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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 (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 (FEV_1), FEV_1%pred, FEV_1/ 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 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.

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 criteria[16]. 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% ≤ FEV1 ≤ 50% predicted) underwent peripheral venous blood sampling to determine full blood count, liver transaminase, total protein (albumin and globulin), thyroid hormone, serum levels of

urea and creatinine. Patients who were subsequently presented with abnormal thyroid hormone values (either high or low), glomerular filtration rate (GFR) below 60mL/min[17] and increased liver enzymes twice the upper limit of normal were excluded. The included patients in the study visited the outpatient clinic within two weeks from the initial visit and performed CPET.

Arterial blood gas analysis and resting pulmonary function measurements

ABG measurements such as PH, partial pressure of oxygen (PaO_2), partial pressure of carbon dioxide ($PaCO_2$) and arterial oxygen saturation (SaO_2) were taken just after 10 minutes of rest. Each subject underwent resting PFT of forced vital capacity (FVC), forced expiratory volume in 1second (FEV_1), inspiratory capacity (IC), diffusing capacity for carbon monoxide (DL_{CO}) corrected by Hb, residual volume (RV) and total lung capacity (TLC) using standard methodology12 and equipment (Jaeger Corp., Hoechberg, Germany). All resting lung function values were reported in absolute terms and normalized to percent of predicted (% pred). Predicted spirometry values were calculated using accepted equations for Chinese adults.[18]

Cardiopulmonary exercise test measurements

All the patients and controls performed on a cycle ergometer using a breath-by-breath system according to the American Thoracic Society/American College of Chest Physicians Statement on CPET.[15] The equipment was calibrated in accordance with manufacturer's specifications using reference and calibration gases before each test. Standard 12 lead electrocardiograms (ECGs) and pulse oximetry were continuously monitored. Arterial blood pressure (BP) was measured every two minutes with an automatic cuff. The protocol was comprised of three minutes of rest, three minutes of unloaded cycling at 55-65 revolutions per minute (rpm), followed by a progressively increasing work rate of 5 to 15 watts (W)/min for PH patients, 20 to 25 W/min for the normal subjects to the maximum tolerance, and four minutes of recovery.[18]

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

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± 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 ± 0.8g/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, DLCO 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 threshod (AT) may not be determined by V-slope method[21] 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 ($\Delta\dot{V}O_2$ /ΔWR 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 FEV_1, FEV_1% pred, FEV_1/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.

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

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.

References Section:

1.Antonelli-Incalzi R., Fuso L., De Rosa M. et al. Co-morbidity con- tributes to predict mortality of patients with chronic obstructive pulmonary disease. Eur. Respir. J. 1997; 10:2794–2800.

2.Zielinski J. Effects of long-term oxygen therapy in patients with chronic obstructive pulmonary disease. Curr Opin Pulm Med 1999; 5: 81–87.

3.John M, Hoernig S, Doehner W, Okonko DD, Witt C, Anker SD. Anemia and inflammation in COPD. Chest 2005; 127: 825–829.

4.Chambellan A, Chailleux E, Similowski T, ANTADIR Observatory Group. Prognostic value of hematocrit in patients with severe COPD receiving long-term oxygen therapy. Chest 2005; 128: 1201–1208.

5. John M, Lange A, Hoernig S, Witt C, Anker SD: Prevalence of anemia in chronic obstructive pulmonary disease: comparison to other chronic diseases. Int J Cardiol 2006, 111:365–370.

6. Martinez FJ, Foster G, Curtis JL, Criner G, Weinmann G, Fishman A, DeCamp MM, Benditt J, Sciurba F, Make B, Mohsenifar Z, Diaz P, Hoffman E, Wise R, NETT Research Group: Predictors of mortality in patients with emphysema and severe airflow obstruction. Am J Resp Crit Care Med 2006, 173:1326–1334.

7. Shorr AF, Doyle J, Stern L, Dolgister M, Zilberberg MD: Anemia in chronic pulmonary disease: epidemiology and economic implications. Curr Med Res Opin 2008, 24:1123–1130.

8. Barnes PJ, Celli BR: Systemic manifestations and comorbidities of COPD.Eur Resp J 2009, 33:1165–1185.

9. Cosio MG, Saetta M, Agusti A: Immunologic aspects of chronic obstructive pulmonary disease. NEJM 2009, 360:2445–2454.

10. Kollert F, Müller C, Tippelt A, Jörres RA, Heidinger D, Probst C, Pfeifer M,Budweiser S: Anaemia in chronic respiratory failure. Int J Clin Pract 2011,65:479–486.

11. Kollert F, Tippelt A, Müller C, Jörres RA, Porzelius C, Pfeifer M, Budweiser S:Hemoglobin levels above anemia thresholds are maximally predictivefor long-term survival in COPD with chronic respiratory failure. RespirCare 2013 Jul;58(7):1204-12

12. Boutou AK, Karrar S, Hopkinson NS, Polkey MI: Anemia and survival in chronic obstructive pulmonary disease: a dichotomous rather than a continuous predictor. Respiration 2013, 85:126–131.

