart disease. The decreased $\Delta VO_2/\Delta WR$ slope is a marker of decreased aerobic
32
33 240 exercise capacity caused by decreased oxygen transport.³¹ In our study, we found that
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35 241 COPD patients with anemia had a lower $\Delta VO_2/\Delta WR$ slope than those without anemia.
36
37 242 This result further confirmed that anemic patients may have a more serious limitation
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39 243 in exercise capacity than non anemic patients, which corroborate with the study by
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41 244 Boutou et al.³² However, we have just included subjects with GOLD III rather than
42
43 245 theirs which included other groups as well. This study also differs in the fact that we
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45 246 have a larger sample size and the anemia cut off values were pertinent to the Chinese
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47 247 population rather than WHO defined general cut off values for anemia. The study by
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49 248 Rutten et al showed the 6MWD and the BODE score were not different among the
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51 249 patients with and without anemia which contradicts our finding.³³ However, the
52
53 250 patient profiles in our study is fundamentally different form that of Rutten et al as we
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55 251 have taken a lower Hb cut off value to define anemia. Nevertheless the patients in our
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57 252 study have much lower Hb levels on average indicating the degree of anemia was
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4 253 more severe. On the other hand, there were no differences in CPET parameters as
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6 254 Peak $\dot{V} E$, Lowest $\dot{V} E / \dot{V} CO_2$, Peak VD/VT (%) between the two groups. Both Peak
7
8 255 VD/VT and Lowest $\dot{V} E / \dot{V} CO_2$ indicate the ventilatory efficiency of oxygen uptake
9
10 256 and carbon dioxide elimination in patients, mainly due to the limitation of blood flow
11
12 257 perfusion, i.e. Q/VA mismatch. Therefore, anaemia may have a negative effect on
13
14 258 exercise capacity of patients with severe COPD, while its influence on ventilatory
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16 259 efficiency is little.

17
18 260 Apart from that, a strong positive correlation was found between Hb
19
20 261 concentration and Peak $\dot{V} O_2$ in anemic patients ($r=0.702$, $P<0.01$), but no statistical
21
22 262 significant correlation was found with non anemic patients. All these results strongly
23
24 263 indicate that oxygen carrying capacity may depend upon the level of haemoglobin for
25
26 264 the anemic patients and oxygen delivery is crucial for the maintenance of oxidative
27
28 265 metabolism. Previous study showed that in normal individuals, 15 g/dl of Hb carry
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30 266 approximately 21 ml of oxygen per 100 ml of blood and a 3g/dl decrease in Hb levels
31
32 267 would result in a reduction of the total oxygen-carrying capacity by 4/100 ml.³⁴
33
34 268 Moreover, anaemia may result in limited oxygen supply with the consequent increase
35
36 269 in ventilatory drive. Among patients with COPD whose ventilatory reserve were
37
38 270 decreased, the accompanying increased ventilatory demand may result in dyspnoea.

39 271 Our study is not free of limitations. The proportion of female patients was small.
40
41 272 The significantly lower prevalence of COPD in females is the possible explanations
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43 273 for this phenomenon. Moreover, the present data were derived from patients with
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45 274 severe (GOLD stage III) COPD and thus cannot be applied to the overall population
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47 275 of COPD patients. The reason why we choose severe (GOLD stage III) COPD
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49 276 patients as our object of study is that patients with anaemia had a tendency to have
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51 277 more severe COPD^{32,35}, while very severe COPD patients may not manage to
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53 278 complete CPET. Efforts should be made to further assess the prevalence of anaemia in
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55 279 other stages of COPD.

280 **Conclusions:**

281 281 Our study demonstrated that COPD can be accompanied by anemia and implied

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3 282 the potential correlations between anaemia in patients with COPD and respiratory
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5 283 physiology utilizing PFT and CPET. The results indicated that both the function of gas
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7 284 exchange and exercise capacity decreased in the severe COPD patients with anaemia
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9 285 compared with the patients without anaemia, while the influence of anaemia on
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11 286 pulmonary ventilation function and ventilatory efficiency was little. Future studies are
12
13 287 needed in order to evaluate the possible therapeutic approaches in COPD patients with
14
15 288 anaemia.