13. Ferrazza AM, Martolini D, Valli G, Palange P: Cardiopulmonary exercise testing in the functional and prognostic evaluation of patients with pulmonary diseases. Respiration 2009; 77: 3–17.

14. Spirometry for Health Care Providers: Quick Guide. Global Initiative for Chronic Obstructive Lung Disease (GOLD). http://www.goldcopd.org. Accessed February 1, 2013.

15, ATS/ACCP: Statement on cardiopulmonary exercise testing. Am J Respir Crit Care Med 2003; 167: 211–277.

16. Wenbin C, Youchi W, Hongda W: Diagnostics. 5th edition. Peking: People's Health Publishing House; 2002

17.Hsu CY, McCulloch CE, Curhan GC. Epidemiology of anaemia associated with chronic renal insufficiency among adults in the United States: results from the Triad National Health and Nutrition Examination Survey. J Am Soc Nephrol.2002;13:504–10.

18. Mu K, Liu S (1990) Summary of Chinese pulmonary function normal values. Beijing Medical University and Peking Union Medical College Press 2:83-86.

19. Wasserman K, Hansen JE, Sue DY, Stringer WW, Whipp BJ (2011) Principles of Exercise Testing and Interpretation, 5th ed. Philadelphia: Lippincott, Williams & Wilkins 568.

20. Sun XG, Hansen JE, Garatachea N, Storer TW, Wasserman K (2002) Ventilatory efficiency during exercise in healthy subjects. Am J Respir Crit Care Med 166:1443-1448.

21 Beaver WL, Wasserman K, WHIPP BJ, et al. A new method for detecting anaerobic threshold by gas exchange. J Appl Physiol. 1986;60:2020-2027.

22.Barnes PJ, Celli BR. Systemic manifestations and comorbidities of COPD. Eur Respir J. 2009;33:1165–85.

23.Weiss G, Goodnough LT. Anemia of chronic disease. N Engl J Med. 2005;352:1011–23.

24. Tassiopoulos S, Kontos A, Konstantopoulos K, Hadzistavrou C, Vaiopoulos G, Aessopos A, et al. Erythropoietic response to hypoxaemia in diffuse idiopathic pulmonary fibrosis, as opposed to chronic obstructive pulmonary disease. Respir Med. 2001;95:471–5.

25. John M, Hoernig S, Doehner W, Okonko DD, Witt C, Anker SD. Anemia and inflammation in COPD. Chest 2005;127: 825–829.

26.Lim VS, DeGowin RL, Zavala D, et al. Recombinant human erythropoietin treatment in pre-dialysis patients. A double-blind placebo-controlled trial. Ann Intern Med 1989; 110: 108–114.

27 Dudgeon DJ, Lertzman M, Askew GR. Physiological changes and clinical correlations of dyspnea in cancer outpatients. J Pain Symptom Manage 2001; 21: 373–379.

28 Mancini DM, Katz SD, Lang CC, LaManca J, Hudaihed A, Androne AS. Effect of

erythropoietin on exercise capacity in patients with moderate to severe chronic heart failure. Circulation 2003; 107: 294–299.

29. Cote C., Zilberberg M.D., Mody S.H., Dordelly L.J., Celli B. Ha- emoglobin level and its clinical impact in a cohort of patients with COPD. Eur. Respir. J. 2007; 29: 923–929.

30 Wasserman K. Cardiopulmonary Exercise Testing and Cardiovascular Health . Armonk, NY: Future. 2002.

31 Sun XG , Hansen JE, Ting H, et al. Comparison of exercise cardiac output by the Fick principle using oxygen and carbon dioxide. Chest. 2000;118:631-640.

32 Stringer WW, Hansen JE, Wasserman K. Cardiac output estimated noninvasively from oxygen uptake during exercise. J Appl Physiol. 1997;82:908–912.

33. Rehabilitation and Prevention. Official document on cardiop ul-monary exercise testing in chronic hear t failure due to left ventricular dysfunction recommen dations for per formance and interpretation. Monaldi Arch Che st Dis 2007; 68:6–12.

34. Culver BH: Physiology; in Albert RK, Spiro SG, Jett JR (eds): Comprehensive Respiratory Medicine, ed 1. St Louis, Mosby Inc, 1999, pp89–150.

35 Watz H, Waschki B, Boehme C, Claussen M, Meyer T, Magnussen H. Extrapulmonary effects of chronic obstructive pulmonary disease on physical activity. Am J Respir Crit Care Med. 2008;177:743–51.

36 Boutou AK, Stanopoulos I, Pitsiou GG, Kontakiotis T, Kyriazis G, Sichletidis L, et al. Anemia of chronic disease in chronic obstructive pulmonary disease. Respiration. 2011;82:237–45.