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17 289

18 290 **contributorship statement**

19 291 Conceived and designed the experiments: JML XGS JG CZ.

20 292 Performed the experiments: JG CZ QX WLY.

21 293 Analyzed the data: JML JG CZ QX GGS QHZ.

22 294 Contributed reagents/materials/analysis tools: JML JG LW JH XS.

23 295 Wrote the paper: JML JG CZ QX .

24
25 296

26 297 **competing interests**

27 298 All of the authors have declared that no competing interests exist.

28 299 **funding**

29 300 There is no specific funding for our work.

30 301 **data sharing statement**

31 302 All the primary data were obtained at Shanghai pulmonary hospital. They are both
32
33 303 secure and available anytime through the medical records section of the hospital, both
34
35 304 in print and digital formats. We the authors are aware about sensitivity of data security.
36
37 305 And with regards to data used from other sources due permission has been obtained
38
39 306 and cited accordingly. We are ready to answer to any queries that may arise in the
40
41 307 future.

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Figure captions

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Figure 1. Figure 1A and 1B show the correlation between Peak $\dot{V} O_2$ and Hb concentration in COPD patients with and without anaemia respectively. Significant positive correlation was found between Hb concentration and Peak $\dot{V} O_2$ in anemic patients ($r=0.702$, $P<0.01$) (Figure1A), but no statistical significant correlation was found with nonanemic patients ($r=0.055$, $P>0.05$) (Figure1B).

441 **Table 1. Demographic and clinical characteristics of patients included in the study**

Variable	Patients with low haemoglobin Concentration (%)	Patients with normal haemoglobin concentration (%)
Number of patients (n = 157) (%)	48 (31%)	109(69%)
Sex	Men (M) 45(94%)	Men (M)99 (91%)
Age (years)	61.32 ± 6.03	60.82 ± 7.13
BMI (kg/m ²)	21.72 ± 2.93	22.18 ± 2.15
Haemoglobin concentration (g/dL)	10.6 ± 0.8*	12.9 ± 1.1*
MCV(fl)	85.9 ± 9.1	87.1 ± 10.2
Smoking index (Pack-years)	43.58 ± 7.70	45.56 ± 4.63
PH	7.39 ± 0.017	7.38 ± 0.023
PaCO ₂ (mm Hg)	44.67 ± 1.32	44.31 ± 1.28
PaO ₂ (mm Hg)	68.11 ± 3.01	67.33. ± 3.62
SaO ₂ (%)	94.44 ± 0.94	94.77 ± 1.13

442 PaO₂= oxygen partial pressure; PaCO₂ = carbon dioxide partial pressure; MCV = Mean

443 corpuscular (erythrocyte) volume;

444 values represent numbers of patients or means+SD, as appropriate.

445 *p < 0.05, COPD patients with anaemia versus patients without anaemia using unpaired t test

450 **Table 2. The comparison of pulmonary function indices in COPD patients with and without**
451 **anaemia**

Variable	Patients with low haemoglobin concentration(n = 48)	Patients with normal haemoglobin concentration(n = 109)
FEV ₁ (L)	1.33 ± 0.27	1.41 ± 0.13
FEV ₁ (% predicted)	42.15 ± 7.53	43.28 ± 5.72
FEV ₁ /FVC (%)	41.32 ± 2.19	43.36 ± 1.38
RV (L)	3.98 ± 1.55	4.11 ± 1.24
TLC (L)	7.82 ± 0.93	7.11 ± 1.78
RV/TLC (%)	50.12 ± 6.78	53.27 ± 6.79
IC (L)	1.52 ± 0.47	1.61 ± 0.43
DLco (L)	15.31 ± 1.94*	17.10 ± 2.05*

452 FEV₁, forced expiratory volume in one second; FVC, forced vital capacity; TLC, total lung capacity; RV,

453 residual volume; IC, inspiratory capacity; DLco, carbon monoxide diffusion capacity corrected for Hb

454 concentration

455 values represent numbers of patients or means+SD, as appropriate

456 *p < 0.05, COPD patients with anaemia versus patients without anaemia using unpaired t test

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462 **Table 3. The comparison of cardiopulmonary exercise testing parameters in COPD patients**
 463 **with and without anaemia**

Variable	Patients with low haemoglobin concentration	Patients with normal haemoglobin concentration
Peak Load (W)	66.36 ± 16.62*	69.46 ± 28.18*
Peak $\dot{V}O_2$ (ml/min)	866.28 ± 274.32**	1180.42 ± 319.18**
Peak $\dot{V}O_2$ (%pred)	56.21 ± 14.79**	69.11 ± 18.72**
Peak $\dot{V}O_2$ /Kg (mL/min/kg)	14.88 ± 3.92*	18.72 ± 3.86*
Peak $\dot{V}E$ (L/min)	37.89 ± 6.33	36.71 ± 5.42
$\Delta O_2/\Delta WR$ slope (ml/min/watt)	8.02 ± 1.03*	9.20 ± 1.42*
Peak O_2 pulse (mL/beat)	7.88 ± 2.63*	11.3 ± 2.19*
Lowest $\dot{V}E/\dot{V}CO_2$	32.15 ± 1.28	31.28 ± 2.90
Peak VD/VT (%)	33.2 ± 7.2	34.9 ± 6.1

464 %pred = percent of predicted; $\dot{V}O_2$ = peak oxygen uptake; $\dot{V}E$ = minute ventilation; $\dot{V}CO_2$ =

465 carbon dioxide output; $\Delta O_2/\Delta WR$ slope = the ratio of $\dot{V}O_2$ increase to WR increase;

466 VD/VT = dead space/tidal volume ratio

467 values represent numbers of patients or means ± SD, as appropriate.

468 *P < 0.05, **P < 0.01, COPD patients with anaemia versus patients without anaemia using unpaired

469 t test

470

Figure 1

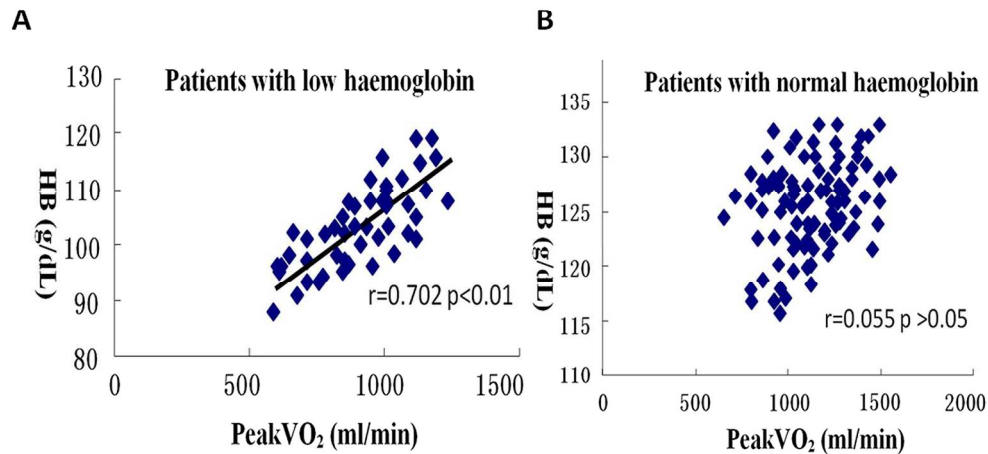


Figure 1A and 1B show the correlation between Peak V. O₂ and Hb concentration in COPD patients with and without anaemia respectively.

Significant positive correlation was found between Hb concentration and Peak V. O₂ in anemic patients ($r=0.702$, $P<0.01$) (Figure1A), but no statistical significant correlation was found with nonanemic patients ($r=0.055$, $P >0.05$) (Figure1B).