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

PaO_2= oxygen partial pressure; $PaCO_2$ = 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*

FEV_1, 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

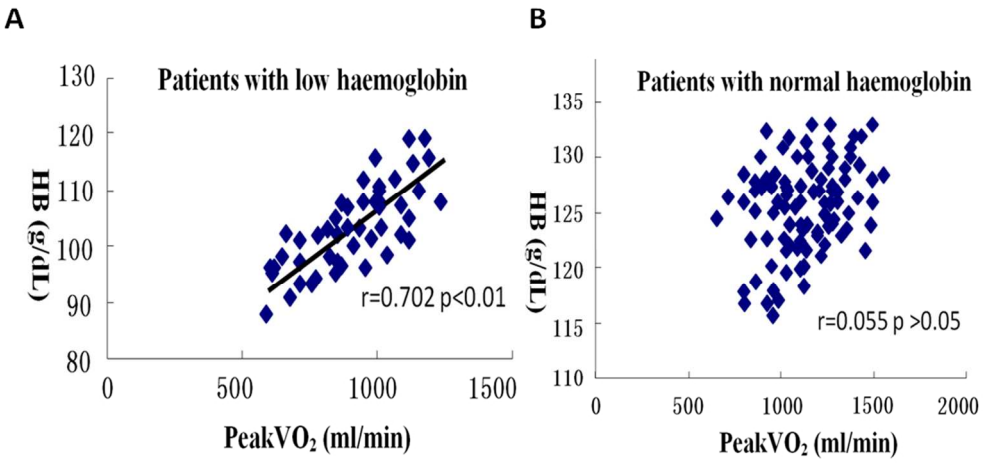
Variable	Patients with low haemoglobin concentration	Patients with normal haemoglobin concentration
PeakLoad (W)	66.36±16.62*	69.46±28.18*
Peak $\dot{V}O_2$ (ml/min)	866.28±274.32**	1180.42±319.18**
Peak $\dot{V}O_2$ (%pred)	56.21±14.79**	69.11±18.72**
Peak $\dot{V}O_2$/Kg (mL/min/kg)	14.88±3.92*	18.72±3.86*
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

%pred = percent of predicted; $\dot{V}O2$ = peak oxygen uptake; $\dot{V}E$ = minute ventilation; $\dot{V}CO2$ = carbon dioxide output; $\Delta O2/\Delta$WR slope =the ratio of $\dot{V}O2$ 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

Figure 1



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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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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: 157 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=48)] 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 (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 (FEV_1), FEV_1%pred, FEV_1/ 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 ($\dot{V}O_2$), Peak $\dot{V}O_2$%pred, Peak $\dot{V}O_2$/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 $\dot{V}E$/ carbon dioxide output ($\dot{V}CO_2$), Peak dead space/tidal volume ratio (VD/VT) were similar between the two groups (P>0.05). (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).

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.

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 criteria[16]. 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% ≤ FEV1 ≤ 50% predicted) underwent peripheral venous blood sampling to determine full blood count, liver transaminase, total protein (albumin and globulin), thyroid hormone, serum levels of

urea and creatinine. Patients who were subsequently presented with abnormal thyroid hormone values (either high or low), glomerular filtration rate (GFR) below 60mL/min[17] and increased liver enzymes twice the upper limit of normal were excluded. The included patients in the study visited the outpatient clinic within two weeks from the initial visit and performed CPET.

Arterial blood gas analysis and resting pulmonary function measurements

ABG measurements such as PH, partial pressure of oxygen (PaO_2), partial pressure of carbon dioxide ($PaCO_2$) and arterial oxygen saturation (SaO_2) were taken just after 10 minutes of rest. Each subject underwent resting PFT of forced vital capacity (FVC), forced expiratory volume in 1second (FEV_1), inspiratory capacity (IC), diffusing capacity for carbon monoxide (DL_{CO}) corrected by Hb, residual volume (RV) and total lung capacity (TLC) using standard methodology12 and equipment (Jaeger Corp., Hoechberg, Germany). All resting lung function values were reported in absolute terms and normalized to percent of predicted (% pred). Predicted spirometry values were calculated using accepted equations for Chinese adults.[18]

Cardiopulmonary exercise test measurements

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

Most CPET values were reported in absolute terms and normalized to percentage of predicted (% pred). Predicted values were calculated using accepted equations.[19] 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± 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-seven 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 ± 0.8g/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 threshod (AT) may not be determined by V-slope method[21] 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 ($\Delta\dot{V}O_2$ /ΔWR 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

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 FEV_1, FEV_1% pred, FEV_1/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,[22-24-] 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.[25] 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 in Chinese population.

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[20,26-28], $\dot{V}O_2$ = 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.[29] 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, which corroborate with the study by Boutou et al.[30] However, we have just included subjects with GOLD III rather than theirs which included other groups as well. This study also differs in the fact that we have a larger sample size and the anemia cut off values were pertinent to the Chinese population rather than WHO defined general cut off values for anemia. The study by Rutten et al showed the 6MWD and the BODE score were not different among the patients with and without anemia which contradicts our finding.[31] However, 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. Nevertheless the patients in our study have much lower Hb levels on average indicating the degree of anemia was more severe.

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

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.[32] 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.

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.[33,34] These mediators increase in patients with COPD and could be regarded as factors related to the onset of anaemia.[35] 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.[36]

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[30,37], 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.

References Section:

1.Antonelli-Incalzi R., Fuso L., De Rosa M. et al. Co-morbidity con- tributes to predict mortality of patients with chronic obstructive pulmonary disease. Eur. Respir. J. 1997; 10:2794–2800.

2.Zielinski J. Effects of long-term oxygen therapy in patients with chronic obstructive pulmonary disease. Curr Opin Pulm Med 1999; 5: 81–87.

3.John M, Hoernig S, Doehner W, Okonko DD, Witt C, Anker SD. Anemia and inflammation in COPD. Chest 2005; 127: 825–829.

4.Chambellan A, Chailleux E, Similowski T, ANTADIR Observatory Group. Prognostic value of hematocrit in patients with severe COPD receiving long-term oxygen therapy. Chest 2005; 128: 1201–1208.

5. John M, Lange A, Hoernig S, Witt C, Anker SD: Prevalence of anemia in chronic obstructive pulmonary disease: comparison to other chronic diseases. Int J Cardiol 2006, 111:365–370.

6. Martinez FJ, Foster G, Curtis JL, Criner G, Weinmann G, Fishman A, DeCamp MM, Benditt J, Sciurba F, Make B, Mohsenifar Z, Diaz P, Hoffman E, Wise R, NETT Research Group: Predictors of mortality in patients with emphysema and severe airflow obstruction. Am J Resp Crit Care Med 2006, 173:1326–1334.

7. Shorr AF, Doyle J, Stern L, Dolgister M, Zilberberg MD: Anemia in chronic pulmonary disease: epidemiology and economic implications. Curr Med Res Opin 2008, 24:1123–1130.

8. Barnes PJ, Celli BR: Systemic manifestations and comorbidities of COPD.Eur Resp J 2009, 33:1165–1185.

9. Cosio MG, Saetta M, Agusti A: Immunologic aspects of chronic obstructive pulmonary disease. NEJM 2009, 360:2445–2454.

10. Kollert F, Müller C, Tippelt A, Jörres RA, Heidinger D, Probst C, Pfeifer M,Budweiser S:

Anaemia in chronic respiratory failure. Int J Clin Pract 2011,65:479–486.

11. Kollert F, Tippelt A, Müller C, Jörres RA, Porzelius C, Pfeifer M, Budweiser S:Hemoglobin levels above anemia thresholds are maximally predictivefor long-term survival in COPD with chronic respiratory failure. RespirCare 2013 Jul;58(7):1204-12

12. Boutou AK, Karrar S, Hopkinson NS, Polkey MI: Anemia and survival in chronic obstructive pulmonary disease: a dichotomous rather than a continuous predictor. Respiration 2013, 85:126–131.

13. Ferrazza AM, Martolini D, Valli G, Palange P: Cardiopulmonary exercise testing in the functional and prognostic evaluation of patients with pulmonary diseases. Respiration 2009; 77: 3–17.

14. Spirometry for Health Care Providers: Quick Guide. Global Initiative for Chronic Obstructive Lung Disease (GOLD). http://www.goldcopd.org. Accessed February 1, 2013.

15, ATS/ACCP: Statement on cardiopulmonary exercise testing. Am J Respir Crit Care Med 2003; 167: 211–277.

16. Wenbin C, Youchi W, Hongda W: Diagnostics. 5th edition. Peking: People's Health Publishing House; 2002

17.Hsu CY, McCulloch CE, Curhan GC. Epidemiology of anaemia associated with chronic renal insufficiency among adults in the United States: results from the Triad National Health and Nutrition Examination Survey. J Am Soc Nephrol.2002;13:504–10.

18. Mu K, Liu S (1990) Summary of Chinese pulmonary function normal values. Beijing Medical University and Peking Union Medical College Press 2:83-86.

19. Wasserman K, Hansen JE, Sue DY, Stringer WW, Whipp BJ (2011) Principles of Exercise Testing and Interpretation, 5th ed. Philadelphia: Lippincott, Williams & Wilkins 568.

20. Sun XG, Hansen JE, Garatachea N, Storer TW, Wasserman K (2002) Ventilatory efficiency during exercise in healthy subjects. Am J Respir Crit Care Med 166:1443-1448.

21 Beaver WL, Wasserman K, WHIPP BJ, et al. A new method for detecting anaerobic threshold by gas exchange. J Appl Physiol. 1986;60:2020-2027.

22.Lim VS, DeGowin RL, Zavala D, et al. Recombinant human erythropoietin treatment in pre-dialysis patients. A double-blind placebo-controlled trial. Ann Intern Med 1989; 110: 108–114.