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STROBE Statement

	Item No	Recommendation
Title and abstract	1	Impact of anaemia on lung function and exercise capacity in patients with stable severe COPD: an observational study
Introduction		
Background/rationale	2	We sought to explore the gas exchange impairment severity in severe COPD patients as such patients present with a lot of functional limitations and comorbidities. We found that decreased haemoglobin has a negative correlation with regards to gas exchange.
Objectives	3	To search for potential correlations between anaemia in patients with severe COPD (GOLD stage 3) and pulmonary function at rest, exercise capacity, as well as ventilatory efficiency, utilizing pulmonary function test (PFT) and cardiopulmonary exercise testing (CPET).
Methods		
Study design	4	Clinical Observational study
Setting	5	Study was undertaken at Shanghai pulmonary hospital, Tongji university. It caters to a large population base within shanghai and serves referrals from centres in other cities as well. Clinically stable outdoor patients of severe COPD (GOLD stage III) between December 2011- November 2013.
Participants	6	<i>Cohort study</i> — The relevant population consisted of consecutive, clinically stable patients with a diagnosis of severe COPD (GOLD stage III), who visited the Respiratory Unit of Shanghai Pulmonary Hospital as outpatients between December 2011 and November 2013. Patients were diagnosed as clinically stable if they had had no hospital admission, exacerbation, respiratory infection or change in medication three months prior to entering the study. They were followed up as outpatients.
Variables	7	During the initial visit, all the patients with a potential or known diagnosis of COPD underwent physical examination, arterial blood gas analysis (ABG) and lung function testing. All stable severe COPD patients (if $30\% \leq FEV1 \leq 50\%$ predicted) underwent peripheral venous blood sampling for a CBC. We used the levels of haemoglobin as per the Chinese criteria, anaemia [haemoglobin (Hb) <12.0 g/dl for males, and <11 g/dL for females.
Data sources/ measurement	8	We used the levels of haemoglobin as per the Chinese criteria, anaemia [haemoglobin (Hb) <12.0 g/dl for males, and <11 g/dL for females. Pulmonary function at rest, exercise capacity, as well as ventilatory efficiency was measured. We used the GOLD criteria to include just GOLD III patients. For the CPET we followed the ATS guidelines. The lab personnel and study authors were never in consultation during or after the selection of subjects.
Bias	9	To limit observer bias lab personnel were never consulted during or after the selection of consecutive subjects. Since we selected consecutive GOLD III stable COPD patients from the outpatient services selection bias was limited as other comorbidities in inpatients were excluded.
Study size	10	We followed selected patients for about two years. Those meeting the defined criteria's and not lost to follow up or died were automatically dropped those

remaining and willing to participate in further testing were included.

Quantitative variables	11	The subjects were divided as those with and those without anemia. We assessed the ABG, PFT, CBC and CPET parameters in both groups to reach our conclusion.
Statistical methods	12	Statistical analysis was performed using SPSS 16.0. Parameters were expressed as mean± SD. Unpaired Student t test was used to identify differences between groups, whereas χ^2 test was used to assess differences in categorical variables between groups. Correlations between CPET parameters and Hb levels were determined by Pearson's correlation test. P value of < 0.05 was considered significant. Since we already had a set number of subjects included into the study with complete relevant study reports we did not encounter missing data problems. Also the study population were selected over a period of over two years no single test parameter was used solely as a defining criteria to include them in the study. Almost all had those test on multiple occasions.