23 Dudgeon DJ, Lertzman M, Askew GR. Physiological changes and clinical correlations of

dyspnea in cancer outpatients. J Pain Symptom Manage 2001; 21: 373–379.

24 Mancini DM, Katz SD, Lang CC, LaManca J, Hudaihed A, Androne AS. Effect of erythropoietin on exercise capacity in patients with moderate to severe chronic heart failure. Circulation 2003; 107: 294–299.

25. Cote C., Zilberberg M.D., Mody S.H., Dordelly L.J., Celli B. Ha- emoglobin level and its clinical impact in a cohort of patients with COPD. Eur. Respir. J. 2007; 29: 923–929.

26 Wasserman K. Cardiopulmonary Exercise Testing and Cardiovascular Health . Armonk, NY: Future. 2002.

27 Sun XG , Hansen JE, Ting H, et al. Comparison of exercise cardiac output by the Fick principle using oxygen and carbon dioxide. Chest. 2000;118:631-640.

28 Stringer WW, Hansen JE, Wasserman K. Cardiac output estimated noninvasively from oxygen uptake during exercise. J Appl Physiol. 1997;82:908–912.

29. Rehabilitation and Prevention. Official document on cardiop ul-monary exercise testing in chronic hear t failure due to left ventricular dysfunction recommen dations for per formance and interpretation. Monaldi Arch Che st Dis 2007; 68:6–12.

30. Boutou AK, Stanopoulos I, Pitsiou GG, Kontakiotis T, Kyriazis G, Sichletidis L, et al. Anemia of Chronic Disease in Chronic Obstructive Pulmonary Disease: A Case-Control Study of Cardiopulmonary Exercise Responses. Respiration 2011;82:237–245

31. Erica P.A. Rutten, Frits M.E. Franssen, Martijn A. Spruit, Emiel F.M. Wouters Anemia is Associated with Bone Mineral Density in Chronic Obstructive Pulmonary Disease COPD 2013 Jun;10(3):286–92,

32. Culver BH: Physiology; in Albert RK, Spiro SG, Jett JR (eds): Comprehensive Respiratory Medicine, ed 1. St Louis, Mosby Inc, 1999, pp89–150.

33.Barnes PJ, Celli BR. Systemic manifestations and comorbidities of COPD. Eur Respir J. 2009;33:1165–85.

34.Weiss G, Goodnough LT. Anemia of chronic disease. N Engl J Med. 2005;352:1011–23.

35. Tassiopoulos S, Kontos A, Konstantopoulos K, Hadzistavrou C, Vaiopoulos G, Aessopos A, et al. Erythropoietic response to hypoxaemia in diffuse idiopathic pulmonary fibrosis, as opposed to chronic obstructive pulmonary disease. Respir Med. 2001;95:471–5.

36. John M, Hoernig S, Doehner W, Okonko DD, Witt C, Anker SD. Anemia and inflammation in COPD. Chest 2005;127: 825–829.

37 Watz H, Waschki B, Boehme C, Claussen M, Meyer T, Magnussen H. Extrapulmonary effects of chronic obstructive pulmonary disease on physical activity. Am J Respir Crit Care Med. 2008;177:743–51.

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 = 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

PaO_2= oxygen partial pressure; $PaCO_2$ = carbon dioxide partial pressure； 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

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*

FEV_1, 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
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

%pred = percent of predicted; $\dot{V}O2$ = peak oxygen uptake; $\dot{V}E$ = minute ventilation; $\dot{V}CO2$ = carbon dioxide output; $\Delta O2/\Delta$WR slope =the ratio of $\dot{V}O2$ 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

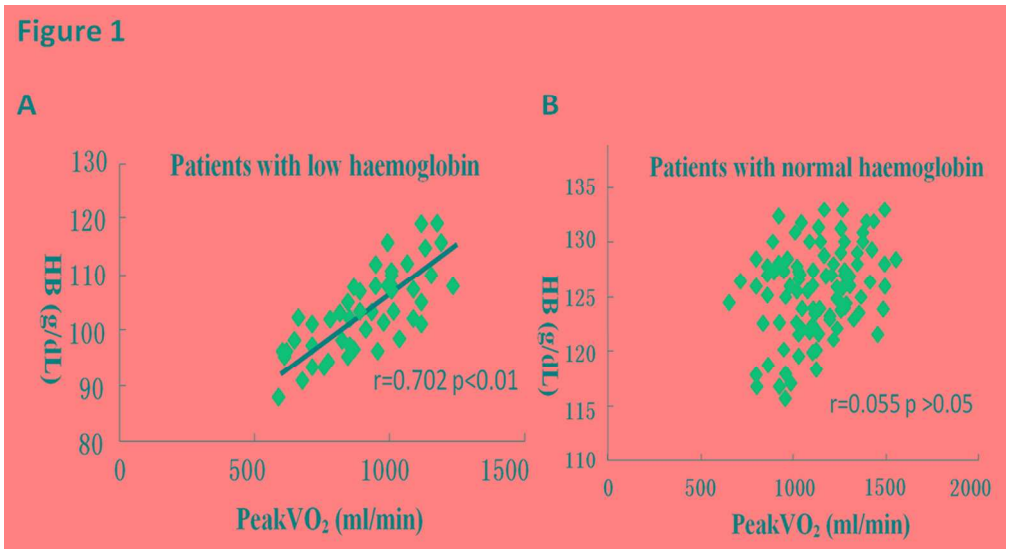

Figure 1A and 1B show the correlation between Peak V. O2 and Hb concentration in COPD patients with and without anaemia respectively.
Significant positive correlation was found between Hb concentration and Peak V. O2 in anemic patients (r=0.702, P<0.01) (Figure1A), but no statistical significant correlation was found with nonanemic patients (r=0.055, P >0.05) (Figure1B).

304x165mm (300 x 300 DPI)

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	*Cohort study*— 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% ≤ FEV1 ≤ 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 co-morbidities 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 $\chi 2$ 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 (VO2), Peak VO2%pred, Peak VO2/Kg, Peak O2 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 ($P>0.05$). (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	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. 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.
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.

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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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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: 157 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=48)] 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 (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 (FEV_1), FEV_1%pred, FEV_1/ 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 ($\dot{V}O_2$), Peak $\dot{V}O_2$%pred, Peak $\dot{V}O_2$/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 $\dot{V}E$/ carbon dioxide output ($\dot{V}CO_2$), Peak dead space/tidal volume ratio (VD/VT) were similar between the two groups (P>0.05). (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).

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. The present study is the first to

investigate the impact of anaemia in COPD patients utilizing CPET in a Chinese population. 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 criteria[16]. 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% ≤ FEV1 ≤ 50% predicted) underwent peripheral venous blood sampling to determine full blood count, liver

transaminase, total protein (albumin and globulin), thyroid hormone, serum levels of urea and creatinine. Patients who were subsequently presented with abnormal thyroid hormone values (either high or low), glomerular filtration rate (GFR) below 60mL/min[17] and increased liver enzymes twice the upper limit of normal were excluded. The included patients in the study visited the outpatient clinic within two weeks from the initial visit and performed CPET.

Arterial blood gas analysis and resting pulmonary function measurements

ABG measurements such as PH, partial pressure of oxygen (PaO_2), partial pressure of carbon dioxide ($PaCO_2$) and arterial oxygen saturation (SaO_2) were taken just after 10 minutes of rest. Each subject underwent resting PFT of forced vital capacity (FVC), forced expiratory volume in 1second (FEV_1), inspiratory capacity (IC), diffusing capacity for carbon monoxide (DLco) corrected by Hb, residual volume (RV) and total lung capacity (TLC) using standard methodology12 and equipment (Jaeger Corp., Hoechberg, Germany). All resting lung function values were reported in absolute terms and normalized to percent of predicted (% pred). Predicted spirometry values were calculated using accepted equations for Chinese adults.[18]

Cardiopulmonary exercise test measurements

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

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

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± 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-seven 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 laboratory 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 ± 0.8g/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, DLCO 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 threshod (AT) may not be determined by V-slope method[21] 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 ($\Delta\dot{V}O_2$ /ΔWR 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

In the current study, anaemia was present in 31% of severe COPD patients. The prevalence of anaemia was higher than in patients from previous studies [3-12], possibly as the study population comprised of patients with more severe COPD. 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 FEV_1, FEV_1% pred, FEV_1/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. Studies examining the effect of just anemia or anemia combined with disorder of the alveolocapillary membrane consistently mention that anemia does affect the diffusion capacity but the defect of the respiratory membrane on diffusing capacity is greater. [22-23] In the present study we have patients with anemia and additionally defect with the respiratory membrane as is common in COPD, both contributing to the reduced DLCO as noted. The initial poor correlation with blood gases in this study we think is because the compensatory mechanisms are not yet fully dysfunctional and the anemia not severe. Also all patients in the study were clinically stable and under adequate medication.

Although anaemia has been associated with dyspnoea and reduced exercise tolerance in patients with chronic kidney disease, heart failure and cancer,[24-26] 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.[27]

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 in Chinese population.

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[20,28-30], $\dot{V}O_2$ = 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.[31] 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, which corroborate with the study by Boutou et al.[32] However, we have just included subjects with GOLD III rather than theirs which included other groups as well. This study also differs in the fact that we have a larger sample size and the anemia cut off values were pertinent to the Chinese population rather than WHO defined general cut off values for anemia. The study by Rutten et al showed the 6MWD and the BODE score were not different among the patients with and without anemia which contradicts our finding.[33] However, 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. Nevertheless the patients in our study have much lower Hb levels on average indicating the degree of anemia was

more severe. 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 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[32,35], 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.

References Section:

1.Antonelli-Incalzi R., Fuso L., De Rosa M. et al. Co-morbidity con- tributes to predict mortality of patients with chronic obstructive pulmonary disease. Eur. Respir. J. 1997; 10:2794–2800.