Results

Participants	13	157 Chinese patients with stable severe COPD, divided into two groups: anemia group [hemoglobin (Hb) <12.0 g/dl for males, and <11 g/dL for females (n=48)] and non-anemia group (n=109) were included and followed up.
Descriptive data	14	157 patients diagnosed with severe COPD (GOLD stage III) in outpatients were included. The demographic and clinical characteristics of patients are shown in the table:

Variable	Patients with low haemoglobin Concentration (%)	Patients with normal haemoglobin concentration (%)
Number of patients	48 (31%)	109(69%)
Sex	Men (M) 45(94%)	Men (M)99 (91%)
Age (years)	61.32±6.03	60.82±7.13
BMI (kg/m ²)	21.72±2.93	22.18±2.15
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Smoking index (Pack-years)	43.58±7.70	45.56±4.63

MCV = Mean corpuscular (erythrocyte) volume;

values represent numbers of patients or means±SD, as appropriate.

*p < 0.05, COPD patients with anaemia versus patients without anaemia using unpaired t test

Outcome data	15*	Forty-eight (31%) patients had anemia that had a negative impact on gas exchange and exercise tolerance during exercise in severe COPD patients.
Main results	16	(1) Diffusing capacity for carbon monoxide (DLCO) corrected by Hb was significantly lower in anemia group [(15.3±1.9) ml/min/mmHg] than in non-anemia group [(17.1±2.1) ml/min/mmHg] (P<0.05). Significant difference did not exist in the level of forced expiratory volume in 1 second (FEV1), FEV1%pred, FEV1/ forced vital capacity (FVC), inspiratory capacity (IC), residual volume (RV), total lung capacity (TLC) and RV/TLC (P>0.05). (2) Peak Load, Peak oxygen uptake (VO ₂), Peak VO ₂ %pred, Peak VO ₂ /Kg, Peak O ₂ pulse

and the ratio of $\dot{V}O_2$ increase to WR increase ($\Delta\dot{V}O_2/\Delta WR$) were significantly lower in anemia group ($P < 0.05$), however, Peak minute ventilation (VE), Lowest VE/ carbon dioxide output ($\dot{V}CO_2$), Peak dead space/tidal volume ratio (VD/VT) were similar between the two groups ($P > 0.05$).

(3) A strong positive correlation was found between Hb concentration and Peak $\dot{V}O_2$ in anemic patients. ($r = 0.702$, $P < 0.01$).

Other analyses	17	N/A
Discussion		
Key results	18	Anemia had a negative impact on gas exchange and impaired exercise tolerance in severe COPD patients.
Limitations	19	The proportion of female patients was small. Moreover, the present data were derived from patients with severe (GOLD stage III) COPD and thus cannot be applied to the overall population of COPD patients.
Interpretation	20	<p>The results strongly indicate that oxygen carrying capacity may depend upon the level of haemoglobin for the anemic patients and oxygen delivery is crucial for the maintenance of oxidative metabolism. Previous study showed that in normal individuals, 15 g/dl of Hb carry approximately 21 ml of oxygen per 100 ml of blood and a 3g/dl decrease in Hb levels would result in a reduction of the total oxygen-carrying capacity by 4/100 ml. Moreover, anaemia may result in limited oxygen supply with the consequent increase in ventilatory drive. Among patients with COPD whose ventilatory reserve were decreased, the accompanying increased ventilatory demand may result in dyspnoea.</p> <p>The significantly lower prevalence of COPD in females is the possible explanations for the small proportion of female patients. The reason why we choose severe (GOLD stage III) COPD patients as our object of study is that patients with anaemia had a tendency to have more severe COPD, while very severe COPD patients may not manage to complete CPET. Our results corroborate with the study by Boutou et al, while contradicts the finding by Rutten et al. The reason is that the patient profiles in our study is fundamentally different form that of Rutten et al as we have taken a lower Hb cut off value to define anemia.</p>
Generalisability	21	The results indicated that both the function of gas exchange and exercise capacity decreased in the severe COPD patients with anaemia compared with the patients without anaemia, while the influence of anaemia on pulmonary ventilation function and ventilatory efficiency was little. Also applicability may be limited as we used a population specific criterion to define anemia. Nevertheless the gas exchange results from this study in such patient groups (stable severe anemic COPD, GOLD III) can serve as a reference for future investigators.
Other information		
Funding	22	No external funding was used for this study. Also all authors declare no financial or other conflicting interests.