2.Zielinski J. Effects of long-term oxygen therapy in patients with chronic obstructive pulmonary disease. Curr Opin Pulm Med 1999; 5: 81–87.

3.John M, Hoernig S, Doehner W, Okonko DD, Witt C, Anker SD. Anemia and inflammation in COPD. Chest 2005; 127: 825–829.

4.Chambellan A, Chailleux E, Similowski T, ANTADIR Observatory Group. Prognostic value of hematocrit in patients with severe COPD receiving long-term oxygen therapy. Chest 2005; 128: 1201–1208.

5. John M, Lange A, Hoernig S, Witt C, Anker SD: Prevalence of anemia in chronic obstructive pulmonary disease: comparison to other chronic diseases. Int J Cardiol 2006, 111:365–370.

6. Martinez FJ, Foster G, Curtis JL, Criner G, Weinmann G, Fishman A, DeCamp MM, Benditt J, Sciurba F, Make B, Mohsenifar Z, Diaz P, Hoffman E, Wise R, NETT Research Group: Predictors of mortality in patients with emphysema and severe airflow obstruction. Am J Resp Crit Care Med 2006, 173:1326–1334.

7. Shorr AF, Doyle J, Stern L, Dolgister M, Zilberberg MD: Anemia in chronic pulmonary disease: epidemiology and economic implications. Curr Med Res Opin 2008, 24:1123–1130.

8. Barnes PJ, Celli BR: Systemic manifestations and comorbidities of COPD.Eur Resp J 2009, 33:1165–1185.

9. Cosio MG, Saetta M, Agusti A: Immunologic aspects of chronic obstructive pulmonary disease. NEJM 2009, 360:2445–2454.

10. Kollert F, Müller C, Tippelt A, Jörres RA, Heidinger D, Probst C, Pfeifer M,Budweiser S: Anaemia in chronic respiratory failure. Int J Clin Pract 2011,65:479–486.

11. Kollert F, Tippelt A, Müller C, Jörres RA, Porzelius C, Pfeifer M, Budweiser S:Hemoglobin levels above anemia thresholds are maximally predictivefor long-term survival in COPD with chronic respiratory failure. RespirCare 2013 Jul;58(7):1204-12

12. Boutou AK, Karrar S, Hopkinson NS, Polkey MI: Anemia and survival in chronic obstructive pulmonary disease: a dichotomous rather than a continuous predictor. Respiration 2013,

85:126–131.

13. Ferrazza AM, Martolini D, Valli G, Palange P: Cardiopulmonary exercise testing in the functional and prognostic evaluation of patients with pulmonary diseases. Respiration 2009; 77: 3–17.

14. Spirometry for Health Care Providers: Quick Guide. Global Initiative for Chronic Obstructive Lung Disease (GOLD). http://www.goldcopd.org. Accessed February 1, 2013.

15, ATS/ACCP: Statement on cardiopulmonary exercise testing. Am J Respir Crit Care Med 2003; 167: 211–277.

16. Wenbin C, Youchi W, Hongda W: Diagnostics. 5th edition. Peking: People's Health Publishing House; 2002

17.Hsu CY, McCulloch CE, Curhan GC. Epidemiology of anaemia associated with chronic renal insufficiency among adults in the United States: results from the Triad National Health and Nutrition Examination Survey. J Am Soc Nephrol.2002;13:504–10.

18. Mu K, Liu S (1990) Summary of Chinese pulmonary function normal values. Beijing Medical University and Peking Union Medical College Press 2:83-86.

19. Wasserman K, Hansen JE, Sue DY, Stringer WW, Whipp BJ (2011) Principles of Exercise Testing and Interpretation, 5th ed. Philadelphia: Lippincott, Williams & Wilkins 568.

20. Sun XG, Hansen JE, Garatachea N, Storer TW, Wasserman K (2002) Ventilatory efficiency during exercise in healthy subjects. Am J Respir Crit Care Med 166:1443-1448.

21 Beaver WL, Wasserman K, WHIPP BJ, et al. A new method for detecting anaerobic threshold by gas exchange. J Appl Physiol. 1986;60:2020-2027.

22 Maras-Simunić M1, Tocilj J, Simunić M. The effect of hemoglobin levels in the blood in pulmonary diffusing capacity Lijec Vjesn. 1994 Jan-Feb;116(1-2):22-5.

23 Petermann W. Effect of low hemoglobin levels on the diffusing capacity of the lungs for CO. Investigations in persons with healthy lungs and in patients with interstitial pulmonary fibrosis. Respiration. 1985;47(1):30-8.

24.Lim VS, DeGowin RL, Zavala D, et al. Recombinant human erythropoietin treatment in pre-dialysis patients. A double-blind placebo-controlled trial. Ann Intern Med 1989; 110: 108–114.

25 Dudgeon DJ, Lertzman M, Askew GR. Physiological changes and clinical correlations of dyspnea in cancer outpatients. J Pain Symptom Manage 2001; 21: 373–379.

26 Mancini DM, Katz SD, Lang CC, LaManca J, Hudaihed A, Androne AS. Effect of

erythropoietin on exercise capacity in patients with moderate to severe chronic heart failure. Circulation 2003; 107: 294–299.

27. Cote C., Zilberberg M.D., Mody S.H., Dordelly L.J., Celli B. Ha- emoglobin level and its clinical impact in a cohort of patients with COPD. Eur. Respir. J. 2007; 29: 923–929.

28 Wasserman K. Cardiopulmonary Exercise Testing and Cardiovascular Health . Armonk, NY: Future. 2002.

29 Sun XG , Hansen JE, Ting H, et al. Comparison of exercise cardiac output by the Fick principle using oxygen and carbon dioxide. Chest. 2000;118:631-640.

30 Stringer WW, Hansen JE, Wasserman K. Cardiac output estimated noninvasively from oxygen uptake during exercise. J Appl Physiol. 1997;82:908–912.

31. Rehabilitation and Prevention. Official document on cardiop ul-monary exercise testing in chronic hear t failure due to left ventricular dysfunction recommen dations for per formance and interpretation. Monaldi Arch Che st Dis 2007; 68:6–12.

32. Boutou AK, Stanopoulos I, Pitsiou GG, Kontakiotis T, Kyriazis G, Sichletidis L, et al. Anemia of chronic disease in chronic obstructive pulmonary disease: A Case-Control Study of Cardiopulmonary Exercise Responses. Respiration 2011;82:237–245

33. Erica P.A. Rutten, Frits M.E. Franssen, Martijn A. Spruit, Emiel F.M. Wouters Anemia is Associated with Bone Mineral Density in Chronic Obstructive Pulmonary Disease COPD 2013 Jun;10(3):286–92,

34. Culver BH: Physiology; in Albert RK, Spiro SG, Jett JR (eds): Comprehensive Respiratory Medicine, ed 1. St Louis, Mosby Inc, 1999, pp89–150.

35 Watz H, Waschki B, Boehme C, Claussen M, Meyer T, Magnussen H. Extrapulmonary effects of chronic obstructive pulmonary disease on physical activity. Am J Respir Crit Care Med. 2008;177:743–51.

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 = 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

PaO_2= oxygen partial pressure; $PaCO_2$ = carbon dioxide partial pressure；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

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 (%)	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*

FEV_1, 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
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

%pred = percent of predicted; $\dot{V}O2$ = peak oxygen uptake; $\dot{V}E$ = minute ventilation; $\dot{V}CO2$ = carbon dioxide output; $\Delta O2/\Delta$WR slope =the ratio of $\dot{V}O2$ 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

Figure 1

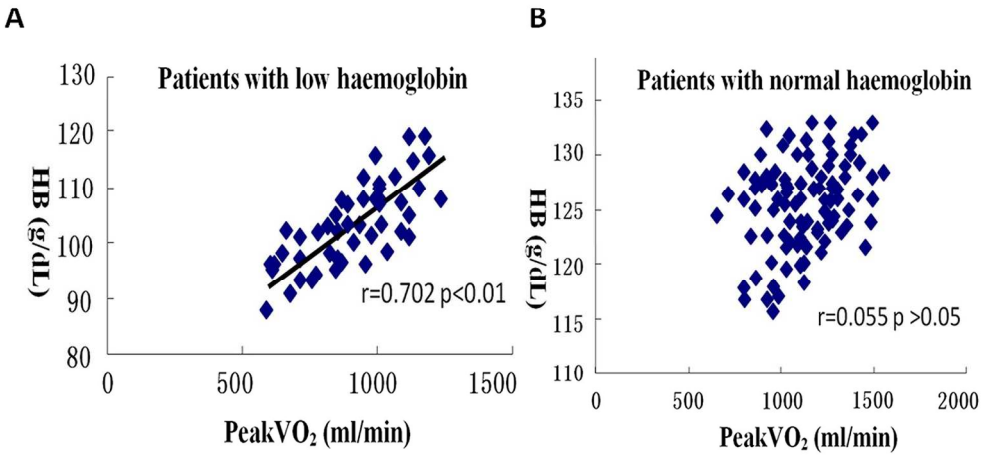

Figure 1A and 1B show the correlation between Peak V. O2 and Hb concentration in COPD patients with and without anaemia respectively.
Significant positive correlation was found between Hb concentration and Peak V. O2 in anemic patients (r=0.702, P<0.01) (Figure1A), but no statistical significant correlation was found with nonanemic patients (r=0.055, P >0.05) (Figure1B).

166x90mm (300 x 300 DPI)

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	*Cohort study*— 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% ≤ FEV1 ≤ 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 co-morbidities 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 $\chi 2$ 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 (VO2), Peak VO2%pred, Peak VO2/Kg, Peak O2 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 ($P>0.05$). (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	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. 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.
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.